DRUG DISCOVERY AND DEVELOPMENT

Volume 2: Drug Development

Edited by

MUKUND S. CHORGHADE

WILEY-INTERSCIENCE

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CONTRIBUTORS

Ian R. Baxendale, Department of Chemistry, University of Cambridge, 225 Lensfield Road, Cambridge CB2 1EW, UK

S. Chandrasekhar, Organic Chemistry Division I, Indian Institute of Chemical Technology, Hyderabad 500007, India

Mukund S. Chorghade, Chorghade Enterprises, 14 Carlson Circle, Natick, MA 01760-4205, USA

Mahendra N. Deshpande, OrgSyn Laboratory, Inc., 2201 West Campbell Park Drive, Chicago, IL 60612, USA

Mukund K. Gurjar, National Chemical Laboratory, Pune 411008, India

Palakodety Radha Krishna, D 211 Organic Chemistry Division III, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, India

Shrikant V. Kulkarni, KulTech Incorporated, 1323 Mellon Court, Cary, NC 27511, USA

Yatendra Kumar, Wockhardt Research Centre, D-4, M.I.D.C., Chikalthana, Aurangabad 431210, Maharashtra, India

Ian C. Lennon, Dowpharma, Chirotech Technology Ltd., Unit 162, Cambridge Science Park, Milton Road, Cambridge CB4 0GH, UK

Steven V. Ley, Department of Chemistry, University of Cambridge, 225 Lensfield Road, Cambridge CB2 1EW, UK

Braj B. Lohray, BHUVID Research Laboratory, 302 Pushkar Tower, Judges Bungalow Road, Ahmedabad 380054, Gujarat, India

Vidya B. Lohray, BHUVID Research Laboratory, 302 Pushkar Tower, Judges Bungalow Road, Ahmedabad 380054, Gujarat, India

Deborah A. Longbottom, Department of Chemistry, University of Cambridge, 225 Lensfield Road, Cambridge CB2 1EW, UK

Raymond McCague, Dowpharma, Chirotech Technology Ltd., Unit 162, Cambridge Science Park, Milton Road, Cambridge CB4 0GH, UK; now at McCague Scientific Consulting, 77 The Footpath, Coton, Cambridge CB23 7PX, UK

Rebecca M. Myers, Department of Chemistry, University of Cambridge, 225 Lensfield Road, Cambridge CB2 1EW, UK

Bipin Pandey, Zydus Research Centre, Ahmedabad 382213, Gujarat, India

Richard J. Pariza, Cedarburg Pharmaceuticals, Grafton, WI 53024, USA

Peter Pollak, Wachtweg 3, CH-4153 Reinach, Switzerland

Sreenivas Punna, Indian Institute of Chemical Technology, Hyderabad 500007, India

G. N. Qazi, Indian Institute of Integrative Medicine (CSIR), Canal Road, Jammu Tawi 180001, India

C. V. Ramana, National Chemical Laboratory, Pune 411008, India

Colin Scott, Novacal Pharmaceuticals, Emeryville, CA 94608, USA

G. V. M. Sharma, D 211 Organic Chemistry Division III, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, India

S. C. Taneja, Indian Institute of Integrative Medicine (CSIR), Canal Road, Jammu Tawi 180001, India

Johan Tijhuis, Specs, Delftechpark 30, 2628 XH Delft, The Netherlands

B. Vijayaraghavan, Ranbaxy, Inc., 600 College Road East, Princeton, NJ 08540, USA

Richard L. Wife, SORD BV, Veldsingel 49, NL 6581 TD Malden, The Netherlands

J. S. Yadav, Organic Chemistry Division I, Indian Institute of Chemical Technology, Hyderabad 500007, India

Andrei A. Zlota, The Zlota Company, LLC, 15 Fairbanks Road, Sharon, MA 02067, USA

PREFACE

The pharmaceutical sector has traditionally been a vibrant, innovation-driven, and highly successful component of industry at large. In recent years, a confluence of spectacular advances in chemistry, molecular biology, genomics, and chemical technology and the cognate fields of spectroscopy, chromatography, and crystallography have led to the discovery and development of numerous novel therapeutic agents for the treatment of a wide spectrum of diseases. To facilitate this process, there has been a significant and noticeable effort aimed at improving the integration of discovery technologies, chemical outsourcing for route selection and delivery of active pharmaceutical ingredients, drug product formulations, clinical trials, and refined deployment of information technologies. Multidisciplinary and multifunctional teams focusing on lead generation and optimization have replaced the traditional, specialized research groups. To develop a drug from conception to commercialization, the biotechnology and biopharmaceutical industries (which have been highly entrepreneurial) have reached out and established global strategic partnerships with numerous companies.

Currently there is no single book in the market that provides an overview of strategies, tactics, milestones, and benchmarks in the entire sequence of operations involved in discovering a drug and delivering it to the armamentarium of clinicians and medical practitioners. There is usually a great gulf between the medicinal and process chemists in industry; neither has the opportunity to delve into the disparate literature of the other. This book is designed to bridge this gap and provide greater understanding of the target areas.

This book is not designed to be a treatise or an encyclopedia. Its scope precludes complete coverage of any defined area. Ideally, it is envisioned to be an advanced-level monograph with appeal to active researchers and investigators in the entire gamut of operations comprising the drug discovery and development process. This two-volume text will be useful to a broad community of academic and industrial chemists.

The introductory chapter in the first volume, by Dr. Richard Pariza, delineates all the essential elements that comprise the development process from the initial conception of a program to the successful marketing of a new drug. A timeline for making critical decisions,

conducting pivotal studies, and the approximate duration of different activities is described. The time line helps to put the entire developmental process into perspective for the reader and serves as a conceptual index unifies all contributions.

Professor Paul Erhardt describes the competition in the pharmaceutical industry to be "first to the market" in a chosen therapeutic area and the strategies currently being pursued. These include research in combinatorial chemistry, collaboration with biopharmaceutical and "virtual companies," and strategies in the licensing of drug candidates, among others. The author takes a futuristic look at what medicinal chemistry is expected to be in the new millennium. Dr. Erhardt's insights, gleaned from expertise and experience, constitute a valuable lesson. Professor Lester Mitscher, an internationally renowned academician and expert, and Professor Apurba Dutta take us through the next critical phase of the drug discovery process: detailed studies of the absorption, metabolism, and excretion of potential drug candidates. Such studies are of pivotal importance in determining the suitability of a new compound for further clinical evaluation.

Combinatorial chemistry has played a highly visible role in the drug discovery effort in several companies; numerous new companies have been set up to partner established companies in the discovery of new molecular entities. Dr. Ian Hughes reviews the state of the art with selected examples from his own research at Glaxo Smith Kline. This is followed by an excellent exposition by Drs. Norton Peet and Hwa-Ok Kim regarding efficient design and development of parallel solution-phase synthesis.

Dr. János Fischer and Dr. Anikó Gere delve into the important area of the timing of analog research in medicinal chemistry. This work is a remarkable synthesis of knowledge of drugs and their functional congeners. Professor Camille Wermuth presents fascinating examples of specific new drugs being derived via the functionalization of old drugs. This approach uses the old drugs as new scaffolds and derives benefit from new molecules already having a propensity to be "drug-like."

Drs. Susan Dana Jones and Peter Warren focus on the impact of proteomics on the discovery of drugs: newer methods for efficient, economical, and safer production, and the development of novel targets and assays for the application of traditional medicinal chemistry methods. A brief survey of novel therapeutic concepts such as gene therapy, antisense, transgenic animals, and pharmacogenomics that have opened new vistas in drug development are surveyed. The authors have familiarized readers with several newer biology-based technologies. Next, Professor Paul Erhardt introduces the concept of using drug metabolism databases during the drug discovery and development process.

Professor C. Robin Ganellin exemplifies the discovery of Tagamet using classical structure-activity relationships and modeling of pharmacophore receptors. This drug was the first "billion-dollar drug." The research work by Sir James Black and Robin Ganellin has long been considered to be a tour de force in modern medicinal chemistry.

The art and science of medicinal chemistry is exemplified and epitomized clearly in the next few chapters. The exponents of the art are highly distinguished and prolific industrial researchers whose work spans the gamut of the therapeutic spectrum. Dr. Bruce Maryanoff brilliantly summarizes research into the discovery of potent nonpeptide vasopressin receptor antagonists. Dr. Paul Feldman presents an informative case study on the discovery of Ultiva (remifentanil). This is an ultrashort-acting analgesic used as an adjunct to anesthesia. Dr. Paul Feldman introduces the rationale for its discovery and discusses how remifentanil fits into the anesthesia drug regimen. The desire to discover an ultrashort-acting analgesic, the group's medicinal chemistry efforts, and the structure-activity relationships are discussed. Drs. Karl Grozinger, John Proudfoot, and Karl Hargrave discuss the discovery

and development of nevirapine. This drug was a key ingredient in our efforts to combat AIDS, and the success of the researchers is an object lesson in creativity and how skills were brought to the forefront of research.

Drs. John Babich and William Eckelman present insights into the applications of nuclear imaging in drug discovery and development; the work is technologically complex and involves radiopharmaceuticals.

Drs. Pradeep Dhal, Chad Huval, and Randal Holmes-Farley take the reader into a new and somewhat unexplored area of polymer therapeutics. The exciting idea of using a polymer as an active pharmaceutical ingredient was introduced in the 1990s and led to the discovery of drugs such as Renagel and Welchol. A large-molecular-weight polymer when used as a drug manifests its action in the gastro-intestinal tract by adsorbing and removing unwanted analytes.

Professor Bhushan Patwardhan and his collaborators demonstrate the utility of botanical immunomodulators and chemoprotectants in cancer therapy. Much of this work has its genesis in the Indian medicine systems of ayurveda; this turns pharmacology "on its head." It starts with plant extracts that have been used extensively in medicine in Asia and identifies the active ingredients from a complex mixture of ingredients. There is considerable scientific debate and discussion about whether the active moieties exhibit their pharmacological action in tandem or singly.

Volume 2 builds on the outstanding contributions by the authors of Volume 1. Drs. S. C. Taneja and G. N. Qazi provide a unique perspective on the therapeutic action of bioactive molecules in medicinal plants. Their group has several years of experience in prospecting natural products in plants and following up with the isolation, characterization and structure elucidation of natural products. The traditional medicinal systems such as Indian and Chinese and those used by African tribes are treasure houses of traditional wisdom, and with the help of modern scientific methods they will continue to be the basis of development of new therapeutic agents. These authors discuss comprehensively how this knowledge can be coupled with diversity-oriented synthesis to discover new therapeutic agents.

Professor Steven Ley, BP 1702 Professor of Chemistry, and his collaborators at Cambridge University enlighten readers as to how natural products have served as inspiration for the discovery of new high-throughput chemical synthesis tools. A salient feature of this masterpiece is the creative use of polymer-supported reagents. The natural world inspires us all—from artist and philosopher to biologist and chemist alike. In our quest for new knowledge, the exquisite and varied architectures of natural products provide a rich pallet for discovery. Synthesis chemists are drawn to these structures as testing grounds for synthetic strategies and for the development of new methods. But we are also drawn to advance the art of molecular assembly of some of nature's most enigmatic creations. However, more is accessible to the synthesis chemists' skills, they can modify natural materials to probe structure activity profiles; they can provide fragment molecules or related structural scaffolds through library generation. Only the synthesis chemist can go beyond the molecule, contemplating macromolecular assemblies and creating unnatural arrangements with awe-inspiring levels of molecular diversity, limited only by their imaginations. Described in this chapter is how methods of immobilizing reagents on polymeric supports or using scavenging agents and catch-and-release techniques can impact on natural product synthesis, and thereby create opportunities for structures mimicking naturally-occurring architectures.

Drs. Braj and Vidya Lohray elaborate on the role of insulin sensitizers in emerging therapeutics. A noteworthy feature of this work is that it was done entirely in India and represents a fast-growing trend: the discovery of new chemical entities in that country.

Drs. Raymond McCague and Ian Lennon at Dowpharma next discuss the criteria for industrial readiness of chiral catalysis technology for the synthesis of pharmaceuticals. They exemplify how and why stereoselective reactions are invented for pharmaceutical researchers: The methodology is applicable in both the discovery and development phases of a drug in making analogs rapidly and by scalable transformations. This chapter specifically addresses chiral catalytic technologies. Chiral technology is an important focus given that many synthetic pharmaceutical candidates are chiral, will need to be produced in the single chiral isomer (enantiomer) form and some specialised *chiral* technology will be needed for the synthesis. Catalytic technologies are of particular interest for the superior economics when applied to the manufacture of intermediates to pharmaceutical agents, and particularly suited are methods of biocatalysis and chemocatalysis. Some of these methods find sufficiently frequent application that significant up-front technology investment is warranted, and there are enough projects on which to apply the technologies, to build up a still higher level of expertise.

Dr. Mukund Chorghade then introduces readers to the field of process chemistry: the quest for the elucidation of novel, cost-effective, and scalable routes for production of active pharmaceutical ingredients. The medicinal chemistry routes used in the past have often involved the use of cryogenic reactions, unstable intermediates, and hazardous or expensive reagents. A case study of the development of a process for an antiepileptic drug is presented; readers will also see how problems in the isolation, structure elucidation, and synthesis of metabolites were circumvented. Described is an interesting application of the technology of metalloporphyrins assisted metabolite prediction, estimation, quantitation and synthesis.

Drs. Mukund K. Gurjar, Mahendra Deshpande, J. S. Yadav, G. V. M. Sharma, P. Radha Krishna, C.V. Ramana, Punna Srinivas, Chepuri Ramana, Yatendra Kumar, Braj and Vidya Lohray and Bipin Pandey have each made seminal contributions to process chemistry. They have invented commercial processes for key pharmaceuticals that have resulted in significant economies in cost, and minimization of waste and have engineered "green chemistry" and the development of eco-friendly processes. These scholars describe their work in the next few chapters with case studies of specific compounds. The work is an eloquent testimony to the collaboration and cooperation inherent in the strategic triad of academic institutions, government, and industry. The work is applicable to the synthesis of both agricultural and fine chemicals. Moreover, the work described has led to the establishment of successful contract research businesses.

Over the last few years, an increasing number of pharmaceutical and biopharmaceutical companies have resorted to outsourcing activities in chiral synthesis, process development, and manufacturing. Dr. Peter Pollack demonstrates this strategy, provides useful pointers about the do's and don'ts, and beautifully elaborates the risks and rewards inherent in outsourcing in the pharmaceutical industry.

Dr. Shrikant Kulkarni exemplifies solving regulatory problems via thorough investigations of processes and processing parameters. Dr. Peter Pollack delineates the fascinating impact of specialty chemicals on drug discovery and development, providing further illustration of the power and utility of outsourcing in drug manufacture.

Chemical engineering plays a central and pivotal role in scale-up operations. Dr. Andrei Zlota discusses chemical process scale-up tools, mixing calculations, statistical design of experiments, and automated laboratory reactors.

Dr Richard Wife explains how some novel initiatives will lead to rescue of "lost chemistry and molecules," how the net will make research results accessible to the entire chemical

world, and how information sharing will lead to better and more efficient research. Thought-provoking and novel studies aimed at predicting compound stability are presented.

In the concluding chapter, Dr. Colin Scott describes some general principles and practices in drug development. A brief review is presented of the history of the requirements for clinical studies leading to the registration of a drug prior to being marketed. This is followed by a discussion of ethical issues related to clinical studies, the phases of drug development, and clinical trial design features. The support operations necessary for the initiation of clinical trials and optimization of results are described. Finally, a global development plan, accelerated development opportunities, international regulatory procedures, and post-marketing requirements are summarized.

There are few courses in academic chemistry departments that deal with drug discovery and development. Graduating students typically have scant exposure to the fascinating world of industrial chemistry. I am confident that the material will excite students interested in careers in the pharmaceutical industry. A salient feature of the book is the inclusion of several case studies that exemplify and epitomize the concepts detailed in each chapter. An instructor interested in developing a course in pharmaceutical chemistry will find the book useful as a teaching text for a one-semester course.

Dr. Raghunath A. Mashelkar, Director-General of the Council of Scientific and Industrial Research, has stated: "Rapid paradigm shifts that are taking place in the world as it moves from super-power bipolarity to multipolarity, as industrial capitalism gives way to green capitalism and digital capitalism, as information technology creates netizens out of citizens, as the nations move from 'independence' to 'interdependence,' as national boundaries become notional, and as the concept of global citizenship gets evolved, will see a world full of new paradigms and new paradoxes; there is no doubt that the rapid advance of science and technology will directly fuel many of these. The global pharmaceutical and, in particular, the contract R&D organizations have seen a dramatic change in their capabilities and sophistication. International pharmaceutical companies should now be ideally poised to seek collaborations to bring innovative drugs to the consumers at an affordable price."

Finally, I wish to thank my wife Veena, my son Rajeev and my parents for their encouragement, emotional support, understanding, and love. They have helped immeasurably during this endeavor.

MUKUND S. CHORGHADE

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BIOACTIVE MOLECULES IN MEDICINAL PLANTS: A PERSPECTIVE ON THEIR THERAPEUTIC ACTION

S. C. Taneja and G. N. Qazi
Indian Institute of Integrative Medicine (CSIR)
Jammu Tawi, India

17.1 INTRODUCTION

Plants have been the companions of humankind since time immemorial. They are an integral part of our lives and provide us with vital oxygen. However, they have been exploited relentlessly, due to their food value, therapeutic effects, and other socioeconomic requirements by cultures across the globe. The traditional belief that all plants grown on the planet have one or another therapeutic or physiological effect is lately gaining better acceptability, thanks to the development of highly sensitive screening technologies as well as inputs from the latest instrumental methods and tools of drug development. It is estimated that an approximate range of the total number of plants growing worldwide may vary between 310,000 and 422,000, and the number of higher (flowering) plants may vary from 150,000 to 250,000 distributed within approximately 320 families. These estimates also warn us that 94,000 to 144,000 plant species are at risk of dying out. Ancient systems of medicine supported by documentations in different cultures and ethnic groups reveal the use of herbal preparations for human benefit. Medicinal systems such as the Indian and Chinese as well as those used by African tribes are the treasure houses of traditional wisdom, and with the help of modern scientific methods, they will continue to be the basis of development of new therapeutic agents.

A vast majority of the world's population still has faith in and dependence on traditional systems of medicine for most of their health problems. It is roughly estimated that 70 to 75% of the population living primarily in the developing world are dependent for their health needs on plants or preparations derived from plants. The principle of discovering

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highly pure single bioactive molecules has been the foremost objective of almost all research programs engaged in drug development. However, the magic bullet approach to curing disease with a single drug has not been as helpful as it was thought to be in complete healing by most prescription drugs. The best healing practices require not only the treatment of symptoms but also the cause of the disease and the minimization of complications for the restoration of health. Accomplishing this goal is often beyond the scope of a single-molecular drug approach but may be achieved using a balanced combination of therapeutic agents or formulations to bring the desired medicinal effect with minimum side effects.

It is also a well-established fact that often, a single pure drug or molecule is more difficult to absorb by biological systems than is a mixture of compounds, which are generally easier to absorb or assimilate than are pure crystalline substances. Ultrapurity, the foremost requirement of modern drug systems, creates more insolubility and less acceptance by biological targets, thus requiring various drug delivery systems or carriers, and because of the presence of polymorphism among pure chemical entities, variations in biological activities have often been observed. In multicomponent systems such as plant preparations, which comprise thousands of molecules on natural matrices, there is no such problem and they require minimum development of special drug delivery systems and carriers. In the following lines we attempt to discover the rationale behind the traditional wisdom of universal use of multiplant formulations or multidrug components for the treatment of diseased conditions, and how this accumulated knowledge can best be supported and complemented by use of the latest scientific and technological advancements.

17.2 EVOLUTIONARY RELATIONSHIPS AMONG PLANTS AND HUMANS

Despite the tremendous progress achieved in the area of phytomedicine, the last few decades have seen a continuing decline in research and investment in the area of natural products, particularly medicinal plants. This may be attributed to diminishing faith by scientists in the efficacy of herbal medicine and an increase in expectations generated by the growth of combinatorial chemistry, high-throughput screening methodologies, and quantum increase in the number of biological targets identified in the last few years. There has been a wide range of views related to the prospects of discovering new drugs from plants. The school of thought that has less faith in natural products believes that there is no scientific evidence for any evolutionary relationship between human and plants, hence less likelihood of discovering new wonder drugs from plants. Generally, this skepticism is based on the premise that natural products have limited relevance to human diseases, as they have not coevolved with human proteins. Stuart L. Schreiber at Harvard University believes that "natural products are highly evolved, highly specific and can be highly effective towards gene products with which they are coevolved. However, it is unlikely that true molecular targets of human disease would have been subjected to natural selection to yield a natural product counterpart. Natural products are simply not there to target the relevant protein for human disease" (Rouhi, 2003). Members of this school of thought also believe that only in infectious diseases, and to some extent in cancer, would it be logical to look toward natural products. Notwithstanding, it is suggested that it would be more appropriate to embark on a diversity-oriented synthesis program to rapidly create natural product libraries of compounds that nature may not have needed to make but which could serve as chemical probes for human disease. On the other hand, views expressed by other experts, particularly Herbert Waldman at the Max-Planck Institute for Molecular Biology, Dortmund, Germany,

are that natural products have coevolved to perform functions by binding to proteins that show conservatism as well as diversity. Thus, they already have been validated to reach and bind specific proteins due to the similarities of their domains even if their number are large, but many have similar types of domains to interact with natural products. A similar view has been expressed by Guy Miller, a senior executive of a pharmaceutical company: that the fundamental reactions of energy metabolism are highly conserved and that the energy apparatus in a single-celled organism is very similar to that in plants. Therefore, how plants or other organisms adapt to biochemical derangement can be instructive in finding treatment for related derangements in humans (Rouhi, 2003). Interestingly, with the recent explosion in genetic knowledge and sophistication of analytical tools, it is now established beyond doubt that all species on this planet are interrelated and that structural genomics has a very important part to play in this understanding. Studies comparing the sea squirts *(Ciona intestinalis)* genome with the human genome indicate that on the evolutionary tree, vertebrates are closely related to each other (Dehal et al., 2002). A recent report also suggests that human beings and plants such as tomatoes have some common evolutionary ancestors (Elder, 2001).

17.3 TRADITIONAL WISDOM

It is an established fact that most modern single-molecular drugs generally vary in their efficacy among the human population, as they are found to be effective in only 30 to 40% of the patients. This figure is not significantly better than placebo effects, where an inert molecule such as sugar or salt is administered in place of a drug without disclosure to patients, which demonstrate an average success rate between 20 and 30%. The studies have also shown that variations in therapeutic response may depend on individual metabolism, sensitivity to various drugs, and even differences among racial groups. To overcome this problem, it has also been suggested that there is a need to develop personalized medicines or person-specific drugs for a single disease based on the response of a group of people: that this approach will significantly enhance the probability of success in treatment. This suggestion supports and substantiates the traditional wisdom that exists in the ancient literature (ayurveda) and also recognized in systems such as homeopathy, where the treatment takes into account people's personalities (prakurti) and lifestyles.

The practice of traditional medicine is widespread in Asian societies such as India, China, Japan, Sri Lanka, Thailand, and Bangladesh. Some 70 to 85 % of the population is dependent on traditional medicine in these countries. In Japan, preparations made from herbal drugs (i.e., the kampo system of medicine) are more in demand than mainstream pharmaceutical products. Similarly, the traditional system of medicine is very popular on the African continent, which is very rich in medicinal herbs. Even in European countries, 1500 plant species of aromatic and medicinal plants are presently being used in countries such as Albania, Croatia, France, Germany, Poland, Spain, and United Kingdom.

Two of the best developed and most widely used traditional medicinal systems, which are both well documented and rich in concept and practice, are the various Indian and Chinese systems of medicines. Among traditional Indian systems of medicine, the ayurvedic system has been the most popular and best known in the West. *Ayurveda* literally means "knowledge or science of life." The system is based primarily on three classic texts known as *samhitas*, which were written by Sushruta, Charaka, and Vaghata in the period between 100 B.C. and 100 A.D. Ayurvedic physiology also views bodily functions in terms of three

essences or *dosas*: vatta (breathing/air), pitta (bile), and kapha (phlegm). Imbalance in any of these three body functions may result in various diseases, many of them manifesting similar types of symptoms.

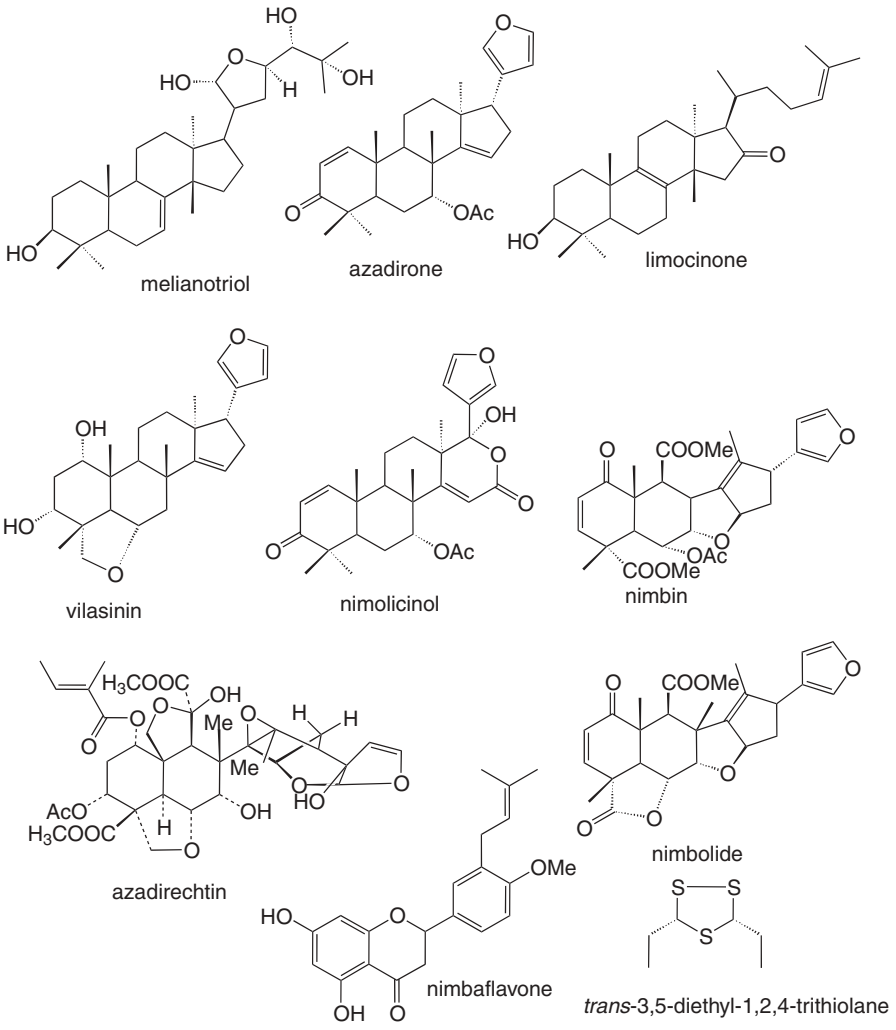
The traditional Chinese system of medicine, believed to be 6000 years old, has developed independent of any other system of medicine. The classical document of Chinese medicine, known as *Nei Ching*, vividly explains the complex action of a composite formulation or "cocktail" of drugs using the example of a hierarchial imperial court in which the major drug acts as a king, synergistic material as courtiers, immunomodulator additives as soldiers, and others as messengers that affect metabolism, stability, and bioavailability.

If we cast a closer look at most ancient and popular systems of medicine and methods of treatment, whether Chinese, Indian, or tribal, they generally all use formulations based on parts of a single plant or a mixture of plants, sometimes as many as 20 to 40. In India alone, 40,000 different formulations comprising about 10,000 plants are being used. In other Asian, South Asian, and African countries, use of multiplant formulations is equally prevalent.

17.4 UNIQUE LIBRARIES FOR PLANTS

According to N.R. Farnsworth, an eminent scientist working in the area of medicinal plants, "each plant is a unique chemical factory capable of synthesizing an unlimited number of highly complex and unusual chemical substances whose structures could escape the imagination of synthetic chemists forever" (Farnsworth, 1984). In nature, both microorganisms and plants are capable of building their own unique libraries of biomolecules in the form of primary and secondary metabolites understood to be meant primarily for propagation, proliferation, and for the purpose of developing their own defense mechanisms. They also have their own in-built metabolic pathway engineering system, capable of producing metabolites that can be regulated and switched on and off whenever necessary. Scientists are slowly learning the art of metabolic pathway engineering, enabling them to produce biomolecules of their choice in higher quantities. The number of molecules in the libraries derived from each plant or other natural organism may vary from hundreds to thousands. Each of these libraries comprises a large variety of metabolites or biomolecules unique to that plant or plant species or microorganism. Each plant also has several unique small libraries comprising natural analogs, which are unique to that plant in every respect. These small libraries of biomolecules may come from different parts of the plant: for example, roots, flowers, stems, and leaves. Each part may have different or the same molecules in variable quantities. These small libraries comprise several analogs or congeners of a particular class of molecules: for example, natural analogs of taxanoids (anticancer agents) in *Taxus brevifolia* (yew), withanolides (adaptogens) in *Withania somnifera* (ashwgandha), azadirechtins (insecticides) in *Azadirechta indica* (Neem) (Scheme 17.1), and lignans (anticancer agents) in *Podophyllum hexandrum* (mayapple). Nature has evolved these libraries of molecules with their varied metabolites as well as simple and complex analogs applying unique combinatorial chemistry and transformation reactions, many of which have been replicated in chemical laboratories, although several remain to be fully understood. The members of these unique libraries are coalition partners in demonstrating their efficacy or activity against external invasions. A similar partnership effect is noticeable in their therapeutic and curative properties or when subjected to bioevaluation studies. The wealth of nature is vast and unparalleled in its diversity and uniqueness. There is therefore a need to understand the genesis of this wealth, developed during a billion years of evolution on

this unique planet, which has survived the onslaught of enormous environmental changes and the attack of poisonous chemicals from environmental pollution through individual defense and propagation mechanisms. If each plant is so rich in both simple and complex molecules (and this number may reach as high as hundreds or thousands), a vast treasure of millions of biomolecules still await discovery by chemists and biologists. The single-molecule approach therefore appears to be out of harmony with nature, which provides us with the unique libraries that may be utilized directly in single- or multicomponent herbal preparations for effective treatment of diseases, generally with few side effects. There is therefore a need to understand the function of multiplant herbal formulations in the treatment of disease in general, together with detailed studies of intricate interactions among a multiple of biomolecules present in those preparations. It may require a nonclassical approach and application of the latest technological tools of separation, identification, and rapid analysis of the large databases generated in these studies.



Scheme 17.1 Members of the library of biomolecules in *Azadirechta indica* (Neem).

17.5 DRUGS AND BIOACTIVE MOLECULES FROM PLANTS

After a brief setback of two to three decades, there has been a resurgence in interest and popularity of herbal drugs in both the developed and the developing world. Traditional systems of medicine hitherto overshadowed by modern Western medicine are staging a comeback, and many more societies are turning to a holistic approach, which includes traditional systems of medicine for health care and remedies. Many herbs and their preparations have become more popular due to their low side effects (e.g., *Hypericum perforatum, Panax ginseng*, *Echnacea angustifolia, Valeriana officinalis, Swertia chirata*), and in some cases their demand has been so high that there is a great risk of their being overexploited and therefore facing extinction in the near future unless remedial measures for their conservation are put in place. The change in perception may be attributed to the fact that the promise of creating a large library database of small synthetic molecules through the use of combinatorial chemistry and parallel synthesis coupled with high-throughput screening has not met with the success predicted initially.

A question that many people associated with the drug industry and actively involved in the area of new drug development based on natural products may find inconvenient to answer is why the number of drugs or established biomolecules isolated or discovered from plants are so limited in number. Although the history of research and development in the area of natural products is more than 200 years old, it is surprising that only some 120 to 130 natural drugs which are being used worldwide have been discovered from even fewer plants. The number of novel natural molecules recorded in the literature is over 150,000, which includes compounds derived from lower plants and microorganisms. It is also estimated that only 5 to 15% of plant species have been investigated for various reasons, not necessarily for bioactivity. The actual figure may be very low when it comes to systematic exploration of these plants for new drug development.

It is understandable that in the past many of these plants were analyzed only for the isolation of pure molecules from a chemical point of view, and few were subjected to detailed biological screening protocols. It cannot be ignored that the rate of discovery of lead molecules (20 in 2001 vs. 37 two decades earlier) has declined steadily despite the introduction of more sophistication in testing as well as separation techniques. There is a need to rethink and reappraise the drug development programs in which so much effort and so many resources have not yielded compatible results. It is estimated that it takes around 10 to 15 years of hard work and approximately $1 billion to develop a synthetic de novo drug. This period is much less in the case of natural products, where the chance of getting a hit is much higher than that for synthetic drugs. Looking at the total number of small molecules introduced as drugs worldwide during the past 20 years, more than 60% are related to natural products (Newman et al., 2003), including 6% natural products and 27% their derivatives. In the anticancer and antibacterial areas, this percentage is 74 and 78%, respectively.

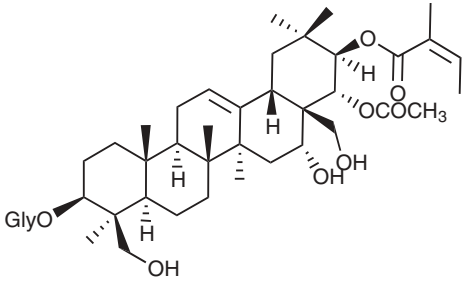
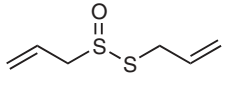
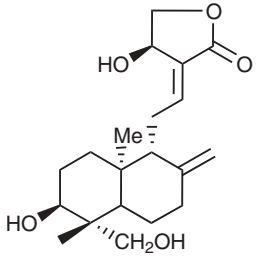
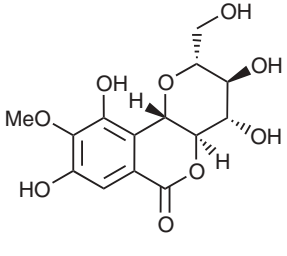
Ironically, we still do not have effective cures with minimal side effects for most of the chronic diseases known to us. In many infectious diseases the problem of drug resistance is a great challenge to biologists. The answer to this problem may lie in traditional wisdom acquired over the ages through systematic experimentation by the great sages and learned people, who were better equipped to understand nature because their physical senses were much more highly developed than those of modern man. It is an undisputed fact that nature, particularly plants, are unmatched in producing small molecules rich in structural diversity as well as bioactivity. The higher plants, particularly, offer great variability compared to

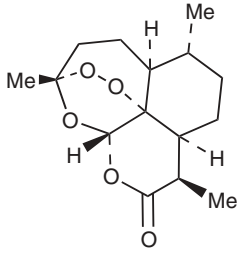
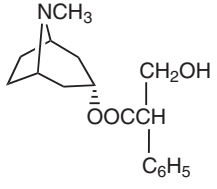
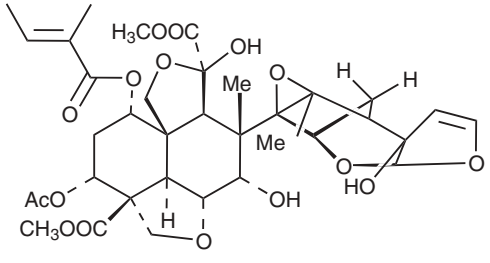
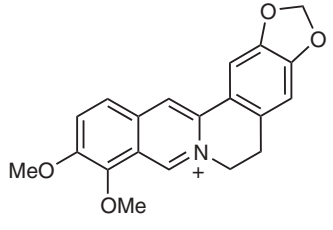
other organisms. Recent studies have also established that approximately 40% of the chemical structures identified in natural products are not represented by synthetic compounds. Moreover, compounds derived from natural products generally have druglike properties, in that they are relatively small and can be absorbed and metabolized.

The scientific study of traditional medicinal use of plants by human beings is known as *ethnopharmacology*, a term that came into existence in 1960s. It was postulated that at least in some therapeutic areas, the use of ethnopharmacological data as a basis of plant selection for screening gives a hit rate considerably higher than when random selection is used (Farnsworth, 1984). Thus, the number of bioactive molecules from various plants identified over the years may be broadly divided into three categories. In the first category are those molecules wherein validation of traditional knowledge or ethnopharmacological data led to the identification of bioactive molecules, notable examples being the *Vinca* alkaloids, quinine, and artemesinine. Table 17.1 catalogs those bioactive natural molecules for which traditional knowledge has been validated by the isolation and identification of bioactive molecules. In the second category are examples of bioactive molecules that displayed more variation in bioactivity than was expected on the basis of their ethnopharmacological uses (Table 17.2). By no means does this list indicate that traditional knowledge has been proven incorrect or contradictory in identifying the true importance of these plants. Perhaps the sophistication and technology, as well as efforts made in their evaluation, are inadequate in identifying the bioactivity expected as well as bioactive molecules. There may also be other factors, such as incorrect identification of plant species and genetic, environmental, and geographical variations. With the advancement in assay methodologies as well as reassessment of the activity of several established molecules earlier regarded as inert or inactive but now displaying useful activities, some molecules (e.g., betulinic acid, resveratrol) are categorized separately. Table 17.3 lists some examples of such natural molecules with their newly discovered bioactivities. In addition, there are important drugs now produced synthetically but derived from natural molecules through structural modification and rational drug design. Table 17.4 lists a few such synthetic drugs.

The biomolecules derived from various herbs and plants listed in Tables 17.1 to 17.3 may not be comprehensive, but they are more or less representative of most of the molecules identified and their reported bioactivity. From these tables it becomes quite apparent that bioactivity is not confined to a specific genre of molecules; rather, almost all classes of compounds, from simple phenolics such as aspirin to alkaloids and complex structures such as azadirechtins and glycosidic saponins, display various forms of bioactivity. It is also significant that only a small number of molecules currently on the market are being used as single-entity or single-molecule drug: aspirin, quinine, morphine, podophyllotoxin, artemesinin, digoxin, colchicine, ergotamine, pilocarpine, taxol, and vincristine, among others. Many bioactive products, such as saponins, ginsenosides, eleutherosides, echnacosides, saikosaponins, and other classes of molecules, including withaferins, gingerols, boswellic acids, allicins, andrographolides, azadirechtins, and sylimarines, are mixtures of several molecules which are not completely defined but which display significant biological activities as a mixture, due to their combined as well as synergistic effects. Many of these drugs are available in the form of standardized extracts wherein individually, the marker molecules may or may not be biologically active. Another important point which is also apparent from Table 17.3 is that the number of active biomolecules which earlier were believed to be inactive are now steadily on the increase, and many of these molecules are in the category of either antioxidants or anticancer agents. This may be attributed to the fact that the anticancer program has got a boost from the National Institutes of Health (NIH) and rapid developments in the area of highly efficient in vitro screening methodologies in the last three decades.

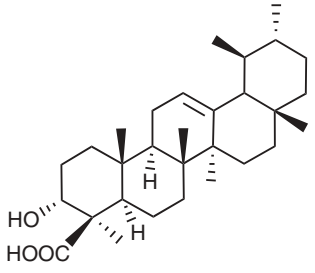
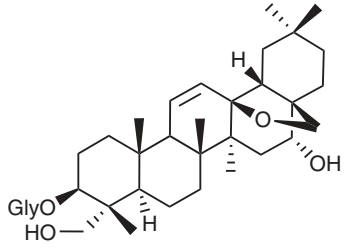
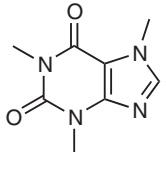
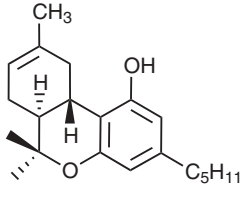
TABLE 17.1 Bioactive Molecules from Plants Identified and Validated on the Basis of Their Traditional Use

Name of Plant Species	Traditional Uses	Bioactive Molecule	Structure	Reference
Aesculus hippocastanum	Anti-inflammatory	Aescin		Sirtori (2001)
Allium sativum	Lipid lowering, antibacterial	Allicin		Jain et al. (1993)
Andrographis paniculata	Hepatoprotective	Andrographolide		Kapil et al. (1993)
Ardisia japonica	Antitussive, antiarrhythmic	Bergenin		Pu et al. (2002)

Artemesia annua	Antimalarial	Artemesinin[a]		Lin et al. (1987)
Atropa balladona	Anticholinergic	Atropine[a]		Brown (1960)
Azadirechta indica	Insecticide, antifeedant	Azadirechtin		Gill and Lewis (1971)
Berberis vulgaris	Antibacterial, antidiarrheal	Berberine		Amin et al. (1969)

(*Continued*)

TABLE 17.1 (*Continued*)

Name of Plant Species	Traditional Uses	Bioactive Molecule	Structure	Reference
Boswellia serrrata	Rheumatoid arthritis	Boswellic acid		Safayhi et al. (1992) Taneja and Dhar (1996)
Bupleurum chinense	Liver tonic	Saikosaponin D		Yen et al. (1991)
Camellia sinesis	CNS[b] stimulant	Caffeine		Zubair et al. (1986)
Cannabis sativa	Analgesic, psychoactive antiemetic	Tetrahydro cannabinol		Coxford (2003)

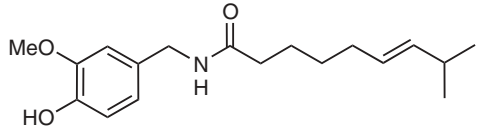
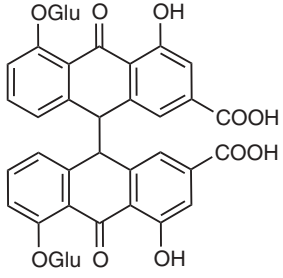
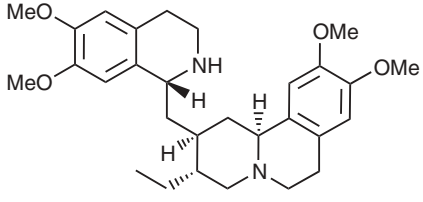
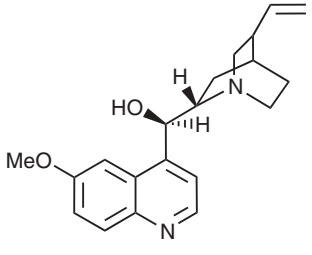
Capsicum annuum	Topical analgesic	Capsaicin		Bernstein et al. (1987)
Cassia acutifolia	Laxative	Sennoside		Franz (1993)
Cephaelis ipecacuanha	Antiamebic, in dysentery	Emetine[a]		Botero (1978)
Cinchona succirubra	Antimalarial	Quinine[a]		Leung (1980)

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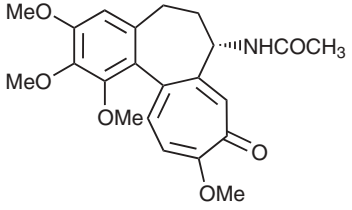
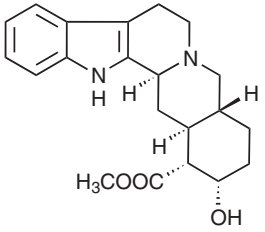
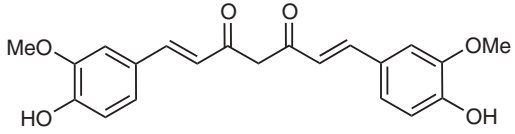
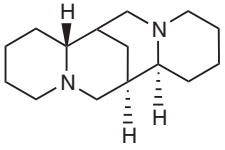
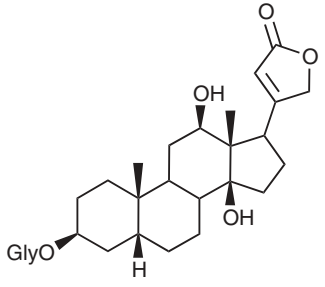
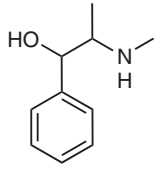
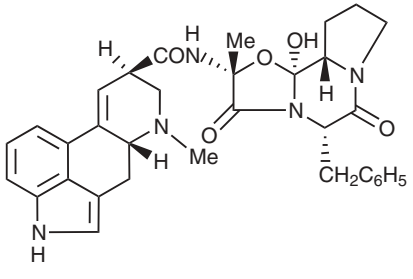
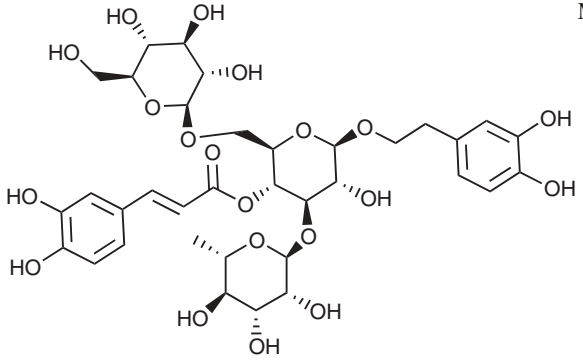
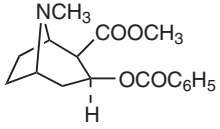
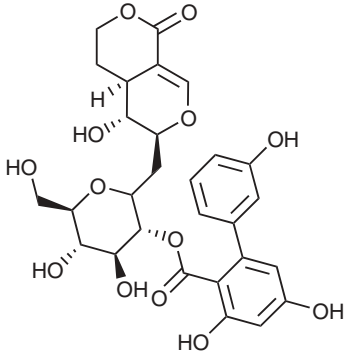
TABLE 17.1 (*Continued*)

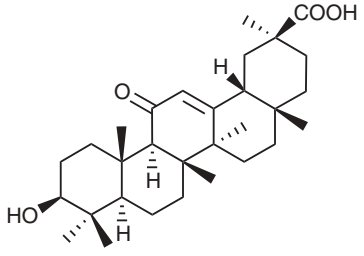
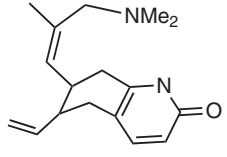
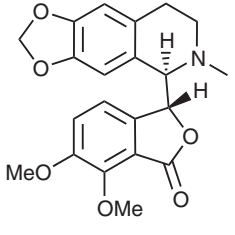
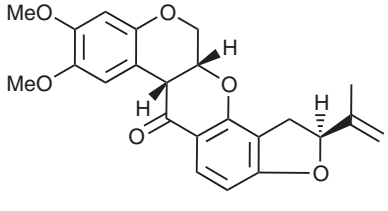
Name of Plant Species	Traditional Uses	Bioactive Molecule	Structure	Reference
Colchicum autumnale	Gout, hepatitis, antitumor	Colchicine[a]		Lange et al. (2001) Warnes (1991)
Corynanthe johimbe	Aphrodisiac, α-adrenergic blocker	Yohimbine[a]		Tam et al. (2001)
Curcuma longa	Antibacterial, anticancer	Curcumin		Nagabhushan and Bhide (1992)
Cystisus scoparious	Oxytocic, diuretic	Sparteine		Gray and Plentl (1958)

Digitalis orientalis	In heart failure, cardiotonic	Digoxin[a]		Campbell and MacDonald (2003) Foss and Benezra (1980)
Ephedra vulgaris	Stimulative, cardiotonic	Ephedrine[a]		Benezra and McRae (1980)
Ergot	Child birth, migraine	Ergotamine[a]		Kreilgard (1977)

(*Continued*)

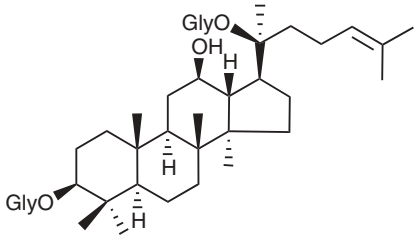
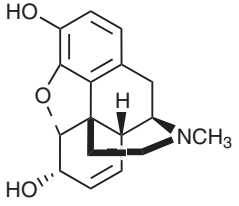
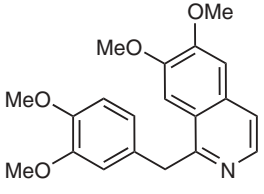
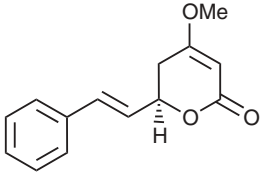
TABLE 17.1 (*Continued*)

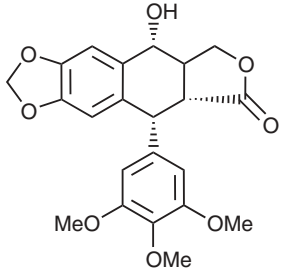
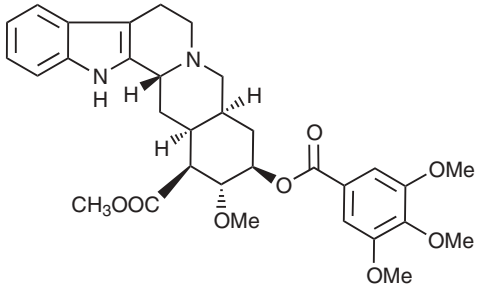
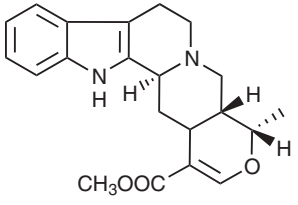
Name of Plant Species	Traditional Uses	Bioactive Molecule	Structure	Reference
Echnacea angustifolia	Immunostimulant	Echnacoside		Melchart et al. (1994)
Erythroxylon coca	Anesthetic	Cocaine[a]		Muhtadi (1986)
Gentiana lutea	Bitter tonic	Amarogentin		Spoerke (1980)

Glycyrrhza glabra	Antiulcerogenic, anti-inflammatory	Glycyrrhetinic acid		Shibata (2000)
Huperzia serrata	Inhibits the breakdown of acetylcholine	Huperzinine[a]		Shu (1998)
Hydrastis canadensis	Hemostatic	Hydrastine		Genest (1969)
Lonchcarpus nicou	Pesticide, insecticide	Rotenone		Fukami and Nakajima (1971)

(*Continued*)

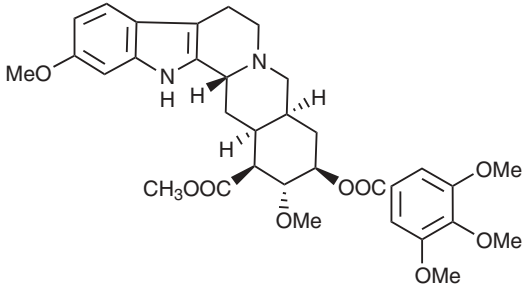
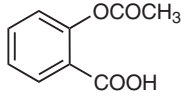
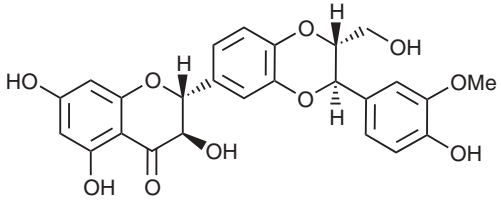
TABLE 17.1 (*Continued*)

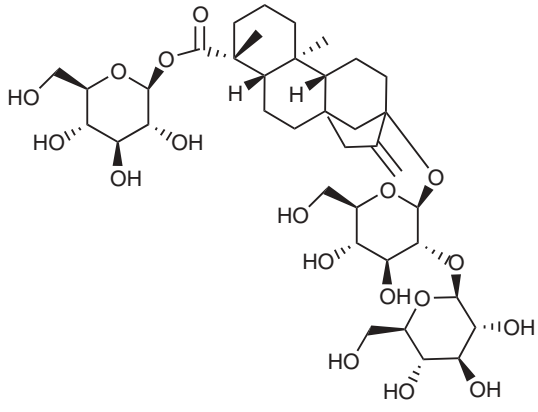
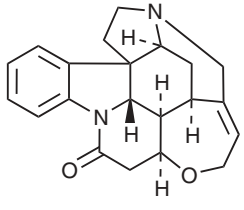
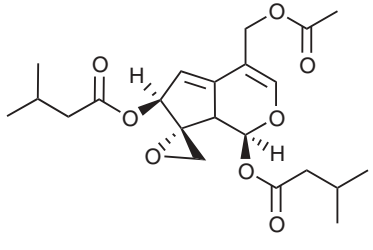
Name of Plant Species	Traditional Uses	Bioactive Molecule	Structure	Reference
Panax ginseng	Adaptogenic	Ginsenoside		Brekhmam and Dardimov (1969)
Papaver somniferum	Painkiller	Morphine[a]		Muhtadi (1988)
	Smooth muscle relaxant, in erectile dysfunction	Papaverine[a]		Whipple (1977)
Piper mythysticum	Tranquilizer	Kawain		Hansel (1996)

Podophyllum peltatum	Treatment of warts, anticancer	Podophllyo-toxin[a]		Canel et al. (2000), Jardine (1980)
Rauwolfia canescens	Sedative, antihypertensive	Deserpidine		Abruzzi (1965)
Rauwolfia serpentina	Antiarrhythmic	Ajmalicine[a]		Cordell et al.(2001)

(*Continued*)

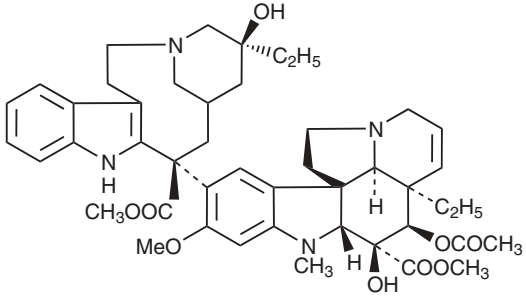
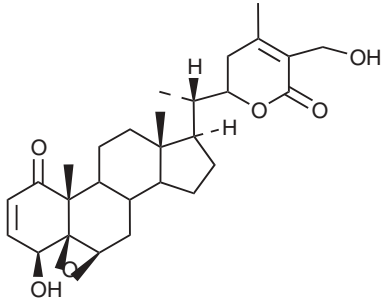
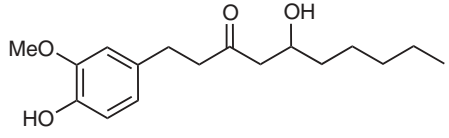
TABLE 17.1 (*Continued*)

Name of Plant Species	Traditional Uses	Bioactive Molecule	Structure	Reference
	CNS disorders	Reserpine[a]		Scriabine (1980)
Salix alba	Antipyretic, pain relief	Aspirin[a]		Kaul et al. (1999)
Silybum marianum	Hepatoprotectant, antioxidant	Silybin		Hobbs (1992) Wellington and Jarvis (2001)

Stevia rebaundiana	Antidiabetic, sweetener	Stevioside		Yamamoto et al. (1985)
Strychnos nux vomica	CNS stimulant	Strychnine[a]		Muhtadi (1986)
Valeriana wallichii	Sedative, tranquilizer	Valtratum		Houghton (1988)

(*Continued*)

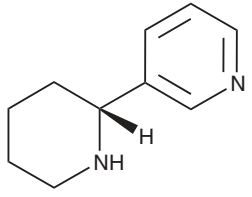
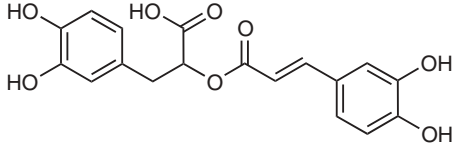
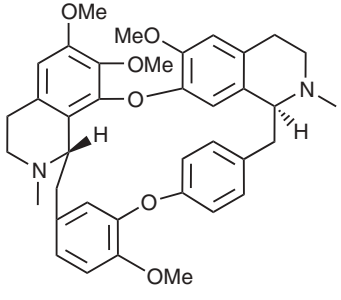
TABLE 17.1 (*Continued*)

Name of Plant Species	Traditional Uses	Bioactive Molecule	Structure	Reference
Vinca rosea	Anticancer	Vinblastine[a]		Van der et al. (2004)
Withania somnifera	Adaptogenic	Withaferin A		Singh et al. (1998)
Zingiber officianale	Motion sickness, anti-inflammatory	Gingerols		Suekawa et al. (1984)

[a]In market use as a single-entity or single-molecular drug.

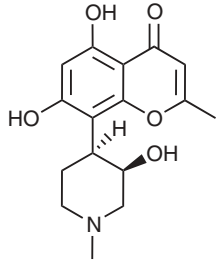
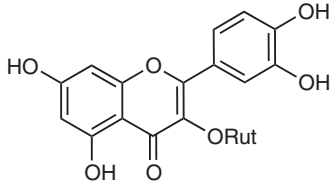
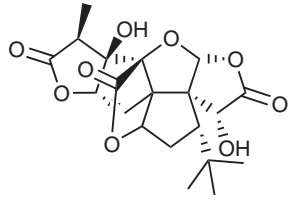
[b]CNS, central nervous system.

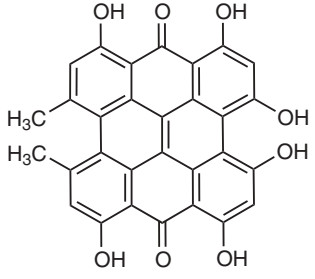
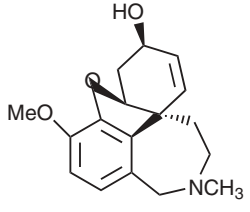
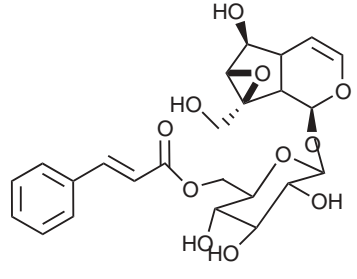
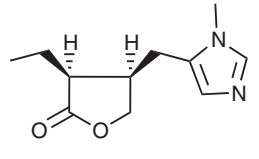
TABLE 17.2 Bioactive Molecules from Plants Identified on the Basis of Their Bioactivity Other Than Traditional Use

Name of Plant Species	Traditional Uses	Bioactive Molecule	Present Use	Structure	Reference
Anabasis aphylla	Pesticide	Anabasine	Relief from nicotine craving		Wolfson et al. (2003)
Anethum graveolens	Flavor-imparting	Rosmarinic acid	Anti-inflammatory, antiviral, antioxidant		Petersen and Simmonds (2003)
Corydalis ambigua	Analgesic	Tetrandrine	Antihypertensive, cytotoxic		Wang et al. (2004)

(*Continued*)

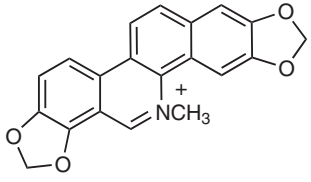
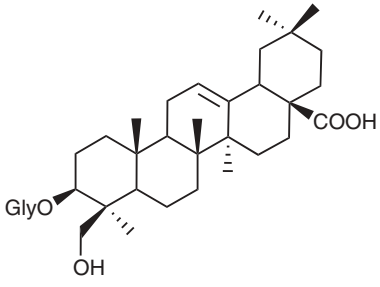
TABLE 17.2 (*Continued*)

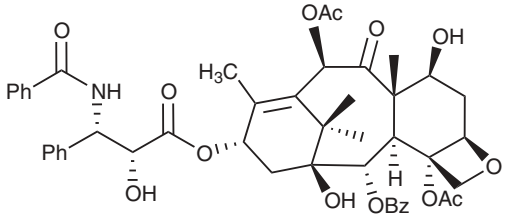
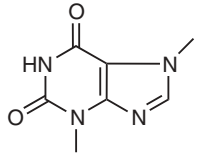
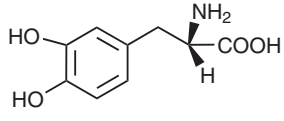
Name of Plant Species	Traditional Uses	Bioactive Molecule	Present Use	Structure	Reference
Dysoxylum binectarifarum	Rheumatic diseases	Rohitukine[a]	Anticancer		Wang (2000)
Eucalyptus macrorhyncha	Insect repellent	Rutin	Capillary fragility		Levitan (1951)
Ginko biloba	Asthma, chillblains	Gingkolide A	Memory aid, Alzheimer's disease		LeBars et al. (1997)

Hypericum perforatum	Antidepressant	Hypericin	Antiviral		Gulick (1999)
Lycoris squamigera	Pesticide	Galanthamine	Antidementia		Raskind et al. (2004)
Picrorhiza kurroa	Bitter tonic, in fever and dyspepsia	Picroside	Hepatoprotective		Vaidya et al. (1996)
Pilocarpus jaborandi	—	Pilocarpine	Sjögren's syndrome		Fox (2003)

(*Continued*)

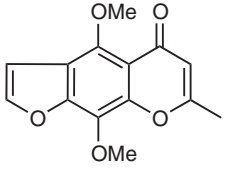
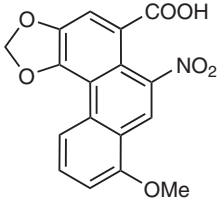
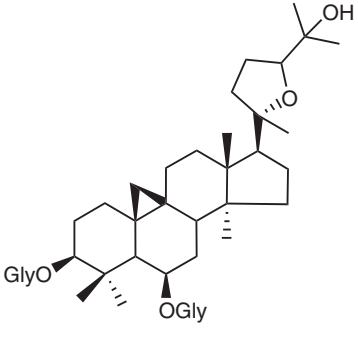
TABLE 17.2 (*Continued*)

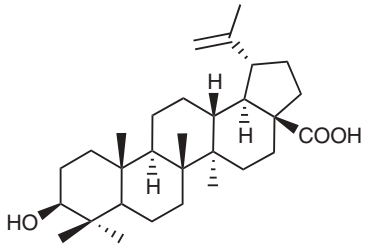
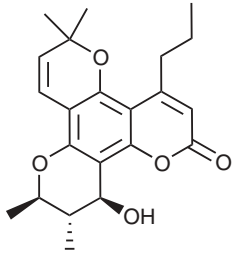
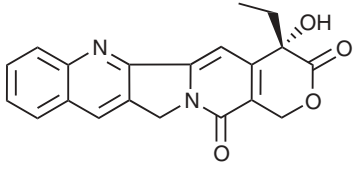
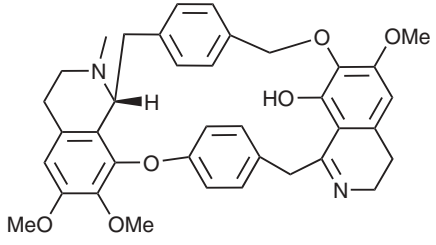
Name of Plant Species	Traditional Uses	Bioactive Molecule	Present Use	Structure	Reference
Sanguinaria canadensis	Skin conditions, blood purifier	Sanguinarin[a]	Antiplaque, gingivitis		Shamma (1972)
Sapindus mukurossi	Soap, antifungal	Sapindosides	Male contraceptive (spermicide), anticancer		Dwivedi et al. (1990)

Taxus baccata	Antispasmodic, rheumatism	Taxol[a]	Anticancer		Legha. (1990) Suffness (1995)
Theobroma cacao	Flavor and aroma	Theobromone[a]	Coronary vasodilator, diuretic		Leung (1980)
Vicia faba	Routing drunkard from stupor	Levodopa[a]	Antiparkinsonian		Lewitt and Nyholm (2004)

[a]In market use as a single-entity or single-molecular drug.

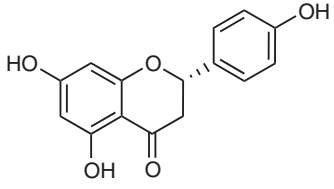
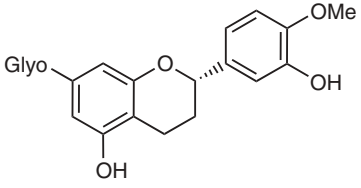
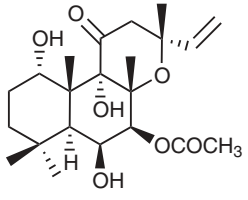
TABLE 17.3 Bioactive Molecules from Plants Identified on the Basis of Their Newly Discovered Bioactivity

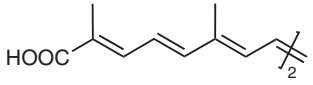
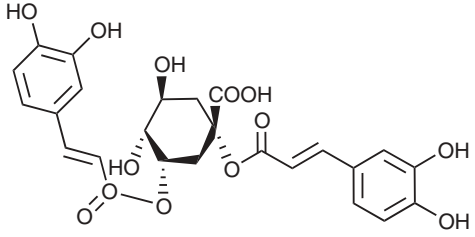
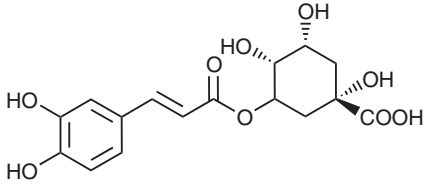
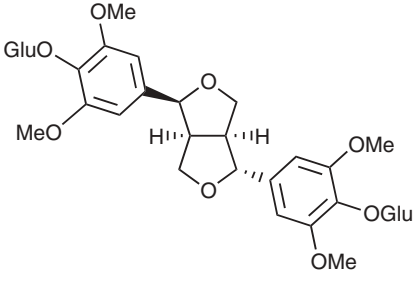
Name of Plant Species	Bioactivity	Bioactive Molecule	Structure	Reference
Ammi visnaga	Vasodilator, vitiligo	Khellin		Ubeda and Villar (1989)
Aristolochia indica	Antifertility	Aristolochic acid		Mathur et al. (1980)
Astragalus membranaceus	Immune modulator	Astragaloside I		Liu et al. (1994)

Betula alba	Anticancer, HIV	Betulinic acid[a]		Cichewicz and Kouzi (2004)
Calophyllum lanigerum	Nonnucleoside reverse transcriptase inhibitor	Calanolide A[a]		Flavin et al. (1996)
Camptotheca acuminata	Anticancer	Camptothecin[a]		Wall and Wani (1980)
Cissampelos pareira	Cytotoxic, skeletal muscle relaxant	Cissampareine		Kupchan et al. (1965)

(*Continued*)

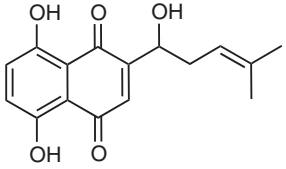
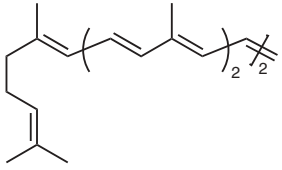
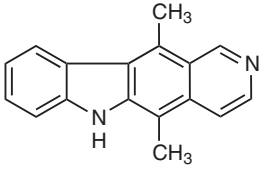
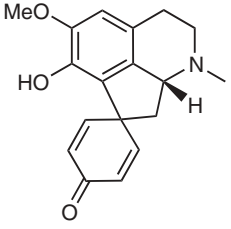
TABLE 17.3 (*Continued*)

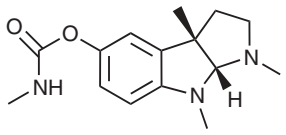
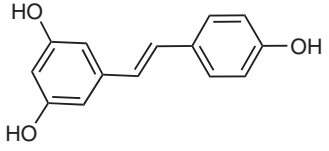
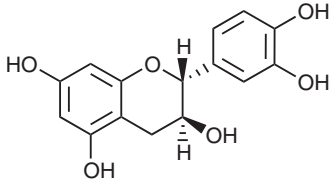
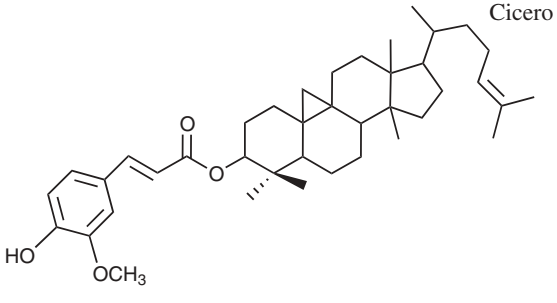
Name of Plant Species	Bioactivity	Bioactive Molecule	Structure	Reference
Citrus paradisi	Anticancer, antibacterial	Naringenin,		Ameer et al. (1996)
Citrus sinensis	Antiviral, aldose reductase inhibitor	Hesperidine		Garg et al. (2001)
Coleus forskolii	Blood pressure lowering antispasmodic effect	Forskolin[a]		Ammon and Mueller (1985) De Souza et al. (1983)

Crocus sativa	Increased oxygen diffusion	Crocetin		Gainer and Chisolm (1974)
Cynara scolymus	Antioxidant, cholerectic	Cynarin		Gebhardt (1997)
	Liver tonic, antioxidant, GI soothing	Chlorogenic acid		Kraft (1997)
Eleutherococcus senticosus	Immunostimulant, antistress	Eleutheroside E		Brekhman (1991) Nikaido (1981)

(*Continued*)

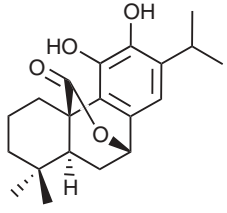
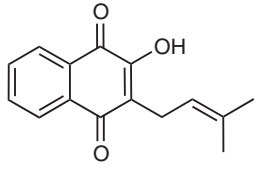
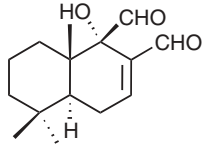
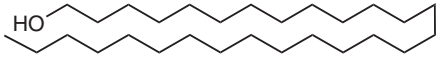
TABLE 17.3 (*Continued*)

Name of Plant Species	Bioactivity	Bioactive Molecule	Structure	Reference
Lithspermum erythrorhizon	Antibacterial, anticancer, antiviral	Shikonin		Chen et al. (2003)
Lycoperscion esculentum	Antioxidant, anticancer	Lycopene		Rao et al. (1998)
Ochrosia elliptica	Anticancer	Ellipticine[a]		Garbett and Graves (2004), Paoletti et al. (1980)
Ocotea glaziovii	Antidepressant	Glaziovine[a]		Buffa et al. (1974), Cordell et al. (2001)

Physiostigma venenosum	Memory enhancer, cholinergic, glaucoma	Physostigmine		Der Marderosian and Beutler (2002)
Polygonum cuspldatum	Antioxidant, antiviral	Resveratrol		Bhat et al. (2001)
Potentilla fragarioides	Hemostatic	(+)-Catechin		Lotito and Fraga (1998)
Rice bran oil	Antihyperlipoprotein-emic	γ-Oryzanol		Cicero and Gaddi (2001)

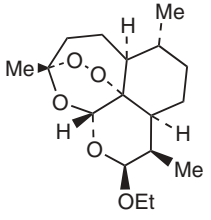
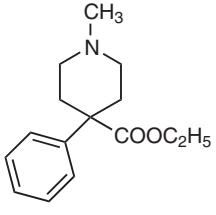
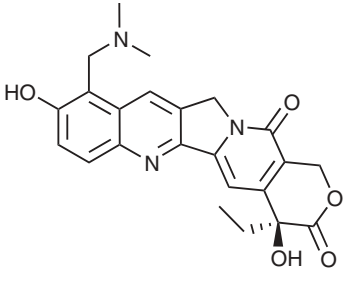
(*Continued*)

TABLE 17.3 (*Continued*)

Name of Plant Species	Bioactivity	Bioactive Molecule	Structure	Reference
Rosmarinus officianalis	Antioxidant	Carnosol[a]		Aruoma et al. (1992)
Tabebuia avenallanedae	Anticancer	Lapachol		Block et al. (1974)
Warburgia ugandensis	Antifeedant	Warburganal		Taniguchi et al. (1983)
Wheat germ oil	Stamina improvement, herpes antiviral	1-Octacosanol		Taylor et al. (2003)

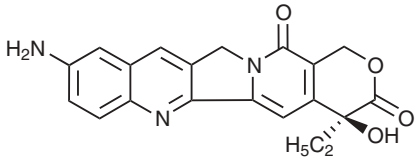
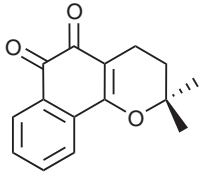
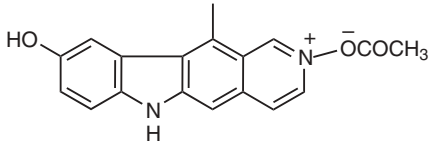
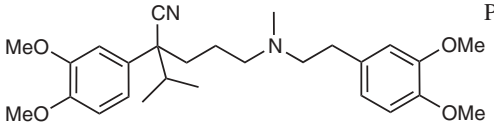
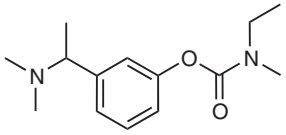
[a]Being used or developed as a single entity.

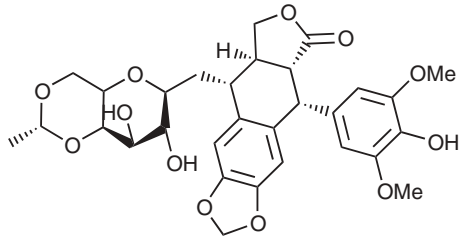
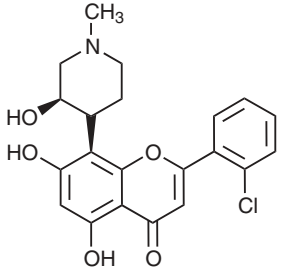
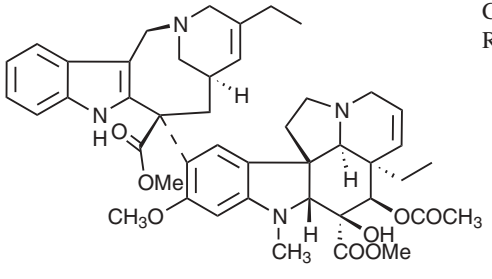
TABLE 17.4 Some Important Structurally Modified Plant Products

Natural Product	Bioactivity	Modified Drug	Structure	Reference
Artimisinine	Antimalarial	Arteether		Li et al. (1981)
Atropine	Analgesic	Meperidine		Latta et al. (2002)
Camptothecine	Anticancer	Topotecan		Fiorica (2003) Forbes et al. (2001)

(*Continued*)

TABLE 17.4 (*Continued*)

Natural Product	Bioactivity	Modified drug	Structure	Reference
	Anticancer	9-Aminocamptothecin		Eder et al. (1996), Ulukan and Swaan (2002)
Lapachol	Anti-HIV	β-Lapachone		Schurch et al. (1978)
9-Methoxyellipticine	Anticancer	9-Hydroxy-*N*-2-methylellipticinium acetate		Garbett and Graves (2004)
Papaverine	Smooth muscle relaxant, calcium channel blocker	Verapamil		Prisant (2003)
Physostigmine	Alzheimer's disease	Rivastigmine		Burns et al. (2004)

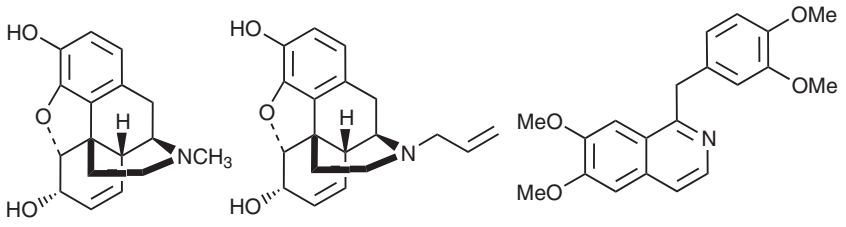
Podophyllotoxin	Anticancer	Etoposide		Clark and Sevin (1987) Clark (1999)
Rohitukine	Antitumor	Flavopiridol		Liu et al. (2004)
Vinblastine	Hodgkin's disease, anticancer	Vinorelbine		Conti and Vici (1998) Rossi et al. (2003)

17.6 SYNERGISM IN HERBAL FORMULATIONS

For those working in the area of ethnomedicine or ethnopharmacology, it is a common observation that the extracts of plants are biologically more active than the pure isolates. It is rather uncommon to find a pure molecule showing more activity than that shown by the parent extract. Most efforts in plant research are therefore unable to reach a critical stage of identification of a prospective biomolecule and its final development to an effective drug. The reason for the loss of activity in a single drug entity is basically explained by the synergistic action displayed by multiple ingredients in herbal preparations. These constituents may or may not individually be sufficiently active to achieve the therapeutic action desired. However, they often seem to cause complete modulation of activity or even increase activity disproportionate to the sum of the individual activity levels. Generally, a substantial decrease in toxicity levels are observed when whole plant extracts are compared with individual molecules derived from a plant even when used in the same proportion as found in the whole plant. Many of the single molecules depicted in Tables 17.1 to 17.3 display a high degree of synergism when used in combination with other natural constituents or in the form of enriched extracts. The benefits of combination drugs may reside not only in enhancing the therapeutic action through bioavailability improvement but also in permitting the use of lower doses. It is simplistic to believe that a disease is caused only through a single receptor or one enzyme or protein. In most cases the cause of a disease may be due to a multiplicity of factors and complications, resulting in both visible and invisible symptoms. Combination drugs may also act on multiple targets at the same time, thus providing thorough relief. It is believed that most such herbal preparations or formulations are generally nontoxic or comparatively less toxic than single-molecular drugs.

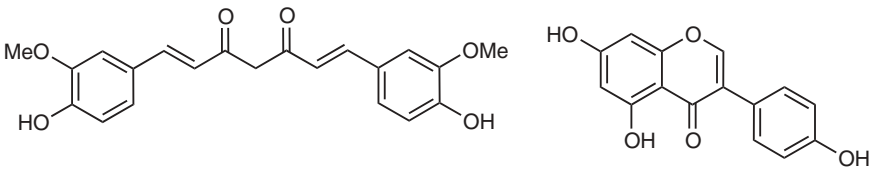
In the last few years, Western medicine pundits have started recognizing the benefit of administering drugs in combination rather than singly. A few examples of synergism among natural molecules in a single plant or from different plants are described here. A simple example is the opium poppy, which is a natural source of the alkaloid morphine (Scheme 17.2), which is used as an analgesic. When administered as a pure isolate, it shows many adverse side effects, which may even be fatal; however, when administered in combination with cooccurring alkaloids such as nalorphine and papaverine, these side effects are countered (http://www.willner.com/Radio/10401.htm).

Verma et al. (1997) studied the synergistic effect of a combination of the plant products curcumin and genistein (Scheme 17.3) and observed that they display synergistic inhibitory effect on the growth of MCF-7 human breast cancer cells induced by estrogenic pesticides. Curcumin is also being tested in combination with another well-known anticancer drug, pacitaxel (Taxol), used in breast and lung cancer metastases in mice (Hoseman, 2002). A recent example is a study of synergistic combination between drugs of diverse origin:



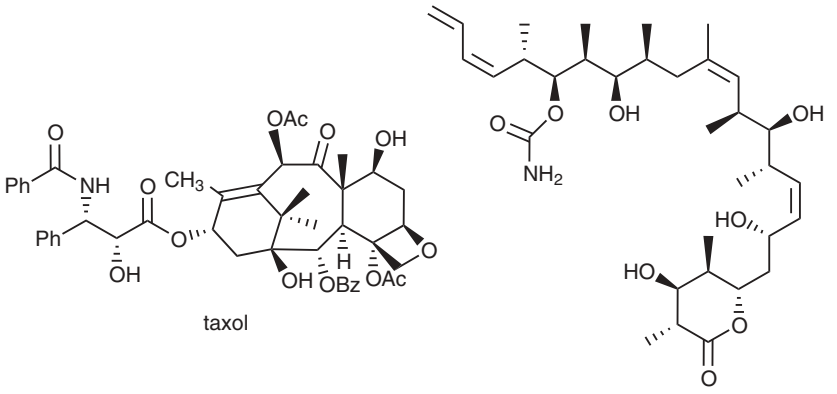
morphine nalorphine papaverine

Scheme 17.2



curcumin genistein

Scheme 17.3



Scheme 17.4

between taxol, a well-known anticancer drug from a plant, and discodermolide, which is isolated from marine sponge (Scheme 17.4). In combination they have reportedly displayed synergistic effects in human carcinoma cell lines (Laura et al., 2000).

Resveratrol is yet other important natural product displaying synergism. It occurs in grapes and red wine and is known as a phytoalexin, which is produced in plants to protect them from the assault of ultraviolet rays and other biological agents. In laboratory studies resveratrol was found to have synergistic effects with drugs such as Videx and Retrovir thus is very helpful in producing anti-HIV effects (Heredia et al., 1999).

Aspirin as a single drug is very effective as a painkiller but causes stomach irritation or ulceration. Therefore, to minimize the side effects a combination with antacids is generally prescribed. An appropriate example of multiherbal formulations is seen in most hepatoprotective preparations. Among the few prescribed herbal preparations in the market, Liv-52, a mixture of more than 20 medicinal plants, is effective in the treatment of common liver disorders. Here a single drug may not be that effective, as liver toxicity may be a manifestation of several metabolic disorders caused by toxins and pollutants.

17.7 INTERACTIONS BETWEEN MODERN DRUGS AND NATURAL PRODUCTS

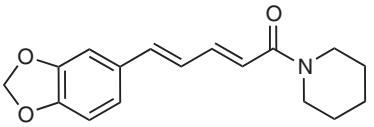
Natural products are known to interact with modern drugs, and these interactions are based on the same pharmacodynamic and pharmacokinetic principles as in the type of drug–drug interactions. These interactions may induce or inhibit drug-metabolizing enzymes

such as cytochrome P450 isoenzymes or may act through impairment of hepatic and other functions. However, if used alone, most herbal preparations are considered to be safe, and the interaction among the herbal preparations and different food supplements may not be that important, although most of these preparations are made by the combination of several herbs or plant parts. Recent studies have shown that ephedrine and pseudoephedrine, the two major alkaloids of the plant ephedra, have additive cardiovascular effects in the presence of caffeine-containing preparations, which may cause serious health problems (Theoharides, 1997). One recently studied example of a drug–food supplement is the interference of garlic (*Allium sativum*), a widely used dietery food supplement, in the metabolism of saquinavir and ritonavir, both used in patients with life-threatening diseases. The combination was suggested to cause serious side effects in patients (Piscitelli et al., 2001).

St. John's wort (*Hypericum perforatum*), a very popular herb used in the treatment of mild to moderate depression, is also known to be a potent inducer of the isoenzyme CYP3A4 and the intestinal P-glycoproteins. It is reported to affect the serum concentration and half-life of indinavir and the P-glycoprotein substrate digoxin (Durr, D., 2000). Another very popular drug, *Ginkgo biloba*, which is used in Alzheimer's disease or as a memory aid and in intermittent claudication, is also reported to be associated with intracerebral hemorrhage in patients stabilized on warfarin for five years (Chung et al., 1987). Episodes of subdural hematoma, subarachnoid hemorrhage, and spontaneous hyphema have occurred in patients using ginkgo along with aspirin or ergotamine. Therefore, the combination of ginkgo and anticogulant drugs or drugs inhibiting platelet adhesion should be avoided (Rowin, 1996). Ipriflavone is a semisynthetic isoflavone soy derivative used to treat osteoporosis. A case report of increased theophyline levels after addition of ipriflavone led to detailed research indicating that ipriflavone or any of its metabolites decreases theophyline metabolism through inhibition of CYP1A2 (Monostory et al., 1998)

17.8 BIOAVAILABILITY AND BIOEFFICACY ENHANCERS

How a drug and an unrelated phytomolecule (derived from a food supplement) interact advantageously for the development of new effective therapies needs to be looked into further. In this context the concept of bioavailability and bioefficacy enhancers developed some years back in the authors' institute needs special mention. In many Indian formulations based on the ayurvedic system of medicine, use of *Trikatu*, a Sanskrit word meaning "three acrids" is well documented. The three herbs: long pepper (*Piper longum*), black pepper (*Piper nigrum*), and ginger (*Gingiber officianalis*) are the essential ingredients of numerous prescriptions and formulations. The studies showed that trikatu and its constituents are capable of enhancing the bioavailability of drug formulations, resulting in enhancement of efficacy or bioactivity. Based on this premise, a group working in the area of drug development proposed that major constituents such as piperine may play an important role for the enhancement of drug bioavailability (Atal et al., 1981). More detailed studies later confirmed that small amounts of piperine increase the bioavailability of specific drugs through several mechanisms, one that could be delaying or affecting their metabolism through inhibition of drug-metabolizing enzymes (Atal et al., 1985; Reen et al., 1993). The direct influence of piperine in the intestinal absorption of specific drug molecules has also been demonstrated (Johri and Zutshi, 1992). Interestingly, recent studies have shown the interactions of piperine with RNA polymerase in *Mycobacterium smegmatis* (Balakrishnan et al., 2001). At specific concentrations in combination with



piperine (bioenhancer)

Scheme 17.5

antibiotics, piperine decreases the minimal inhibitory concentration of several drugs against pathogenic bacteria and fungi.

The concept of bioefficacy enhancement was utilized successfully in combination with a well-known antitubercular drug rifamycin, for which it was shown experimentally that small amounts of piperine significantly increase the bioavailibity of the drug. This observation helped in substantial reduction of doses of rifamycin (by 60%) in experimental animals. Subsequently, in human volunteers, too, the effect of piperine (Scheme 17.5) at reduced doses of rifamycin (200 mg as against 450 mg) was confirmed (Kapil et al., 1995). The traditional wisdom thus appears to be a treasure house of many such concepts, which may go a long way to help patients who require long-term treatment with large doses of drugs, causing several side effects and toxicity. The rifamycin–piperine combination has successfully been demonstrated to be efficacious in multicentric phase III clinical trials and may soon hit the market. Later studies showed that when used along with selected drug molecules such as phenytoin, piperine displayed a similar type of bioavailability enhancement. Besides, Several other classes of drugs, such as anti-inflammatory and many antibacterial, have also displayed some degree of enhancement of bioavailability in experimental animals. No wonder several herbal preparations have appeared in the market containing small doses of Bioperine (piperine). The present work on piperine as a bioenhancer opens a new area of research for scientists looking for new combinations of drugs and methods of treatment. Novel bioenhancers from food supplements and medicinal plants may find use in reducing the doses of many drugs used in long-term treatments such as those for cancer and HIV. In this direction some progress has already been made by our institute, and three potent phytomolecules have been identified. The concept of adding phytomolecules to known drugs to enhance their efficacy may also find acceptability with the Food and Drug Administration and other regulatory agencies thoughout the world in the near future.

17.9 COMBINATION THERAPIES IN MODERN DRUGS

Combination therapies are accepted and now well established. Even in modern (Western) drug systems particularly in several chronic diseases, these are much more effective than single-drug therapies. An NIH news release discloses that in anti-HIV therapy, use of triple-drug combinations, such as either protease inhibitors or nonnucleoside reverse transcriptase inhibitors with any two nucleoside(-tide) reverse transcriptase inhibitors, which include abacavir, lamivudin, tenofovir, and others, greatly reduces the chances of disease progression and death in patients suffering from AIDS. HIV can become resistant to the effects of anti-HIV drugs; it is therefore also recommended that to prevent it one should include at least two new drugs that have not been taken before. Or ideally, all the three drugs should be new. In other cases, such as tuberculosis, cancer, inflammations, and many chronic diseases, combination therapy is prescribed worldwide with proven benefits.

17.10 ROLE OF DEVELOPMENTS IN TECHNOLOGIES AND ANALYTICAL TOOLS

The combination of traditional knowledge with the latest developments in science and technology will have a catalytic effect on the development of effective new therapies. Judicious application of these techniques will help not only in the generation of new databases for the understanding of traditional systems of medicine, but will also shorter the time it takes to realize new leads and their further development. Standardization and safety evaluation of complex herbal formulations is a very important aspect where these techniques are of immense help. Much information is already available in the literature on methods of standardization of herbal extracts and evaluations of their safety. However, the problem of standardization of plant or herbal formulations comprising more than one plant or extract is quite challenging. With the advancements made in the area of separation technologies as well with the introduction of new combination techniques, many of these problems can be tackled effectively. Some new developments in these techniques that may find wider use in the area of drug development based on natural products are described briefly below.

17.10.1 Developments in Separation Technologies

Developments in the area of natural product research are often affected by the inadequacy of technologies for the separation of complex mixtures obtained from plant extracts. However, new developments in the field of separation technologies have simplified the work of chemists. We are now better equipped to explore and harness these new technologies to our advantage.

HPLC–SPE Combination Techniques The need to obtain pure molecules in the shortest period of time led to the development of new techniques in the area of chromatography. In addition to sophistication and added efficiency in the area of preparative high-performance liquid chromatography (HPLC) and more efficient flash chromatographic techniques, a useful concept based on a combination of HPLC and solid-phase extraction (SPE), or SEPBOX, is now being employed successfully for the separation of complex mixture of natural products (Simpson, 2000). In this technique, moderately pure natural molecules ranging from less polar to more polar fractions (300 to 600) can be effected, through two-dimensional separation depending on the capacity of the battery of columns used. A solvent evaporation unit can be coupled to provide fractions or semipure molecules, which may be subjected directly to medium- or high-throughout screening.

Novel analytical techniques such as forced-flow planar chromatography (FFPC) and optimum pressure laminar chromatography (OPLC) are other additions to ever-refined tools for separation on a preparative scale, wherein small amounts of complex mixtures may be separated more efficiently on thin-layer chromatography plates operating at fast medium-pressure development with continuous collection of mobile phase at the end of chromatographic plates (Nyredy, 2000, 2003).

Simulated Moving-Bed Chromatography (SMBC) As the name suggests, this technique is based on the principle of liquid mobile phase and chromatography phase moving in opposite directions. Establishment of equilibrium helps in the improved efficacy of separation of the component of a mixture—often, a mixture of two enantiomers—which are fed into the middle of the column. The advantages are the continuous feeding of the sample

mixture and the reuse and recycling of both separation phase and solvents. In practice, it is generally difficult to implement the concept of a moving bed, although recycling of the moving phase is possible. Therefore, a simulated moving-bed chromatography system is designed with fixed-bed columns connected such that by switching the inlet and outlet points on and off from one column to another periodically the moving-bed effect is simulated. SMBS is more effective than normal fixed-batch chromatography techniques and reduces the use of solvent substantially. Moreover, it is very useful in the separation of materials in large quantities. The technique is now used not only in preparative-scale separation of enantiomers but also in the natural product, pharmaceutical, and flavor industries (Nicoud, 1998): for example, in the separation of sugar mixtures such as fructose and sucrose and xylose and rabinose, stereoisomers of phytol, and fractionation of human serum albumin.

Thermal Gradient in Separation Technologies Separation of natural products using new technologies that yiled improved separations at higher temperatures, such as high-temperature liquid chromatography (HTLC), utilizes temperature gradients rather than the more common solvent gradients (Marin et al., 2004). It has been observed that peak separation and sharpness improve significantly at high temperatures (Fields et al., 2001). Some occasions where this technology has been used successfully include natural products such as the major constituents of green tea, ginseng, and cocoa. Chromatographic techniques have also been used successfully in the separation of enantiomers and diastereomers of many drugs.

Supercritical Fluid Extraction of Herbal Plants Supercritical fluid extraction (SCFE) processes utilize dense gases such as carbon dioxide and ammonia at their critical temperature and pressure (31°C and 200 to 500 bar for CO_2). The compressed gas has the density of a liquid, but is able to penetrate deeply into plant cells and dissolve the lipophilic constituents. The pressure is then carefully released, the gas dissipates harmlessly into the atmosphere, and all that is left behind is the pure, concentrated extract; no pollution, no heat stress or damage, and no solvent residue results. If made carefully the extract can be a broad, virtually complete representation of a plant's lipophilic constituents. The extracts are free of undesirable components such as solvent residue, heavy metals, and inorganic materials. With increasing necessity to eliminate solvent residues in pharmaceuticals and medical and nutraceuticals, and stricter regulations on volatile organic compound (VOC) and ozone depleting compounds (ODC) emissions, the use of SCFE is proliferating rapidly in industrial sectors. Most nonpolar and medium-polar components up to 500 molecular weight are extracted in supercritical carbon dioxide. However, this property of CO_2 can be used advantageously for the extraction and fractionation of the desired material by making use of mixed solvents such as ether–carbon dioxide, water–carbon dioxide, and ethanol–carbon dioxide for the fractionation of plant extracts into nonpolar and medium-polar fractions. By clever variation of mixed solvents, separation of natural product mixtures into their components can be achieved with respect to their polarity (Catchpole et al., 2003). In general, SCFC processes are faster and more efficient than conventional methods as well as green in nature.

17.10.2 Developments in Combined Techniques and Advanced Technologies

Often, chemists working in the area of natural products face problems of separation, purification, and identification or structural assignment of the isolates. It is a very difficult task to purify a natural molecule to complete homogeneity. The presence of small amounts

of impurities impedes structure elucidation work to a great extent. These problems can now be tackled easily with combinations of separation and spectral techniques known as "hyphenated techniques." Thus, a combination of HPLC with electron spray ionization tandem mass spectrometery (ESI–MS/MS) or with nuclear magnetic resonance (NMR) has become a convenient tool for rapid identification and structure elucidation. The LC–circular dichroism (CD) combination helps not only in identification of biomolecules but also in solving the configurational and stereostructural aspects. HPLC–NMR has become a very handy and potent tool in the hands of a chemist, due the introduction of powerful solvent suppression techniques and their coupling with two-dimensional homo- and heteronuclear experiments, such as TOCSY, NOESY, and ROESY (Bringmann et al., 1999, 2000, 2001). These techniques are highly sensitive and it is possible to obtain satisfactory data at the nanogram level. The combination of LC with MS, NMR, and CD, also called the LC-triad, has been very helpful in determination of structure as well as well as metabolite profiling of several natural molecules. Information about absolute configuration is generally not available directly from NMR or MS data. LC–CD coupling with quantum chemical calculation can, however, bridge this gap effectively in natural biomolecules, where stereochemistry plays an important role with respect to three-dimensional structures. Such is the strength of these new and powerful techniques that it has made the task of structure elucidation very rapid, with high throughput. AnalytiCon, a German firm, claims to have used these instruments very successfully in combination with a known natural product database in the structural assignment of thousands of molecules. In the past five years they claim to have solved the structures of over 27,000 molecules from nature: among them, 15,000 never reported before (Rouhi, 2003).

17.10.3 Molecular Farming and Bioengineering of Medicinal Plants

The biogenetic pathways of metabolites in plants or other natural species are often complex and multibranched. Biogenetic pathway engineering or genetic manipulation can help to increase or decrease the content of important biomolecules in these plants. Thorough understanding of the biosynthesis or pathways is essential for genetic modifications leading to increased production of desired secondary metabolites. Knowledge of regulatory genes and enzymes together with the latest bioengineering techniques may help to develop "designer plants" with modified properties. Through directed evolution methods such as optimized DNA shuffling and the staggered extension process in vitro recombination method, it is possible to generate novel libraries of biomolecules and to produce desired molecules in plants in large quantities (Locher et al., 2002). In other developments it is now also possible to grow plants that may increase immunity toward infectious diseases. In fact, human antibodies produced in certain plants are already at clinical trial stages. By transplanting human or other genes into commercial plants such as corn, large-scale production of antibodies (also termed "plantibodies"), drugs and vaccines manufactured in specific plants may become possible in the near future (Stoger et al., 2002). However, producing them affordably will be a big challenge to biotechnologists as well as to drug manufacturers.

17.10.4 High-Throughput Screening of Natural Products

High-throughput screening (HTS) is a powerful analytical tool for assaying large and small libraries of molecules. In these assays, molecular targets for diseases are adapted to a variety of conventional assay platforms, such as binding assays or enzyme inhibition

assays used with automated instrumentation to screen libraries of both natural and synthetic compounds used as binders, activators, or inhibitors of target molecules (Entzeroth, 2003). Disciplines other than biology have now entered the screening scene; bioinformatics, microtechnology, and analytics provide us with powerful combinations and applications hitherto difficult to achieve. Advancements in the area of HTS due to highly sensitive high-speed detection methods, automation, and miniaturization have enabled screening of up to 100,000 compounds per day against a given assay. These natural product libraries can be made available in standard form ready for HTS microtiter plates. A wide range of assay systems are already available that are applicable to a range of therapeutic areas. It is being recognized that approximately 5000 of 31,000 genes in the human genome will provide targets for therapeutic intervention in disease. To serve these highly efficient search batteries, it is necessary to have access to a large number of test compounds. Combinatorial chemistry has played a significant role in this respect; however, natural products remain the most expensive source of test molecules in the pharmaceutical industry (Butler, 2004). In the last few years, thanks to developments in the separation technologies as well as to the introduction of hyphenated techniques for faster identification, the creation of medium-sized libraries of natural products has become much easier.

With the developments made in the area of biosensors, the future of HTS for drug discovery looks even brighter (Keusgen, 2002). A biosensor is an analytical device consisting of a biological system (e.g., enzymes, antibodies, cells, DNA) and a physical transducer (e.g., electrode, optical device). The selectivity of a biosensor is determined by the integrated biological component. These systems, which until now have been used for routine analysis, such as clinical diagnosis, are now being employed for the screening of new drugs.

17.11 HERBAL MEDICINE: THE BEST POSSIBLE ROUTE TO HEALTH CARE

Traditional knowledge accumulated through the ages has now become accessible to the common man. Digitalization of this knowledge, particularly for herbal drugs, will make it accessible at the click of a button. Attempts in this direction have been initiated in India with the inauguration of a digital library of traditional knowledge (TKDL, 2003). This will also help to solve problems related to intellectual property rights faced by many countries. This large and valuable reservoir with its vast diversity makes a strong base for the development of new and effective treatments of complicated diseases that will help to improve the quality of life. Plants, particularly the higher plants, provide secondary metabolites of wider molecular diversity than that of most other classes of organisms. There is a growing realization about the benefit of using crude plant extracts containing mixtures rather a single entity. However, there are still many challenges and obstacles to the acceptance of plant extracts in modern medicinal concepts. One of the foremost challenges faced is that of standardization or the determination of precise chemical composition of various extracts derived from a single or a mixture of plants. There may be variations in compositions that have their origin in genetic and environmental factors. There is also much concern about safety regulations, toxicity, and hazard identification of herbal preparations. There is also some concern, though it is not that important, that many of the extracts that show interesting activity may contain a mixture of mostly known molecules. Or the major activity may rest in polyphenols or polysaccharides or similar polymeric constituents that may be difficult to standardize. Many of these concerns can be defeated by utilization of the

latest technological tools. Successful attempts in this direction have already been made. The standardized Japanese drug for liver cancer, Sho-saiko-to, a mixture of seven herbal drugs, is now undergoing clinical trials and may soon become a prescription drug (Bayes et al., 2003; Shimizu, 2000). However, there is need to have parallel approaches, including standardized single or composite formulations or their extracts and conventional lines of attack to isolate and identify the pure and active ingredients and molecules, including lead optimization and a diversity-oriented approach using a combinatorial or classical approach if required. This will help us to better understand the bioactivity, metabolism, and mechanism of action. With the introduction of new and sophisticated hyphenated techniques as well as advancements in screening methodologies, the future of natural products appears to be very promising.

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18

NATURAL PRODUCTS AS AN INSPIRATION FOR THE DISCOVERY OF NEW HIGH-THROUGHPUT CHEMICAL SYNTHESIS TOOLS

STEVEN V. LEY, IAN R. BAXENDALE, DEBORAH A. LONGBOTTOM, AND REBECCA M. MYERS

University of Cambridge
Cambridge, UK

18.1 INTRODUCTION

The natural world inspires us: from artist and philosopher to biologist and chemist. In our quest for new knowledge, the exquisite and varied architectures of natural products provide a rich pallete for discovery. Whether these are used to probe biological mechanisms or to provide the basis for pharmaceutical drug discovery, natural products continue to command attention. For many of these reasons, synthesis chemists are drawn to these structures as testing grounds for synthetic strategies and for the development of new methods. But we are also drawn to advance the art of molecular assembly of some of nature's most enigmatic creations. However, more is accessible to the synthesis chemists' skills: They can modify natural materials to probe structure–activity profiles, and and they can provide fragment molecules or related structural scaffolds through library generation.[1] Yet the synthesis chemists can go beyond the molecule, contemplating macromolecular assemblies and creating unnatural arrangements with awe-inspiring levels of molecular diversity, limited only by their imaginations.

Multistep organic synthesis remains a difficult endeavor, however, requiring extensive knowledge and appreciation of both the mechanism and reactivity of molecules. In a synthesis pathway, complex decision making must be balanced against the logic and creativity required for the process. The pathway chosen must provide products with the desired structures and properties in a timely fashion and at an acceptable cost. Increasingly, we expect more step and atom efficient processes to evolve to achieve better and more sustainable practices for the

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future. As we move forward, new tools that can be used for chemical synthesis are becoming available, especially those founded on automation and miniaturization technologies. Synthesis is changing and we must embrace these new techniques and exploit their full potential.

In this chapter we review how methods of immobilizing reagents on polymeric supports[2] or using scavenging agents and catch-and-release techniques can affect natural product synthesis and thereby create opportunities for structures mimicking naturally occurring architectures.[3] In this chapter we also discuss how these new methods can generate derivatives and analogs of a diverse range of compounds.[4]

At this point, preliminary remarks concerning the process of immobilization and its potential application in synthesis are appropriate. First, immobilization is not a new concept since it has been practiced since 1946.[5] Having said this, tremendous advances have been made in this field, and our group in particular has sought to develop the application of immobilized systems to complex synthetic challenges in a multistep mode,[6] harnessing all the aspects and opportunities that attachment to a polymeric support has to offer.

Second, in situations where highly toxic or obnoxious materials must be used, by virtue of the attachment process such unpleasant compounds can be rendered much easier to manipulate and recover, and therefore, once immobilized, safer to use. Similarly, the immobilization of expensive catalysts and ligands greatly improves the chances that they can be recovered, then recycled, and the spent reagents can be recovered by filtration and recycled. Using supported reagents by-products and unwanted components can also be scavenged and taken out of circulation by filtration. This obviates the need for more conventional and often more expensive or wasteful procedures, such as chromatography, distillation, or crystallization.

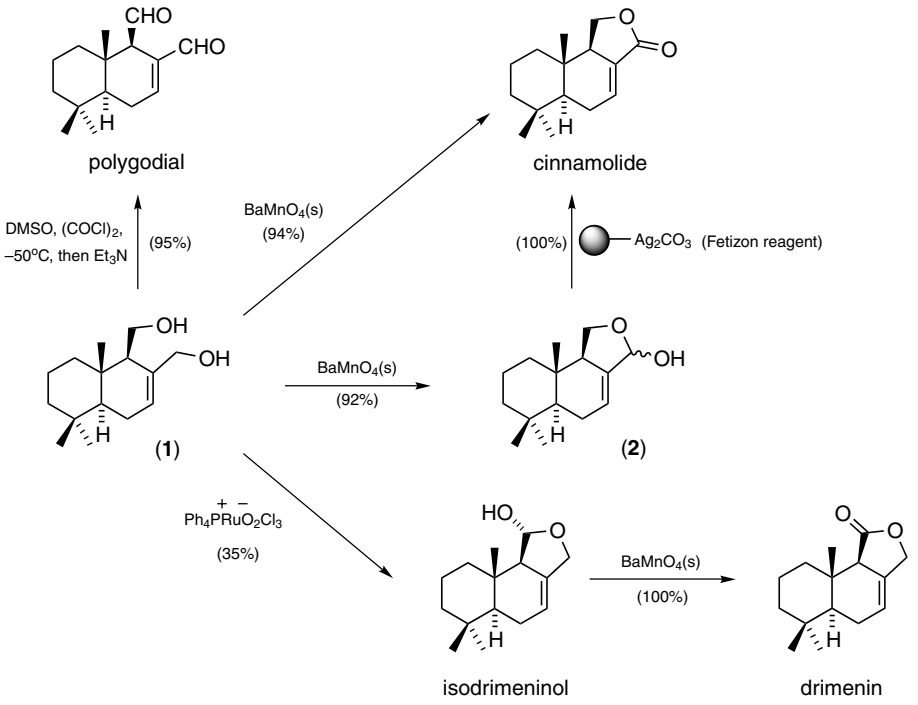
Polymer-bound species can be added to reactions that are programmed to recognize only the target product. This, of course, is not a serendipitous process but requires specific chemical design. Products can then be captured and washed, then rereleased to give clean compounds, a procedure otherwise known as the catch-and-release technique for compound purification.

Effective syntheses, convergent as well as batchsplitting, are possible using these methods, which can provide both combinatorial arrays and fragment sets of new molecules. The act of immobilization can be accomplished in a variety of ways, such as on beads, surfaces, colloids, plugs, and laminates. Many materials are available that can be used (e.g., polymers, cellulose, silica gels). Microencapsulation methods can also be employed, and these are discussed later.

Immobilization intrinsically results in reagents becoming site-isolated; that is, immobilized reagents are able to react with substrates in a solution but not readily with one another. This means that multireagent processes such as oxidation and reduction or other mutually incompatible transformations can be conducted simultaneously in single-reaction vessels. This is an extremely attractive concept and constitutes a new way of thinking about synthesis. One-pot processes are of great interest in modern synthetic practice, as they minimize solvent use and workup procedures such as extensive water washes or quenching steps, which can affect yields adversely. The inherently slower reactivity of some immobilized systems can even be enhanced when these methods are used in conjunction with ionic liquids and microwave dielectric heating protocols.[7]

18.2 SOLID-SUPPORTED REAGENTS AS TOOLS IN NATURAL PRODUCT SYNTHESIS

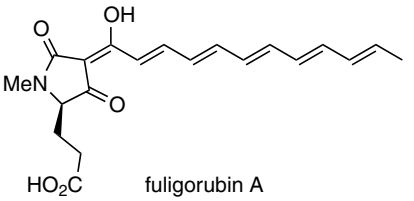
Supported chemical reagents give opportunities for improving automation,[8] information feedback, and self-optimization processes, especially using design of experiment



Scheme 18.1 Synthesis of sesquiterpenes: polygodiol, cinnamolide, drimenin, and isodrimeninol.

(DoE), principal component analysis (PCA), and ReactArray software.[9] In recent years, supported reagents have played a prominent role in a number of our own synthesis programs as well as those emanating from other research groups; a selection of these is now discussed.

During our work on the synthesis of various drimane-related compounds[10] we encountered a difficult problem concerning selective oxidation of the diol (**1**, Scheme 18.1), which we believed would serve as a versatile precursor for a number of natural products. Double oxidation of **1** worked well under Swern conditions to give the insect antifeedant polygodiol in 95% yield. However, selective oxidation of the allylic alcohol to the corresponding cyclic hemiacetal, and subsequent oxidation to the natural product cinnamolide, was more problematic when conventional oxidants were used, as low yields of mixed products were typically obtained. A solution to this problem was finally found in the form of a two-step oxidation protocol. First, treatment of the diol with solid barium manganate facilitated the desired hemiacetal formation of lactol (**2**) in 92% yield. Subsequent reaction of the lactol with silver carbonate adsorbed on to celite (the Fetizon reagent) gave cinnamolide in quantitative yield simply by filtering away the spent reagent. In fact, solid barium manganate alone at room temperature was even better. By manipulating the number of equivalents used in the reaction, the lactol (**2**, or cinnamolide) could be delivered selectively in excellent yields. To convert **1** to the natural product isodrimeninol, we used dioxotetraphenylp hosphonium ruthenium trichloride in methylene chloride. Further oxidation to drimenin with solid barium manganate was achieved at room temperature and worked up by filtration through a celite pad; other oxidants required chromatographic purification of the product.

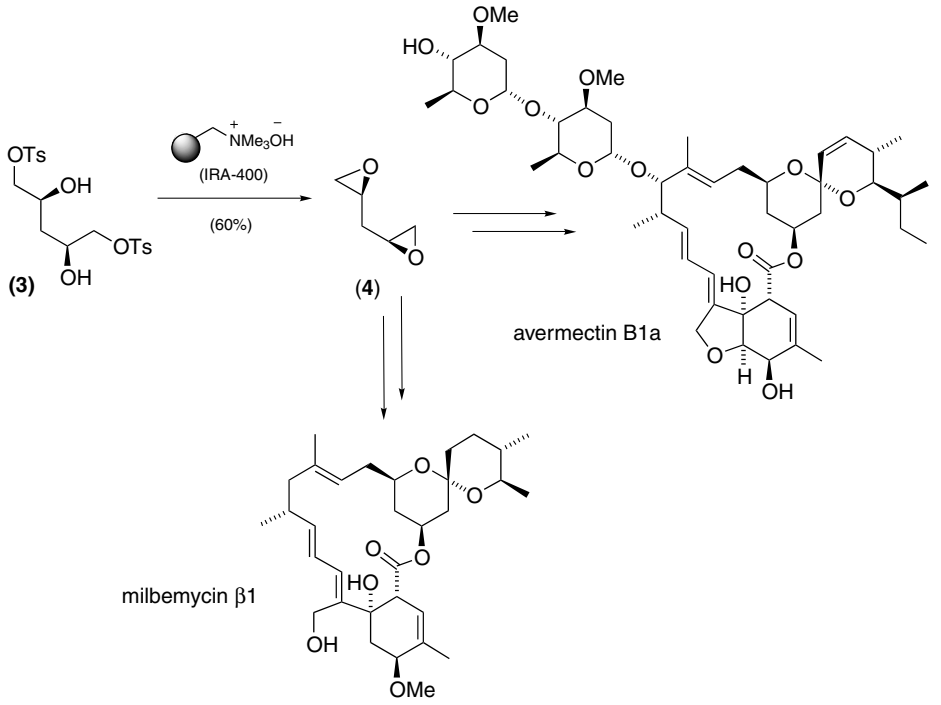


Scheme 18.2 Fuligorubin A, a polyene acyltetramic acid pigment isolated from the yellow slime mold *Fuligo septica* (L).

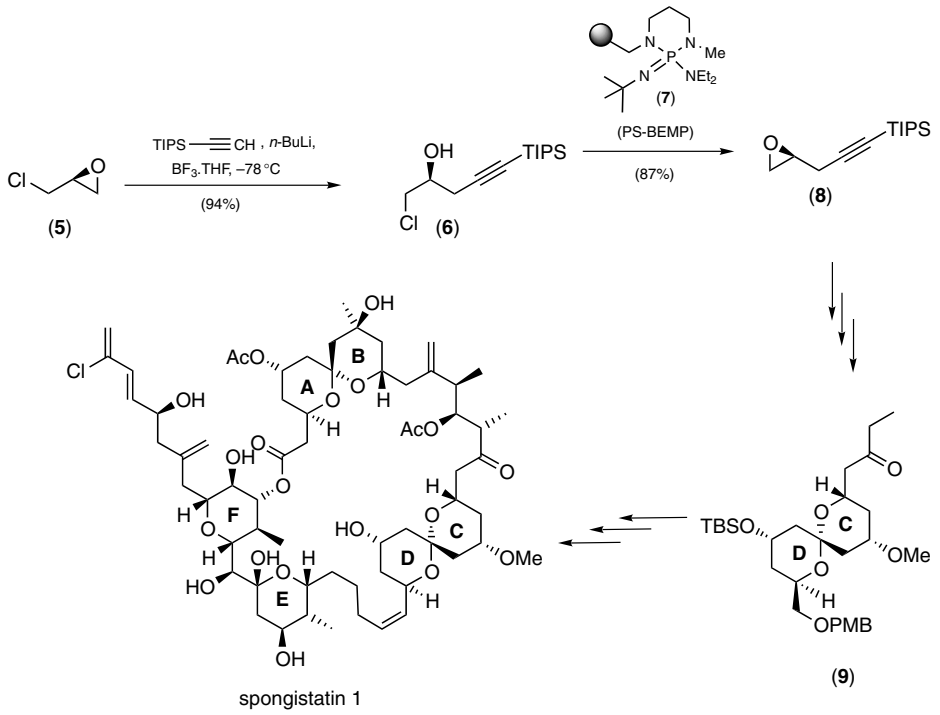
Similar insoluble oxidants were also used in the preparation of volatile, unstable polyene aldehydes during work on the plasmodial pigment fuligorubin A,[11] while comparable solution-phase oxidants were markedly less successful (scheme 18.2).

One further instance where product volatility or its instability had been problematic was during the preparation of the bisepoxide (**4**), a lynchpin for the spiroketal components in the synthesis of the antiparasitic agent milbemycin β1 and the insecticide avermectin B1a (Scheme 18.3).[12] Here the epoxide was formed by treatment of the precursor bistosylate (**3**) with a basic ion-exchange resin (IRA-400 in OH^- form) to give the highly volatile bisepoxide in 60% yield; simply filtering away the spent resin was sufficient to remove the by-products of the reaction. In contrast, all of the solution-phase bases that were examined gave very poor yields, owing to difficulties in the workup due to the volatility of the product.

A more recent example from our group, where the application of supported reagents was crucial to the success of a synthesis, occurred en route to spongistatin 1



Scheme 18.3 Key step in the synthesis of avermectin B1a and milbemycin β1.

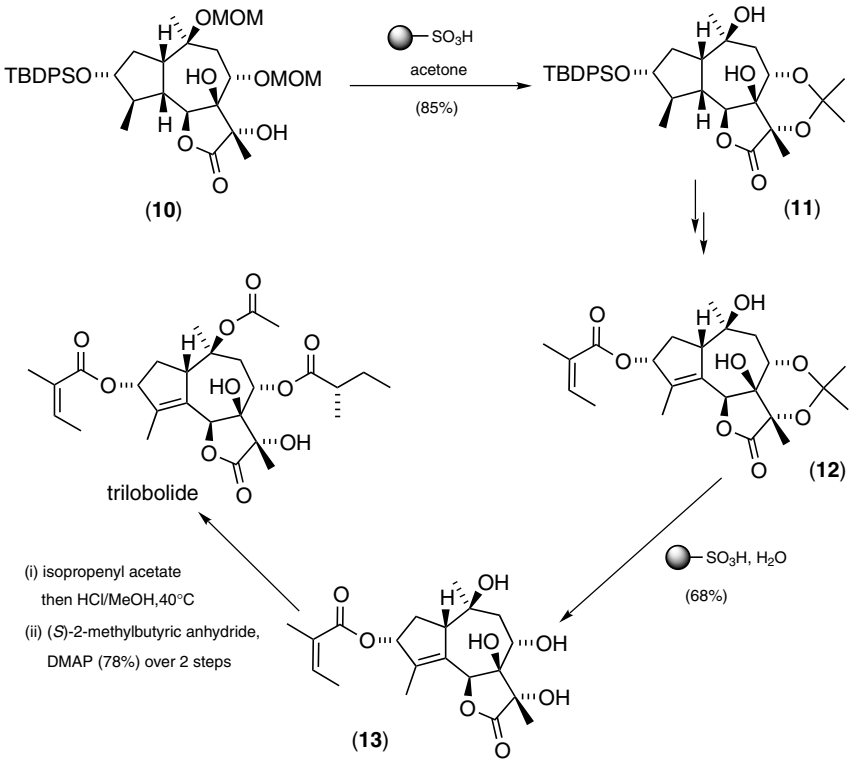


Scheme 18.4 Use of a polymer-supported phosphazene base in a key step in synthesis of the C/D fragment of spongistatin 1.

(Scheme 18.4).[13] For preparation of the C/D spiroketal fragment (**9**), we needed to transform the chloroepoxide (**5**) to a second homologated epoxide (**8**) via an intermediate chlorohydrin (**6**) (Scheme 18.4). Solution-phase bases were used initially but led to alternative allylic alcohol products; however, the polymer-supported phosphazene base (**7**; PS-BEMP) was more successful and allowed the epoxide (**8**) to be isolated following filtration in 87% yield.

During synthesis of the thapsigargin family member trilobolide, a difficult acid-catalyzed process was necessary. Again, an immobilized reagent, sulfonic acid in this case, came to the rescue (Scheme 18.5).[14] The step required the subtle selective removal of MOM-protecting groups of the intermediate (**10**), without concomitant loss of silicon and simultaneous acetonide protection to give **11**. Supported sulfonic acid was also used in the deprotection of **12** to **13**, which then underwent selective acylation to give trilobolide.

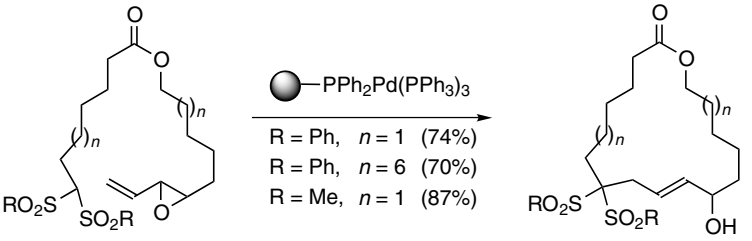
Although it would be possible to cite many other examples where immobilized reagents have played a key role in complex molecule synthesis, this would be outside the scope of the chapter. However, three further examples do deserve special mention. First, the pseudodilution that is encountered as a result of immobilization can be important when applied to solution reactions that are normally possible only under high dilution. This is as a result of intermolecular reactions being restricted, owing to isolation of specific reaction sites on the support material. Reactions with supported reagents can therefore be conducted at much higher concentrations. This phenomenon is particularly beneficial in macrocyclization events. A good example demonstrating this principle can be found in how



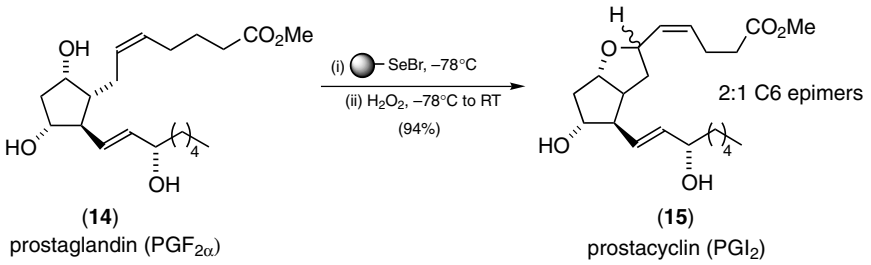
Scheme 18.5 Steps in the synthesis of trilobolide requiring orthogonal protection and deprotection using polymer-supported sulfonic acid.

application of a polymer-bound palladium coupling agent circumvented the need for high dilution (Scheme 18.6).[15] Here, the reactive functionality is effectively "switched on" only at active sites on the polymer, thus favoring intramolecular ring closure over dimerization or oligomerization.

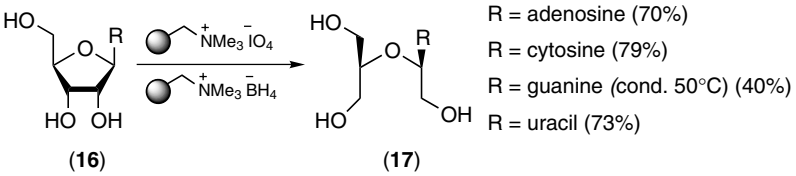
Second, the ability to immobilize obnoxious sulfur or selenium reagents is very appealing, as they become air-stable and odorless, unlike their pungent solution-phase counterparts. One of many applications of these otherwise unpleasant reagents employs a polymer-supported selenyl bromide. The reagent had been prepared simply by the lithiation of a polystyrene resin and subsequent quenching with dimethyl diselenide and exposure to bromine. This reagent has been used in a number of successful transformations, including, for example, a



Scheme 18.6 Palladium-catalyzed allylic substitution: an example of pseudodilute conditions.



Scheme 18.7 Immobilized selenium species for cyclo-addition reactions as used for the transformation of $PGF_{2\alpha}$ to PGI_2.



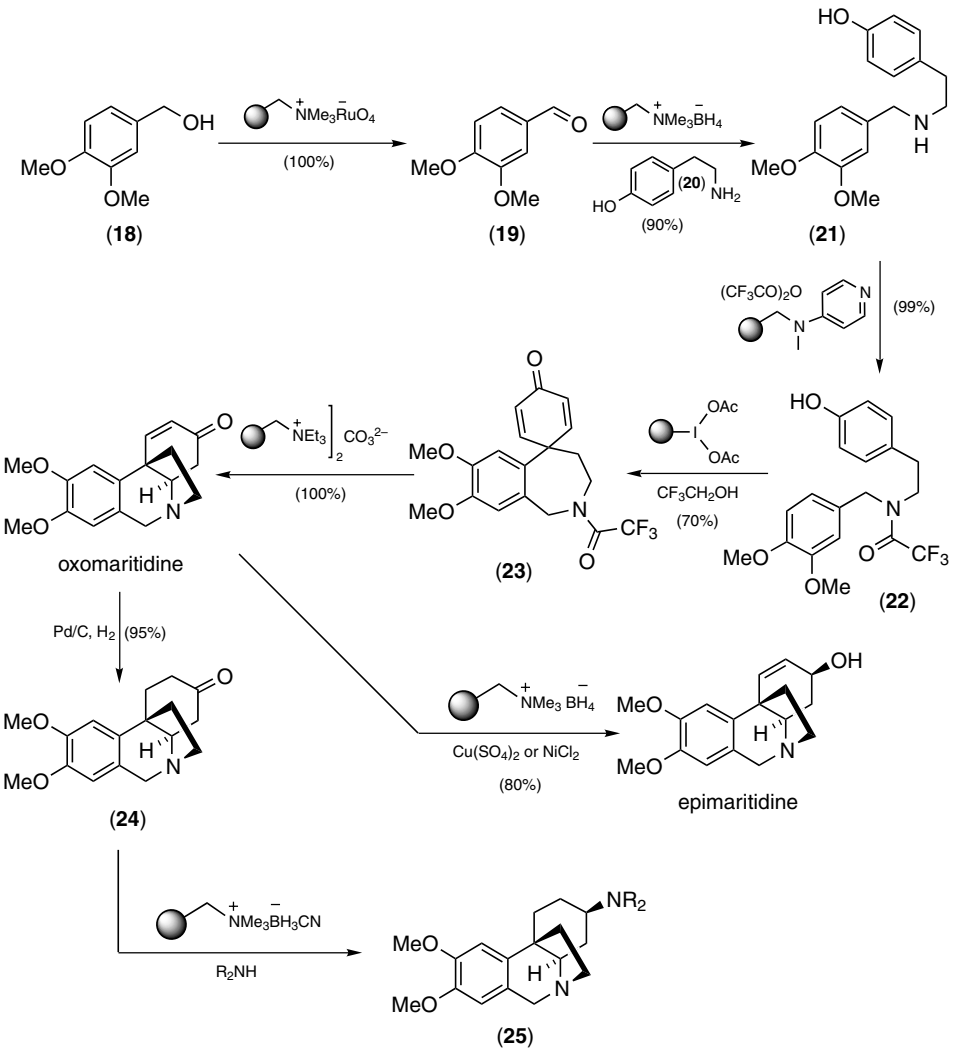
Scheme 18.8 Simultaneous use of otherwise mutually incompatible reagents in the synthesis of trihydroxy nucleosides using both a polymer-supported oxidant and a polymer-Supported reductant.

selenium-mediated ring closure of the methyl ester of prostaglandin $PGF_{2\alpha}$ (**14**). This generated a supported product, which on oxidation and *syn*-elimination is detached as the final unsaturated prostacyclin (PGI_2) analog (**15**) (Scheme 18.7).[16]

Finally, as mentioned briefly earlier, site isolation in polymer-supported reagents is an extremely important feature and thus can be a great advantage in synthesis. An early example of this is seen in the synthesis of the otherwise low-yielding and difficult-to-purify acyclovir-related (Zovirax) nucleosides. These practical inconveniences were overcome by taking advantage of site-isolation properties. In this instance, a polymer-supported oxidant (periodate) and a polymer-supported reductant (borohydride) were used mixed together as dry resins, while a nucleoside (**16**), dissolved in a small amount of water, was pumped through a column packed with the resins. This resulted in selective cleavage of the vicinal diol to give four trihydroxy nucleosides (**17**).[17] The unstable intermediate dialdehyde resulting from diol cleavage with periodate was reduced immediately in situ by the borohydride (Scheme 18.8).

18.3 MULTISTEP USE OF SUPPORTED REAGENTS IN NATURAL PRODUCT SYNTHESIS

Although these examples illustrate opportunities for using a variety of immobilized reagents for individual transformations, a more imaginative use of these methods is in multistep syntheses. In this respect our group's approach has been to minimize the use of conventional workup procedures, yet be able to prepare complex natural products and related compounds simply and efficiently. In this chapter we refrain from commenting on the use of on-bead synthesis of natural compounds or on the diversity-oriented procedures,



Scheme 18.9 First orchestrated and successive application of solid-supported reagents in the synthesis of natural products: synthesis of epimaritidine and oxomaritidine.

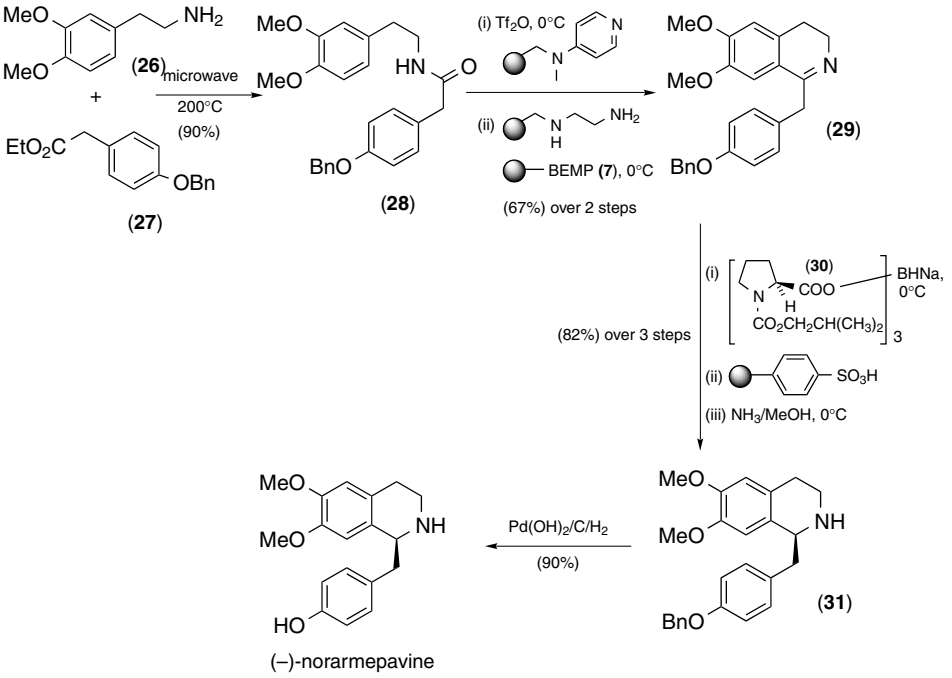
as these are described adequately elsewhere.[1,18] It will, however, concentrate on how the innovative and creative use of solid-supported reagents and scavengers is beginning to influence the synthesis of natural products in a more general manner.

The first serious multistep application of supported reagents for natural product synthesis was reported by our group in 1999 when we reported concise routes to the amaryllidaceae alkaloids oxomaritidine and epimaritidine in five and six steps, respectively (Scheme 18.9).[19] Supported reagents featured in each of the steps in sequential fashion, leading to pure products by filtration of spent reagents. The route began with the oxidation of a benzylic alcohol (**18**) to an aldehyde (**19**) using polymer-supported perruthenate (PSP), an oxidizing catalyst that we had developed previously.[20] This aldehyde was reductively aminated with a phenolic amine (**20**) using a polymer-supported borohydride,[21] again under

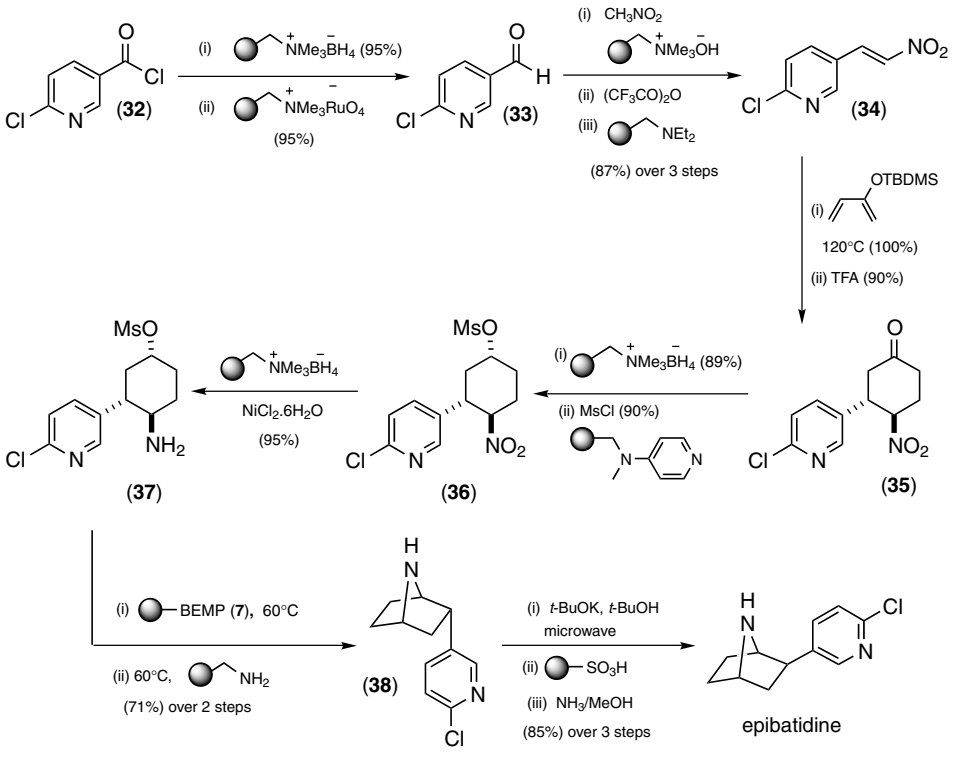
conditions that we had optimized earlier.[22] The secondary amine (**21**) was then protected as its *N*-trifluoroacetate using immobilized dimethylaminopyridine (PS-DMAP) as a base and a catalyst. Oxidative coupling of **22** to form a spirodienone (**23**) was accomplished using a polymer-supported hypervalent iodine reagent that we developed specifically for this task.[23] The last steps required removal of the trifluoroacetoxy using a wet carbonate resin. This caused simultaneous cyclization by conjugate addition of the released amine to the enone to give oxomaritidine. Treatment of this with an immobilized copper boride (or nickel boride) delivered the second natural product, epimaritidine, in an excellent 50% yield over the six steps.

Other analogs were also prepared from oxomaritidine by hydrogenation to give **24**, which on reductive amination with polymer-supported cyanoborohydride afforded a small library of unnatural analogs based on the structure of **25**. Obviously, many other derivatives would be possible given the efficiency of this route to the natural products, and clearly many of the intermediates, such as the spirodienone **23**, could usefully be diverted to other combinatorial chemistry programs for further elaboration and decoration.

Using these methods we more recently reported the synthesis of another isoquinoline alkaloid, (−)-norarmepavine (Scheme 18.10).[24] This was accomplished by coupling the amine **26** with the ester **27** to give an intermediate amide (**28**). This was best done using focused microwave irradiation under solvent-free conditions. Next, **28** was converted to a Bischler–Napieralski product by using a combination of triflic anhydride and PS-DMAP. This was followed by scavenging with polymer-supported *N*-(2-aminoethyl)aminomethyl polystyrene to remove any excess anhydride. The intermediate triflate was then converted to the free base **29** with PS-BEMP (**7**), mentioned earlier. Finally, a proline-derived reductant



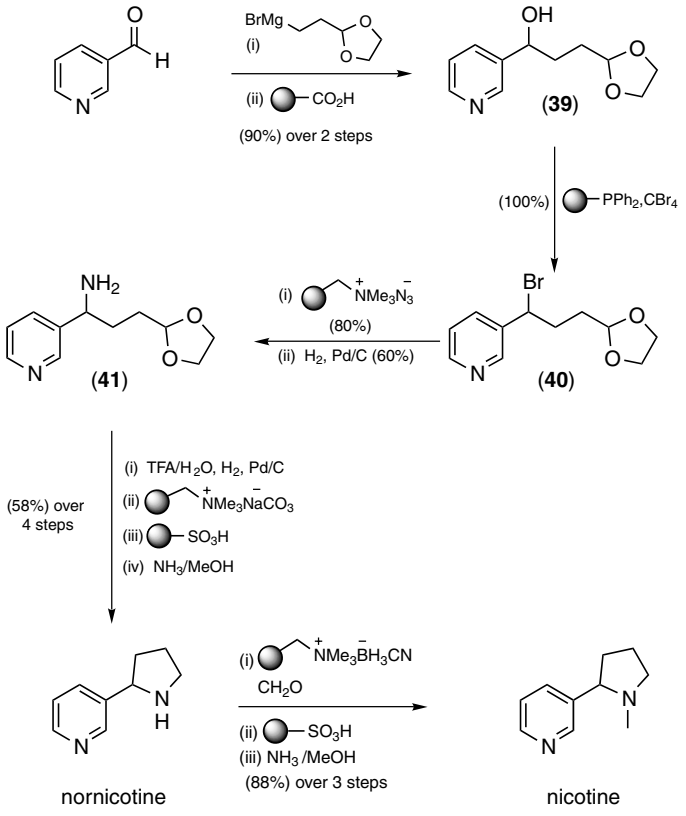
Scheme 18.10 Enantioselective synthesis of the isoquinoline alkaloid (−)-norarmepavine using polymer-supported reagents.



Scheme 18.11 Synthesis of alkaloid epibatidine using 10 polymer-supported reagents or scavenger-assisted steps.

(**30**) effected an asymmetric stereoselective reduction to give **31**, which was purified by a polymer catch-and-release technique using a polymer-supported sulfonic acid capture and a methanolic ammonia release step. After appropriate benzyl deprotection, the natural product (−)-norarmepavine was obtained.

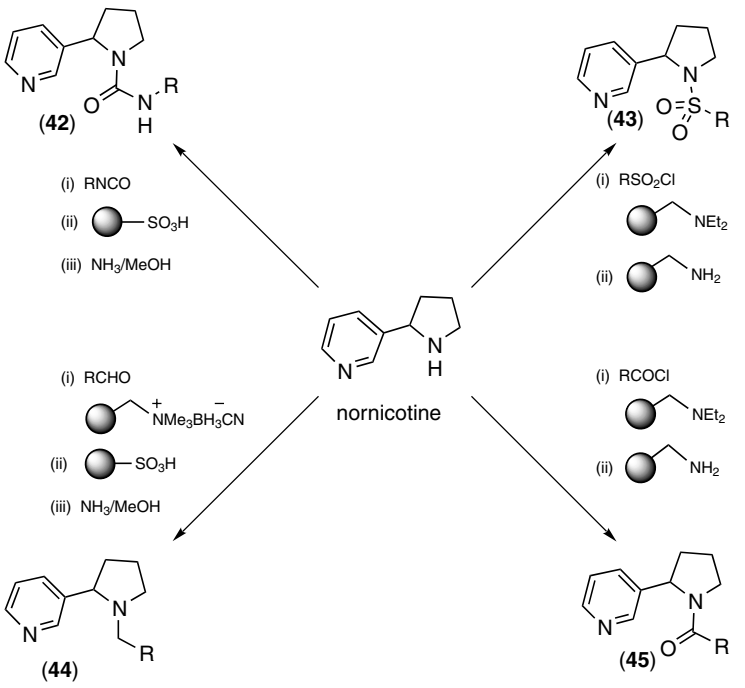
A more challenging synthesis is that of the potent analgesic alkaloid epibatidine, a compound isolated from the Equadorian poison frog, *Epipedobates tricolour*. This synthesis made use of no less than 10 supported reagent and scavenger systems (Scheme 18.11).[25] This early total synthesis of the natural product merits further comment. First, the aldehyde **33** was prepared from the commercially available acid chloride **32** by a two-step process comprising a polymer-supported borohydride reduction followed by oxidation with PSP. The aldehyde then underwent straightforward conversion to the nitrostyrene via a Henry reaction catalyzed by polymeric base, followed by elimination of an intermediate trifluoroacetate with a polymer-bound diethylamine base. The product (**34**) underwent a Diels–Alder reaction to a cyclohexanone (**35**), which after reduction and conversion to a mesylate using polymer-supported reagents, gave **36**. To achieve selective reduction of the nitro group in this compound without affecting the aromatic chloride, we found nickel boride generated on a polymeric support to be particularly effective. Indeed, this selectively formed the desired amine (**37**) in excellent 95% yield and in a 7 : 1 ratio of diastereomers. Cyclization of the natural product precursor (**37**) occurred efficiently in the presence of PS-BEMP **7**, using a polymer-supported amine base to scavenge the cyclized minor mesylate diastereomer.



Scheme 18.12 Synthesis of nornicotine and nicotine.

Finally, compound **38** had to be epimerized to epibatidine, and this was best done using microwave heating in the presence of potassium *t*-butoxide in *t*-butanol. This was one of the early examples of focused microwave heating. The natural product was then isolated by catch-and-release: caught with a polymer-supported sulfonic acid followed by release using methanolic ammonia.

A somewhat simpler alkaloid, nicotine, can also be made from pyridine precursors (Scheme 18.12).[26] In the first step a 1,3-dioxolane Grignard reagent was added to pyridine 3-carboxyaldehyde, working the reaction up by adding Amberlite IRC-50, a carboxylic acid–functionalized resin, to give an alcohol (**39**). This was elaborated further to the corresponding bromide using an immobilized triphenylphosphine in the presence of carbon tetrabromide.[27] This very convenient procedure for halide production has been used in many syntheses of reactive halides.[28] Next, the bromide thus formed (**40**) was displaced by a polymer-supported azide. It is noteworthy, however, that the azido product (which are often hazardous) does not need a conventional workup; rather, after filtration it can be reduced in situ to give the amine **41**. After acid-mediated acetal removal an intermediate cyclic imine is formed, which is reduced further in situ, with palladium on carbon, to nornicotine. Purification again employs a catch-and-release protocol to give the natural product in gram quantities suitable for further modification. Finally, conversion of nornicotine to nicotine was achieved by reductive methylation with formaldehyde and polymer-supported cyanoborohydride.



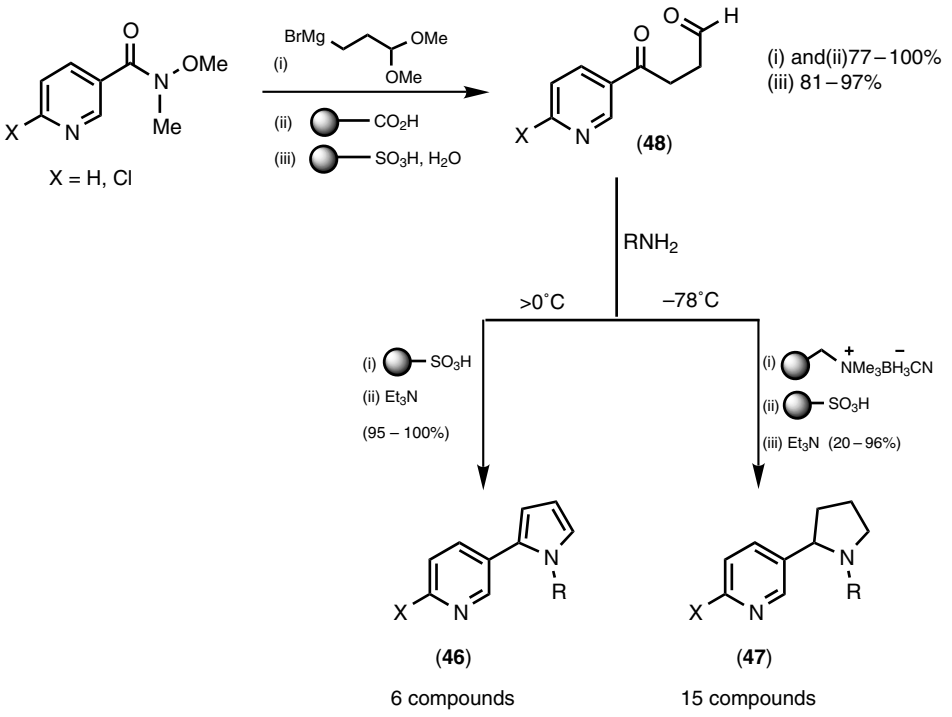
Scheme 18.13 Combinatorial decoration of the nornicotine core.

Nornicotine and nicotine have potent biological activity, especially as ligands at the nicotinic acetylcholine receptor as well as cognitive function as neuroprotecting agents, so analogs of these compounds are of considerable interest for biological studies.[29] A number of analogs have been prepared in a library format, starting from nornicotine using immobilized systems (Scheme 18.13).[26] A range of ureas (**42**), sulfonamides (**43**), and alkyl (**44**) and acyl (**45**) derivatives were all synthesized efficiently using polymer-supported reagents and catch-and-release purification techniques. Yields were good to excellent (44 to 100%), and purities of the products were in excess of 90% according to liquid chromatographic/masss spectrometric analysis.

In our original article on this work,[26] alternative sequences to pyridinopyrroles were developed, one of which also gave easy access to aryl pyrroles (**46**) and pyrrolidines (**47**) from an intermediate keto aldehyde (**48**) (Scheme 18.14).

Pyrroles have their own importance in natural product chemistry: for example, dioxapyrrolomycin, which is an interesting insecticide, and pyrrolonitrin, a broad-spectrum antibiotic (Scheme 18.15).[30] A route to various analogs based on these structures was developed that used a five-step chromatography-free polymer-supported synthesis (Scheme 18.16).[31]

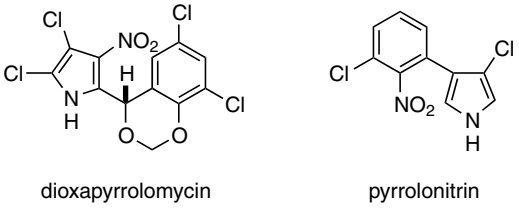
Here aldehydes (**50**) were formed by polymer-supported permanganate oxidation of commercially available benzylic alcohols (**49**), and after transformation to nitrostyrenes (**51**), they acted as excellent precursors for pyrrole formation using the polymer-supported guanidine base 1,5,7-triazabicyclo[4.4.0]dec-5-ene (PS-TBD). The products were converted to other materials useful for biological evaluation by a number of transformations, including alkylation and decarboxylation (**52** to **53**). By making appropriate changes to the building blocks, arrays of other compounds may be obtained with variations in R^1, R^2, and R^3.



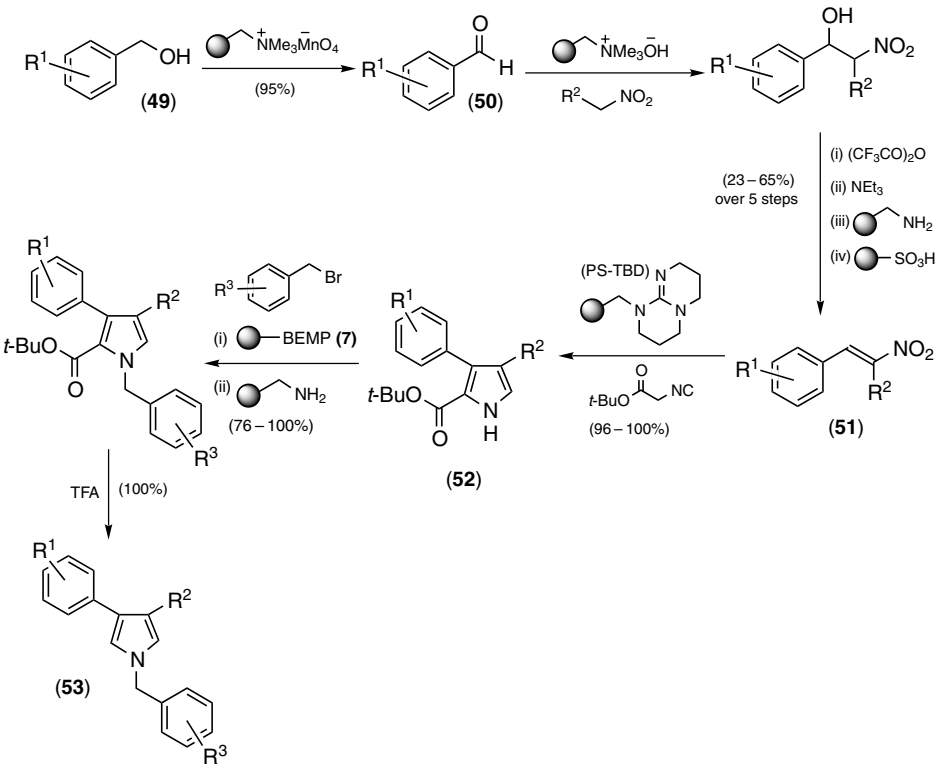
Scheme 18.14 Short synthetic sequence for nicotine derivatives.

As an ultimate example of how polymer-supported reagents can be applied to alkaloid synthesis, their multiple applications in the synthesis of (+)-plicamine truly stands out (Scheme 18.17).[32] In this synthesis, extensive use was made of parallel optimization methods in order to progress the forward route more rapidly. Again, microwave techniques worked especially well to achieve fast reaction times. The entire route, including optimization, was complete in just six weeks, without the need to rehearse the reactions using conventional solution-phase reagents or the requirement of separation methods.

Many of the steps employed concepts that we established earlier during the synthesis of simpler alkaloids; therefore, they are not discussed in detail. However, some steps are noteworthy. First, it should be noted that the use of immobilized hypervalent iodine once again served us well in the oxidative formation of the spirodienone (**54**). The conjugate addition of the amidic nitrogen atom to complete the tricyclic scaffold (**55**) proceeded nicely when a Nafion-H fluorosulfonic acid resin was used as a source



Scheme 18.15 Structures of the pyrrole-containing natural products dioxapyrrolomycin and pyrrolonitrin.

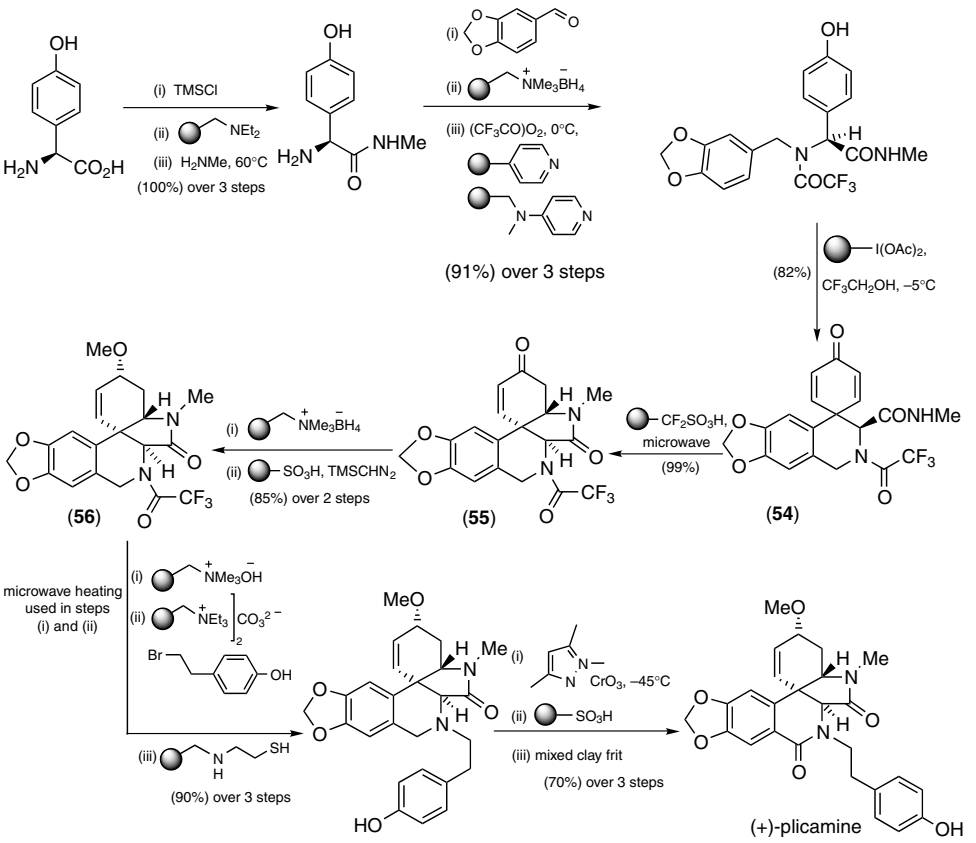


Scheme 18.16 Library synthesis of tetra-substituted pyrroles.

of protons. These intermediate products, produced in multigram quantities, were split and diverted to other analog programs. Another noteworthy transformation is the use of a supported sulfonic acid, which with trimethylsilyl diazomethane, turns out to be an extremely efficient way to methylate the very hindered alcohol to give **56**. This procedure is general and could be applied widely to other systems. Not only did this synthesis deliver the natural product (+)-plicamine; by choice of the enantiomeric amino acid precursor, the unnatural antipode (−)-plicamine was prepared with equivalent yields and selectivities.

In the original article describing the synthesis of plicamine, extensive use was also made of the intermediate structures to deliver a range of new structural analogs for evaluation (Scheme 18.18).[32b]

Several classes of compound have been studied to illustrate the wider applicability of the immobilized reagent concept to the synthesis of other natural product types: the spiroketal motif being a good example of a common structural feature of many natural products, especially in polyketide-derived materials. In fact, spiroketals can be categorized as one of nature's most privileged building blocks, and they display extensive structural diversity as well as biological properties.[33] Spiroketals are characterized by a spiro-fusion of acetal rings—most commonly with 6,6 and 6,5 arrangements. By way of illustration, they can range from the simple insect pheromone to more functionalized spiroketals, such as routiennocin,[34] and finally, up to highly complex arrays such as those found in the potent phosphatase inhibitor okadaic acid[35] (Scheme 18.19).

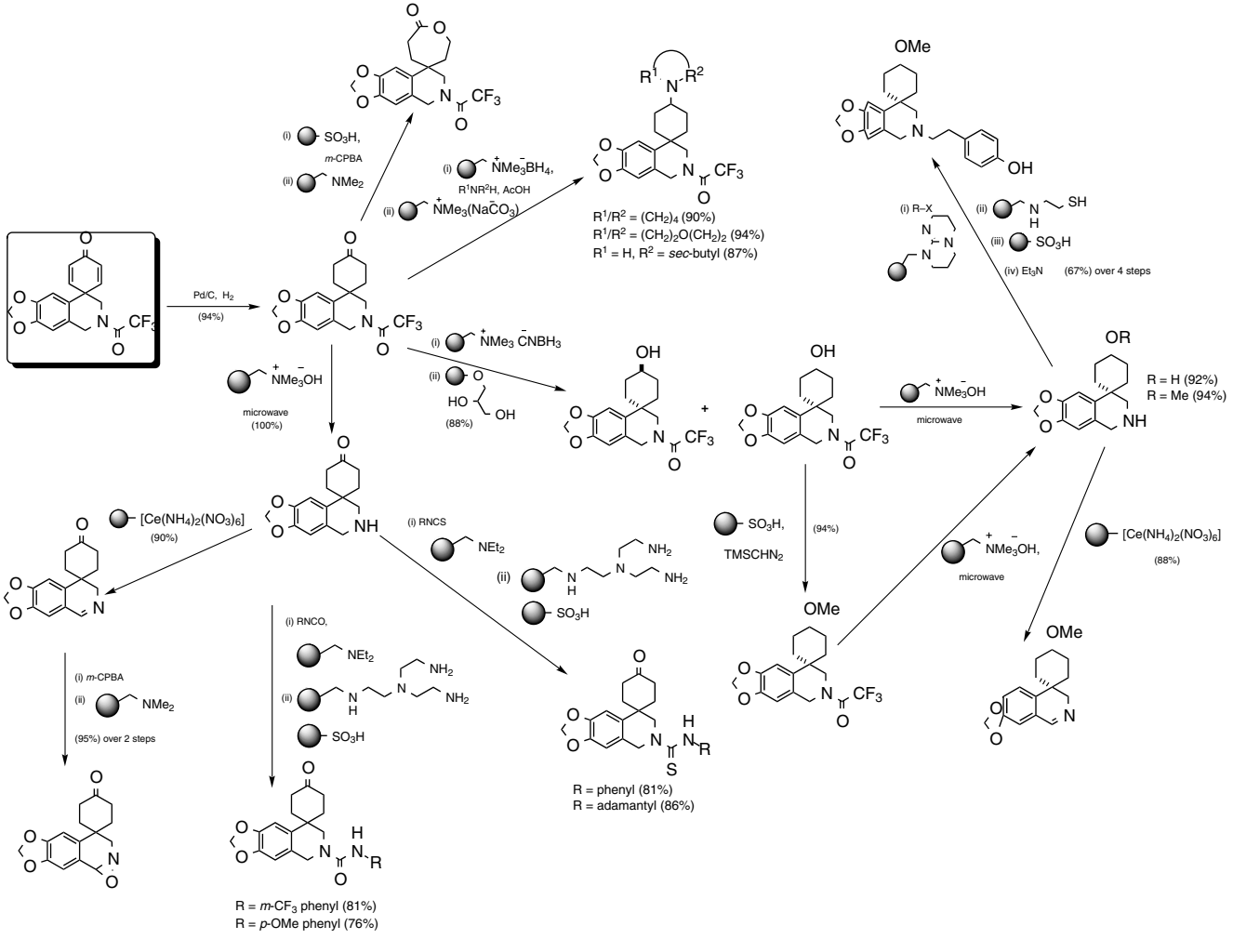


Scheme 18.17 Synthesis of (+)-plicamine using an extended array of polymer-supported reagents.

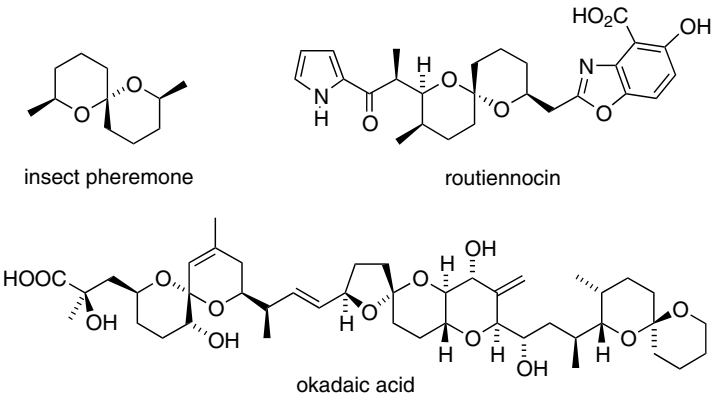
One approach to the spiroketal motif used glycerol immobilized on a polyethylene glycol support (PEG), the glycerol acting as a "capturing" functional group (Scheme 18.20). This was reacted with a ketone such as **57** to give a trapped acetal (**58**). Terminally differentiated ketones could clearly be used in a similar fashion to give varying substitution patterns. Epoxidation with *m*-CPBA provided a product which was then suitable for further elaboration via incoming nucleophiles (both carbon and heteroatom), providing bound systems that upon acid-catalyzed cleavage led to spiroketal products based on structure **59** (Scheme 18.20).[36]

The natural product trichostatin A is known to be a potent inhibitor of histone deacetylase (HDAc).[37] There is increasing evidence that the acetylation and deacetylation of proteins is an important regulatory modification in many cellular processes,[38] and an imbalance of histone acetylation has been associated with malignant disease. HDAc inhibitors lead to a reversal of transcriptional repression and associated upregulation of tumor sequences.[39] They have also been observed to result in the inhibition of angiogenesis.[40]

Largely for these reasons, a range of structural analogs based on the trichostatin A structure were prepared as potential HDAc inhibitor drug candidates — the particular structural element clearly important for activity being the hydroxamic acid group. However, in terms



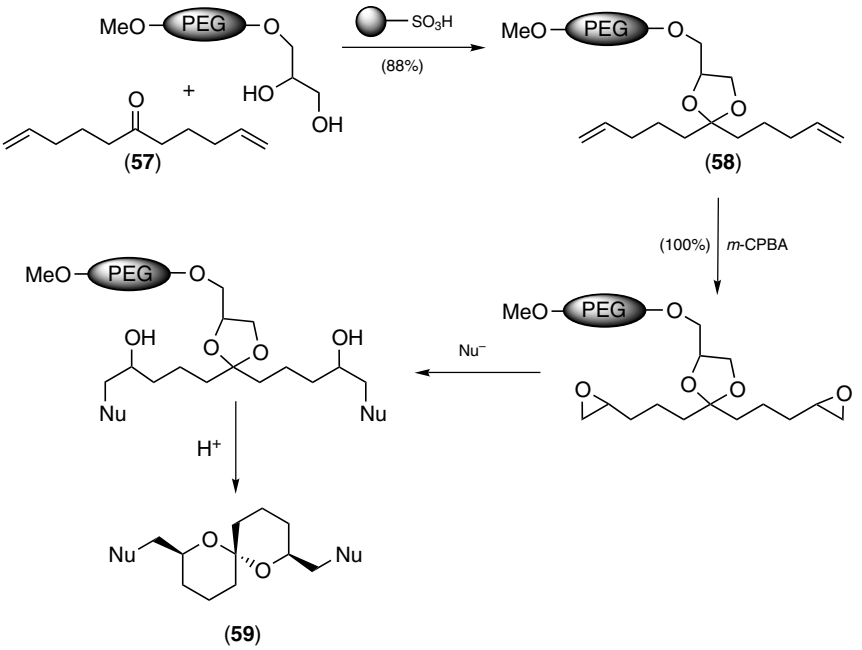
Scheme 18.18 Plicamine-related analogs.



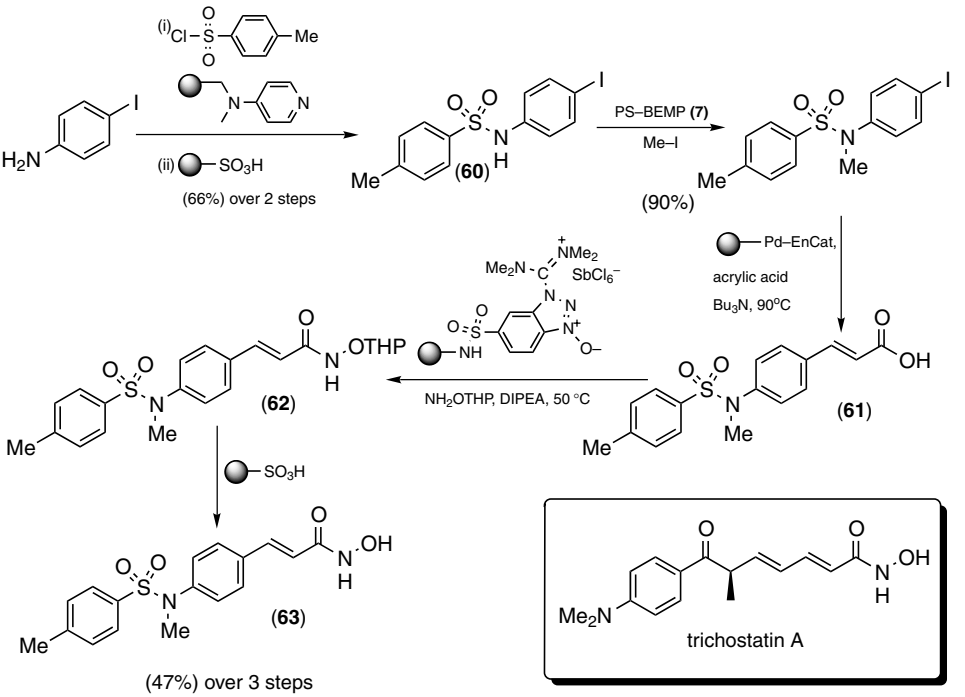
Scheme 18.19 Spiroketal motifs in nature.

of novel chemical library synthesis, this functional group can cause problems due to its propensity to bind metal atoms: inconveniently, the nature of the reagents often used is to catalyze carbon–carbon bond-forming reactions. A route amenable to automation and minimal metal contamination employs supported reagents and scavengers and presented a sensible choice.

A five-step process was planned to provide a focused array of compounds incorporating three points of diversity: arising from sulfonamide formation, *N*-alkylation, and substituent variation (Scheme 18.21).[41] The route makes use of established reliable reactions such that the entire process was readily automated using a Zinsser Sophas robotic platform. The first reaction uses a polymer-supported sulfonyl transfer reagent, derived from the immobilization



Scheme 18.20 Synthesis of spiroketals.



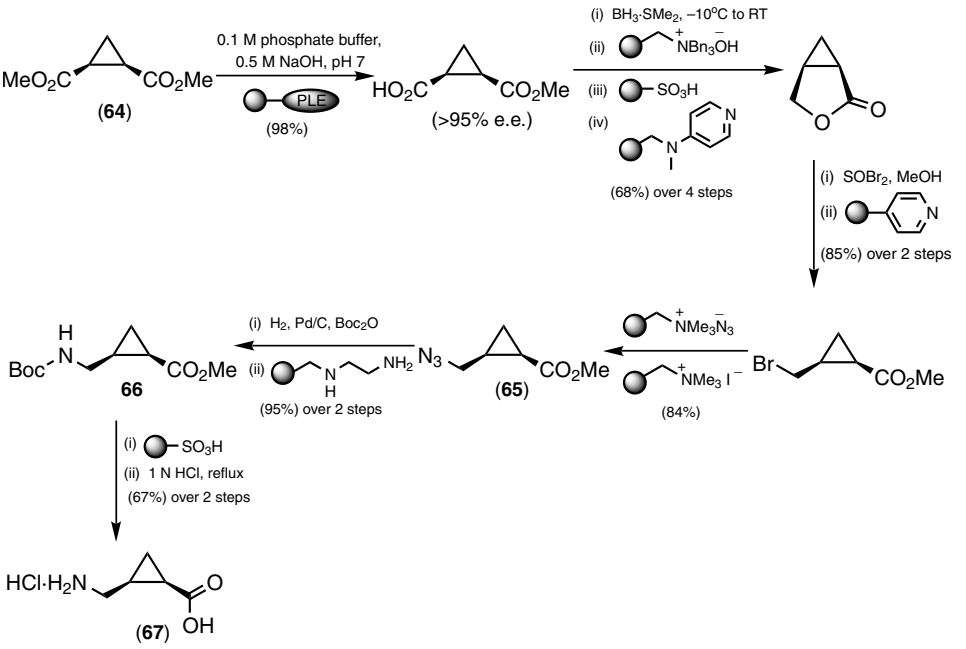
Scheme 18.21 Synthesis of trichostatin A analogs.

of an appropriate aryl sulfonyl chloride on polymer-supported DMAP. This reagent transformed the 4-iodoaniline into the sulfonamide (**60**), and excess aniline was removed by polymer-supported sulfonic acid scavenging. Clearly, variation is possible in either the iodide or sulfonyl partner. After alkylation of the nitrogen atom using an alkyl halide and PS-BEMP (**7**), the most significant reaction in the process involves a Heck coupling reaction with acrylic acid to furnish **61**.

Heck reactions can lead to considerable metal contamination of the product. Here, however, the metal catalyst is trapped inside a polyurea microcapsule,[42] allowing the substrates to diffuse into the encapsulated catalyst, react, and then allow the product to diffuse back out into solution. The metal remains entangled inside the microcapsule because of the strong binding to the highly polar urea functional groups that act as a chemical barrier. The encapsulated Pd-EnCat catalysts have performed well in other palladium-mediated processes, such as Suzuki and Stille reactions. The Heck reaction in this case was optimized using a ReactArray SK233 automated reaction workstation.

The final steps in synthesis of the HDAc inhibitor analogs progressed in a straightforward fashion using a polymer-supported coupling reagent to couple the THP moiety to the carboxylic acid, resulting in the protected hydroxamic acid (**62**). Purification was achieved through an amino-SPE cartridge. Finally, removal of the THP-protecting group with a sulfonic acid resin revealed the trichostatin A analog (**63**). Using this methodology, an array of 36 compounds were prepared in good purity in an automated robot synthesis.

γ-Aminobutyric acid (GABA), an important natural neurotransmitter,[43] serves to illustrate further how these immobilized chemical tools are useful in synthesis. It particularly highlights the wide range of classes of natural product and natural-product-like compounds that are compatible with and amenable to immobilized techniques.

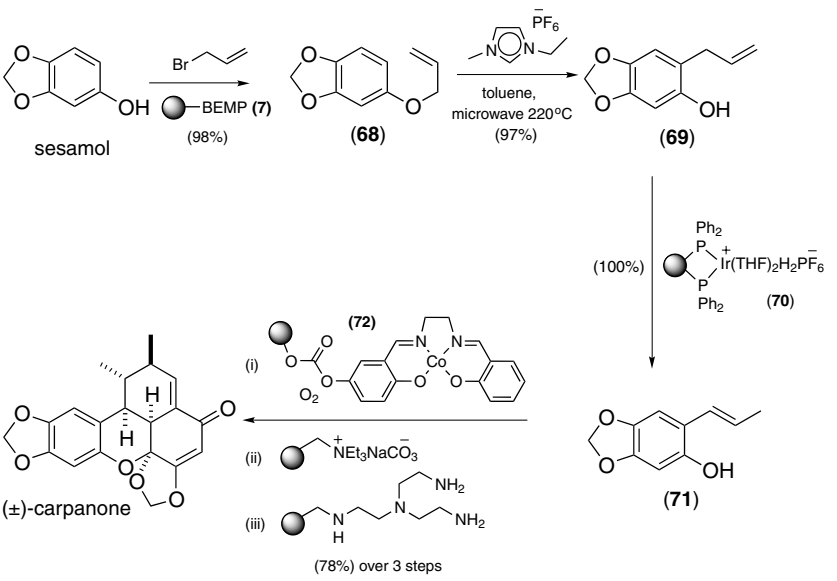


Scheme 18.22 Synthesis of a γ-aminobutyric acid analogs.

GABA analogs have been used to explore the binding mode and structure–activity relationships of the parent compound, and they have also been investigated as therapeutic agents for a number of central nervous system disorders.[44] It is therefore pertinent to prepare conformationally restricted analogs of GABA. However, as these molecules tend to be very polar, their conventional syntheses can be fraught with difficulties. An attractive alternative is, of course, to make use of supported reagents (Scheme 18.22).[45]

First, *meso*-diester **64** may be terminally differentiated in an asymmetric fashion using commercially available Eupergit-supported pig liver esterase (PLE). While supported enzymes are commonly used, even on a large scale, their application in conjunction with other multistep immobilized reagents had not been reported. Yet they are readily accommodated in this situations.

During the synthesis of cyclopropane analogs of GABA, a few conventional solution-phase reagents, such as borane dimethyl sulfide and thionyl bromide, are used (Scheme 18.22). However, by appropriate polymer-supported scavenging, all waste by-products or excess reagents were readily removed without recourse to chromatography. Also of interest is the use of a supported azide to introduce the required nitrogen substituent by displacement of a bromide. In a manner analogous to a key step in the synthesis of nornicotine and nicotine (Scheme 18.12), the resulting hazardous azide product (**65**) is not isolated but is reduced directly with palladium on carbon to the amine derivative. In situ butoxycarbonyl(Boc) protection and supported amine scavenging of the excess reagents then provided the desired protected amine (**66**) in excellent 95% yield. An acid catch-and-release affords the hydrolyzed hydrochloride salt of the cyclopropanyl GABA analog (**67**). It is also possible to prepare four-, five-, and six-ring analogs by similar sequences of events.[45]



Scheme 18.23 Synthesis of carpanone.

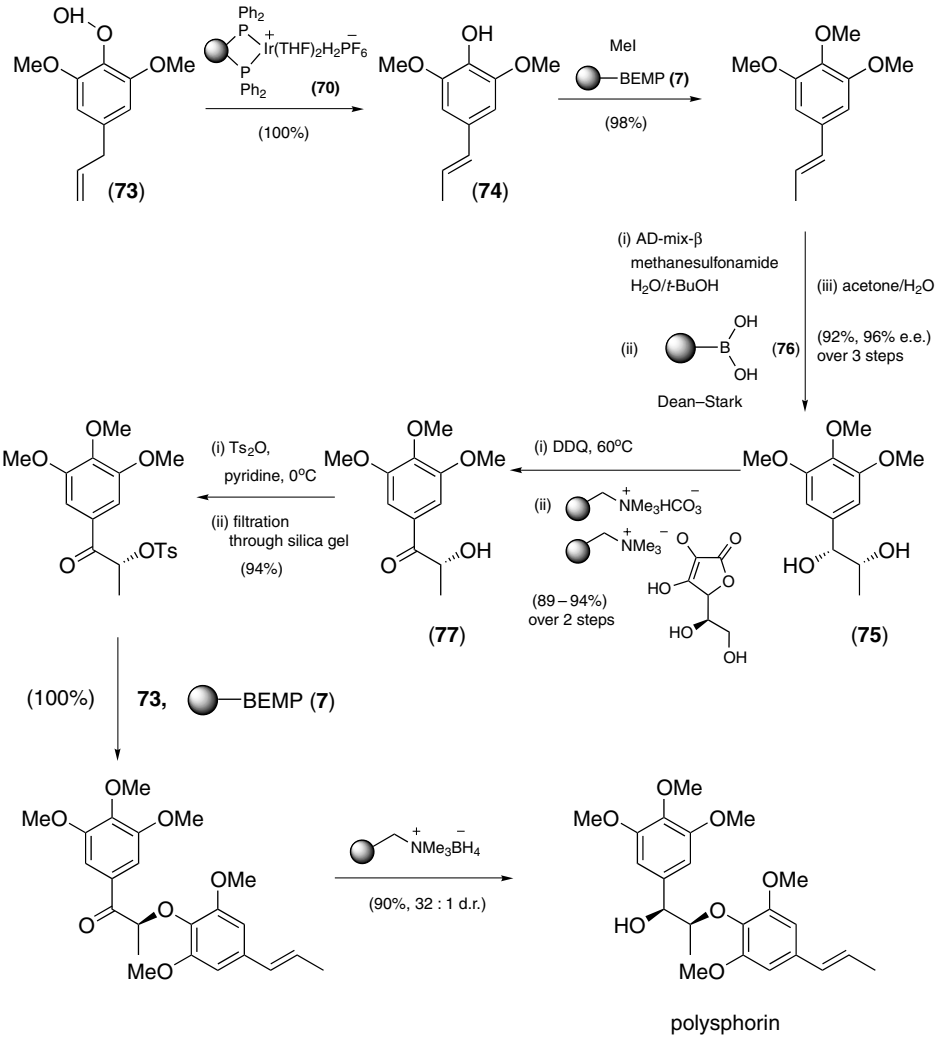
Yet another deceptively complex natural product, carpanone, can be prepared in a relatively simple set of reactions from commercially available sesamol (Scheme 18.23). In this route, again using the immobilized reagent concept, several interesting aspects of synthesis were developed.[46] Sesamol is readily O-alkylated with allyl bromide in the presence of PS-BEMP (**7**) to give **68**. This product then underwent a clean Claisen rearrangement to **69** in virtually quantitative yield, using a combination of ionic liquids and toluene, then heating the reaction to 220°C for three 15-minute periods in a focused microwave. The combination of ionic liquids to absorb the microwave energy and an organic solvent, toluene, had been developed earlier and is a very convenient binary heating mixture.[47] The product-containing toluene solution was readily separated using a Gilson liquid-handling robot and was used directly in the next reaction step. Following a degree of investigation, double-bond isomerization was achieved using an immobilized iridium catalyst (**70**) that was specially developed for this synthesis.[48] Both the yield and the selectivity in this process were extremely good, yielding the phenolic styrene (**71**) with a *trans*/*cis* ratio of 11 : 1. Conversion of the styrene to carpanone was achieved effectively with a polymer-supported cobalt catalyst (**72**) in the presence of molecular oxygen; following original work by Chapman,[49] **71** could then be oxidatively coupled, and via a subsequent intramolecular Diels–Alder reaction, afforded the natural product carpanone. Clean product was obtained in excellent 78% yield after filtration of the spent catalyst and brief scavenging with both a polymer-supported trisamine and a carbonate resin to remove unreacted phenol and aldehyde by-products. The efficiency of this sequence of reactions means that gram quantities of the natural product can be prepared for more extensive modification and biological evaluation.

Phenols and phenolic residues are also present in many other natural products. Consequently, the procedures used for the synthesis of carpanone above could readily be adapted, for example, in the preparation of polysphorin and related neolignins.[48] Interest in these

compounds stems from their antimalarial activity; some also show activity against other vectorborne diseases and display potent antifungal properties.[50]

The synthesis shown in Scheme 18.24 uses supported reagents to prepare polysphorin and also corrects a previous structural misassignment. The route also illustrates some other useful advances in the application of these systems to natural product synthesis.

Use of the immobilized iridium double-bond isomerizing catalyst (**70**) developed for the carpanone synthesis again worked well to convert commercially available **73** to the conjugated product (**74**). This was then methylated in the presence of PS-BEMP (**7**), a reaction that proceeds in almost quantitative yield. Next, an asymmetric dihydroxylation was performed according to methods developed by Sharpless.[51] However, instead of using a conventional workup procedure for the vicinal diol (**75**), a new catch-and-release protocol was developed. This made use of the immobilized boronic acid (**76**)[52] to trap the chiral diol

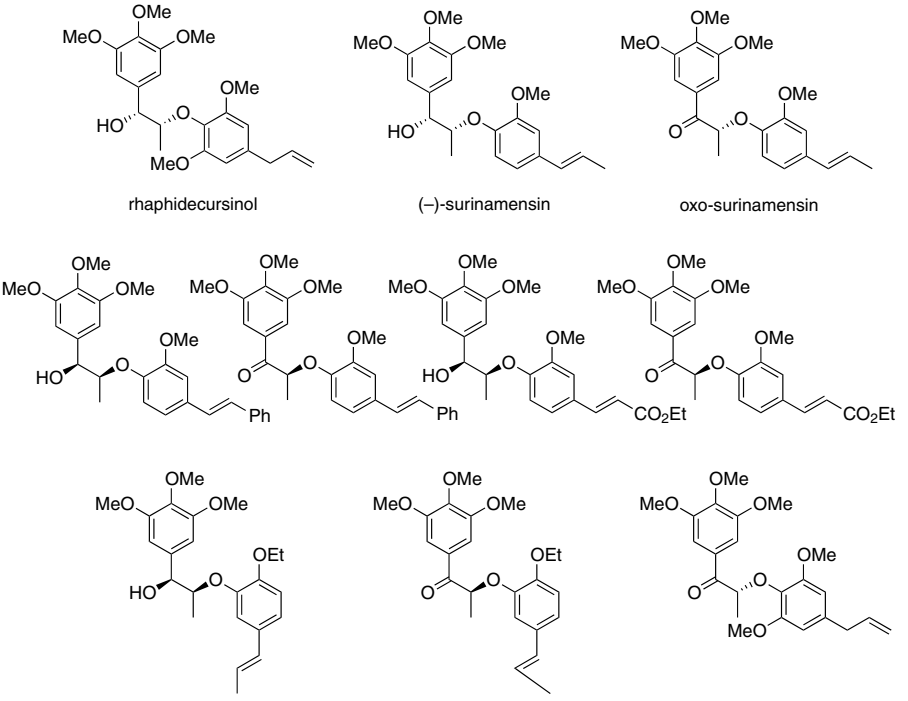


Scheme 18.24 Synthesis of polysphorin utilizing the polymer-supported iridium catalyst used in a key step in the synthesis of carpanone.

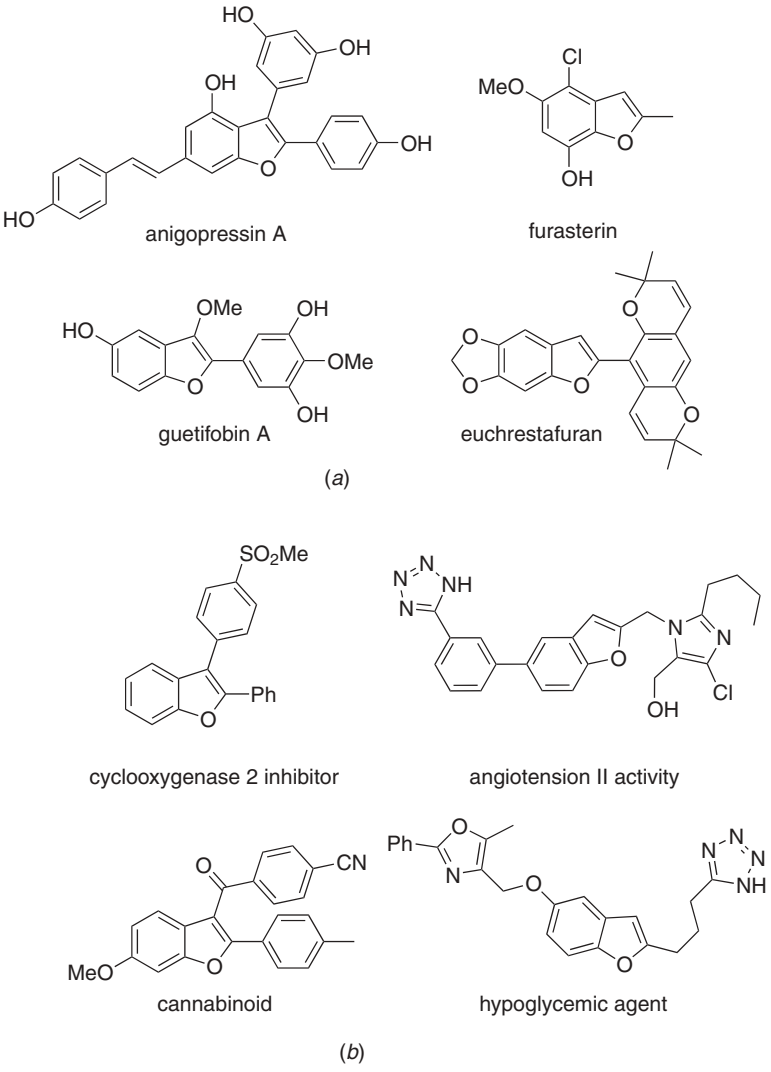
as its cyclic boronic ester using a Dean–Stark apparatus. After washing, the pure diol (**75**) was released by treatment with aqueous acetone in 92% yield (96% e.e.) (Scheme 18.24).

Selective benzylic oxidation of **75** was achieved using dichlorodicyanoquinone (DDQ). These oxidations are known to be procedurally troublesome, as they usually result in multiple by-products, many of which are colored. However, it was found that using an immobilized ascorbic acid in the workup to convert DDQ completely to DDQH, as well as concurrent addition of polymer-supported carbonate to scavenge the DDQH, led to a completely clear and colorless solution of the desired product (**77**). The final steps in the synthesis, involving tosylation and displacement with phenol (**73**), proceeded in a straight forward fashion. In addition to polysphorin, other natural neolignons [rhaphidecursinol, (−)-surinamensin, and oxo-surinamensin] were prepared along with several other analogs that are being investigated currently for their antimalarial properties (Scheme 18.25). The benzofuran core happens to be another structural motif that occurs in many natural products and is an especially prevalent feature in a diverse range of pharmaceutical substances (Scheme 18.26).

A recurrent theme in the synthesis to these molecules is the use of phenolic precursors, and without doubt a highly effective approach to making these uses solid-supported reagents. They can be used to construct core structures efficiently with a wide range of structural and chemical diversity (Scheme 18.27). Halogenation of commercially available acetophenones using polymer-supported pyridinium perbromide provides the desired α-bromo derivatives (**78**), key building blocks for many applications.[53] When reacted with a variety of phenols (where the R groups include H, Me, Br, OMe, CF_3, and NO_2 in various substitution patterns) using a supported 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) equivalent as a base, the ether products (**79**) undergo ready cyclization to a range of benzofurans



Scheme 18.25 Other natural neolignons and analogs prepared using protocols developed for the synthesis of polysphorin.

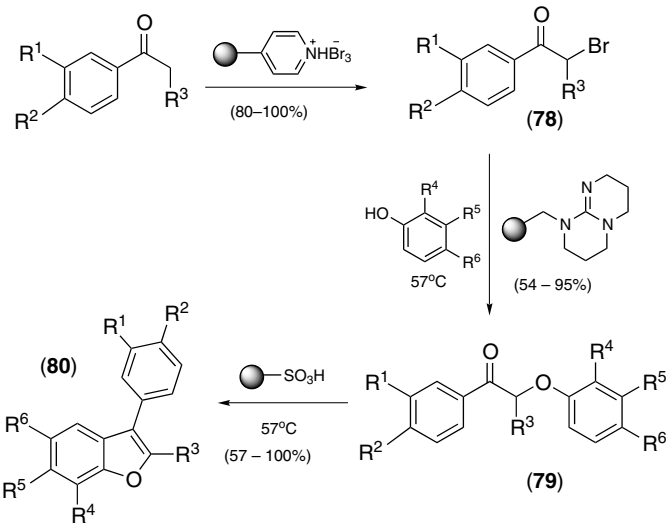


Scheme 18.26 Natural products *(a)* and pharmaceutical agents *(b)* containing the benzofuran structural motif.

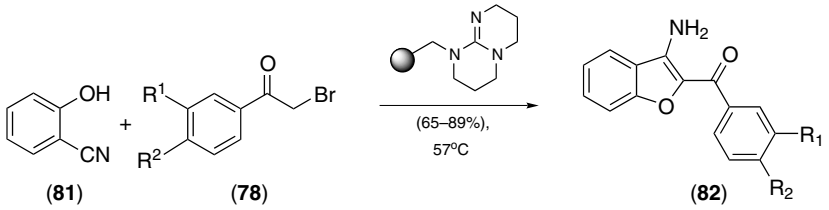
(**80**), where sulfonic acid acts as the catalyst.[53] In this manner 25 benzofurans were synthesized in excellent yields ranging from 57 to 100% and generally in excellent purity.

Using the protocols above, α-bromo derivatives (**78**, where R^1 is either H or CF_3 and R^2 is *p*-MeO or F) were coupled with *o*-cyano phenols (**81**), readily introducing additional structural variation, as when warmed with the supported base in acetone, they provide 3-aminobenzofuran derivatives (**82**) (Scheme 18.28).

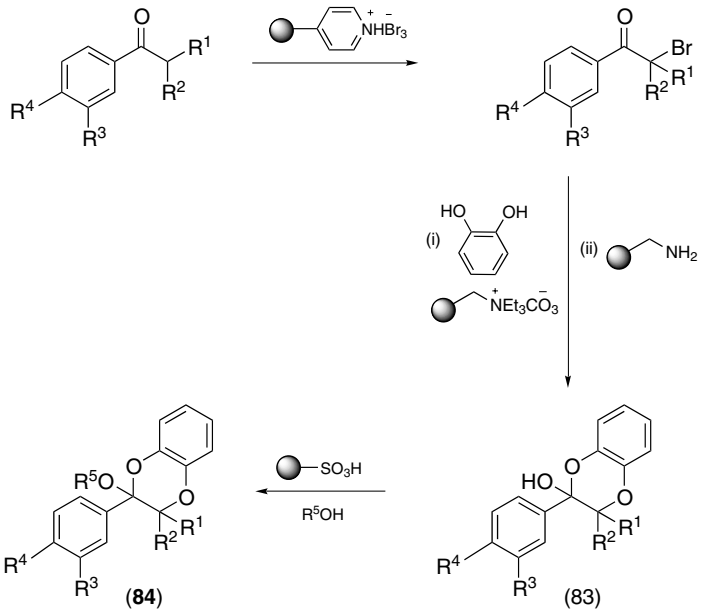
When 1,2-diphenols are used instead of *o*-cyano phenols, benzo-1,4-dioxanes ensue (**83**). These can be modified by acid activation of the hemiacetal and formation of the mixed acetal **84**, now presenting five sites of functionality, offering extensive scope for further elaboration and increased diversity (Scheme 18.29).[54]



Scheme 18.27 Synthesis of an array of substitited benzofurans.



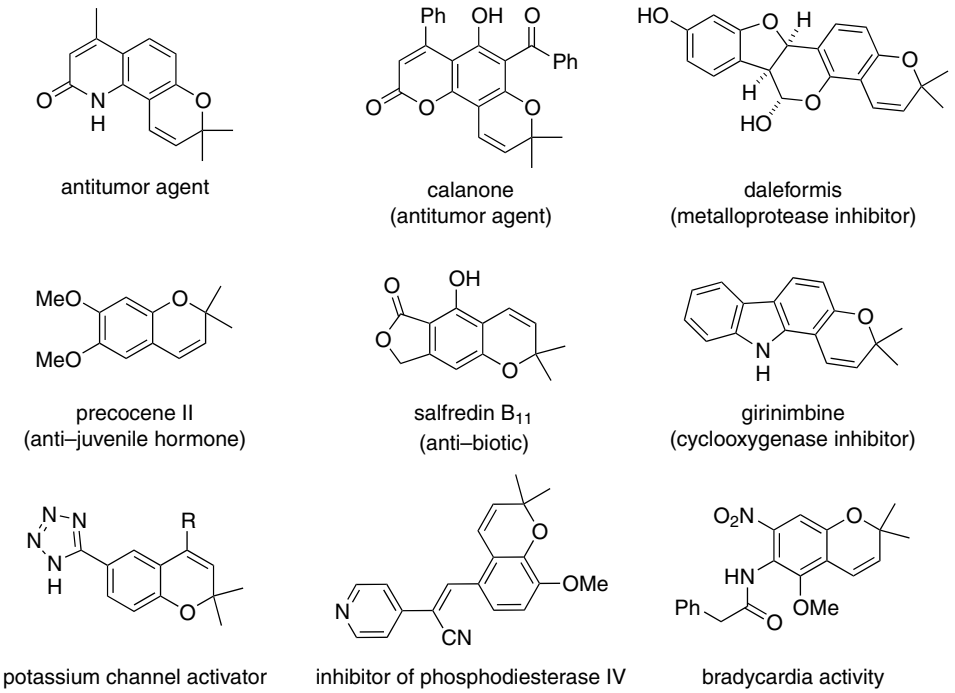
Scheme 18.28 Introduction of variation to the benzofuran scaffold.



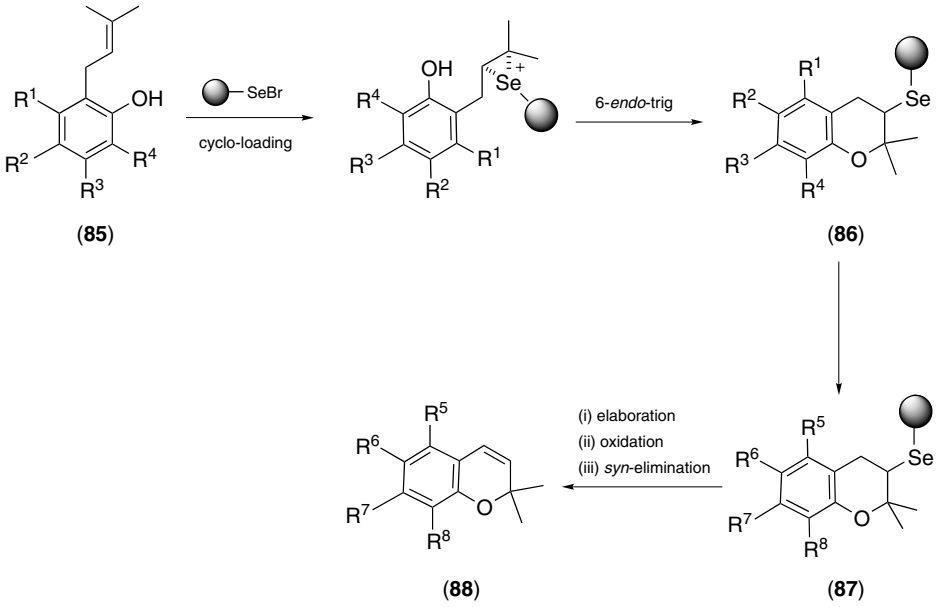
Scheme 18.29 Synthesis of an array of benzodioxanes.

The Nicolaou group has examined extensively natural product libraries based on privileged structures, such as the benzopyrans, which are found as common motifs in a vast range of biologically active natural products and also feature prominently in many pharmaceutical agents with impressive biological function (scheme 18.30).[55] Here substrates are immobilized onto solid supports using a specific reaction such as cyclo-addition. The products supported are then further elaborated "on-bead" with a succession of steps and ultimately released by appropriate transformations such as elimination or reduction reactions. Very large libraries (10,000 members) have been generated by the application of IRORI NanoKan technology, using optical encoding to assist in sorting of the library members. The process described leads to "libraries from libraries" as a method for enhancing structural diversity.

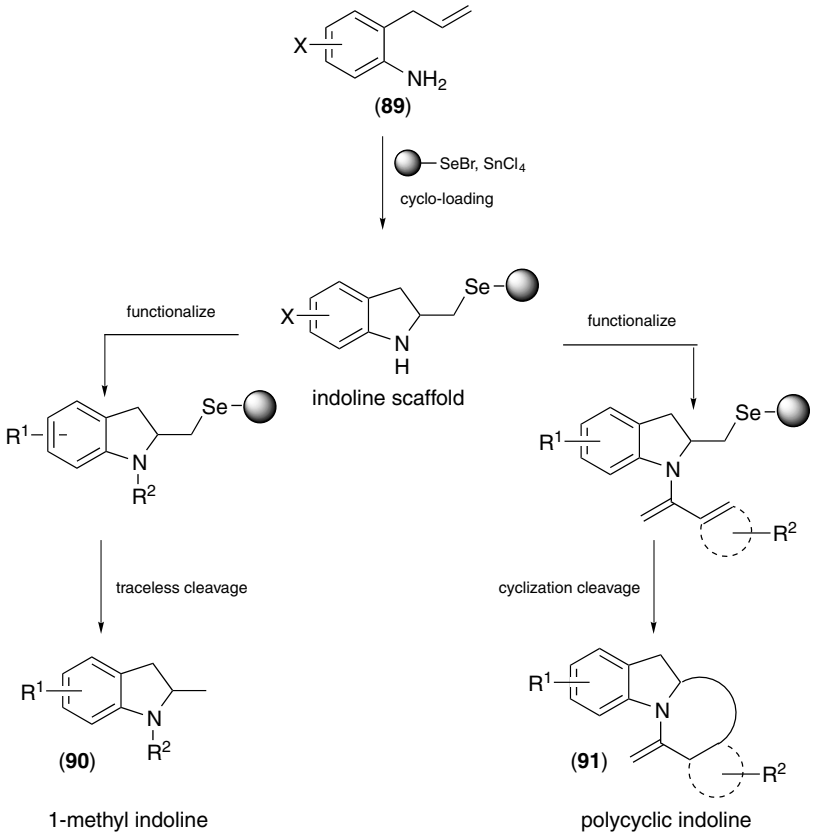
The general approach to these molecules makes use, once again, of an immobilized selenyl bromide reagent that reacts with *o*-allyl phenols (**85**) to give the corresponding supported benzopyran scaffolds (**86**). These were elaborated further through a variety of reactions whereby R-group functionality was modified (**87**). Following oxidation and syn-elimination from the support, a large number of benzopyrans (**88**) were released in such high purity that they could immediately be screened for biological activity (Scheme 18.31). This scheme implies a greatly simplified picture, and it is important to emphasize the variation actually achieved during the chemical elaboration stage. Moreover, the products released can be modified still further by supported-reagent-mediated processes such as epoxidation and ring opening to give an entirely new set of library members.



Scheme 18.30 Pharmaceutical agents containing the benzopyran structural moiety.



Scheme 18.31 Synthesis of a library of benzopyrans employing the use of a polymer-supported selenium resin.

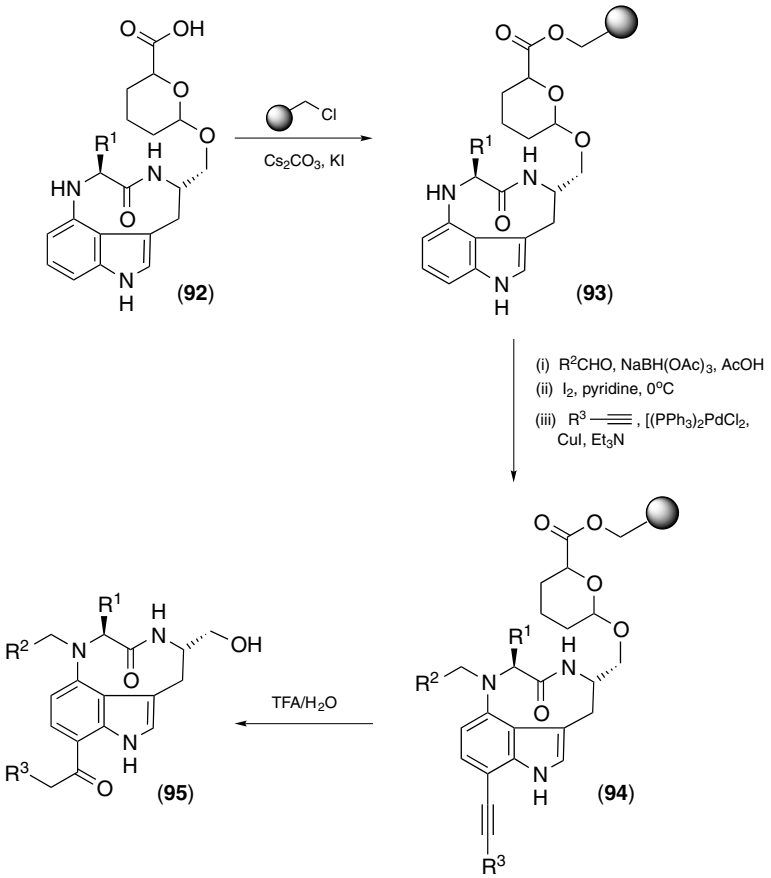


Scheme 18.32 Synthesis of a library of bicyclic and polycyclic indolines.

The real beauty of this chemistry lies in its adaptability, as the chemistry is not restricted to intramolecular ring-forming reactions through oxygen atoms; nitrogen substituents perform similar processes with considerable ease when treated with *o*-allyl anilines (**89**) and the same selenium-based resin. Rapid access to indolines (**90** and **91**) is achieved (Scheme 18.32). The resulting scaffold can then undergo numerous transformations, and with a traceless cleavage of the final product gives a library of natural product-like molecules.[56]

Although not strictly part of this review, another process using a trap–react–release protocol was reported by Waldmann et al. during their preparation of an indolactam library (Scheme 18.33).[57] In this work, a fully formed indolactam compound (**92**) with variation at the R^1 site, prepared by traditional solution-phase methods, was loaded onto Merrifield resin. Subsequent manipulation of **93**, including reductive amination of the C3 nitrogen to vary the R^2 group and modification at C6 to append a range of substituted acetylene units (R^3), ultimately provided a precursor (**94**) for the keto compounds desired. After detachment using aqueous TFA, new teliocidin and indolactam derivatives (**95**) were obtained.

Many other trap–react–release sequences similar to natural product analogs are known. These processes greatly enhance the opportunities provided by immobilization, and they also demonstrate further that these new approaches to synthesis, whether utilizing



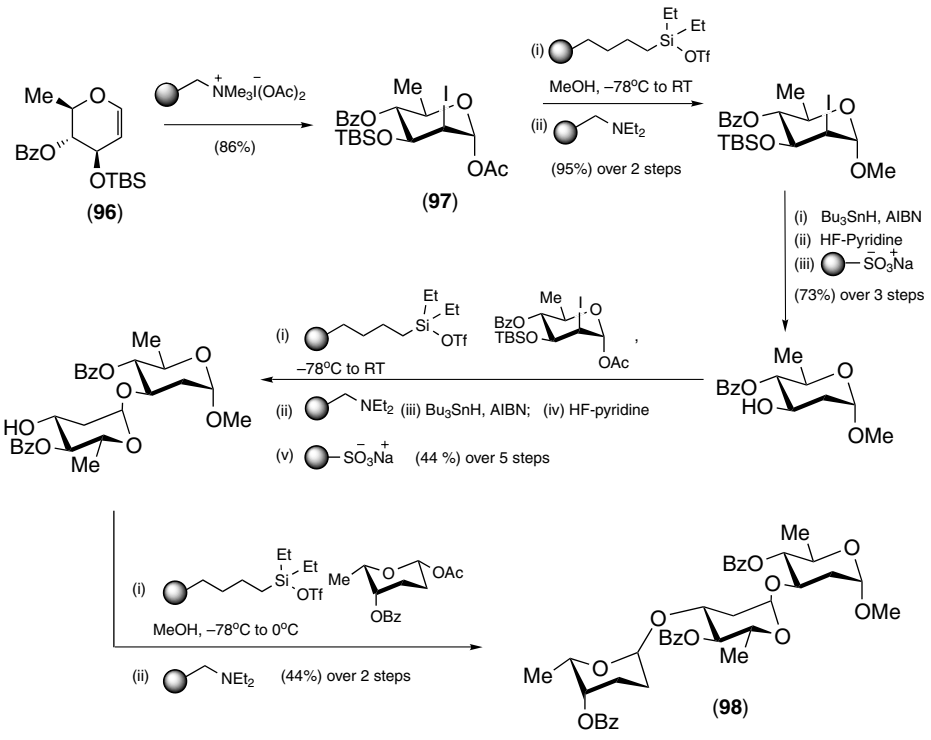
Scheme 18.33 Synthesis of novel teliocidin and indolactam derivatives.

immobilized reagents and scavengers or catch-and-release protocols, add considerably to the repertoire of the synthetic chemist.

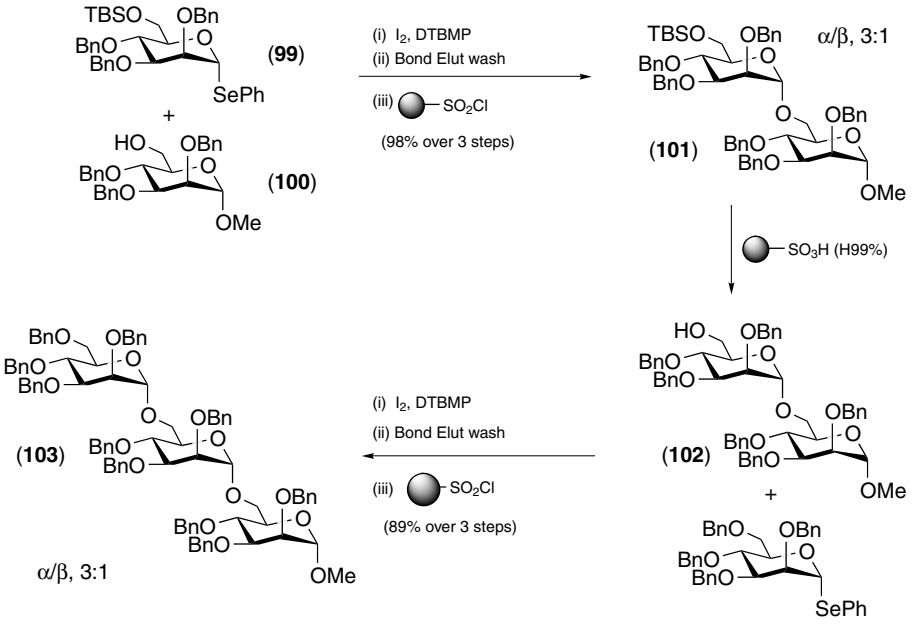
Complex oligosaccharides, in both natural and unnatural arrangements, have long provided a stimulus for organic chemists because of their challenging structures and can range from linear to multibranched systems; their formation also necessitates precise anomeric glycosyl bond construction.

Kirschning has developed a general strategy for solution-phase glycosylation in the formation of glycoconjugates and oligosaccharides using polymer-assisted reactions.[58] Carbohydrates containing anomeric acetates were activated using a supported silyl triflate to provide the glycosyl donor; these were then coupled with a range of sugars in an iterative process giving remarkably high selectivity and good yields, a process that can readily be extended to trisaccharide synthesis (Scheme 18.34).[59] The protected gylcal (**96**) was transformed to the iodogylcosyl acetate (**97**) using an immobilized bisacetoxyiodate resin. This was then progressed through a series of coupling steps involving integration with some classical solution-phase techniques to afford a trisaccharide (**98**). Kirschning had also shown that glycosyl phenylsulfides[60] can be activated using the hypervalent iodate resin by coupling a number of hindered alcohols using these methods.[61]

Selenophenyl glycosyl donors are particularly attractive for oligosaccharide synthesis, as they can be activated in a variety of ways, depending on the appropriate coupling environment. However, nearly all of the methods generate fairly obnoxious by-products that have to be removed by chromatography or other techniques that require significant practical expertise.



Scheme 18.34 Synthesis of trisaccharides using polymer-supported silyl triflate as an activator.



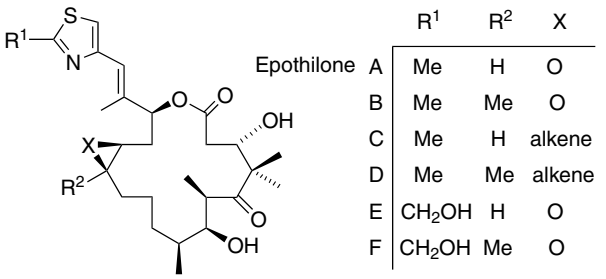
Scheme 18.35 Trisaccharide synthesis using polymer-supported tosyl chloride.

However, the conditions developed by Field et al.[62] that use iodine activation are especially interesting, particularly when they are integrated with new workup methods involving polymer-supported scavenging, using a sulfonyl chloride resin to remove unwanted hydroxyl-containing by-products or excess starting glycosyl acceptor (Scheme 18.35).[63] In this way, it becomes possible to prepare trisaccharides without chromatography.

Simply by treating the TBDMS-protected selenyl donor (**99**) with iodine, a glycosyl donor (**100**) and the hindered organic base di-*tert*-butyl-4-methylpyridine (DTBMP), the product desired is formed in a 3 : 1 α/β ratio. Passing the mixture through a Bond Elut cartridge, then treating it with polymer-supported tosyl chloride, scavenges the hydroxylated materials and furnishes a disaccharide (**101**) in 98% yield—very cleanly without chromatography. The silicon-protecting group was removed using polymer-supported sulfonic acid to give pure disaccharide (**102**), which was then coupled with the next glycosyl selenyl donor using the same procedure to provide the trisaccharide **103**, also in excellent yield. Global deprotection using palladium on carbon in the usual way removed the benzyl protection. Obviously, these methods can be adapted to produce a range of oligosaccharides in good α/β ratios, excellent yields, and excellent purity.

The epothilones are a family of natural products that have received special attention in recent years (Scheme 18.36).[64] The interest surrounding these compounds comes from their ability to induce mitotic arrest by stabilizing microtubules; hence they are of great importance as potential anticancer agents. Epothilone structures lend themselves well to convergent total synthesis, which brings together modular building blocks or fragments. These approaches can be adapted quite readily to provide epothilone analogs, which ultimately assist researchers in probing their structure–activity profiles more comprehensively.

Although total syntheses of these biologically intriguing molecules are now known,[65] it is the application of solid-phase methods—and primarily supported reagents and scavengers—that have been used for their construction that merits discussion here. In

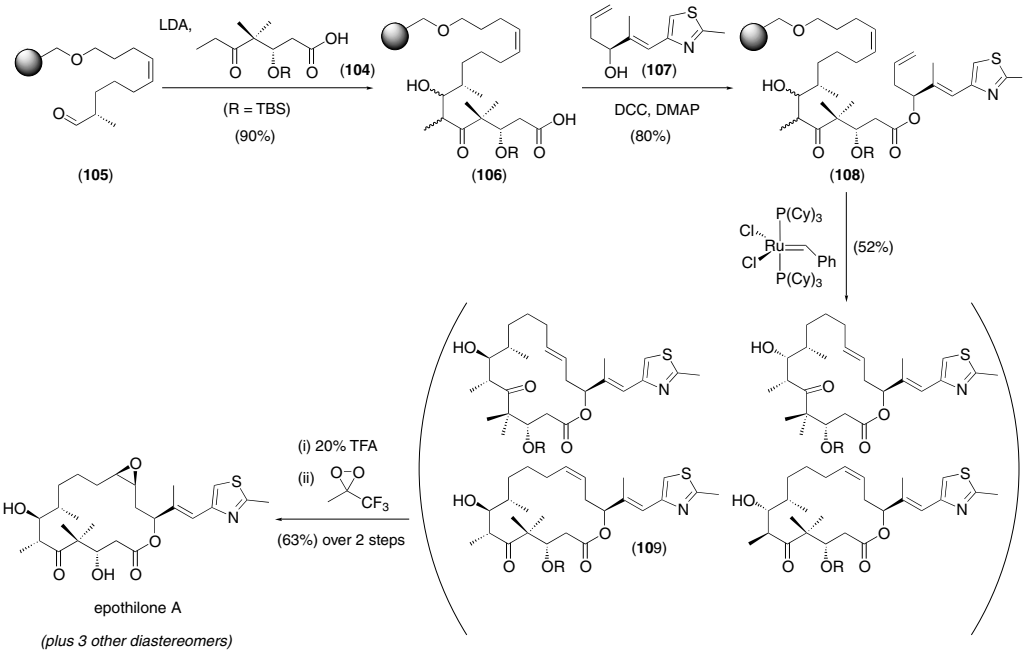


Epothilone	R^1	R^2	X
A	Me	H	O
B	Me	Me	O
C	Me	H	alkene
D	Me	Me	alkene
E	CH_2OH	H	O
F	CH_2OH	Me	O

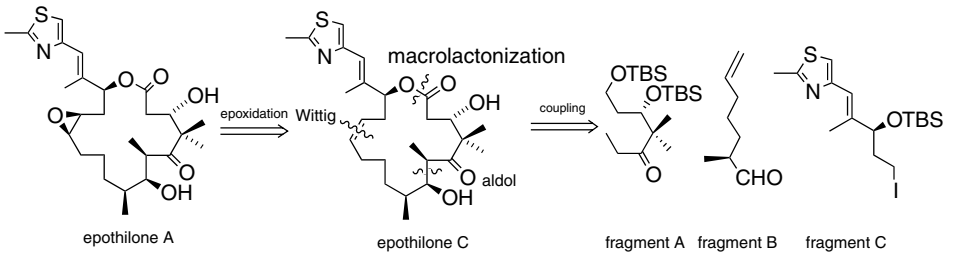
Scheme 18.36 Epothilone family.

the first approach, epothilone A was made by the Nicolaou group in 1997. As shown in Scheme 18.37, representing steps farther along in the synthesis, the keto acid (**104**) was reacted as its dianion [generated by treatment with lithiodiisopropyamine(LDA)] with the immobilized aldehyde fragment (**105**) to give the aldol condensation product (**106**).[66] The acid of this product, on dicyclohexylamine(DCC) coupling with the heterocyclic allylic alcohol (**107**), furnished the polymer-supported ester (**108**). The macrocyclic ring-closing metathesis of **108** also acted simultaneously to detach the products from the supporting polymer. In this particular instance, four products were obtained as a result of the formation of *cis* and *trans* double-bond isomers, together with diastereoisomers from the unselective aldol coupling in an earlier step. Under DMDO oxidation this mixture was transformed into mixed epoxide products, of which the minor diastereoisomer (**109**) led to the natural product epothilone A. This route also formed the basis for a more extended analog program that defined many of the key parameters essential for good biological activity.[55b]

The epothilones have provided an excellent platform to explore the full potential of supported reagents and scavengers in complex molecule synthesis.[67] A convergent route to



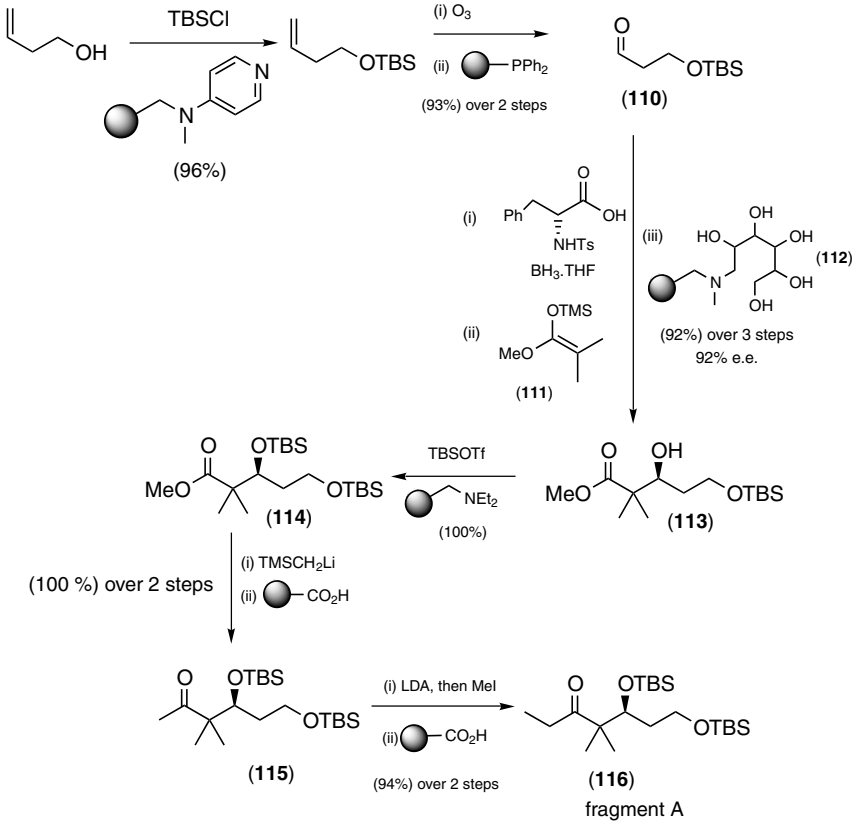
Scheme 18.37 Solid-phase synthesis of epothilone A and stereochemical analogs.



Scheme 18.38 Retrosynthetic analysis of epothilones A and C.

epothilones A and C was devised based on fragment coupling and using knowledge gained from previous synthesis studies. However, the goal was to conduct all steps in a clean and efficient manner using only immobilized reagents and scavengers to effect the required transformations. In this way, chromatography, crystallization, distillation, or water washing—common to a conventional synthesis approach—could be avoided.

The synthesis plan in terms of the three coupling fragments desired is illustrated in Scheme 18.38. A number of alternative sequences to these key fragments were investigated and are described in the original article,[68] but here only the most efficient routes are discussed, as they more than adequately reinforce the state of the art of these methods. The preparation of fragment A is shown in its entirety in Scheme 18.39.

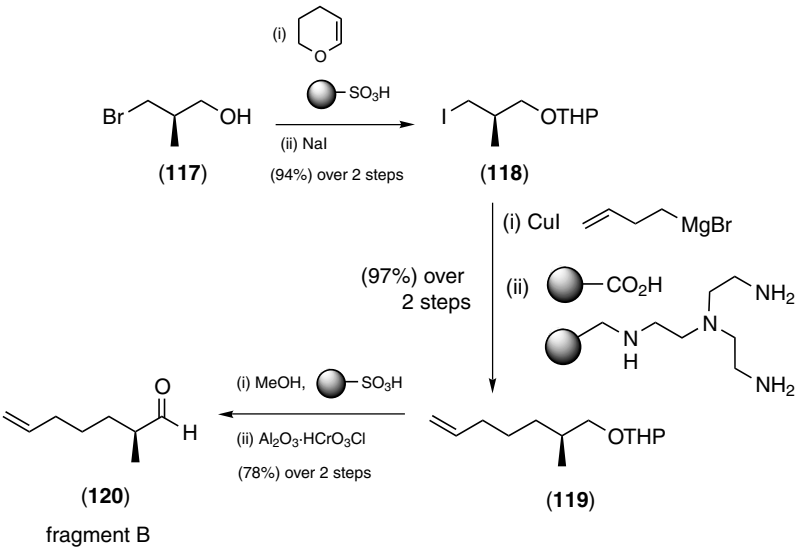


Scheme 18.39 Synthesis of fragment A of epothilone A.

This synthesis uses a modified Mukaiyama aldol reaction to set up the required C3 stereochemistry (Scheme 18.39). The reaction was mediated by formation of a chiral borane complex, which catalyzed the coupling of an aldehyde (**110**) with the trimethylsilyl enol ether (**111**). This reaction proceeded extremely well, giving the product in excellent yield and high enantiomeric excess ($\geq 92\%$). Workup necessitated addition of a minimum amount of water and Amberlite IRA 743 (**112**) to quench the reaction and remove boric acid contaminants. Filtration and solvent removal provided a suspension of amino acid and the aldol product **113**. Subsequent filtration allowed the amino acid residue to be recovered and recycled, while concentration of the filtrate allowed isolation of the pure adduct (**113**). Following TBS protection of the secondary alcohol to give **114**, homologation was achieved in two steps. First, treatment with (trimethylsilylmethyl)lithium followed by a supported acid quench provided **115**; this was monomethylated and quenched again with a polymer-supported acid, furnishing fragment A (**116**) in excellent yield, requiring no chromatographic purification.

The preparation of the second key coupling partner, fragment B, was achieved in just five steps from the known bromide **117** (Scheme 18.40). Following supported acid-catalyzed tetrahydropyranyl protection and conversion to the iodide **118**, displacement with the cuprate derived from 3-butenylmagnesium bromide gave the product desired. Subsequent addition of a carboxylic acid resin and a trisamine resin simultaneously quenched the reaction and scavenged the dissolved copper salts. Filtration through a pad of silica gel and evaporation gave the clean product **119**. Finally, deprotection was effected by methanol and catalytic sulfonic acid resin, and the resulting alcohol was then oxidized to the required aldehyde fragment B (**120**) using chlorochromate on basic alumina as the supported reagent system.

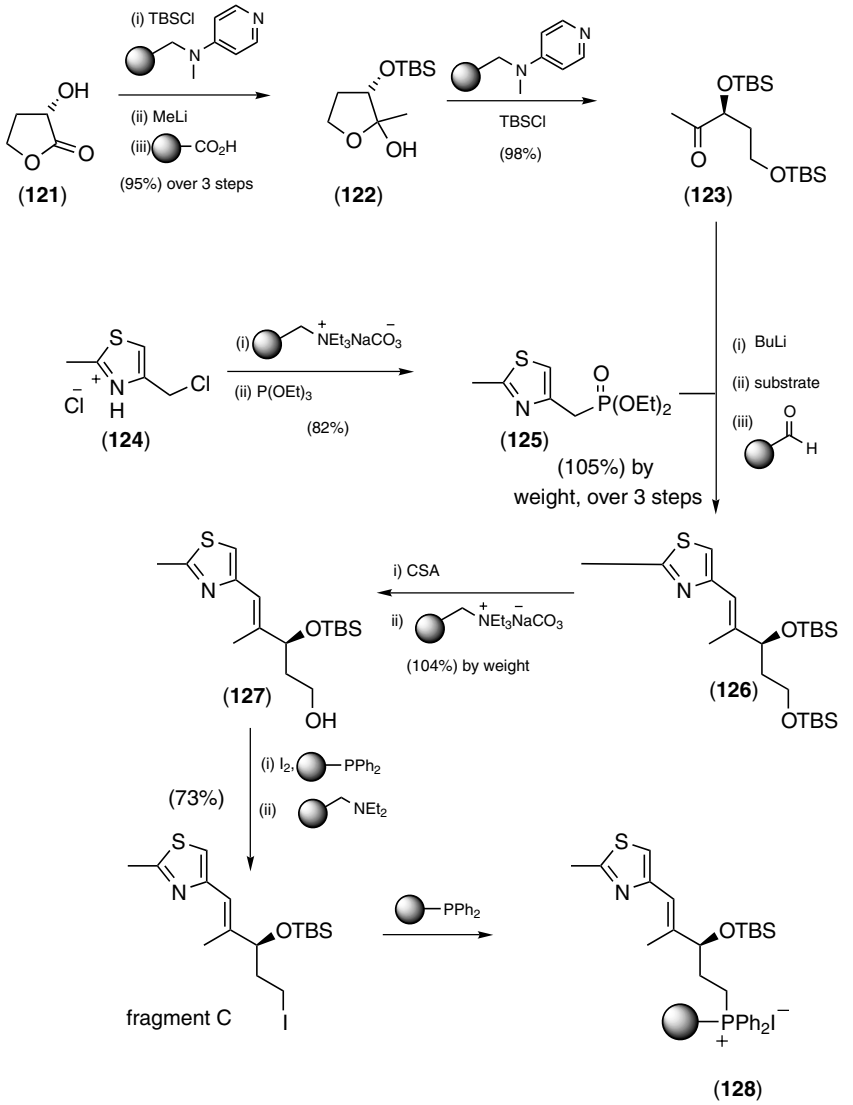
The final key fragment, fragment C, was obtained as its immobilized phosphonium salt (**128**) using a convergent coupling process (Scheme 18.41). The first component was prepared from a commercially available lactone (**121**) via protection and subsequent addition



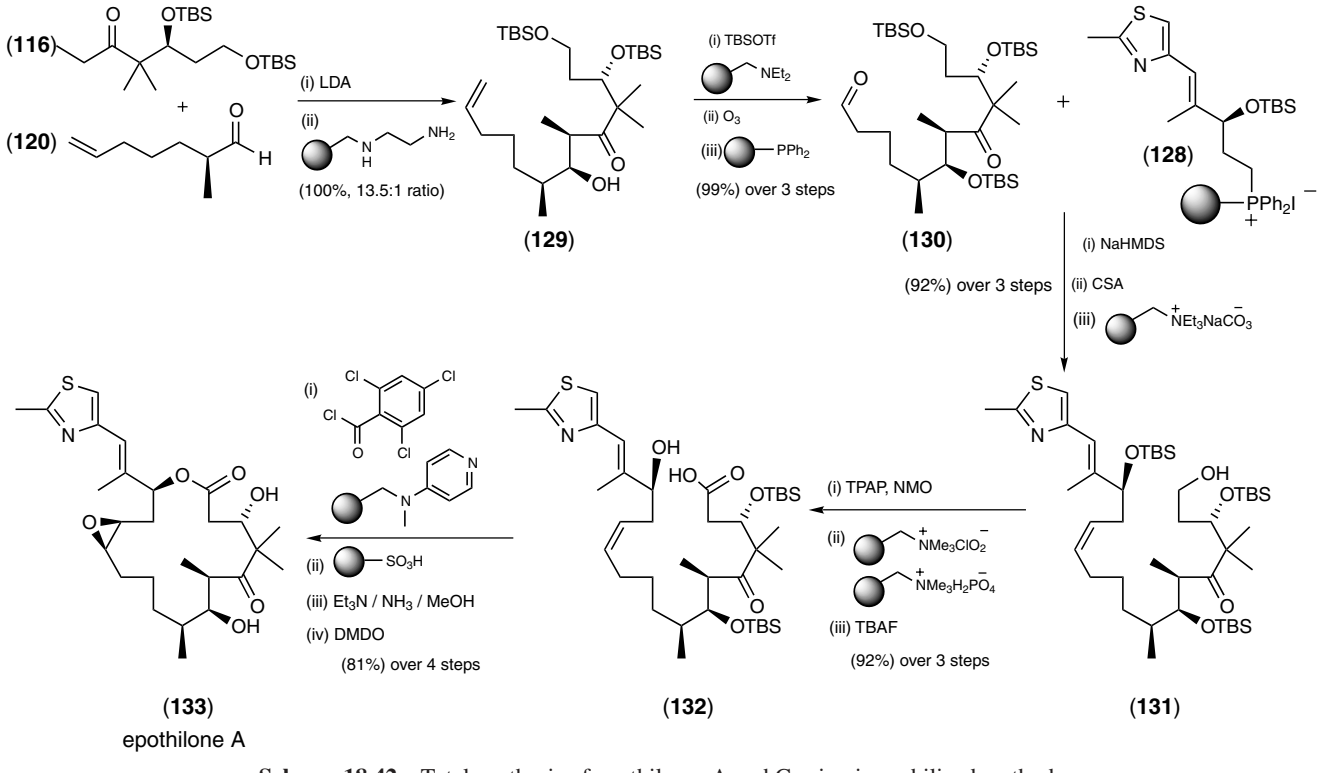
Scheme 18.40 Synthesis of fragment B of epothilone A.

of methyl lithium followed by immobilized acid quench to provide a lactol (**122**). This was then treated with *t*-butyldimethylsilyl chloride and polymer-supported DMAP to give the protected ketone (**123**). The other partner was derived from the chlorothiazole hydrochloride (**124**).

After transforming this to the free base with an immobilized carbonate resin, conversion to a phosphonate (**125**) gave a substrate ready for Horner–Wadsworth–Emmons coupling. This reaction proceeded well with very high stereoselectivity to give the coupled material (**126**). Any slight excess of the phosphonate was scavenged with polymer-supported benzaldehyde. Selective removal of the primary silyl group with camphor sulfonic acid followed by carbonate resin workup afforded an alcohol (**127**). This was



Scheme 18.41 Synthesis of fragment A of epothilone A.



Scheme 18.42 Total synthesis of epothilones A and C using immobilized methods.

converted to **128** by treatment with iodine and polymer-supported triphenylphosphine. Fragment C was then captured onto a phosphine resin to give phosphonium salt (**128**) with a loading of 0.9 mmol/g ready for later release from the resin by a Wittig reaction (Scheme 18.41).

With all the fragments now in place, the more difficult task of coupling fragments together to form the natural product could begin (Scheme 18.42). Deprotonation of fragment A (**116**) with LDA as the base and coupling with the aldehyde, fragment B (**120**), gave the aldol product **129** with greater than 13 : 1 stereoselectivity in quantitative yield. Brief quenching with a small quantity of acetic acid was followed by addition of a diamine-functionalized polymer (this effectively removed any residual acid and any unreacted aldehyde). Simple filtration and evaporation afforded the product **129**. Following TBS protection, alkene cleavage by ozonolysis with a polymer-supported triphenylphosphine gave an aldehyde (**130**).

The key coupling of the two major fragments was then addressed. The resin-bound phosphonium salt of fragment C (**128**) was treated with sodium hexamethyldisilylazide (NaHMDS) and washed with dry THF to give a salt-free ylid, which was then coupled successfully with an aldehyde (**130**) to give the *cis*-olefin exclusively. Selective deprotection of the primary TBS ether (**131**) was achieved with camphor sulfonic acid (CSA), followed by a polymeric carbonate workup to quench and scavenge unwanted acidic residues, yielding **131**. Oxidation of the primary alcohol in **131** was best achieved in two steps: TPAP oxidation to the aldehyde and then a modified Pinnick oxidation process. This oxidation was addressed using an immobilized reagent combination with polymer-supported chlorite,[69] which gave the required carboxylic acid (**132**). Selective deprotection of this compound using immobilized catalysts such as supported fluorides was unfortunately not achieved, and there were no immediate alternatives to using an aqueous tetrabutylammonium fluoride (TBAF); however, no further purification of the product was necessary. Finally, successful Yamagachi macrolactonization using polymer-supported DMAP gave the macrocycle epothilone C. As the final product contained minor impurities, a product capture-and-release protocol was used to provide the pure product, which was released from the sulfonic acid resin with ammonia and triethylamine in methanol. This product was identical, in all respects, to epothilone C. One final oxidation with dimethyldioxirane took epothilone C to epothilone A.

This synthesis demonstrates the immense scope and power of supported reagent systems in complex molecule synthesis, in this case effecting 29 steps with great efficiency, with a longest linear sequence a mere 17 steps from available starting materials.

18.4 CONCLUSIONS

In this chapter we have described the multistep application of supported reagents, scavengers, and catch-and-release techniques to the construction of various natural products and natural product–like libraries of compounds. These methods of immobilization can aid considerably the rapid optimization of complex chemical transformations. They avoid or minimize the need for more time-intensive skill-based workup procedures, such as chromatography, distillation, and crystallization, and are therefore valuable new tools to add to the repertoire of the synthesis chemist.

In the future one can anticipate that these procedures will be conducted in a flow mode with full use of automation, and control of informatics feedback, leading to entire new arrays of molecules suitable for full biological evaluation.

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19

INSULIN SENSITIZERS: EMERGING THERAPEUTICS

BRAJ B. LOHRAY AND VIDYA B. LOHRAY
BHUVID Research Laboratory
Ahmedabad, Gujarat, India

19.1 INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hyperglycemia. Clinically, patients can be classified as having either insulin-dependent diabetes mellitus (IDDM or type 1 diabetes) or having non-insulin-dependent diabetes mellitus [NIDDM or type 2 diabetes (T2D)]. The World Health Organization (WHO) has designated diabetes mellitus as an epidemic, although it is a noninfectious disease.[1] Nearly 2.3% of the world's population is estimated to be suffering from this disease, with an increasing trend of 4 to 5% every year. WHO has reported 154.4 million diabetes patients worldwide in 2000. There may be still more people who are undiagnosed or have prediabetic symptoms but have not been diagnosed clinically as being diabetic. IDDM is an autoimmune disease that is treated with exogenous insulin administration and patients have an absolute deficiency of insulin, caused by defective pancreatic β-cell function. NIDDM has a more complex etiology, including reduced sensitivity of tissues to insulin, and is characterized in the later stages by hyperglycemia, hyperinsulinemia, and perhaps defects in insulin secretion by the pancreatic β-cells. It is a multifactorial disease, including obesity and cardiovascular disease, and involves a combination of genetic and environmental factors. Untreated T2D leads to several complications, such as retinopathy, nephropathy, and cardiovascular diseases including atherosclerosis, leading to increased mortality.[2] The United Kingdom Prospective Diabetes Study (UKPDS) has shown that rigorous management of blood glucose levels [HbA_{1c} (glycosylated hemoglobin)] and blood pressure can reduce the complications.[3] Early stage T2D may be managed well with diet and exercise. However, in addition to lifestyle and dietary changes, insulin or oral hypoglycemic agents (OHA) either alone or in combination are needed for good control over blood glucose levels.

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19.2 THERAPEUTIC INTERVENTIONS

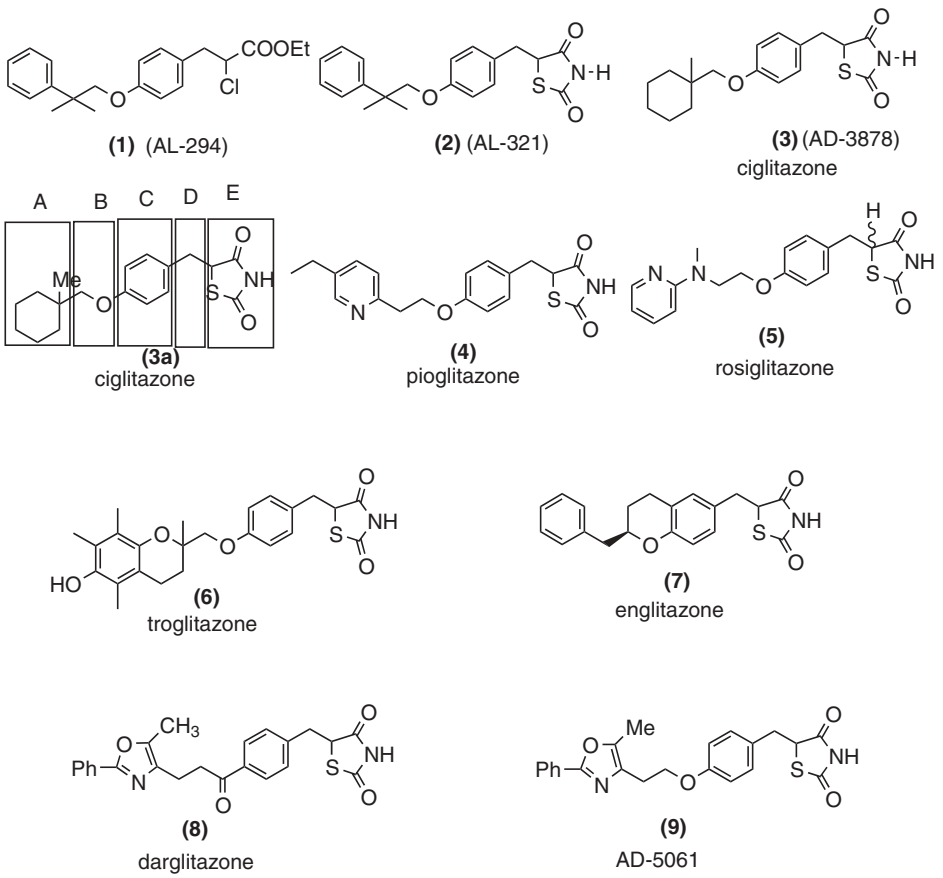
Although there have been several drugs for the treatment of T2D, none of them alone is most effective, including insulin, due to the complexity of the disease, involving defects in different targets, causing insulin resistance (or impaired insulin action) or other factors.[4,5] Therefore, one would expect several therapeutic agents to manage the disease. These may be broadly classified as follows:

1. Enhancers of insulin release, which include sulfonylureas (e.g., tolbutamide, acetohexamide, glyburide, glipizide, gliclazide, glimepiride), glinides (e.g., repaglinide, netaglinide), GLP-1 (glucagon-like peptide-1) and analogs (e.g., Exendin-4, a GLP-1 mimetic recently been approved in the United States), DPP-IV (dipeptidyl peptidase IV) inhibitors (which can extend the lifetime of GLP-1), and imidazolines, potassium channel openers, and glucokinase (GK) activators
2. Inhibitors of hepatic glucose production, such as biguanides (e.g., metformin), glucagon receptor antagonists, glycogen phosphorylase inhibitors, pyruvate dehydrogenase kinase (PDHK) inhibitors, fructose-1,6-bisphosphatase (FBPase) inhibitors, glycose-6-phosphatase (G-6-Pase) inhibitors, glycogen synthase kinase-3 (gsk-3) inhibitors, liver-selective glucocorticoid (GC) receptor antagonists, adenosine A2B receptor antagonists, phosphoenolpyruvate carbokinase (PEPCK) inhibitors, and 11β-hydroxysteroid dehydrogenase-1 (11-β-HSD-1) inhibitors
3. Inhibitors of glucose uptake, such as glucosidase inhibitors (e.g., acarbose, voglibose), and sodium-glucose transporter (SGLT) inhibitors
4. Enhancers of insulin action, such as peroxisome proliferator-activated receptor (PPAR) ligands [e.g., troglitazone (withdrawn from clinical use), pioglitazone, rosiglitazone], retinoid X receptor (RXR) modulators, and protein tyrosine phosphatase 1B (PTP 1B) inhibitors

Except for a few targets against which drugs have been developed and marketed successfully (e.g., sulfonylureas, glinides, biguanides, thiazolidinediones), intense drug discovery efforts are under way against several targets. A number of drug candidates are in various stages of clinical development. The recent developments have been compiled in *Annual Reports in Medicinal Chemistry* (2000 and 2004).[6,7]

19.3 DISCOVERY OF INSULIN SENSITIZERS

The concept of insulin sensitizers emerged with the discovery of the thiazolidinedione (TZD) class of compounds. The first compound in the TZD class was discovered in 1982 in Japan by Sohda et al. at Takeda.[8] While searching for a potent agent for reducing cholesterol and triglyceride, Takeda scientists discovered compound **1** (AL-294) (Scheme 19.1), which was also tested for antidiabetic activity in KK mice for their ability to reduce the glucose level. It was found that **1**[9] was able to reduce the glucose level by 18 to 41%. Search for a suitable acid functionable equivalent of **1** led to the discovery of TZD's head group in **2** (AL-321), which was slightly more potent than **1**.



Scheme 19.1 Development of glitazones.

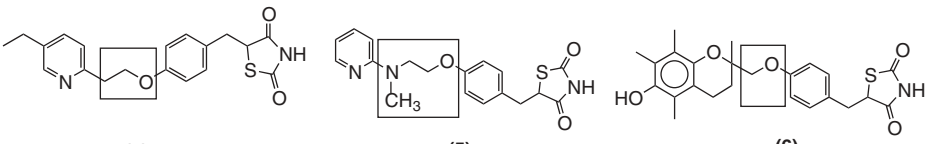
Change of TZD to rhodanine or 2-iminothiazolidinediones led to weaker compounds. *N*-Methyl compound **2** was totally inactive, suggesting the importance of an acidic proton at the 3-position of the TZD ring. Further modification on the lipophilic tail of the molecule lead to ciglitazone (**3**), which was found to be more potent than **1** or **2** and possess no liver toxicity. This compound was tested in several animal models of diabetes, such as db/db, ob/ob, and KKAy mice. In all the models, the glucose level was found to decrease without an increase in insulin secretion. Also, the insulin level decreased in the hyperinsulinemic model, indicating the potency of ciglitazone to improve insulin sensitivity. On the other hand, ciglitazone had no effect in insulin-deficient diabetic animals,[10] nor did it reduce the glucose level below the normal plasma glucose level (euglycemic agent). This was a breakthrough discovery for the treatment of type 2 diabetes, and that followed the discovery of several glitazones and other heterocycles, which were found to be insulin sensitizers[8] and distinguished themselves from insulin secretagogs such as sulfonylureas and from biguanides. The initial lead molecule, ciglitazone, was modified in five regions (A to E), as shown in **3a** in Scheme 19.1, which led

to a number of active molecules: (1) modification of the lipophilic tail on the A region, (2) modification in the linker chain in the B region, (3) modification in the central aryl group in the C region, (4) variation in the linker between the pharmacophore E and aryl group C, and (5) the pharmacophore group. Of all the heterocycles used in A, substituted oxazoles (as in **8** and **9**) and pyridyl groups (as in **4** and **5**) have been found to give potent compounds. The preferred linker B is a two-carbon ether or *N*-alkylethyleneoxy group as in **4**, **5**, or **9**. Bivalent phenylene is a preferred C group and the D group is a one-carbon methylene group. These variations gave rise to several glitazones, which were developed for clinical evaluation, such as pioglitazone (**4**) (Takeda[11]), rosiglitazone (**5**) (GlaxoSmithKline[12]), troglitazone (**6**) (Sankyo[13]), englitazone (**7**) (Pfizer[14]), darglitazone (**8**) (Pfizer[15]), and AD-5061 (**9**) (Takeda[16]), as listed in Scheme 19.1, of which pioglitazone and rosiglitazone have been developed and marketed successfully. Due to the unacceptable toxicity of troglitazone, it was withdrawn from the market, whereas englitazone, ciglitazone, and darglitazone were not developed for clinical use. The mechanism of thiazolidinediones is not well understood. They have been found to induce gene expression in adipocytes and to enhance adipocyte differentiation in cells[17], however, the molecular basis for the adipogenic effect remained unclear. The development of these glitazones through rational modifications has been reviewed by Hulin et al.,[18] and more recently, Lohray and Lohray[19] have reviewed the development of insulin sensitizers.

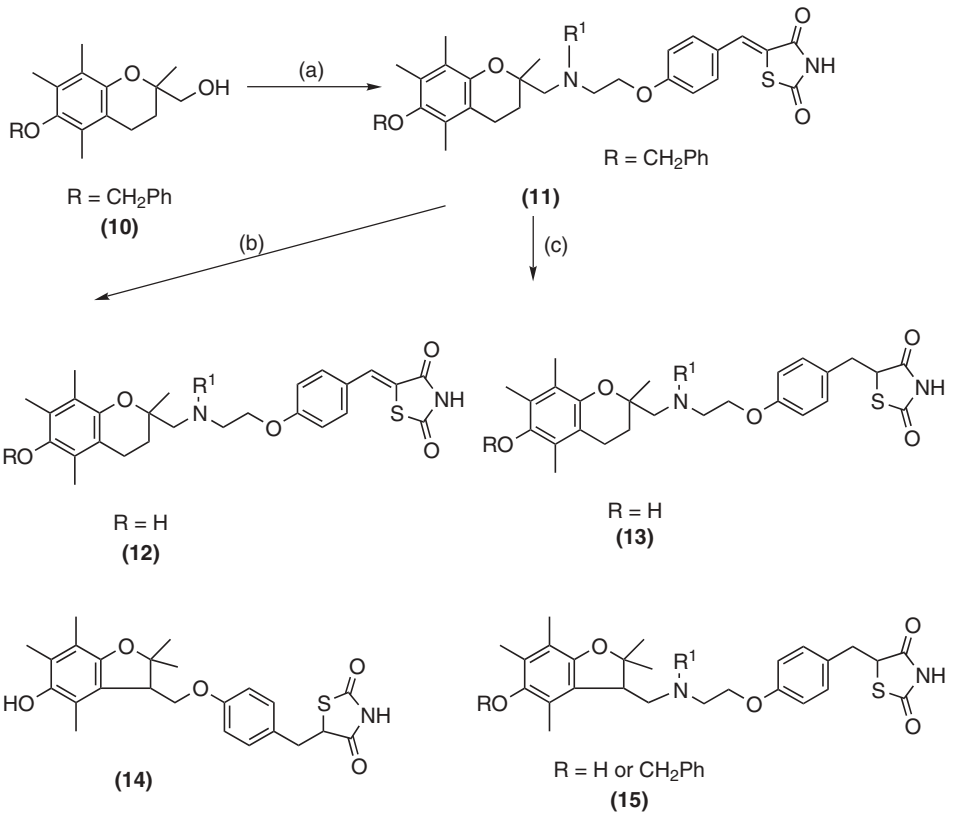
Lehmann et al.[20] demonstrated that the antidiabetic thiazolidinediones are high-affinity ligands for peroxisome proliferator activated receptor γ (PPARγ), indicating that PPARγ is a molecular target for the adipogenic effects of thiazolidinediones, and further may be a target for the action of this class of compounds. Since then, PPAR has become a hot target for research, along with its three isoforms α, β (or δ), and γ, in the field of metabolic syndromes.[21]

19.4 JOURNEY TOWARD NEW DRUGS

Need for new insulin sensitizers was felt when troglitazone was withdrawn from the market. The antioxidant moiety of the troglitazone was thought to be responsible for the safety and beneficial effect of troglitazone until that time. Our initial strategy was based on comparison of euglycemic activities of pioglitazone (**4**) and rosiglitazone (**5**), which suggested that introduction of an N—(CH_3) group between the pyridine ring and the phenoxyethyl moiety of pioglitazone would lead to a several-fold increase in potency (Scheme 19.2). We therefore introduced an —NR group between the chroman ring and the phenoxyethyl moeity (**12** and **13**) (Scheme 19.3) in troglitazone as well as in its correspondingly similar dihydrobenzofuran analog (**14** and **15**).[22]



Scheme 19.2 Modification of troglitazone.



Scheme 19.3 (a) (i) $MeSO_2Cl$ (98%), (ii) $R^1NHCH_2CH_2OH$, Δ, neat, 120°C (95%), (iii) $SOCl_2$ (98%), (iv) 4-hydroxybenzaldehyde (97%), (v) 2,4-thiazolidinedione, piperidine, benzoic acid, toluene, Δ (98%); (b) AcOH–HCl, Δ, 2 h, 60°C (98%); (c) Mg–MeOH, (ii) AcOH–HCl, 60°C, 2 h (90%).

Both unsaturated and saturated thiazolidinedione derivatives were evaluated in db/db mice at 200 mg/kg per day per os for 9 days. The results are summarized in Table 19.1. The study of antioxidant properties of OH free and protected compounds **11** vs. **12a** and **15a** vs. **15d** indicated the absence of any antioxidant property in O-benzylated compounds. However, comparing the in vivo activities of compound **12a** with **11**, we did not observe differences in plasma glucose-lowering activity (Table 19.1, entry **12a** vs. **11**), although there is considerable change in triglyceride-lowering activities. Similar effects on plasma glucose and triglyceride were observed for the dihydrobenzofuran analog (Table 19.1, entry **15a** vs. **15d**). However, contrary to our expectations, saturation of the C=C bond **12a** (i.e., **13**) leads to the complete loss of plasma glucose- and triglyceride-lowering activities. Thus, we find that compounds **12a** and **15a** are superior to troglitazone in both plasma glucose- and triglyceride-lowering activities.

It is also interesting to note that the methyl group is an optimum substituent on N in both the chroman and benzofuran series of compounds. Replacement of N—Me by N—H (Table 19.1, entry **12c**) or N—Et (Table 19.1, entry **12b**) leads to a reduction of plasma glucose-lowering activities.

TABLE 19.1 Thiazolidinediones (12 and 15) and Their Biological Activity[a]

Compound	R	R^1	DB	Dose (mg/kg)	X^D	PG Reduction[b] (%)	TG Reduction (%)
11	Bn	Me	Yes	200	—	39	32
12a	H	Me	Yes	200	—	43	70
12b	H	Et	Yes	200	—	2	76
12c	H	H	Yes	200	—	6	29
12d	H	Me	Yes	200	CH_3COO	11	31
12e	H	Me	Yes	200	HCl	31	30
12f	H	Me	Yes	300	Maleate	20	60
12g	H	Me	Yes	200	$CH_3SO_3^-$	20	70
13	H	Me	No	200	—	4	N.A.
	Troglitazone	Ñ	—	200	—	24	50
14	H	Ñ	No	200	—	19	40
15a	H	Me	Yes	200	—	46	71
15b	H	Me	No	200	Maleate	35	33
15d	Bn	Me	Yes	200	—	23	N.A.

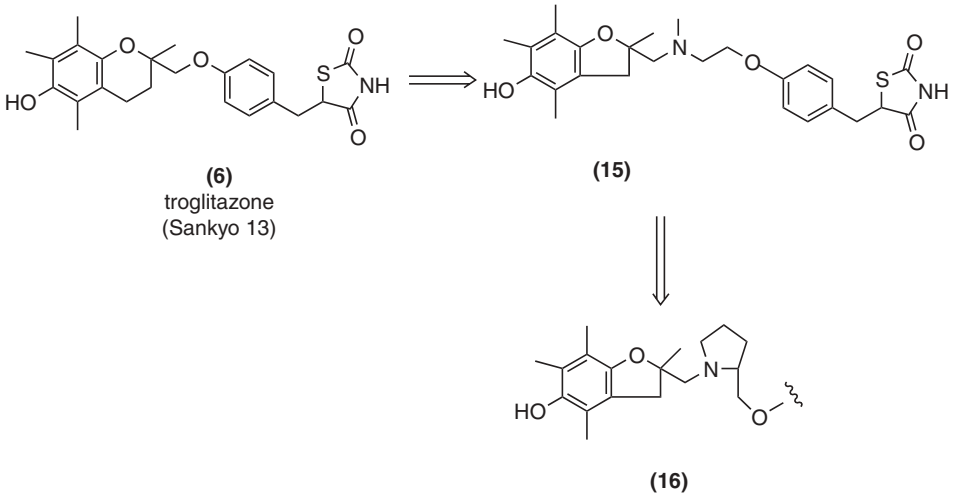
[a]DB, double bond; X^D, counterion for salt formation; PG, plasma glucose; TG, plasma triglyceride. N.A., not applicable.

[b]After 9 days of treatment.

Further, we cyclized the methyl group on the nitrogen with the CH_2 of the ethoxy linker to form a five- or six-membered ring (Scheme 19.4) to obtain compounds that showed activity superior to that of their parent molecules.[22b] The synthesis of these molecules is outlined in Scheme 19.5. The activity of these compounds to reduce blood glucose and triglyceride in db/db mice is shown in Table 19.2. It is observed that the cyclic linker moiety improved the antidiabetic activity of the compounds compared to the N—Me ethoxy linker. The unsaturated analogs showed better activity than that of their saturated counterparts. Benzyl-protected compounds have been found to be better than free OH compounds, despite losing antioxidant activity. Further, the benzopyran moiety in these molecules were shrunk to dihydrobenzofuran and benzofuran derivatives, which showed modest antidiabetic activities.

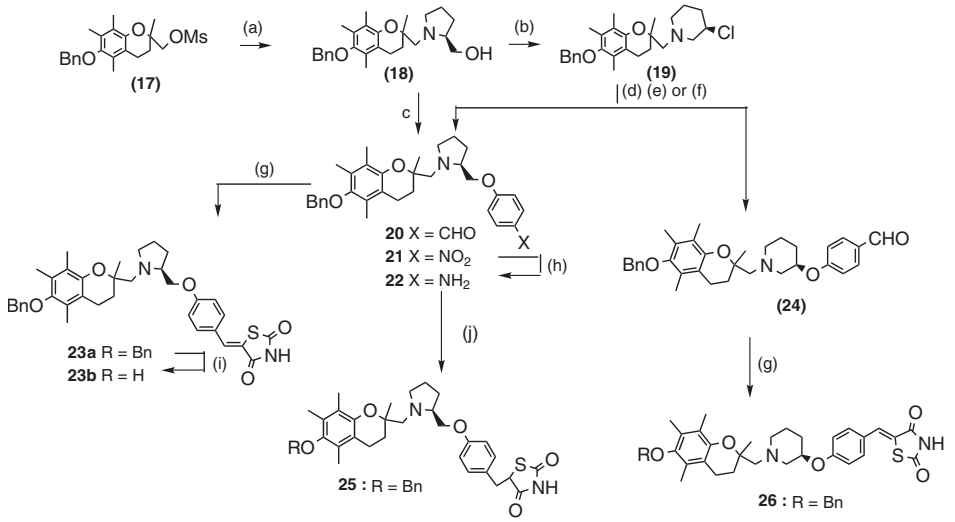
Similarly, the methyl group on nitrogen of rosiglitzone was incorporated in a ring with or without the help of a carbon or heteroatom, as shown in **27** (Scheme 19.6).

We prepared a series of indole and azaindole analogs by a synthesis outlined in Scheme 19.7 and evaluated their antihyperglycemic activity.[23] We first examined several unsaturated 2,4-thiazolidinediones (**29a** to **29c**) and compared their activities against those of troglitazone or unsaturated rosiglitzone (entry 13 in Table 19.3). In cases where the antihyperglycemic activities were comparable to that of unsaturated rosiglitazone**,** we synthesized the corresponding saturated analogs (**30a**, **30d**, **30g**, and **30l**) and compared their euglycemic activities with those of troglitazone or rosiglitzone (Table 19.3).



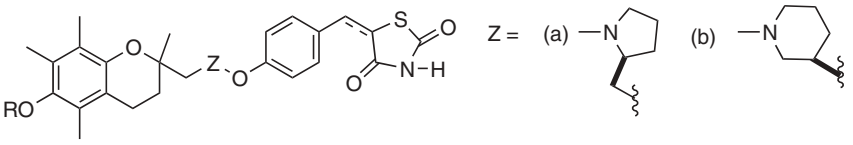
Scheme 19.4

To obtain further insight into the structure–activity relationship, we examined several indole derivatives (**29e** to **29k**). The effect of various substituents on the indole ring was examined. The presence of an electron-withdrawing group such as COOH or COOMe (**29e** and **29f**) or electron-donating group such as methyl (**29i**, and **29j**) has a



Scheme 19.5 Reagents and conditions: (a) (*S*)-prolinol, 120°C, 6 h, 75%; (b) $SOCl_2$, C_6H_6, 25°C, 1 h, 73%; (c) 4-fluoronitrobenzene, NaH, DMF, 26°C, 2 h, 81%; (d) 4-hydroxybenzaldehyde, K_2CO_3, DMF, 80°C, 2 h, **20** + **24**, 33% + 35%; (e) 4-hydroxybenzaldehyde, DEAD, Ph_3P, THF (**20** + **24**, 41% + 40%); (f) 4-fluorobenzaldehyde, KOBu^t, DMF, 36 h, 25°C, 60%; (g) 2,4-thiazolidinedione, piperidine, $C_6H_5CO_2H$, toluene, 120°C, 2 h, 57 to 93%; (h) Pd/C (10%), H_2 (60 psi), EtOAc, 6 h, 91%; (i) CH_3COOH–HCl (3 : 1), 70 to 80°C, 98%, (j) (i) $NaNO_2$, aqueous HBr; MeOH–acetone, ethyl acrylate, Cu_2O, 38°C, 47%, (ii) thiourea, NaOAc, EtOH, Δ, 5 h, (iii) 2 N HCl, EtOH, Δ, 12 h, 85%.

TABLE 19.2 Euglycemic and Hypotriglyceredemic Activities of Thiazolidinediones



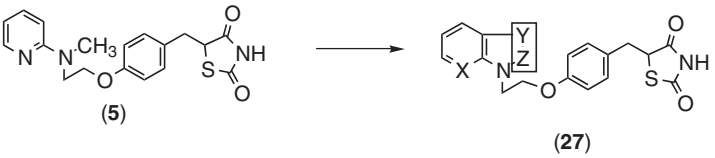
						Sixth Day	
Entry	Compound	R	DB[a]	Z = (a)/(b)	Dose[b] (mg/kg)	PG Reduction[c] (%, mean±E)	TG Reduction[d] (%, mean±SE)
1	**23a** + **26a**	Bn	Yes	(a)/(b) (21 : 4)	200	52±7	N.D.
2	**23a** + **26a**	Bn	Yes	(a)/(b) (3 : 7)	200	53±6	N.D.
3	**23a**	Bn	Yes	(a)	100	68±4	N.D.
4	**25**	Bn	No	(a)	100	26±3	N.D.
5	**26**	Bn	Yes	(b)	100	5±7	24±7
6	**23b**	H	Yes	(a)	100	37±5	36±4

[a] DB, double bond; (dashed line), optional double bond.

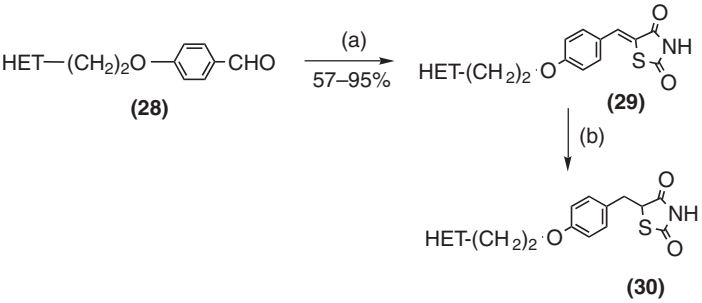
[b] Daily dose given per os.

[c] Percent reduction of plasma glucose (mean S.E.; $n = 4$) after 6 days of treatment.

[d] Percent reduction of plasma triglyceride (mean S.E.; $n = 4$) after 6 days of treatment; N.D. not done.

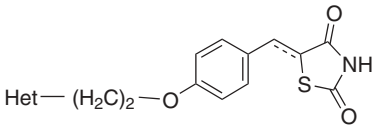


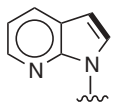
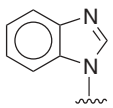
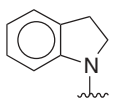
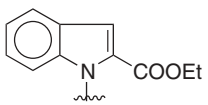
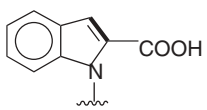
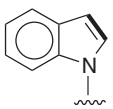
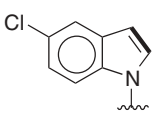
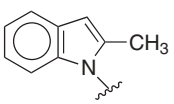
Scheme 19.6



Scheme 19.7 (a) 2,4-Thiazolidinedione, piperidine, benzoic acid, toluene, Δ; (b) H_2/10% Pd/C, 70 to 96% yield.

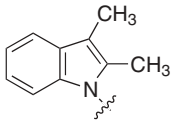
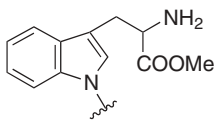
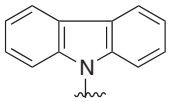
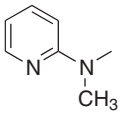
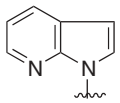
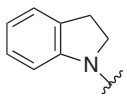
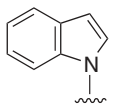
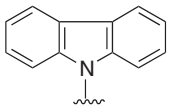
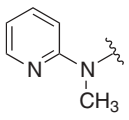
TABLE 19.3 Euglycemic and Hypolipidemic Activities of Thaiazolidinediones



Entry	Compound	Het	DB[a]	Yield (%)	Melting Point (°C)	Dose (mg/kg)	PG Reduction[b] (%)	TG Reduction[b] (%)
1	**29a**		Yes	81	205	200	36	79
2	**29b**		Yes	92	258–260	200	33	76
3	**29c**		Yes	78	210	200	13	72
4	**29d**		Yes	94	179	200	61	83
5	**29e**		Yes	82	184	200	0	13
6	**29f**		Yes	84	115	200	12	0
7	**29g**		Yes	78	216	200	33	37
8	**29h**		Yes	91	206	200	39	11
9	**29i**		Yes	95	235	200	16	41

(*Continued*)

TABLE 19.3 (*Continued*)

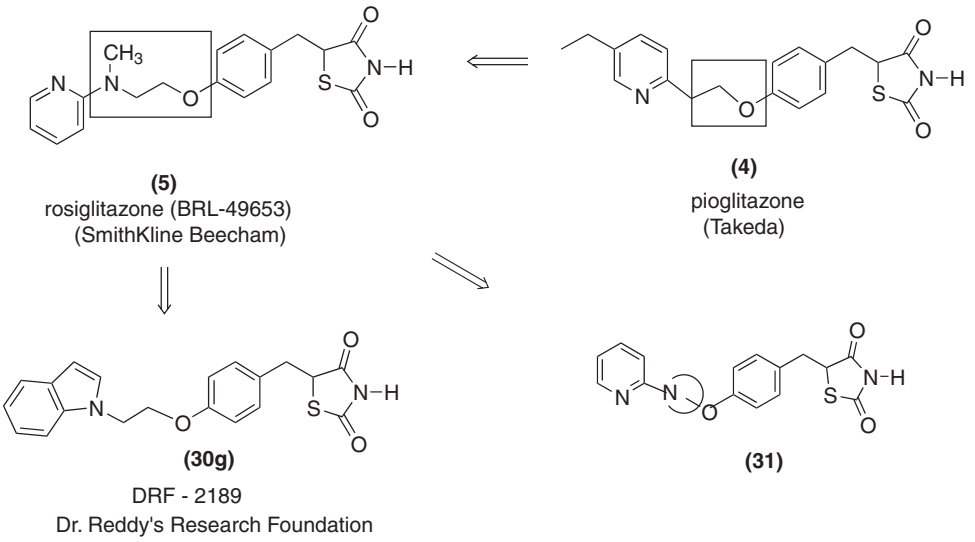
Entry	Compound	Het	DB[a]	Yield (%)	Melting Point (°C)	Dose (mg/kg)	PG Reduction[b] (%)	TG Reduction[b] (%)
10	**29j**		Yes	94	254	200	40	2
11	**29k**		Yes	71	180	100	27	64
12	**29l**		Yes	95	277–278	100	0	44
13	Unsaturated rosiglitazone		Yes	87	193–194	10 100	15 56	17 32
14	**30a**		No	70	195	200	33	74
15	**30d**		No	83	143–144	200	33	79
16	**30g**		No	92	103	100	74	77
17	**30l**		No	80	214	100	0	21
18	Rosiglitzone		No	52	153–155	10 100 200	15 71 67	11 46 55
19		Troglitazone	No	77	180–181	200	41	
20		Rosiglitazone maleate	No	76	123	10	55	37

[a]DB, double bond; dashed line, optional double bond.
[b]After 9 days of dosing in db/db mice via oral gavage.

deleterious effect on the euglycemic as well as hypolipidemic activities of these thiazolidinediones. It appears that substituents at the C2 and C3 positions of the indole ring are not tolerated. We selected thiazolidinedione (**29g**), which has no substituent on the indole ring, for further study and synthesized saturated thiazolidinedione (**30g**; DRF-2189), which was very effective in reducing blood glucose (74%) and triglyceride (77%), with the blood glucose level reaching levels close to that of the lean littermate (8 ± 1 mM). At 10 mg/kg, we observed that DRF-2189 and rosiglitazone maleate reduced the plasma glucose (PG) by 51 to 55% (PG: 12.5 ± 1 mM), whereas rosiglitzone free base (entry 18, Table 19.3) did not produce a significant reduction at the same dose. DRF-2189 was studied in a number of animal models in comparison to rosiglitzone maleate and was found to be similar or a shade better. Although all the compounds showed good euglycemic and excellent-to-good triglyceride (TG) lowering activities, and the indole derivative (**30g**; DRF-2189) was found to be the most potent euglycemic and hypolipidemic agent. Comparative studies were carried out in db/db mice using DRF-2189 (**30g**) and rosiglitazone (**5**) at doses of 1, 3, and 10 mg/kg per day, and the results were compared with those for troglitazone at doses of 100, 200, and 800 mg/kg [(DRF-2189: (1 mg/kg dose) PG↓ 24 ± 6%, TG↓ 55 ± 10%; (3 mg/kg dose) PG↓ 52 ± 8%, TG↓ 58 ± 8%; (10 mg/kg dose) PG↓ 51 ± 11%; TG↓ 59 ± 8%; rosiglitazone: (1 mg/kg dose) PG↓ 24 ± 6%; TG↓ 16 ± 7%; (3 mg/kg dose) PG↓ 55 ± 8%, TG↓ 3 ± 8%; (10 mg/kg dose) PG↓ 55 ± 8%, TG↓ 39 ± 10%; troglitazone: (100 mg/kg dose) PG↓ 32 + 6%, TG ↓52 ± 10%; (200 mg/kg dose) PG↓ 42 ± 10%, TG↓ 65 ± 8%; (800 mg/kg dose) PG↓ 50 ± 8%; TG ↓60 ± 12%)]. The dose-dependent reductions in plasma glucose and TG levels were observed. While DRF-2189 and rosiglitazone were approximately equipotent, even at 800 mg/kg, troglitazone was less effective in reducing plasma glucose. The reductions in plasma glucose for DRF-2189 (**30g**) and rosiglitazone (**5**) were not significantly different, whereas DRF-2189 was significantly better in reducing plasma triglyceride in db/db mice. The oral ED_{50} value for plasma glucose lowering for DRF-2189 was 3 mg/kg, and higher doses (10 and 30 mg/kg) did not reduce the blood sugar level further.

Similarly, plasma TG lowering activity was found to be the highest (approximately 53% reduction in plasma TG) with 1 mg/kg; once again, higher doses did not further reduce TG levels. Furthermore, DRF-2189 also reduced plasma insulin levels by 79%, similar to that of rosiglitazone (10 mg/kg). DRF-2189 was evaluated further in ob/ob mice at 20 mg/kg for 14 days. Treatment resulted in reductions in PG and TG levels of 56 and 50%, respectively. In addition, a 22% reduction in cholesterol was also observed, an effect not found with rosiglitazone. A glucose tolerance test performed in ob/ob mice after 15 days of treatment led to a 68% reduction in AUC (area under the curve). In contrast, troglitazone-treated animals showed only a 30% improvement in glucose clearance. When high-fat-fed Sprague–Dawley rats were treated with DRF-2189 (10 and 30 mg/kg), significant reductions in TG (47 and 30%), total cholesterol (11 and 41%), LDL cholesterol (13 and 47%), and VLDL (47 and 36%) were observed, as well as an increase in HDL cholesterol (80 and 79%). In contrast, rosiglitazone at the same doses did not have any effect on these parameters. It should be pointed out that improved management of lipid profiles is essential for patients with a high risk of coronary artery disease, and in this regard, DRF-2189 was superior to rosiglitazone. DRF-2189 also showed quite favorable pharmacokinetic and toxicological profile compared to rosiglitazone.[24] The compound was found to be a PPARγ agonist.

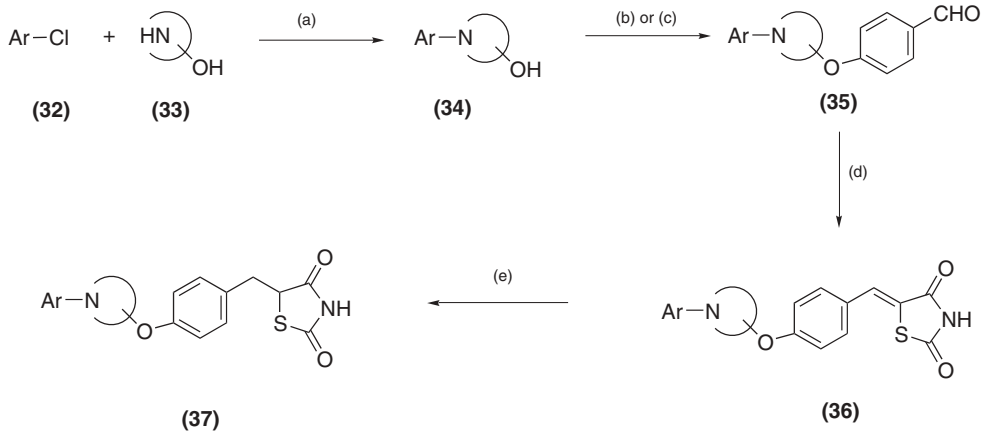
Additionally, we envisaged incorporating a N—Me group of rosiglitzone in the side chain of the linker with the help of a carbon atom or other heteroatom, as shown in



Scheme 19.8 Approach to novel thiazolidinidiones.

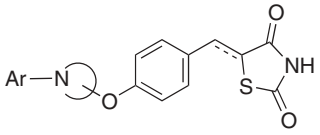
Scheme 19.8 (structure **31**) and studied the structure–activity relationship of this class of thiazolidinediones.[25] Several pyridyl- and quinolinyl-containing thiazolidinediones were prepared. A general synthetic route is shown in Scheme 19.9. The antidiabetic activity of the compounds of this class is shown in Table 19.4.

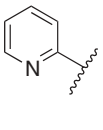
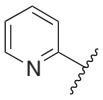
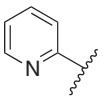
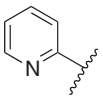
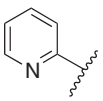
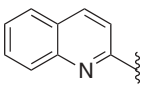
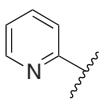
Finally, we selected the maleate and hydrochloride salts of **36a**, the maleate salt of saturated TZD (**37a**), and the sodium salt of **37b** for dose–response studies in db/db mice and compared them with rosiglitazone maleate. The animals were treated with different doses



Scheme 19.9 (a) Neat, 160°C, 50 to 100% yield; (b) 4-fluorobenzaldehyde, NaH, DMF, 40 to 76% yield; (c) (i) methanesulfonyl chloride, triethylamine, CH_2Cl_2, 83 to 90% yield, (ii) 4-hydroxybenzaldehyde, K_2CO_3, DMF, 80°C, 36 to 45% yield; (d) 2,4-thiazolidinedione, piperidine, C_6H_5COOH, toluene, Δ, 63 to 100% yield; (e) Mg-MeOH, 48 to 65% yield.

TABLE 19.4 Euglycemic and Hypolipidemic Activities of Unsaturated TZDs



Entry	Compound	Ar	DB[a]	Dose (mg/kg)	PG Reduction[b] (%)	TG Reduction[b] (%)
1	**36a**		Yes	100	57±3.37	77.75±9.37
	37a		No	30	52.75±6.67	65.54±15.95
2	**36b**		No	100	38±9.51	40.25±13.77
	37b					
3	**36c**		Yes	100	N.S.[c]	27.33±8.21
	37c					
4	**36d**		Yes	100	35.75±18.18	69.75±16.92
	37d					
5	**36b**		No	100	55.25±6.34	77.75±9.37
	37e		No	30	72.25±3.45	49.0±9.06
6	**36f**		Yes	100	63.75±5.22	N.S.[c]
	37f		No	30	40.5±9.63	66.25±4.55
7	**11**		Yes	100	55.5±3.39	35.0±2.24
			No	30	35.0±2.75	40.21±8.69

[a] DB, double bond; dashed line, optional double bond.
[b] After 6 days of treatment in db/db mice via oral gavage.
[c] N.S., not significant.

for 14 to 15 days, and plasma glucose and triglyceride levels were measured in each case. Rosiglitazone showed euglycemic activity at a 3-mg/kg dose (PG, 42±2%; TG, 47±15%) superior to that of **36a** (maleate), **37a** (maleate), and **37b** (sodium salt); however, at higher doses (i.e., 10 and 30 mg/kg) all four compounds were equipotent or sometimes superior

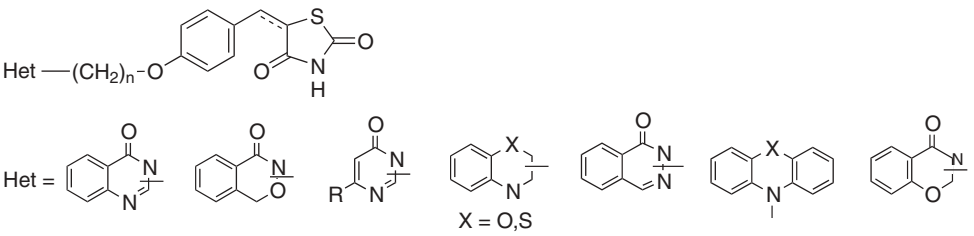
to rosiglitazone maleate. In addition, **36a** especially showed better triglyceride-lowering activities at 10- and 30-mg/kg doses than did rosiglitazone.

Further, to understand the mechanism of action of **36a** and **37a** (maleate), we carried out a receptor transactivation assay. Thus, we carried out transactivation studies of PPARγ at 0.1, 1, and 10 μM concentration) for **36a** and **37a**. We were rather gratified to find that neither **36a** nor **37a** showed significant PPARα or PPARγ activation.[25] (PPARγ activation for **36a** at 0.1, 1.0, and 10 M showed 0.9-, 1.1-, and 1.0-fold transactivation). We also examined the PPARγ transactivation of corresponding saturated TZD (**37a**) at similar concentrations (i.e., 0.1, 1.0, and 10 μM). Interestingly, the saturated compound **6a** showed 3.5-, 7.4-, and 7.2-fold transactivation of PPARγ, although **37a** is not so impressive in controlling plasma glucose in db/db mice. It is interesting to note that **36a** (which is later referred to as PAT5A and found to be a PPARγ partial agonist) is the first unsaturated thiazolidinedione showing such impressive euglycemic- and triglyceride-lowering activities.

Encouraged by the results obtained with DRF-2189 and PAT5A, we modified the heterocycle further with insertion of heteroatoms as well as an oxo group on the heterocycle (Scheme 19.10). The novel compounds generated are the subject matter of several patents.[26] The activities of the compounds when the Het moiety is phthalazinone and benzoxazinone[27] are compiled in Table 19.5. PHT46 (entry 1, Table 19.5) was found to be a better PPARγ activator than pioglitazone and was found to normalize the PG levels at a dose of 10 mg/kg per day and to reduce TG levels by 57% in db/db mice treated for 10 days.

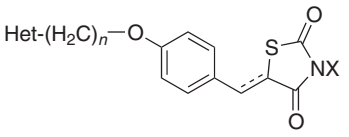
More recently, we have reported[28] pyrimidinone containing TZDs (**38**), which showed remarkable PG- and TG-lowering activities in db/db mice. One of the compounds was found to be far superior to pioglitazone and rosiglitazone in both PG- and TG-lowering activities, although in in vitro transient transactivation assay of PPARγ, the compound was as effective as rosiglitazone (Table 19.6). Compound **38b** also showed a favorable pharmacokinetic profile (AUC_{0-8}, 206.8 μg/mL; C_{max}, 39 μg/mL; T_{max}, 1.25 h; $T_{½}$, 3.0 h). TZDs (**39**) having oxime as part of the heterocycle moiety have also been reported.[29] Some of the results listed in Table 19.7 show interesting antidiabetic activities in KKA^y mice.

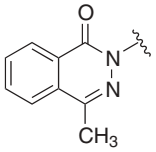
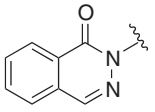
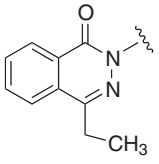
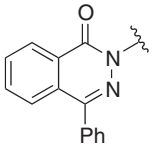
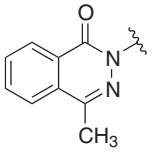
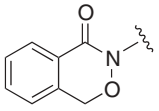
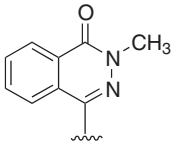
Oguchi et al.[30] developed imidazopyridine-containing TZDs (**41**) as a hypoglycemic agent, which can again be visualized as modification (Scheme 19.11) of DRF-2189. The latter showed very potent antihyperglycemic activity.[23,24] Several imidazopyridine derivatives were screened in KK mice at a 1-mg/kg dose, and a few of them showed interesting euglycemic properties (Table 19.8). Compounds **41a** and **41b** were found to be most potent; however, these compounds caused cardiac hypertrophy in rats after multiple dosing for 2 weeks at 50 mg/kg and tend to accumulate in rats.



Scheme 19.10 Variation in heterocycle.

TABLE 19.5 SAR Studies of Phthalazinone and Benzoxazinone–Thiazolidinediones

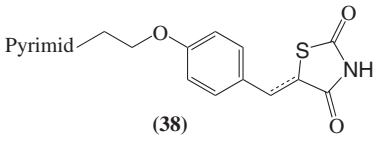


Entry	Het	DB[a]	*n*	X	Melting Point (°C)	Dose (mg/kg)	PG Reduction (%)	TG Reduction[b] (%)
1		No	2	H	170	10	72	68
2		No	2	H	165	10	54	N.E.
3		No	2	H	174	10	7	N.E.
4		No	2	H	198	10	12	N.E.
5		Yes	2	Na	280	10	21	N.E.
6		No	2	Na	255	10	37	60
7		No	1	H	234	10	15	N.E.

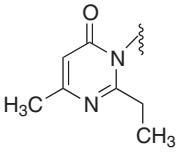
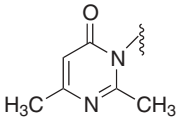
[a] DB, double bond; dashed line, optional double bond.

[b] N.E. not evaluated.

TABLE 19.6 PG- and TG-Lowering Activities in db/db Mice

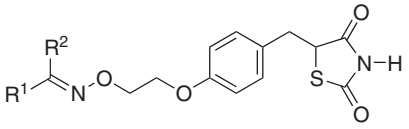


(38)

		DB[a]	Dose (mg/kg)	PG Reduction (%)	TG Reduction (%)	PPARγ (1 μM) Fold Activation
 Pyrimid	**38a**	Yes	30	55	63	
	38b	No	30	73	85	16.6
			10	72	59	
			3	58	54	
	**38c**	No	100	56	76	
Rosiglitazone		—	10	47	40	15.0
			3	43	24	
Pioglitazone		—	10	46	46	3.8
			3	38	36	

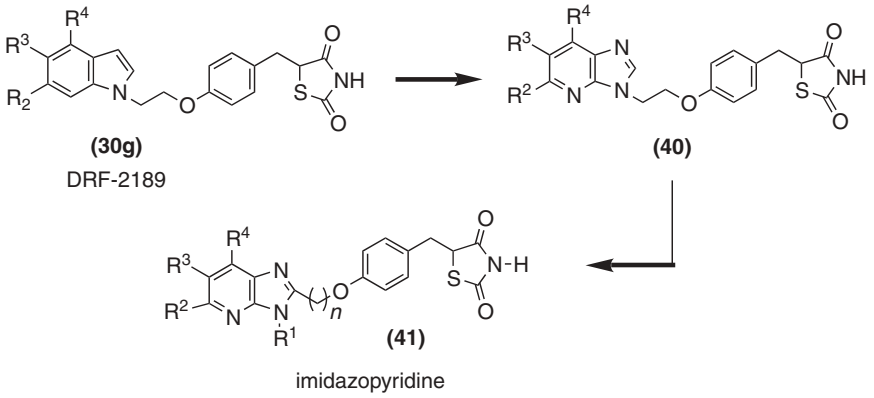
[a] DB, double bond; dashed line, optional double bond.

TABLE 19.7 Antidiabetic Activities of TZDs (39) in KKA^y Mice



(39)

R^1	R^2	Dose (mg/kg)	PG Reduction (%)	PPARγ EC_{50} (μM)
2-Pyridyl	Me	10	67	2.8
4-Ph-Ph	Me	10	67	0.40
4-Ph-Ph	Et	10	67	1.6
Rosiglitazone		10	66	0.73
Pioglitazone		10	42	7.6



Scheme 19.11

TABLE 19.8 Antidiabetic Activities of Imidazopyridine TZDs (41) in KK Mice at a 50-mg/kg Dose

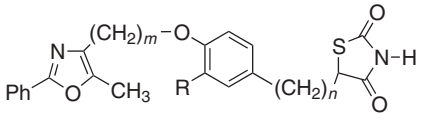
Compound	R^1	R^2	R^3	R^4	*n*	PG Reduction (%)
41a	Me	H	H	H	1	24.7±9.1
41b	Me	Cl	H	H	1	37.1±7.7
41c	Me	OEt	H	H	1	29.7±7.4
41d	Me	OMe	H	H	1	3.6±5.4
41e (HCl salt)	Me	OMe	H	H	1	14.8±10
Rosiglitazone (**5**)						21.7±18.5

Momose et al.[31]synthesized several thiazolidinediones and oxazolidinediones and evaluated their antidiabetic activities in KK mice. Some of the results are shown in Table 19.9. However, none of the TZDs were superior to rosiglitazone in their profile. In general, most thiazolidinediones have been found to be PPARγ agonists. However, KRP-297 has been reported to be a PPARα and PPARγ dual agonist.[32]

Miyachi et al. at Kyorin Pharmaceuticals[33] developed a new type of thiazolidinedione in which a hydrophobic moiety is located at the 3-position of phenyl ring (**43b**, Table 19.10) instead of the 4-position of the phenyl ring. A series of 3-[(2,4-dioxothiazolidin-5-yl)methyl]benzamide derivatives of formula **43** were prepared by changing R^1 and R^2 groups and varying the value of *m* and *n*, a structure–activity relationship was developed in ob/ob mice.

Keeping in mind the acidic nature of glitazone class of compounds, scientists at Pfizer visualized the ring opening of thiazolidinedione, which led to a dihydrocinnamic acid class of compounds (Scheme 19.12).[34] In fact, the parent dihydrocinnamic acid (**45a**) was found to be inactive even at a dose of 5 mg/kg in db/db mice; however, various α sulfur-substituted analogs of dihydrocinamic acid were remarkably active (Table 19.11).

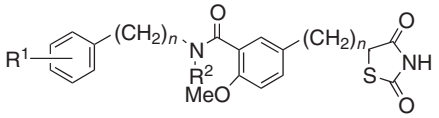
TABLE 19.9 Antidiabetic Activities in KK Mice



(42)

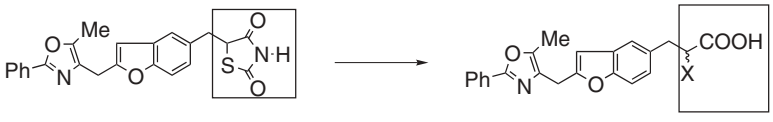
Compound	R	*m*	*n*	PG Reduction (%) at 0.005% in Diet
42a	H	2	2	14
42b	H	2	3	26
42c	H	1	2	24
42d	H	1	3	48
42e	H	1	4	22
42f	H	1	5	19
42g	OMe	1	3	54 (ED_{25} = 3.17 mg/kg)

TABLE 19.10 3-[(2,4-dioxothiazolidin-5-yl)methyl] Benzamide Derivatives (43) and Their Antidiabetic Activities in ob/ob Mice



(43)

Compound	R^1	R^2	*m*	*n*	PG Reduction (%) at the Dose (mg/kg): 10	3	1
43a	H	H	1	1	37	31	19
43b (KRP-29)	4-CF_3	H	1	1	58	53	35
43c	4-CF_3	H	0	1	48	36	27
43d	4-CF_3	H	2	1	43	20	15
43e	4-CF_3	H	3	1	39	29	10



(44) **(45)**

Scheme 19.12

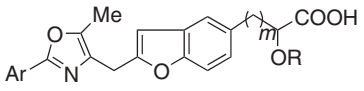
TABLE 19.11 Antidiabetic Activities of α-Alkylthiocarboxylic Acid Derivatives (45) in db/db Mice

		Percent Glucose Normalization in db/db (Mice[a] at the Dose) (mg/kg):				
Compound	X	5	2	0.5	0.25	0.1
45a	H	i.a.	—	—	—	—
45b	SMe	—	89	100	16	26
45c	SEt	86	60	80	3	N.T.
45d	S-Pr	94	69	82	39	N.T.
45e	SBn	100	88	93	72	I.A.
45f	SPh	70	90	100	92	86

[a] Normalization relative to 50 mg/kg dose of ciglitazone per day to db/db mice, which lowers the plasma glucose level to that of lean littermates; I.A., inactive; N.T., not treated.

Compound **45f** was nearly 500 times as potent as ciglitazone. The corresponding alkoxy analogs (**46**) have been also studied and it was found that the ethoxy group is the most optimum, although α-benzyloxy also shows potent antidiabetic activities (Table 19.12). The α-benzyloxy analog (**46d**) was nearly 1000 times as active as ciglitazone and showed significant hypoglycemic effects at a 0.01-mg/kg dose. It has been found that the entire activity of the α-ethoxy analog (**46b**) resides in its *S*-enantiomer (100% at a 0.1-mg/kg dose),

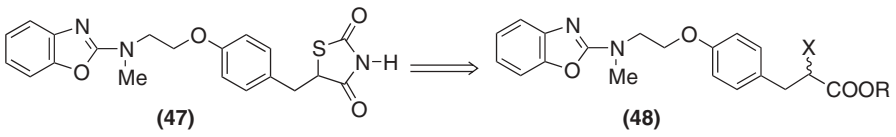
TABLE 19.12 Antidiabetic Activities of α-Alkoxycarboxylic Acid Derivatives (46) in db/db Mice



(46)

				Percent Glucose Normalization in db/db Mice[a] at the Dose (mg/kg):			
Compound	*m*	R	Ar	5	1	0.1	0.01
46a	1	Me	Ph	94	100	83	44
46b	1	Et	Ph	100	N.T.	100	69
46 [(*R*)-b]	1	Et	Ph	N.T.	74	19	16
46 [(*S*)-b]	1	Et	Ph	N.T.	—	100	78
46c	1	N—Pr	Ph	100	100	100	43
46d	1	Bn	Ph	N.T.	N.T.	100	74
46e	1	Et	2-thienyl	N.T.	100	100	98
46f	1	Et	2-furyl	N.T.	100	100	63
46g	0	Et	Ph	9	N.T.	N.T.	N.T.
46h	2	Et	Ph	100	75	N.T.	N.T.

[a] N.T., not tested.



(47)
BRL-48482

(48)

Scheme 19.13

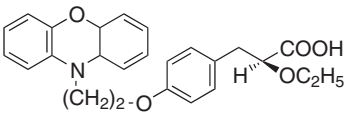
whereas the *R*-enantiomer was much less active. It is clear from Table 19.12 that compound **46g**, which is an acetic acid analog, is virtually inactive, whereas compound **46h** has reduced activity. A similar approach was used by scientists from SmithKline Beecham, in which they modified BRL-48482 (TZD derivative **47**) to give an open-chain dihydrocinnamic acid analog (**48**)[35] (Scheme 19.13).

From Table 19.13 it is seen that the α-ethoxy-β-phenylpropionic acid derivative (**48i**) is the most potent compound (SB 213068) and also shows excellent PPARγ transactivation activity ($K_i = 2.5$ nM). These results also showed a good correlation in the glucose-lowering activities of the compounds in db/db mice with their transactivation of PPARγ.

We have also synthesized a number of alkoxypropionic acid derivatives[36] and examined their glucose-lowering activities in animal models of diabetes. Interestingly, we found that

TABLE 19.13 Antidiabetic Activities of Compound 48 in ob/ob Mice

Compound	X	R	PG Reduction (%) [ED_{25} (mg/kg)]	PPARγ K_i (nM)
48a	F	H	10	—
48b	Cl	H	10	33
48c	SH	H	300	—
48d	SMe	Me	100	600
48e	SPh	H	30	37
48f	NHMe	Me	1000	—
48g	NHPh	H	30	—
48h	OMe	H	3	15
48i (SB 213068)	OEt	H	0.1	2.5
48j	OPr	Na	0.3	—
48k	OPh	H	1	0.4
48l	OBn	Na	0.3	—
48m	H	Me	>2000	—
48n	COOMe	Me	10	—
48o	CN	H	10	—
48p	Et	H	30	—
48q	$(CH_2)_3Ph$	H	3	—
BRL-48482	—	—	3	22
Troglitazone	—	—	400	3800



(49)

(–)DRF-2725 (ragaglitazar)

Scheme 19.14

most α-alkoxypropionic acid derivatives were activators of both PPARα and PPARγ and thereby showed both glucose- and triglyceride-lowering activities.[37] From a large pool of compounds, DRF-2725 (**49**) was selected as a development candidate (Scheme 19.14).[37]

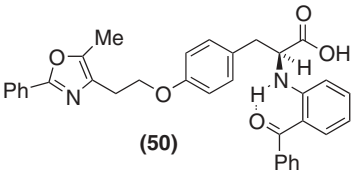
The *S*-isomer of DRF-2725 was found to be more potent for both PPARα (10.87 ± 1.2-fold, at 50 μM) and PPARγ (16.67 ± 1.2-fold at 1 μM) than either racemic or (*R*)-isomer. This compound was found to be superior to rosiglitazone in both glucose- and triglyceride-lowering activities and therefore taken for further clinical development. An arginine salt of DRF 2725 (NN-61-0029) was further developed as ragaglitazar as a clinical candidate. Pharmacokinetic parameters were evaluated in healthy as well as in type 2 diabetic subjects. Ragaglitazar was absorbed rapidly and eliminated slowly with linear pharmacokinetics (T_{max}, 1.4 to 2.5h; (*t*½, 68 to 97 h; dose, 1 to 120 mg/day in male subjects).[38] A study was conducted for 12 weeks in 177 hypertriglyceredemic (>150 mg/dL) subjects with type 2 diabetes (age 18 to 70 years) randomly assigned to receive ragaglitazar (0.1, 1, 4, or 10 mg/day), placebo, or pioglitazone (45 mg/day). The mean baseline (BL) values of TG (244 to 307 mg/dL) and fasted plasma glucose (FPG, 170 to 216 mg/dL) were similar in all groups. Ragaglitazar treatment improved the fasting lipid profile (decrease in TG, LDLC, TC, and increase in HDLC) and glycemic control (decrease in FPG). Results are summarized in Table 19.14. Ragaglitazar also showed some cardiac edema on long-time treatment. In two-year toxicity studies, ragaglitazar showed the development of tumor in mice and rats and has been discontinued from phase III clinical trials. It remains to be seen if the tumor formation after long-term treatment is rodent specific.

TABLE 19.14 Comparison of Ragaglitazar and Pioglitazone in Healthy Human Subjects

Human Male Subjects:	26	30	32	31	28
	Ragaglitazar Dose				Pioglitazone Dose
	0.1 mg	1 mg	4 mg	10 mg	45 mg
TG %ΔBL[a]↓	−18	−45	−67	−56	−45
HDL %ΔBL&↓	3	17	28	8	12
LDL %ΔBL↓	10	−6	−14	−19	11
TC %ΔBL↓	3	−5	−17	−16	0
FPG %ΔBL↓	−32	−71	−97	−100	−66
Weight gain (kg)	1	4	7	8	3

Source: Ref. 39.

[a] ΔBL, change from baseline.



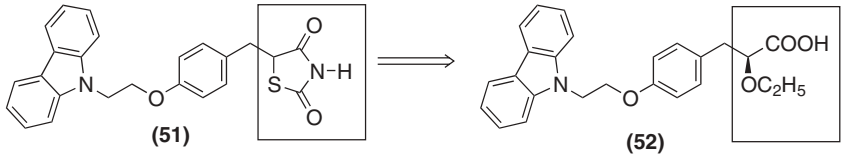
(50)

Scheme 19.15

Scientists from GlaxoSmithKline (GSK) have examined several α-amino- and α-alkoxyphenyl propionic acid derivatives. They have also reported that α-alkoxy phenyl propionic acids are dual PPARα and PPARγ agonists,[40] whereas α-amino derivatives are a more selective PPARα agonist.[41] Several oxadiazole-substituted α-isopropoxyphenylpropionic acids were evaluated.[40] GSK scientists also made several L-tyrosine-based analogs.[42] The compound selected (farglitazar, **50**) was discontinued after phase III clinical trials, due to safety concerns (Scheme 19.15).

Sauerberg et al.[43] prepared α-alkoxyphenylpropionic acid analogs of several TZDs reported by us[23] to see if they show improved glycemic and dyslipidemic control over their TZD counterpart (Scheme 19.16). Among all the heterocycles used in the α-alkoxyphenylpropionic acid, carbazole analog (**52**) was found to be the most promising. Sauerberg et al. also measured the transactivation of several analogs of α-alkoxypropionic acid and compared them with rosiglitazone and WY14643 for PPARγ and PPARα, respectively, which were taken as the standards. The lead compound showed a 58% reduction in PG and 52% in TG in db/db mice and was superior to rosiglitazone.

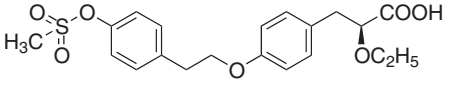
Cronet et al.[44] reported a novel PPARα and PPARγ dual agonist and cocrystallized **53** (AZ-242) (Scheme 19.17) with PPARγ and determined the x-ray structure. AZ-242 (**45**) activates PPARα and PPARγ, both in vitro and in vivo. In cell-based assay, compound **53** (AZ-242) activated PPARα (EC_{50}, 2.8 μM) and PPARγ (EC_{50}, 0.30 μM), respectively.



(51) (Lohray et al. [23])

(52) (Sauerberger et al. [43])

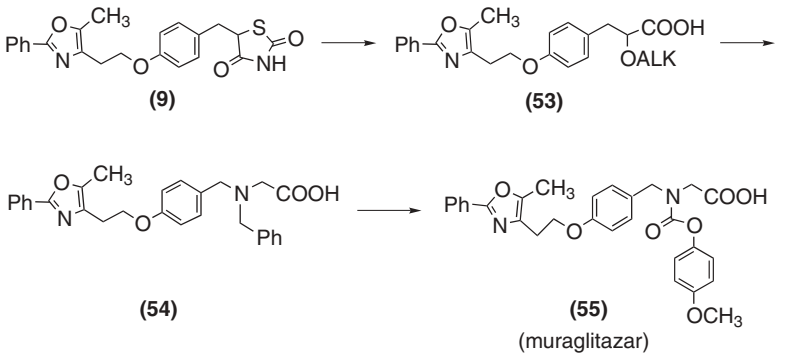
Scheme 19.16



(53)

AZ-242 (tesaglitazar)

Scheme 19.17

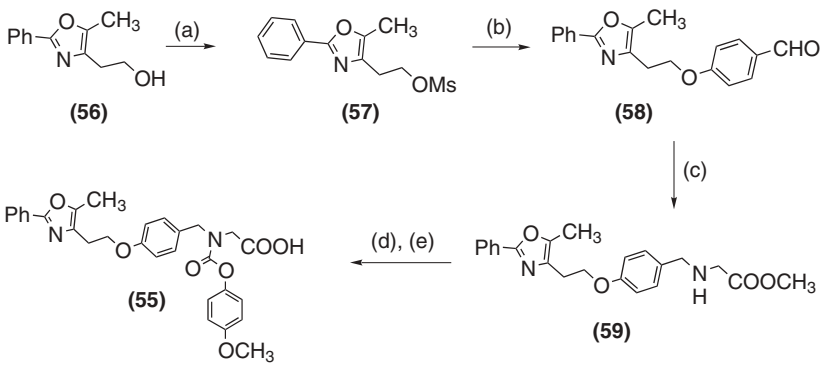


Scheme 19.18

AZ-242 was evaluated in obese Zucker rats[45] at 3 mg/kg per day for 4 days and was found to improve glucose, insulin, and triglyceride very effectively. PG, insulin, and TG were measured in a group of ob/ob mice ($n = 14$ per group) that received AZ-242 (0.01 to 100 mg/kg per day) for 8 days. AZ-242 in a dose-dependent manner reduced elevated PG, insulin, and TG in ob/ob mice to or below the level of lean littermates without increasing the body weight.[45b] The effect of AZ-242 was found to be seven fold higher than rosiglitazone[45a] and 250-fold higher than pioglitazone in lowering PG, TG, and insulin in an ob/ob mouse model. Similar results were observed in Zucker fa/fa rats. AZ-242 (**53**, tesaglitazar), which was in phase III evaluation for a long time, was discontinued recently.

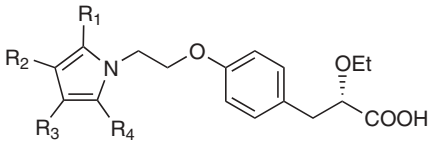
More recently, scientists from Bristol-Myers Squibb have prepared oxybenzylglycines from known α-alkoxy- and α-aminoarylpropionic acids (Scheme 19.18).[46] Members of this class of compounds possess no chiral center. The optimized candidate (**55**) has been shown to possess PPARα and PPARγ dual agonist activity (PPARα, EC_{50}-0.32 ± 0.1 μM; PPARγ, EC_{50}-0.11 ± 0.06 μM). In male db/db mice, **55** reduced the glucose level 54%, triglyceride 33%, nonesterified fatty acids 62%, and insulin 48%. Compound **55**, which has an excellent ADME (absorption, distribution, metabolism, excretion) profile with oral bioavailability of 88% in rats, 18% in beagle dogs, and 79% in cynomologus monkeys, has completed phase III clinical trial and was discontinued due to lack of superiority over pioglitazone. This compound has been reported to cause species-specific tumor at a dose 40 times higher than the human therapeutic dose. The synthesis of **55** is outlined in Scheme 19.19.

With a view to introducing cholesterol-lowering activity in PPARα and PPARγ activator templates, we selected the pyrrole-containing compound **60–61** (a heterocycle moiety present in atorvastatin) as a starting point.[47] Compounds **60a** and **60b** have bulky substituents which are not favorable for binding to the PPARα and PPARγ receptors (unpublished results). Therefore, we carried out a gradual structural modification to generate a structure–activity relationship. This led us to arrive at suitable substituents around the pyrrole ring. The results, which are compiled in Table 19.15, show that tetra- and trisubstituted pyrroles (**60a**, **60b**, and **60d**) do not reduce serum TGs in Swiss albino mice. Thus, we synthesized 1,4-disubstituted pyrrole derivates. Although compounds **60c** and **60f** did not show serum TG reduction, the TG reduction shown by **60e** (26% at a 10-mg/kg dose) and **60g** (31% at a 10-mg/kg dose) were encouraging. At present, we are not able to understand the rationale for certain 1,4-disubstituted compounds (**60d** and **60f**) to increase serum TG and TC levels, whereas certain other 1,4-substituted compounds reduce the TG



Scheme 19.19 (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 25°C, 1 h, >98%; (b) 4-hydroxybenzaldehyde, K_2CO_3, CH_3CN, 95°C, 24 h, 71%; (c) H_2NCH_2–CO_2Me–HCl, Et_3N, 4 Å molecular sieves, anhydrous $MgSO_4$, MeOH, 25°C, 12 h; $NaBH_4$ 25°C, 3.5 h, 80%; (d) 4-methoxyphenyl chloroformate, Et_3N, CH_2Cl_2, 25°C, 2 h, 98%; (e) LiOH–H_2O, THF/H_2O (1:1), 25°C, 2.5 h, 94%.

TABLE 19.15 TG- and TC-Lowering Activity in Male Swiss Albino Mice After 6 Days of Treatment



(60–61)

Compound	Substitution				Dose (mg/kg)	TG Change (%)	TC Change (%)
	R_1	R_2	R_3	R_4			
60a	*i*-Pr	PhNHCO	C_6H_5	4F-C_6H_4-	50	−15	−3
60b	*i*-Pr	H	C_6H_5	4F-C_6H_4-	50	−3	−8
60c	*i*-Pr	H	H	4F-C_6H_4-	10	—	+69
60d	*i*-Pr	PhNHCO	H	4F-C_6H_4-	10	+10	+9
60e	C_6H_5	H	H	4F-C_6H_4-	10	−26	−19
60f	*i*-Pr	H	H	C_6H_5	3	+30	+36
60g	*i*-Pr	H	H	4-$MeOC_6H_4$	10	−31	+42
60h	*i*-Pr	H	H	CH_3	10	−53	−4
					1	−6	+9
60i	*i*-Pr	H	H	C_2H_5	3	−41	+24
						−50	−2
60j	H	H	H	H	10	−16	+27
60k	CH_3	H	H	CH_3	50	−55	−18
					3	−39	+33
					1	−27	+10
60l	CH_3	H	H	C_6H_5	10	−65	−31

and TC levels found in Swiss albino mice. We also studied the activity of 1,4-dialkylated pyrrole derivatives **60h** and **60i,** which showed a 53% reduction in TG at a dose of 10 mg/kg and 41% reduction in TG at 3 mg/kg, dose, respectively. The unsubstituted pyrrole derivative (**60j**) showed very poor activity. When the isopropyl group in **60f** is replaced with a methyl group, the resulting compound (**60e**) reduced serum TG up to 27% even at 1 mg/kg, in contrast to compound **60h** (6% TG reduction at 1 mg/kg). We further studied compounds with a methyl group intact at R_1 and varying the substituents on the phenyl ring at R_4 in **60**–**61**. The results are compiled in Table 19.16. These 2,5-disubstituted pyrrol derivatives showed very good hypotriglyceredemic activity (except **61i**, **61j**, **61l**, **61o**, and **61q**). Dose–response studies in Swiss albino mice of the selected compounds gave very good ED_{50} values: 1.2 mg/kg for **60l**, 1.4 mg/kg for **61f**, 0.24 mg/kg for **61h**, and 0.97 mg/kg for **61k** (Table 19.17). The compounds were tested further in db/db mice at a dose of 1 mg/kg for 6 days. The excellent plasma glucose and triglyceride reductions of **61f**, **61h**, and **61k** are listed in Table 19.18. These compounds are undergoing preclinical evaluation.

TABLE 19.16 TG and TC-Lowering Activity in Male Swiss Albino Mice After 6 Days of Treatment

	Substitution				Dose	TG	TC
Compound	R_1	R_2	R_3	R_4	(mg/kg)	Change(%)	Change(%)
61a	CH_3	H	H	4F-C_6H_4-	1	−53	+18
61b	CH_3	H	H	4CH_3-C_6H_4	3	−30	+43
61c	CH_3	H	H	3CH_3-C_6H_4	1	−56	+25
61d	CH_3	H	H	2CH_3-C_6H_4	1	−56	+9
61e	CH_3	H	H	3-MeOC_6H_4	3	−58	−17
61f	CH_3	H	H	4-MeOC_6H_4	10	−54	−38
61g	CH_3	H	H	4-HO-C_6H_4	3	−33	+9
61h	CH_3	H	H	4-BnOC_6H_4	3	−52	−32
61i	CH_3	H	H	3-BnOC_6H_4	10	−13	+11
61j	CH_3	H	H	3,4-di$OMeC_6H_3$	3	−13	+14
61k	CH_3	H	H	3,4-dioxymeth-enephenyl	3	−63	−26
61l	CH_3	H	H	Cy CH_2-O-C_6H_4	3	−4	+22
61m	CH_3	H	H	CH_3-SO_3-C_6H_4	3	−34	+28
61n	CH_3	H	H	2-pyridyl	3	−60	+5
61o	CH_3	H	H	3-pyridyl	3	+5	+4
61p	CH_3	H	H	4-pyridyl	3	−29	+5
61q	CH_3	H	H	1-napththyl	10	−5	+10
61r	CH_3	H	H	2-naphthyl	3	−38	+34

TABLE 19.17 Hypotriglyceredemic and Hypocholesterolemic Effects of Selected Compounds in Swiss Albino Mice After 6 Days of Treatment

Compound	Dose (mg/kg)	Triglyceride Reduction (%)	Cholesterol Reduction (%)	Triglyceride Reduction
60l	0.1	25.2±3.9	−13.7±5.7	ED_{50} = 1.2 mg/kg
	1.0	46.8±3.0	−9.8±6.2	
	10.0	64.6±2.4	20.7±4.7	
61f	0.1	18.0±7.4	−8.9±4.6	ED_{50} = 1.4 mg/kg
	1.0	38.7±5.3	2.1±7.9	
	10.0	64.5±1.5	46.9±3.2	
61h	0.1	41.3±6.8	−11.7±3.8	ED_{50} = 0.24 mg/kg
	1.0	67.2±4.0	18.9±2.0	
	10.0	74.7±1.3	38.7±4.7	
61k	0.1	27.8±6.2	−26.5±7.7	ED_{50} = 0.97 mg/kg
	1.0	50.7±1.6	−0.7±9.1	
	10.0	63.5±1.2	45.6±2.9	

TABLE 19.18 Antihyperglycemic and Hypotriglyceredemic Effects of Compounds in db/db Mice After 6 Days of Treatment

Compound	Dose (mg/kg)	Glucose Reduction (%)	Triglyceride Reduction (%)
61f	1	61.9±1.7	48.9±6.3
61h	1	58.2±1.5	50.5±5.7
61k	1	65±2	54.9±3.2

19.5 CONCLUSIONS

PPAR agonists have shown great promise as new therapeutics for the treatment of diabetes, dyslipidemia, and related metabolic disorders. Safety has been an issue and a concern, especially due to lack of proper understanding of the mechanism of toxicity and side effects. The compounds are being discontinued from clinical development due to nonspecific tumors seen in a two-year carcinogenicity study in rodents. Pioglitazone, which has been on the market since 2000, had also shown tumors in rodents. However, as the understanding of concern becomes clear, insulin sensitizers would become important therapeutic tools for the treatment of diabetes.

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20

CRITERIA FOR INDUSTRIAL READINESS OF CHIRAL CATALYSIS TECHNOLOGY FOR THE SYNTHESIS OF PHARMACEUTICALS

RAYMOND MCCAGUE AND IAN C. LENNON

Dowpharma, Chirotech Technology Ltd.
Cambridge, UK

20.1 INTRODUCTION

Technological development is a chicken-and-egg situation. Which should come first? To sit and wait until a commercial opportunity comes along and then design the technology to act on it, or to develop the technology first in the hope that one day a commercial opportunity arises that will need it?

In developing new technology to service the pharmaceutical pipeline, the answer is somewhere in between, in that elements of both approaches are needed. A knowledge of emergent needs in the sector needs to be researched and understood as a way to guide which technologies should receive investment. Then, if judgment is good, the appropriate technologies can be developed to just the extent where they will be available to use when opportunities come along. The need is to develop technology to the state of *industrial readiness*.

In this chapter we specifically address chiral catalytic technologies. Given that many synthetic pharmaceutical candidates are chiral, they, will need to be produced in single chiral isomer (enantiomer) form and some specialized chiral technology will be needed for the synthesis. Within chiral technology the focus here is on catalytic technologies, although many of the principles we present below regarding industrial readiness are equally applicable to other synthetic technologies. Catalytic technologies are of particular interest for their superior economics applied to the manufacture of intermediates to pharmaceutical agents, and in this regard, methods of biocatalysis and chemocatalysis are particularly well

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suited. Some of these methods find sufficiently frequent application that significant up-front technology investment is warranted, and there are enough projects on which to apply the technologies to build up a still higher level of expertise.

20.2 CRITERIA FOR TECHNOLOGY READINESS

The following six criteria have been identified for determining if a technology is ready for industrial applications. All six criteria should be met. Then, if a commercial opportunity arises from a customer, it can be acted upon immediately. To meet the needs of the pharmaceutical pipeline, the technology may need to be ready to produce hundreds of kilograms of an intermediate within a few months of learning of the opportunity. If any one of the six criteria is not met, there could be an impasse regarding application of the technology. If the technology is not ready, the window of opportunity to act may pass.

1. *A sufficiently well-established and reproducible catalyst preparation.* A need to produce a particular amount of a pharmaceutical intermediate will translate into the need for a particular amount of catalyst. If the catalyst has not been made before in the necessary quantity and cannot be made quickly or reproducibly, the opportunity to use the catalysis may well be lost. At the other end of the scale, if a technology has already been worked at a large scale, it is likely that appreciable amounts of catalyst or intermediates will already be at hand, and that will create a readiness to take on other opportunities quickly. Although this chapter focuses on chiral catalytic technologies, the essence of the criterion can be applied more widely. If there a stoichiometric reagent is used (either as well as, or instead of, a catalyst), its availability or preparation could become the key factor in determining whether the technology is ready for industrial use.

2. *Acceptable catalyst utilization.* A prominent factor as to whether a catalyst technology will be effective, and in particular whether it will be economical, is the molar substrate/catalyst ratio (S/C), so important to process economics. Chiral catalysts are often expensive, and in the case of chemical catalysts based on precious metals, a minimum S/C of 1000 : 1 will usually be needed to meet economic demands. Moreover, the catalyst utilization links with the first criterion above. If catalyst utilization is poor, more catalyst has to be sourced, and the possibility of making that amount of catalyst quickly enough may be called into question even if the process economics are not at issue. On the other hand, if a very high substrate/catalyst ratio can be achieved (say, 100,000 : 1 of a chemical catalyst), the catalyst contribution will fall out of the overall process cost equation and the likelihood will be that enough of the catalyst or its precursors will have already been made. It is the same in biocatalysis, where enzymes can become costly to produce if their activity is poor. Indeed, in biocatalysis a useful ratio although not easily revealed from most literature) is the number of liters of fermentation broth needed to produce the amount of enzyme necessary to transform a kilogram of substrate in the biocatalysis. A ratio of 1 L of fermentation broth per kilogram of substrate is usually a very satisfactory result, and if 0.1 L of fermentation broth supplies enough enzyme to convert 1 kg of substrate, the economics are likely to be excellent.

3. *Process conditions that can be operated on scale.* To use catalysis technology for the manufacture of pharmaceutical agents, as with any chemistry, the process conditions need to be workable industrially. This means that the process needs to be operable in an

available plant; materials such as substrate, reagents, and solvents need to be obtainable economically; the conditions should not be too extreme (e.g., very high or low temperatures, very high pressures); the reaction should be complete within a reasonable time and be safe to operate; and the workup for recovery of the product should be straightforward. Various process conditions are highlighted in the examples below, although it should be observed that a key factor for economy in manufacture is the volumetric productivity: that is, the volume of reaction mixture (or total volume of all materials, including the workup) needed per kilogram of product output. Features of the catalytic reactions described in the examples below are that high volumetric productivity can be achieved (e.g., 100 g of substrate per liter of solution) and the reagents are simple. Thus, in bioresolution of an ester, the reagent is effectively water, and in asymmetric hydrogenation it is hydrogen gas.

4. *A Technology that delivers sufficient selectivity.* In identifying a suitable chiral catalysis method (asymmetric synthesis or catalytic kinetic resolution), the enantiomeric excess of the product will almost always be the first result noted and is clearly important for the catalysis to be feasible. However, it is only one of many criteria for success, and it is often the case that the stereoisomeric purity of the product can be raised relatively easily and in high yield (e.g., by recrystallization). Indeed, in designing a process, it could be worthwhile to identify how easy it is to raise the stereopurity of the product before choosing the catalyst for development. If stereopurification is difficult, it will be known that a very highly selective catalyst will be necessary, or it may warrant redesign of the substrate structure (e.g., a different ester used) so that the product is easier to purify. Conversely, if the product is easy to raise to stereopurity, the demand for selectivity in the catalysis can be relaxed so that the process chemist can choose a method that is more economical overall. In the case of bioresolution, where for example a racemic ester is hydrolyzed by an enzyme and one isomer is hydrolyzed in the presence of the other (a kinetic resolution), the enantiomeric excesses of both product and starting material will depend not only on the enantiospecificity of the catalyst but also on the extent of conversion. Generally, the enantiomeric excess of the unconverted substrate increases with conversion and that of the product decreases.[1] In particular, for a reasonably enantiospecific biocatalyst, the enantiomeric excess of the substrate can be raised to a very high value by taking the transformation to beyond 50% conversion. With this knowledge, the need for biocatalyst enantiospecificity can be balanced against such factors as the enzyme cost, value of substrate, and operating concentration.

5. *A technology that has a sufficient breadth of application.* If a commercial opportunity is sufficiently large, an entirely new technology discovery and development program could be pursued to meet that one need. However, the nature of the pharmaceutical pipeline is that such an opportunity will rarely arise. Once the application is evidently large enough, development of the pharmaceutical agent, including its process of manufacture, may already be so far advanced that options will have narrowed and it will be too late. Consequently, it is necessary to develop a technology in anticipation of future requirements coming along. Then, to make the technology development program worthwhile, there is a need for a sufficient likelihood that the technology, once ready, will actually be used and not just left on the shelf. For this to happen, the technology must have a sufficient breadth of application. As will be evident, the specific technologies used with the examples we give below meet this need. However, the breadth of application must take into account whether competing technologies exist. It is not sufficient that a new technology can address a high percentage of target structures if simpler and more readily accessed methods are available for the

same materials. Somehow, investment to develop a new technology eventually has to be recouped in added commercial profits, and this will not happen if the technology is no more than equal to preexisting alternatives. Therefore, for a technology to have sufficient breadth of application, it has to address a good proportion of the likely structural types in pharmaceutical agents and to have features that will give it a process advantage (e.g., if it is a catalytic technology replacing a stoichiometric technology). It is of crucial importance to consider carefully, before embarking on it, whether a contemplated technology investment in catalysis is ever likely to have sufficient commercial applicability. It is not unusual for a conceptually very elegant methodology to come to one's attention that is so restricted to particular structural types that the chance of ever actually using it, if developed commercially, fades to almost zero.

6. *Freedom to operate over intellectual property.* Last, but important if a technology is to become industrially ready, there must be freedom to operate over any intellectual property in the area. In this regard it is important to realise that having a patent on a technology does not create the right to use it commercially; it only gives the right to prevent others from using it. In a competitive market a new technology will often need to be designed to work carefully around a picket fence of intellectual property created by competitors. In particular, even if a supplier is able to patent its own technology advances, their use may be restricted by dominating patents of others. In industry, a common way to access the necessary rights to operate a technology is by in-licensing from the party that discovered it and holds the patent rights. This often has the added advantage that the technology may be partially developed at the time that it is licensed. Nonetheless, usually when a technology is licensed (e.g., from a university to an industrial organization) it will still be a laboratory method that is far away from being ready for application to an industrial process. Then a degree of investment will be needed to bring it to the status of being industrially ready.

20.3 EXAMPLES OF INDUSTRIALLY READY CHIRAL CATALYTIC TECHNOLOGIES AND THEIR APPLICATION

The discussion in Section 20.2 considered the various criteria needed for a technology to be considered industrially ready. To visualize the importance of these needs, it is appropriate to reflect on a number of catalytic processes that have been used industrially. In this regard, a range of examples is presented below of processes employing chiral catalysis, developed in our laboratories and process plant. Both biocatalysis and chemocatalysis processes are covered, and indeed, the criteria for industrial readiness apply equally well to both.

20.3.1 Lipase Bioresolution: Ethyl 3-Amino-3-Phenylpropionate

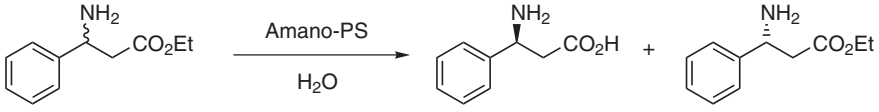
Biocatalysis is very well suited to meet the criteria needed for industrially ready technology since, addressing each of the aforementioned criteria:

1. Biocatalysts, when derived from microbes, can be obtained in any quantity desired by fermentation of the microorganisms that produce them.

2. Rates of enzyme turnovers can be extremely high in favorable cases, so that only a very small amount of biocatalyst can supply a large amount of biocatalysis product.
3. The process conditions are very favorable for industry, often using water as the solvent, at or near ambient temperature.
4. Selectivities between enantiomers can be high, with enzymes often only converting the isomer that is structurally closest to that of its natural substrate.
5. A given enzyme will often be effective for a wide class of substrates having a particular functionality distribution, and usually an array of enzymes can be made available to screen to optimize the selectivity.
6. Several commercially available enzymes exist where the manufacturer does not need to be concerned with the intellectual property position on the catalyst.

An example where a commercially available enzyme may be used to good effect is in the resolution of ethyl 3-amino-3-phenylpropionate (Scheme 20.1), a method that we published originally[2]; subsequently, we developed a process to a manufacturing scale along similar lines. In the published work, Amano-PS lipase was shown to be valuable for this resolution, hydrolyzing one enantiomer of the racemic substrate with high enantiospecificity. A consequence of carrying out such a resolution is that half of the material (the wrong enantiomer) has to be discarded, and the maximum attainable yield is 50%. However, in this case the racemic substrate is obtained very cheaply from benzaldehyde by simple condensation chemistry, and the bioresolution will take place without any need to protect the amino function. The result is a resolution process that gives better economics than a more circuitous route that fits an asymmetric synthesis method. Thus, for asymmetric hydrogenation[3] there needs to be additional chemistry to generate a suitable substrate, and the product tends to be an *N*-acyl derivative, the deprotection of which can be problematic. With regard to the process conditions in the biocatalysis as reported, they are very simple. An enzyme is just added to a stirred buffered aqueous suspension of the ester at a concentration of 200 g/L and the mixture maintained at a given pH. At the end, the acid precipitates and the ester remains in solution and can be extracted out with organic solvent. Nonetheless, in biocatalysis it is usually necessary to optimize the reaction parameters sufficiently. In this case it was found that the Amano-PS enzyme gave only 73% e.e. amino acid product at pH 7 compared with the 99% e.e. observed at pH 8.[2]

It is evident that such lipase biocatalysis is very ready for industrial use, and indeed, the example above is just one of numerous examples carried out by us as well as by others. With regard to the production of fine chemicals more generally by biotransformations, an analysis has been reported of 134 such industrial processes.[4] This reveals that on average there is a volumetric productivity of 15.5 g/L per hour and a final average product concentration of 108 g/L, figures very suitable for high economy.



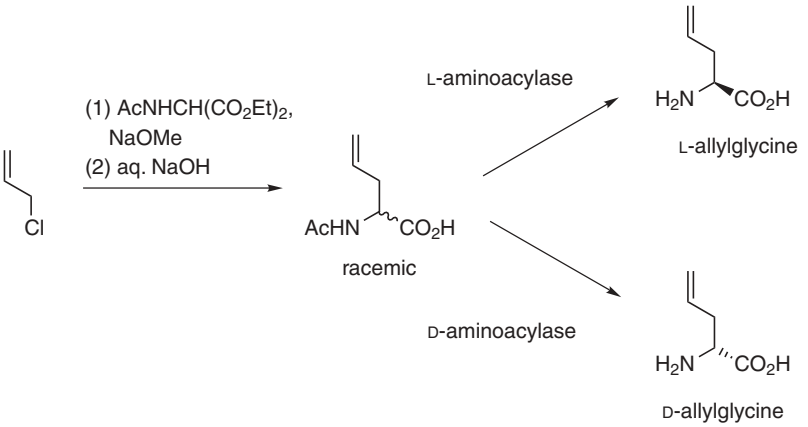
Scheme 20.1 Enzymatic resolution of ethyl 3-amino-3-phenylpropionate.

20.3.2 Aminoacylase Bioresolution of *N*-Acylamino Acids

For the pharmaceutical industry, unnatural amino acids are important components of new drug structures. Sometimes the need is for the natural L-configuration, to mimic closely a natural biological component that is being used as a lead. Alternatively, the need might be for the unnatural D-configuration, where there are benefits such as reduced metabolic cleavage of a derived peptide. This means that any technology directed toward unnatural amino acids should preferably be applicable to either configuration. An example where biocatalysis has been used for the synthesis of unnatural amino acids is shown in Scheme 20.2 for access to either L- or D-allylglycine, which can then be used as an intermediate for more advanced amino acid structures by judicious reactions onto the olefin function.

For instance, allylglycine has been used as a precursor for 4-hydroxypipecolates.[5] The synthesis starts with inexpensive raw materials. A condensation between diethyl acetamidomalonate and allyl chloride in the presence of alkoxide base, followed by hydrolysis and monodecarboxylation of the malonate unit, can be conducted in a one-pot procedure and delivers the racemic acetylamino acid ready for the bioresolution. Although one cannot realistically obtain mirror-image forms of a biocatalyst (unlike with asymmetric chemical catalysis), nonetheless, acylases are available that act specifically on either l- or d-substrate, each with a very high degree of enantioselectivity. Using straightforward process conditions, a volumetric productivity in the range 50 to 100 g/L of substrate is typical.

Although it is a resolution approach, there is the option to improve raw material utilization by recycling the unwanted isomer back to racemate that can be treated with further biocatalyst. Racemization of *N*-acylamino acids can be conducted by heating in the presence of acetic anhydride, where there is a reversible formation of an azlactone intermediate that racemizes spontaneously. Regarding the breadth of the approach, it generally works well whenever the acetamidomalonate can be alkylated efficiently. It is particularly well suited for generating amino acids with structurally simple alkyl chains. The chain can then be elaborated subsequently. A further example is to attach a propargylic residue, as in the formal synthesis of the natural product bulgecinine developed by us.[6] It is also a valuable approach for producing phenylalanine analogs in cases where an asymmetric hydrogenation method is problematic (cf. Section 20.3.3). Another benefit is that the amino acid is produced in the mixture in its zwitterionic form, and in favorable



Scheme 20.2 Access to L- and D-allylglycine by bioresolution.

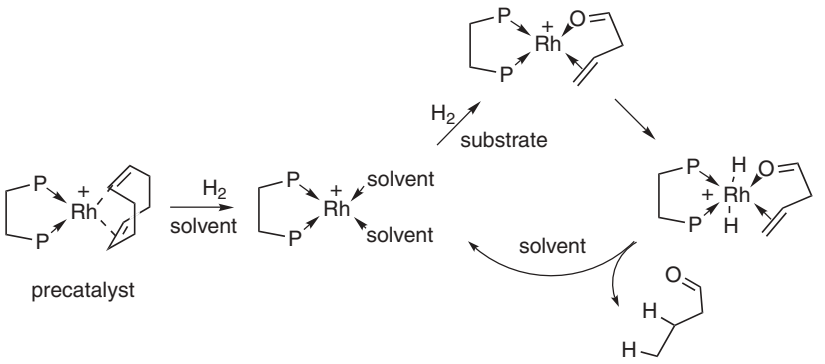
cases it may precipitate directly from the bioresolution mixture to be harvested by simple filtration.

Although, especially for the L-isomer, it is possible to use commercial aminoacylases for the purpose, we felt that for the technology to be industrially ready, we had to be assured of the catalyst supply. To achieve this, we wanted to produce the catalyst ourselves and embarked on a program of biocatalyst discovery and development.[7] Through this we identified and characterized our own cloned proprietary L- and D-aminoacylase enzymes,[8] which we can now use to manufacture products in ton quantities if needed. Collaboration with Littlechild at the University of Exeter characterized a particularly useful theromostable and therefore robust L-aminoacylase from *Thermococcus litoralis* that can optionally be immobilized to generate a reusable industrial biocatalyst.[9]

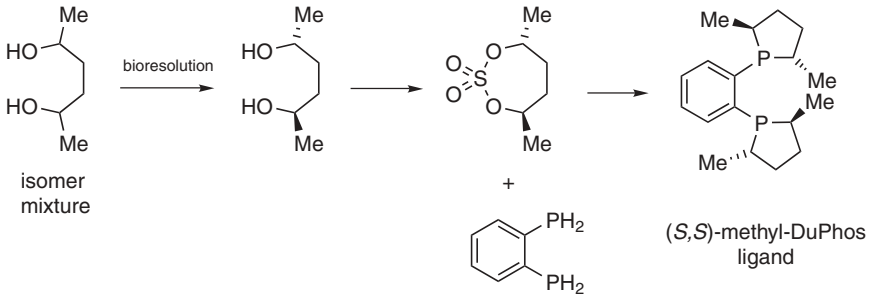
20.3.3 Asymmetric Hydrogenation of Prochiral Olefins by Rhodium–DuPhos Catalysts

Asymmetric hydrogenation can be an ideal technology for industrial use because at the end of the reaction there is simply a solution of the desired product containing a trace of catalyst, the only reagent used being hydrogen gas. It was some 30 years ago that catalytic asymmetric hydrogenation was initiated industrially by Monsanto through the work of Knowles for the manufacture of L-dopa from a dehydroamino acid precursor by a rhodium catalyst with the chiral bisphosphine ligand DIPAMP.[10] Knowles was awarded a share of the 2001 Nobel Prize for Chemistry for this landmark contribution.[11] However, the rhodium–DIPAMP catalyst delivers high enantioselectivity for only a narrow range of prochiral olefin substrates. Subsequently, rhodium catalysts containing chiral bisphospholane ligands (e.g., DuPhos) were discovered by Burk to give high enantioselectivities in the hydrogenation of a wide range of prochiral olefin substrate types.[12] In particular, the catalysis was very effective when the substrate contained, in addition to the olefin to be hydrogenated, a coordinating carbonyl function that orients the catalyst with respect to the substrate. Scheme 20.3 indicates the mechanism of the catalysis, starting from a precatalyst (e.g., cyclooctadiene complex) form that is convenient to use.[13]

In 1995, Chirotech was successful in licensing the DuPhos technology from DuPont for commercial pharmaceutical applications, satisfying the industrially ready criterion of the intellectual property position, but at the time only gram quantities of a few catalysts had been made and applications were limited. It was our challenge to move this methodology



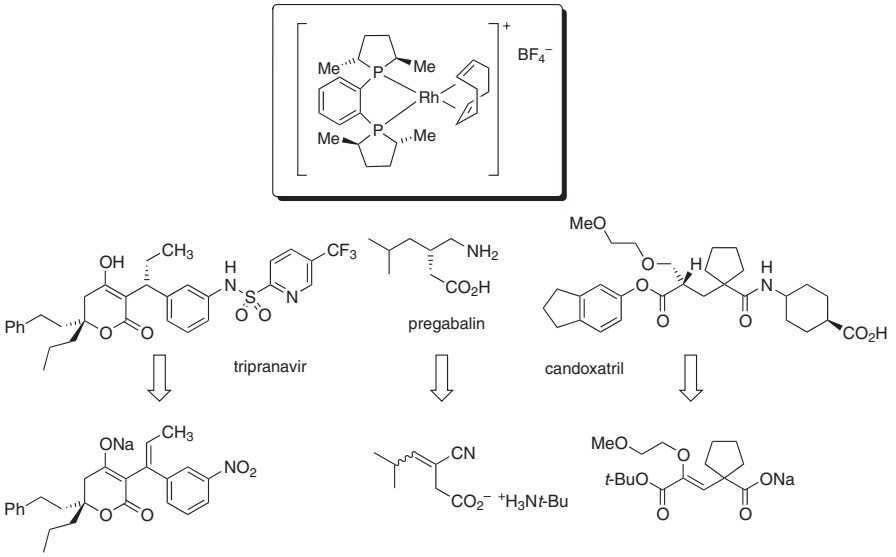
Scheme 20.3 Mechanism for olefin hydrogenation with rhodium–DuPhos catalysts.



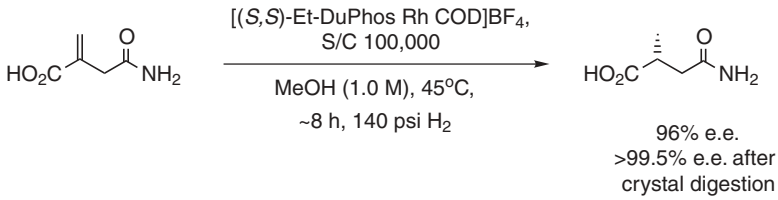
Scheme 20.4 Route to (*S*,*S*)-methyl-DuPhos ligand.

from a promising laboratory tool to a technology that was industrially ready for commercial manufacture. Therefore, a crucial development for industrial readiness was to progress from having gram amounts of ligand and catalyst available to having kilograms sufficient for manufacture of multiple hundreds of kilograms of asymmetric hydrogenation product. This is the typical scale of need for intermediates by pharmaceutical companies as they seek clinical trial amounts of their products. The route to the bisphospholane ligands requires a chiral 1,4-diol (Scheme 20.4). Originally, a Kolbe electrolysis of a single isomer 3-hydroxycarboxylic acid was used to access the diol, but this approach proved problematic to scale and we switched to bioresolution of the easily sourced mixed isomers of the 1,4-diols.[7] So this is an interesting case where the chirality that is eventually established in the asymmetric hydrogenation product was derived originally by the enantiospecificity of a natural biocatalyst. Although it was a resolution approach and much material was discarded, it matters little because only kilogram amounts are needed to produce enough catalyst so that a ton or so of eventual product can be manufactured. So with the improved synthesis, one of the industrially ready criteria was satisfied, at least for a core range of catalysts.

With sufficient catalyst, we readily demonstrated application of the catalysis to the manufacture of a range of pharmaceutical products; examples are shown in Scheme 20.5.[14]



Scheme 20.5 Industrial applications of rhodium–DuPhos catalysts.



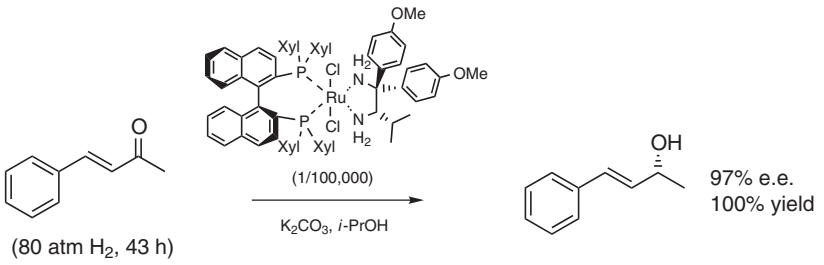
Scheme 20.6 Application of a rhodium–DuPhos catalyst for 3-methylsuccinamic acid.

One of the benefits of using the rhodium–DuPhos catalysts are that the process conditions required are readily applied on an industrial scale. Typically, only moderate hydrogen pressures (e.g., 8 atm) are needed to give good rates of reaction, and therefore conventional hydrogenation process equipment can be used. A recent example where we have sought to optimize process conditions is in the hydrogenation shown in Scheme 20.6 to give 3-methylsuccinamic acid as a possible precursor to 4-amino-2-methylbutanol, which in turn is an established pharmaceutical intermediate.[15]

It is in studying the process parameters in detail that important findings can be made toward improving the readiness to apply the technology to future targets. On the methylsuccinamic acid target it was discovered that chloride salts contaminating the substrate severely impaired the reaction and early reactions were slow. The best substrate/catalyst ratio that we could achieve for the hydrogenation to proceed at a satisfactory rate and go to completion was 1000 : 1. This catalyst utilization would have been unlikely to deliver the necessary economics for such a low-molecular-weight substrate. However, upon ensuring that the substrate was free of chloride, the reaction rate was increased by a factor of 30, and an excellent substrate/catalyst ratio of 100,000 : 1 could be achieved.[15]

20.3.4 Asymmetric Hydrogenation of Prochiral Ketones by Ruthenium–Bisphosphine–Diamine Catalysts

In 2001, Ryoji Noyori won a share of the Nobel Prize in Chemistry for his work in asymmetric hydrogenation, recognising the major industrial applicability of his methodology. His pioneering studies included the use of ruthenium–chiral bisphosphine complexes in asymmetric hydrogenation of such substrates as β-ketoesters to give the corresponding 3-hydroxyesters, examples of which are important intermediates in the synthesis of pharmaceutical agents. This work demonstrated the great value of the chiral bisphosphine ligand BINAP for establishing an asymmetric environment around the ruthenium atom.[16] Key specific applications have been developed by the Takasago company, including synthesis of the carbopenem antibiotics.[17] More recently, Noyori discovered a reaction whereby ruthenium diamine complexes can catalyze the hydrogenation of aldehydes and ketones selectively in the presence of other functionality, such as olefins. When the ligands on the metal are nonchiral, the reaction serves as a valuable catalytic alternative to the use of stoichiometric reducing agents such as sodium borohydride, but can be environmentally cleaner and more economical.[18] Then when the substrates are ketones and the ligands are chiral, the approach offers a uniquely efficient route to the single enantiomers of secondary alcohols, frequently sought after as pharmaceutical intermediates. In one version of the technology,[19,20] the ruthenium catalysts contain not only a diamine but also a bisphosphine, both of which may be chiral, and by selecting a matching combination of the two ligands, the selectivity and rates of the catalysis can be optimized (Scheme 20.7).

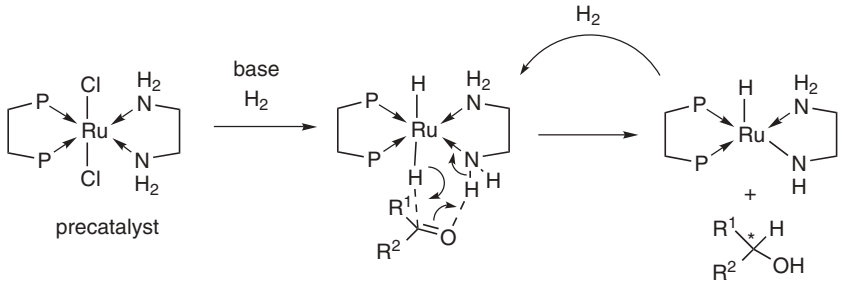


Scheme 20.7 Example of Noyori asymmetric ketone hydrogenation technology.

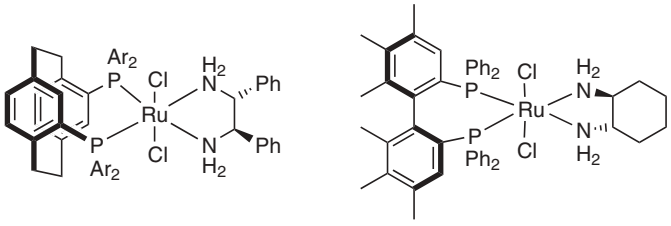
The mechanism of the catalysis (Scheme 20.8) is quite unlike that of the rhodium–DuPhos catalysis of prochiral olefins described above, since the ketone substrate does not bind to the metal (ruthenium) atom. When a substrate binds the metal, as in the rhodium–DuPhos systems, there are opportunities for unwanted pathways that terminate the catalysis. On the other hand, a consequence of the metal being protected by its ligands in the Noyori–Ikariya catalysis in principle reduces the likelihood of catalyst deactivation and increases the expectation for achieving very high catalyst utilization (substrate/catalyst ratios). Thus, in the asymmetric hydrogenation of acetophenone to (*R*)-1-phenylethanol, Noyori et al. reported an astounding molar substrate/catalyst ratio of 2,400,000 : 1.[19]

Our company was successful in securing a license under patents to use the technology from the Japan Science and Technology Corporation, thus meeting the need to have freedom to operate the intellectual property. However, we needed to bear in mind that there was third-party patent cover on ruthenium–BINAP complexes, and that required us to develop the technology with a bisphosphine ligand other than BINAP. In this regard we were fortunate to have previously secured a license to produce a cyclophane-based ligand known as PhanePhos developed by Merck for various hydrogenations.[21] Combining the two technologies it was discovered that the PhanePhos–ruthenium diamine complexes were highly effective in the asymmetric hydrogenation of aromatic ketones into the corresponding secondary alcohols.[22] Subsequently, we discovered that a proprietary bis(aryl)phosphine system of our own known as HexaPhemp (Scheme 20.9) was also very effective in this technology, giving faster reaction rates than those of corresponding catalysts with the BINAP ligand.[23]

To assure ourselves that we could meet all the criteria required for the technology to be ready for industrial application, we investigated the practicalities in the asymmetric hydrogenation of acetophenone derivatives, using catalysts based on PhanePhos. Note that

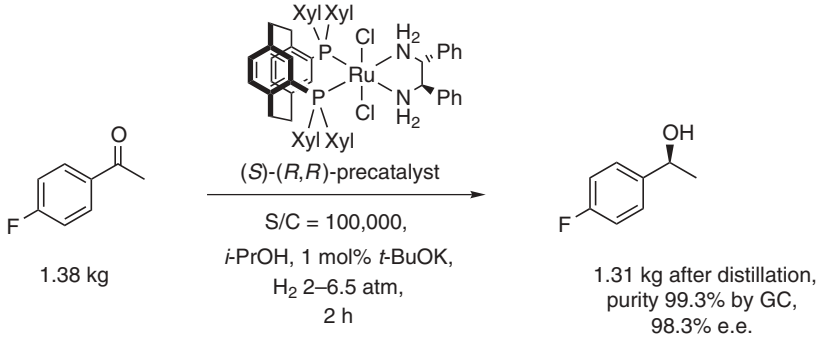


Scheme 20.8 Mechanism of hydrogenation of ketones with ruthenium–diamine–bisphosphine complexes.



Scheme 20.9 Structures of ruthenium–diamine catalysts with PhanePhos and HexaPhemp ligands.

as for rhodium–DuPhos, the actual ruthenium species added to the reactor is in this case a precatalyst in the form of a dichlororuthenium complex. To convert the precatalyst into active catalyst requires the presence of hydrogen and a base wherein an actual catalytic species, a dihydridoruthenium complex, is generated (see Scheme 20.8). In a study to develop industrially feasible conditions for the catalysis,[24] we found that the catalysts derived from the precatalyst [(*S*)-(xylyl)-PhanePhos)$RuCl_2$[(*R*,*R*)-DPEN] and its enantiomer were so active that 4′-fluoroacetophenone could be hydrogenated at a molar substrate/catalyst ratio of 100,000 : 1 with complete conversion in just over 2 hours, to afford the 2-(4-flurophenylethanol) in the range 95 to 99% e.e. (Scheme 20.10). In industrial practice, the choice to use extremely low catalyst loadings has to take into account the risk to the robustness of the reaction, the possible need and expense to use specially purified materials, and the need for a longer reaction time or more forcing conditions. Thus, in the reported example of the hydrogenation of acetophenone at S/C 2,400,000 : 1 (2.2 mg of precatalyst for 601g of ketone substrate),[19] a hydrogen pressure of 45 atm was used to achieve reaction completion in 48 hours, whereas comparable reactions at 100,000 : 1 substrate/catalyst ratios in this work of Noyori needed only 10 atm hydrogen to achieve a similar reaction rate. Industrially, using a higher hydrogen pressure has a significant impact on the equipment costs for a process. Also, the consequence of large-scale reaction stalling because of insufficient catalyst could be more expensive than using a higher catalyst loading. Typically for pharmaceutical targets, once molar substrate/catalyst loadings of around 10,000 : 1 are achieved, the catalyst becomes only a small part of the overall process cost.



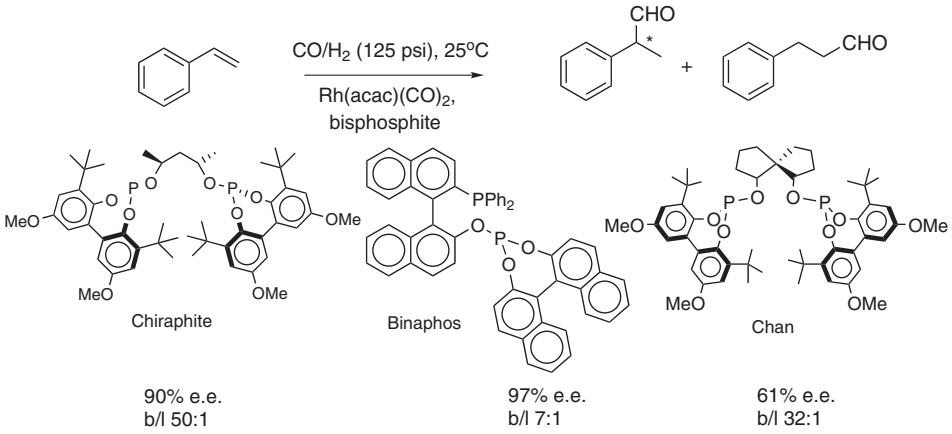
Scheme 20.10 Asymmetric hydrogenation of 4′-fluoroacetophenone.

Against the 4′-fluoroacetophenone target, we found that with more catalyst, a nonetheless very acceptable substrate/catalyst ratio of 5000 to 10,000 : 1 was tolerant to a range of commercial-grade solvents, and thus a robust process is assured.[24] We concluded that the Noyori method has a significant advantage over other enantioselective reactions.

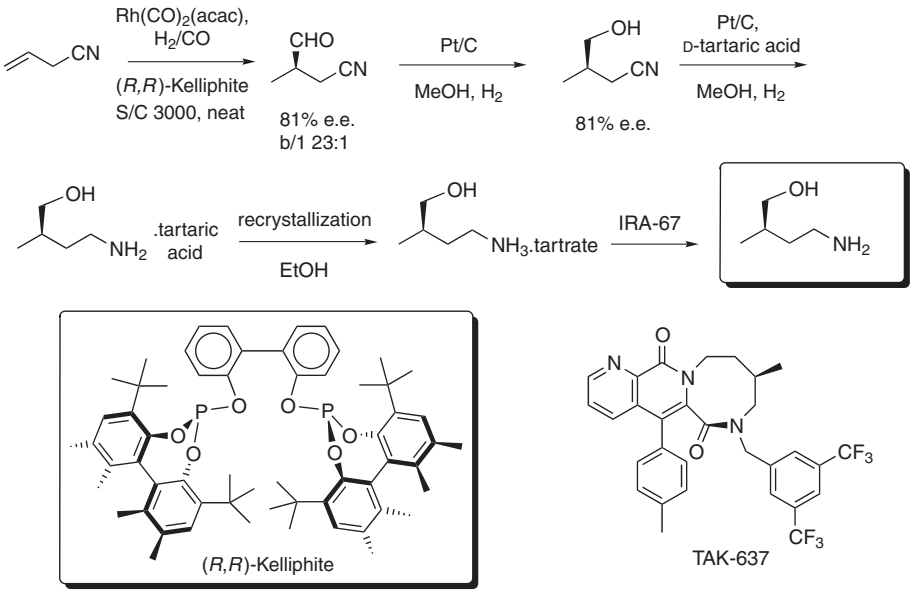
20.3.5 Asymmetric Hydroformylation with Rhodium–Phosphite Catalysts

In contrast to achiral hydroformylation, which is used in the production of butyraldehyde and ultimately *n*-butanol, asymmetric hydroformylation has been underutilized and not practiced on an industrial scale. This is due partially to the need to control several factors: regioselectivity, enantiomeric excess, and hydrogenation of the starting olefin or product aldehyde. The early literature examples of asymmetric hydroformylation used primarily vinylarene substrates, as these are biased electronically to give the desired chiral-branched product.[25] The products from this type of hydroformylation provide access to the profen class of drugs, such as naproxen and ibuprofen. This approach never became commercializable, due mainly to the effectiveness of alternative resolution-based methods and to market forces. Thus, naproxen is a generic drug selling at an extremely low price, while the sales of single-enantiomer (racemic-switched) profens such as ibuprofen and ketoprofen have not been substantial. In addition, many catalyst systems have failed to give high regioselectivity. Workers from the Dow Chemical site in South Charleston (formerly Union Carbide) discovered ligands that provided exceptional branched/linear (b/l) ratios. In the asymmetric hydroformylation of styrene, the rhodium–Chiraphite system gave an astounding 50 : 1 ratio of branched to linear products in an acceptable 90% e.e. (Scheme 20.11).[25] Binaphos gave a superior enantioselectivity (97%) but with poor regiocontrol (7 : 1 b/l). The Chan ligand afforded an acceptable b/l ratio (34 : 10), but with poor enantioselectivity (61%).

As vinylarene substrates do not provide an entry to commercially interesting products, many research groups have been investigating asymmetric hydroformylation of more useful olefins. Independently, workers from DSM and from Dow investigated the asymmetric hydroformylation of allyl cyanide, which provides access to (*R*)-4-amino-2-methylbutanol, an intermediate in the synthesis of TAK-637, a drug in development



Scheme 20.11 Asymmetric hydroformylation of styrene.



Scheme 20.12 Asymmetric hydroformylation of allyl cyanide.

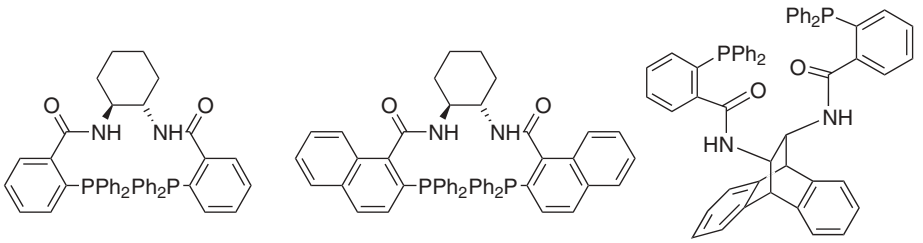
by Takeda for urinary continence.[26] For the reaction with allyl cyanide, Binaphos gave a 66% e.e. with b/l 2.6 : 1.[27] By comparison, Kelliphite was highly effective at controlling selectivity to the branched aldehyde considering that allyl cyanide has no electronic factors that lead to branched selectivity (Scheme 20.12). Ultimately, a process that was operated without solvent at 45°C, 10 bar, CO/H_2 1 : 1, 3,000 : 1 molar S/C, and 1:1.1 rhodium/ligand ratio was developed. This provided products in quantitative yield, 81% e.e. and 23 : 1 b/l.[28]

The Chiraphite and Kelliphite ligands are modular, and in our research program well over 200 variants have been synthesized, allowing fine-tuning for a range of substrates with respect to enantio- and regioselectivity. With the manufacturing expertise in hydroformylation at the former Union Carbide site at South Charleston, the expertise in screening catalysts at Dow's Michigan facility and the ability to manufacture ligands and catalysts provided by Chirotech in Cambridge, UK, all aspects of the industrial readiness equation have been met for asymmetric hydroformylation.

20.3.6 Asymmetric Allylic Substitution with Palladium-Based Catalysts

In 1992, Barry Trost introduced a family of chiral ligands for palladium(0)-catalyzed asymmetric allylic alkylation (AAA),[29] and Chirotech acquired an exclusive license to this technology in 1997. The first challenge was to develop syntheses that could provide the desired ligands in good yield and on a kilogram scale. From the plethora of chiral products that can be produced by this technology[30] it was obvious that three ligands were used for the majority of examples; the standard phenyl ligand, the naphthyl ligand, and the anthracenyl ligand (Scheme 20.13).

In the case of the phenyl ligand we need to improve the synthesis of the phosphine component, *o*-diphenylphosphinobenzoic acid, develop a scalable coupling method, and

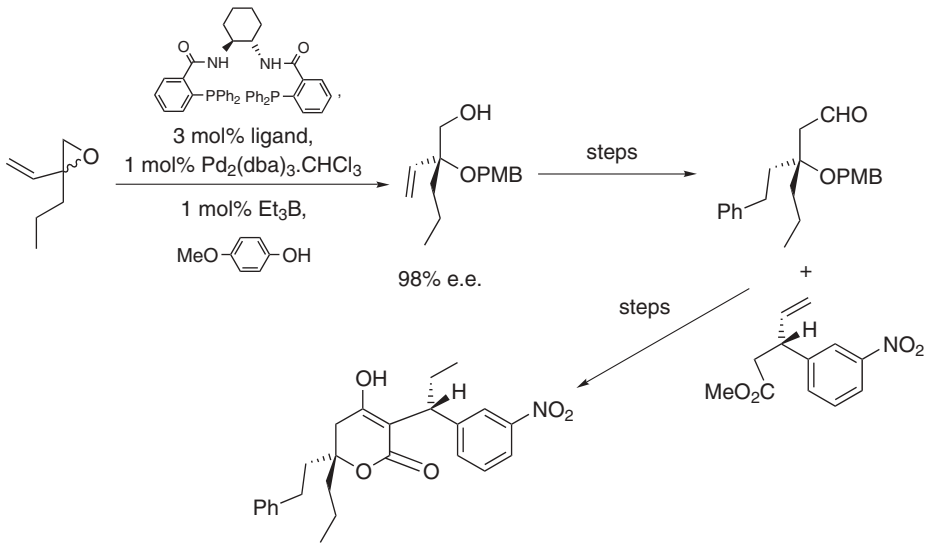


Scheme 20.13 Ligands for use in Trost asymmetric palladium-catalysis technology.

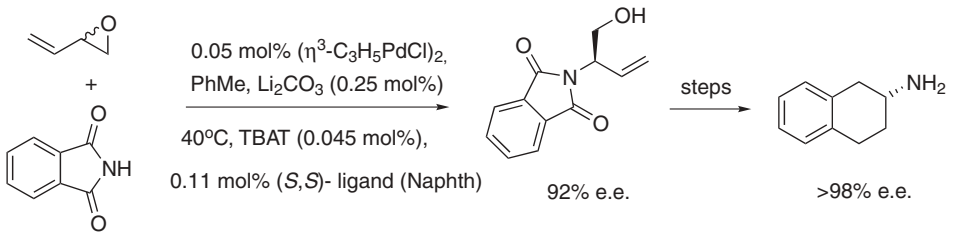
remove chromatography. With these objectives met, we have been able to produce kilogram quantities of the ligand.[31]

One good example of the application of this technology is in the AAA reaction of a racemic vinyl epoxide. The epoxide undergoes a dynamic kinetic asymmetric transformation (DYKAT) by reaction with *p*-methoxybenzyl alcohol, the standard ligand, and a palladium source. The product is obtained in 69% yield and 98% e.e. After further manipulations a key building block for the nonpeptidic protease inhibitor tipranavir was produced. Coupling of this intermediate with a synthon obtained using a molybdenum-catalyzed DYKAT process led to an advanced intermediate in a total synthesis of tipranavir (Scheme 20.14).[32]

The naphthyl ligand provided more challenges in developing a scalable process, but this was achieved.[33] This ligand is superior for the amination of racemic epoxybutene, by a DYKAT process. We have developed this reaction to work on a kilogram scale at an acceptable S/C ratio of 1000 : 1. The product from this reaction, phthalimidovinylglycinol



Scheme 20.14 Synthesis of tipranavir by Trost technology methods.

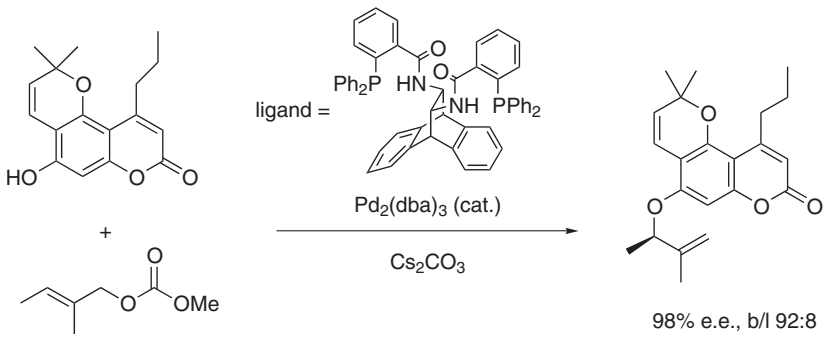


Scheme 20.15 Route to 2-aminotetralins.

(PVG), is a key intermediate in a synthesis of (*S*)-vigabatrin, an antiepileptic drug.[34] We have developed a concise approach to pharmaceutically interesting 2-aminotetralins using this building block. A Heck coupling of PVG with a bromo or iodobenzene, followed by hydrogenation, cyclization, and phthalimide removal, provides facile access to a range of 2-aminotetralins (Scheme 20.15).[35]

The anthracenyl ligand is by far the optimum for controlling regioselectivity and enantioselectivity in the palladium-catalyzed allylic alkylation of tiglyl methyl carbonate with a sterically demanding phenol nucleophile. This is demonstrated in the synthesis of calanolide A, a potent inhibitor of HIV-1 reverse transcriptase.[36] Using the anthracenyl ligand, a 98% e.e. with an 11 : 1 ratio of branched to linear product is obtained. However, the standard ligand gave an 81% e.e. and a 3.7 : 1 branched/linear ratio; and the naphthyl ligand afforded only a 45% e.e. and 3.6 : 1 branched linear ratio (Scheme 20.16). This clearly demonstrates the need to have a diverse range of ligands and catalysts for any particular asymmetric transformation, including hydrogenation and hydroformylation.

We have developed the synthesis of all three ligands so that they can be produced on a scale that can quickly deliver multiple kilograms of product. A range of substrates have been investigated and the optimum ligand and palladium source identified for each. Representative products have been produced on a scale of 1 to 2 kg. Although we would not class this an industrial-scale technology, producing multiple hundreds of kilograms of product, it is definitely industrially ready technology.



Scheme 20.16 Intermediate for calanolide A using Trost asymmetric allylic displacement technology.

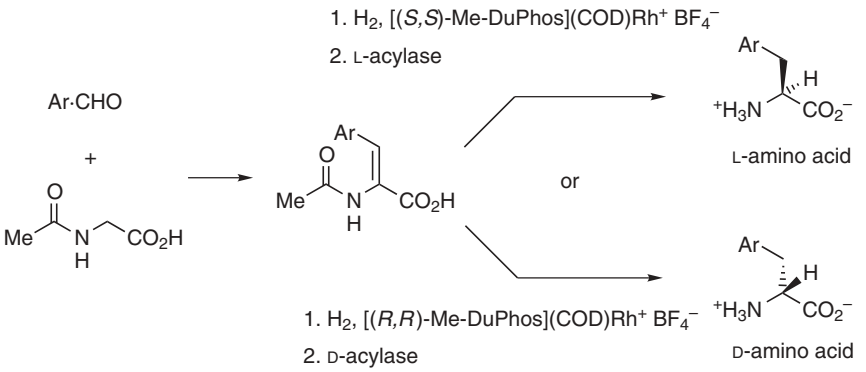
20.4 HOW INDUSTRIALLY READY TECHNOLOGY CAN DELIVER COMMERCIAL ADVANTAGES

Having industrially ready technology creates responsiveness when a new opportunity arises. This gives much commercial benefit. The industrially ready status means that the technology can be applied rapidly to a new opportunity, and it is sufficiently robust that confidence in delivery of product can be assured. Consequently, long-term economic production can be anticipated if the pharmaceutical agent makes it into the marketplace. Nonetheless, the cost of investment of a new technology has to be recovered, and to achieve this, it is best to harness every possible opportunity for profitable commercial exploitation, by ensuring that the technology has a wide breadth of application.

Three particular mechanisms can exist whereby the technology, once made industrially ready, may deliver a particular commercial advantage:

1. *It may solve a specific synthetic process problem.* Much of the account above considers the need to have technology ready for industrial application in order to have a platform to respond to specific problems of customers as they need them. Because of the high attrition rate in the pharmaceutical pipeline, wherein only a small proportion of drug candidates will eventually make it to the market, there is a tendency for drug companies not to expend any more effort on the process chemistry than is necessary. Such effort would be wasted if development of the drug were discontinued. That leaves a pharmaceutical company to push the route used in their medicinal chemistry department some way into the development program. However, if the drug candidate is taken into clinical trials successfully, the route used for the medicinal chemistry will often not be amenable to produce the larger quantities of material are necessary. At this point the pharmaceutical company is under considerable time pressure and becomes very receptive to having a process problem solved by a technology that is ready to use industrially. Moreover, if the industrially ready technology can also deliver an economic route of production, it is likely to become the method of choice for manufacture of the active pharmaceutical ingredient if the drug makes it all the way to the market.

2. *It may create synergies with other capabilities.* Frequently, having one technology industrially ready can increase the value of another technology. A good example is our synergistic use of asymmetric hydrogenation with acylase biocatalysis as a means to manufacture single-enantiomer amino acids. The result is that greater commercial benefit can accrue than by using either technology alone. Scheme 20.17 represents the synthesis of L- or D-amino acids of the arylalanine type. Earlier in this chapter we mentioned a bioresolution approach to amino acids exemplified by allylglycine, but there is the disadvantage that either the maximum possible yield is 50% or there have to be additional processing steps to recycle the unwanted isomer. In the case of arylalanines, where the aldehyde is an available raw material, straightforward Erlenmeyer condensation chemistry provides a dehydroamino acid as a substrate for asymmetric hydrogenation, which can be carried out highly effectively with the rhodium–DuPhos catalysts. However, the resulting product is the *N*-acetylamino acid, and the acetyl group will generally need to be removed. Chemical hydrolysis (e.g., heating with excess hydrochloric acid) is a brutal procedure, and neutralization to recover the amino acid from its hydrochloride generates large volumes of salt. Furthermore, in some cases the hydrolysis step causes some racemization of the amino acid, undermining the selectivity of hydrogen catalyst.



Scheme 20.17 Approach to arylalanines that combines asymmetric hydrogenation and acylase biocatalysis.

On the other hand, by using the acylase to remove the acetyl group assures a clean reaction and delivers the amino acid directly as its neutral zwitterionic form, which in favorable cases crystallizes out of the mixture (Scheme 20.17). In addition, the biocatalyst assures enantiomerically pure product even if the chemical catalyst has imperfect stereoselectivity. A consequence is that although rhodium–*ethyl*-DuPhos gives slightly better selectivity in these hydrogenations, the more readily available rhodium–*methyl*-DuPhos can be preferred. Overall, the combination of technologies provides all the benefits of clean reaction conditions, high overall yield, choice of either l- or d-isomer, and assured enantiomeric purity for a wide range of amino acid products. The combination provides a truly industrially ready technology.

In other cases it will be the synergy between the catalytic chiral technology and another process technology feature that will enable a commercial benefit. So, for example, if a crystallization method is effective to enhance the stereopurity of a pharmaceutical intermediate but the racemate itself cannot be resolved this way, then using an enzyme to get good but imperfect stereopurity, followed by the crystallization method, might allow production of product in a quality superior to what competitors could produce.

3. *It may be used to seed the marketplace or open a new business approach.* There may be other ways in which the benefits of having an industrially ready technology can be realized in the marketplace. One is to use the technology to supply a contract screening service of catalysts for customers. Then if a particular catalyst system is shown to be effective, the customer may wish to see it used in their process: that is, if the customer is assured that the technology is sufficiently ready for the purpose. If a catalyst is shown highly effective from a screen on a customer's substrate but cannot be made in any quantity, or if the intellectual property position is suspect, the business opportunity may not proceed further. Beyond the screening service, in certain cases it might be appropriate to supply catalysts to customers so that they can carry out the production themselves. To this end we have made catalyst systems available through the Strem catalog. Given the expertise base in our company with the catalyst technology, we should eventually be well placed to take on the manufacture of product for the customer using the catalyst. Finally, with an industrially ready technology, we have found it worthwhile to place examples of products generated with the technology into a suitable catalog, such as Acros. Although only small amounts of material will be sold from there, it does encourage the customer to adopt the materials into their emerging drug

candidates, and if any of them are successful, the customer will need greater quantities of material. Having the technology ready to use at large scale is a key factor in securing such follow-up business.

20.5 CONCLUSIONS

It is evident that the examples presented above cover catalytic technologies for which all the criteria for readiness for industry are met, but these are techniques with proven commercial application. The considerations given are nonetheless of particular value to those planning to develop a new technology. Having technology industrially ready is also of much value to pharmaceutical companies that are attracted to the elegance of a method for use on one of their targets but need to be assured that the technology is sufficiently developed that the supply of their product in the quantity and quality needed will be assured.

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21

ENANTIOSELECTIVE SYNTHESIS OF PROPARGYL ALCOHOLS AS MULTIFUNCTIONAL SYNTHONS

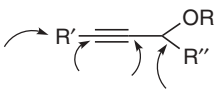
J. S. YADAV AND S. CHANDRASEKHAR

Indian Institute of Chemical Technology
Hyderabad, India

21.1 INTRODUCTION

The science of organic synthesis revolves around the development of newer strategies and methodologies which are more efficient, greener, practical, and safe. Despite the fact that there has been dramatic success in the past few decades in the area of development of newer and environmentally friendly methods, one is not able to synthesize everything that is needed. In fact, in the literature there are more unconquered complex targets than already synthesized targets. There are several complex bioactive and "most wanted" natural products that are either conquered but not with optimum efficiency or that have never been synthesized.[1]

To achieve these goals, several novel strategies and approaches are being explored continuously. These can be discussed under various heads: catalysis, domino/cascade/one-pot process (MCC), and design of multifunctional synthons, to name a few. All these areas of research have attracted synthetic organic chemists, and whose efforts of these researchers have benefited humankind immensely in the pharmaceutical, materials, agricultural, and other sectors. Despite the plethora of methods available, more efficient techniques are still welcome. In this chapter we highlight various synthetic methods available in the preparation of chiral propargyl (acetylenic) alcohols, which constitute an important multifunctional synthon.[2] Logically, propargyl alcohols make it possible to functionalize at four sites:



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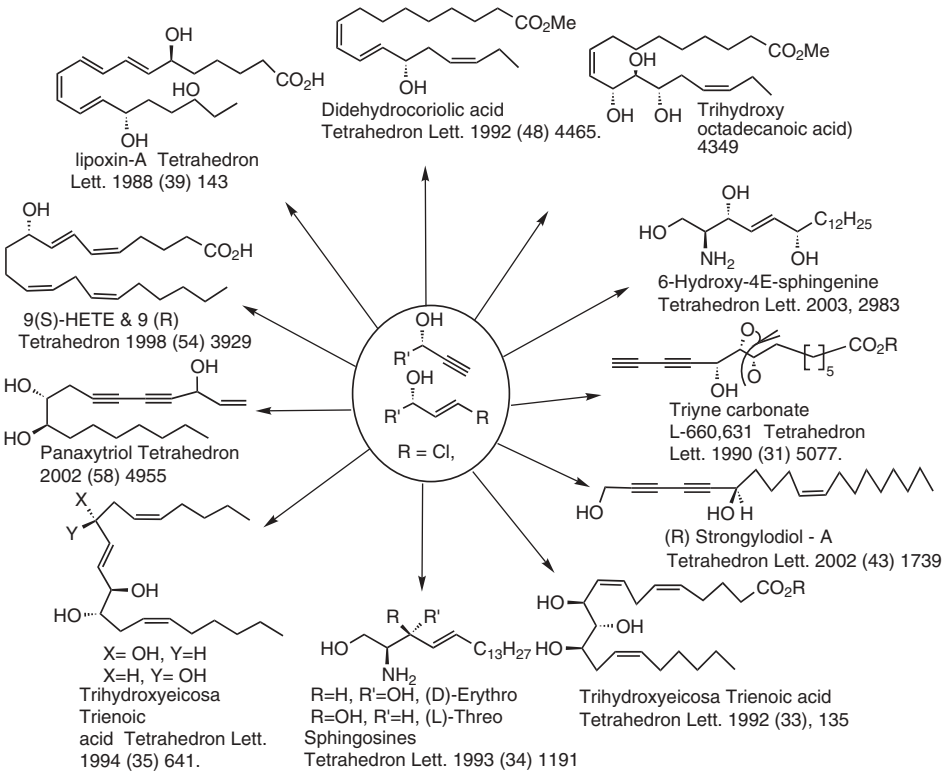


Figure 21.1

Before venturing into describing various strategies developed for the synthesis of propargyl alcohols in optically pure form, it would be interesting to look at various bioactive natural products and derivatives synthesized starting from this multifunctional synthon.

Figures 21.1 to 21.3 show several arachidonic acid–derived and other bioactive natural products synthesized using chiral propargyl alcohol (CPA) in the authors' own group (Fig. 21.1) and other groups (Figs. 21.2 and 21.3).

The major strategies for constructing chiral propargyl alcohol can be classified as follows:

1. Asymmetric reduction of prochiral α,β-alkynyl ketones
2. Addition of acetylenic anion to aldehydes under chiral catalysis
3. Desymmetrization and enzymatic strategies
4. β-Elimination and miscellaneous strategies

21.2 ASYMMETRIC REDUCTION OF PROCHIRAL α, β-ALKYNYL KETONES

The asymmetric reduction of prochiral ketones is one of the best methods available today for the preparation of optically active propargyl alcohols. The first report of this sort was made by Brinkmeyer and Kapoor,[3] in which Mosher's (2*S*,3*R*)-(+) - 4 -(dimethylamino)-1,2-diphenyl-3-methylbutanol–lithium aluminum hydroxide (LAH) (Darvon alcohol)

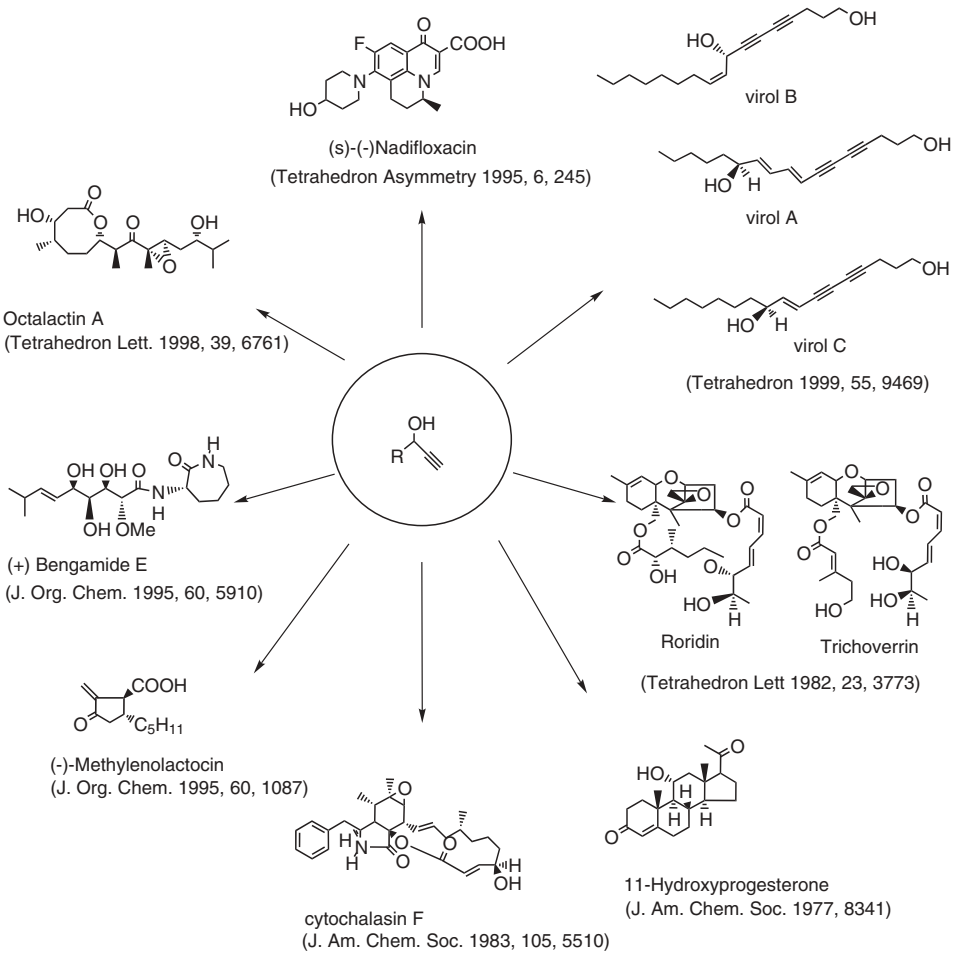
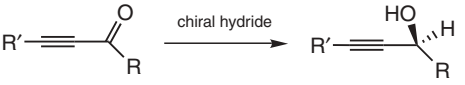


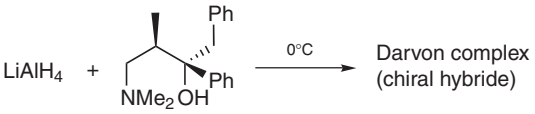
Figure 21.2

complex could reduce ketone to alcohol with 62 to 84% e.e. (Scheme 21.1). In most cases studied, the absolute stereochemistry was found to be R, with a few exceptions.

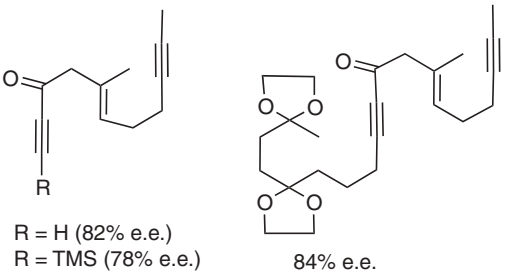
Several prochiral ketones were subjected to reduction using this protocol (Scheme 21.2) and reasonable to good e.e. values were obtained in all the cases studied. Interestingly, acetylenic groups, olefinics, and ketals survived the reaction conditions (Scheme 21.3).



Scheme 21.1



Scheme 21.2



Scheme 21.3

Prior to this work, Mosner and Yamaguchi[4] reported similar reduction with an LAH–quinine combination; however, no example of acetylenic ketone was attempted. In a later study, Midland et al.[5] developed α-pinene–9-Borabicyclo [3.3.1] nonane complex as an excellent reagent for the reduction of α,β-acetylenic ketones and observed high asymmetric induction in aliphatic systems (Table 21.1).

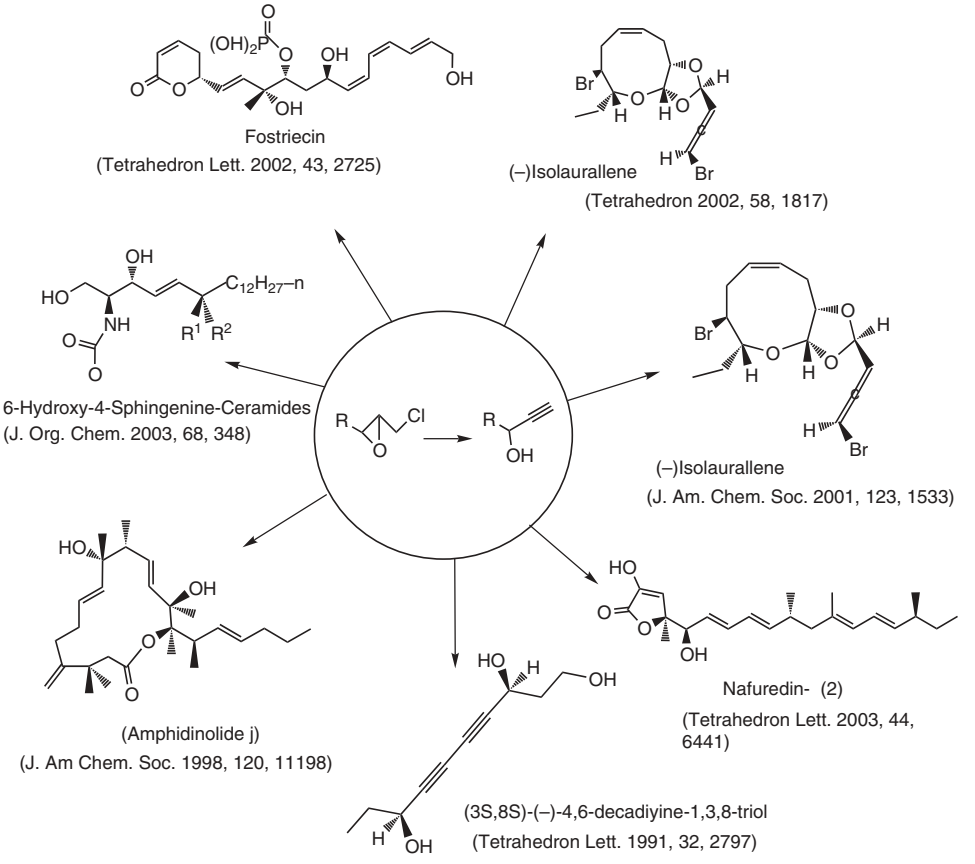
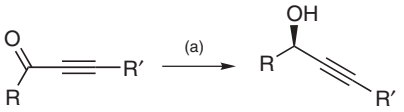
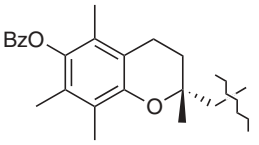
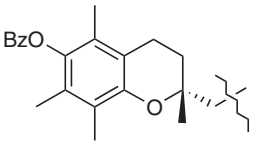


Figure 21.3

TABLE 21.1 α-Pinene-9-BBN Complex

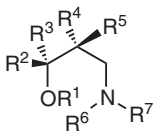


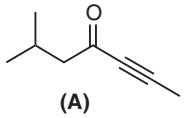
R	R′	e.e.(%)
C_6H_5	*n*-C_4H_9	89
CH_3	C_6H_5	72
	CH_3	70
	H	82

Typical Experimental Procedure An oven-dried 50-mL round-bottomed flask equipped with a septum-capped sidearm, magnetic stirring bar, reflux condenser, and stopcock adaptor connected to a mercury bubbler was assembled while hot and flushed with a stream of nitrogen. Then 18.5 mL of a 0.54 M 1,2,3,4-tetrahydro-9-fluorenone (THF) solution (10.0 mmol) of 9-BBN was added by syringe followed by 1.78 mL (11.0 mmol) of (+)-α-pinene, $[\alpha]_D^{25} = +47.28^\circ$, 92% e.e., distilled in $LiAlH_4$. The solution was stirred at reflux for 2.5 hours. The solution was cooled to room temperature and 0.73 mL of 4-phenyl-3-butyn-2-one was injected into the flask. A yellow-orange color at this stage is characterized in these reductions. Stirring at room temperature was continued for 48 hours, then 0.5 mL of acetaldehyde (excess) was added to the solution and stirring was continued for another 15 minutes. With the flask in a water bath, the solvent was removed by applying a water aspirator and stirring vigorously as a stream of nitrogen was passed over the solution. This operation was completed by stirring the residue at 40°C under aspirator pressure for 10 minutes. The flask was then filled with nitrogen and the liquid was dissolved in 12 mL of anhydrous diethyl ether. This solution was cooled in an ice bath and then treated with 0.66 mL (11 mmol) of ethanolamine. A white precipitate formed and the mixture was stirred for 15 minutes at 0°C. The flask was then opened to air and the mixture was filtered under suction. The solid was washed with 4 mL of ether. The combined filtrate was then washed with 20 mL of saturated aqueous sodium chloride, dried over a magnesium Kugelrohr oven (pot temperature), 100°C (0.02 mmHg), to provide 0.72 g (98%) of 4-phenyl-3-butyn-2-ol ($[\alpha]_D^{25} = +51.8^\circ$).

Taking a lead later from the work of Brinkmeyer and Kapoor, several amino alcohols have been exploited for the asymmetric reduction of alkynones. One such publication appeared from the group of Cohen,[6] who have also used Darvon alcohol–LAH complex at −70°C to furnish various propargyl alcohols in 34 to 90% e.e. In addition to Darvon alcohol, other chiral ligands were explored (Table 21.2).

TABLE 21.2





(A)

	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	H	H	H	CH_3	H	CH_3	CH_3
2	H	H	H	CH_3	H	CH_3	(*S*)-$CH(CH_3)Ph$
3	H	H	H	H	CH_3	CH_3	CH_3(*S*)-$(CH_3)Ph$

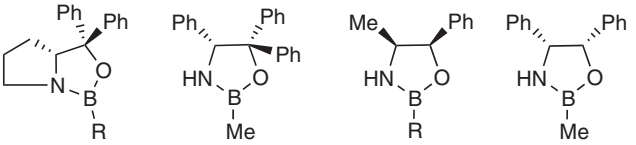
Reduction of **A** with **1**–LAH complex furnished the opposite enantiomer, (*S*)-carbinol, in 36% e.e., and acetophenone furnished the corresponding (*S*)-carbinol in 60% e.e.

More recently, oxazaborolidines have emerged as the most productive chiral ligands in asymmetric reductions, especially when using borane as the hydride source. Some of the more commonly used borolidines are shown in Scheme 21.4.

Based on this strategy, Garcia et al.[7] have used oxazaborolidine, **(R)-2**, and effectively reduced several α,β-acetylenic ketones. The e.e. value of the products was in the range 90 to 97%. To improve the results further, the monobranched ketones were complexed with hexacarbonyl dicobalt complexes[8] and subjected to reduction. Unfortunately, the reactions were sluggish and under forced conditions or modifications of oxazaborolidine resulted only in low yields and enantioselectives (Table 21.3).

General Procedure for Reduction of Acetylenic Ketones with Borane Catalyzed by **R-(2)** A solution of **1a** (115 mg, 0.50 mmol) in THF (0.5 mL) was slowly (ca.1 mmol/h) added to a solution of **(R)-2** (0.5 mmol) and $BH_3{\cdot}SMe_2$ (60 μL, 0.60 mmol) in THF (1 mL) at 0°C under an argon atmosphere. Upon completion of the addition, TLC revealed the disappearance of the starting ketone. Reaction was cautiously quenched by addition of MeOH (1 mL) at 0°C. The solution was stirred for 15 minutes at room temperature and then concentrated under vacuum. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2) to yield 107 mg (92%) of (*R*)-5-phenyl-1-(trimethylsilyl)-1-pentyn-3-ol (**2a**).

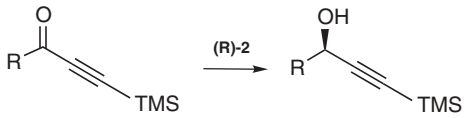
The electronic effects of remote substituents on the oxazaborolidine-catalyzed enantioselective reduction of ketones were studied in detail by Corey and Helal.[9] Unlike the earlier case, this group was able to complex the cobalt octacarbonyl with acetylene and



(R)-1 **(R)-2** **(3)** **(4)**

Scheme 21.4

TABLE 21.3

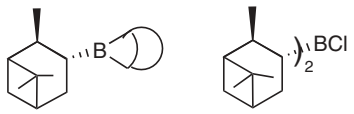


	Yield (%)	e.e. (%)
1a, R = $PhCH_2CH_2$	92	90
1b, R = cyclohexyl	65	93
1c, R = 1-adamantyl	80	71

using **R-(1)**–oxazaborolidine and catechol borane as a hydride source, reduced the ketone in over 90% e.e.

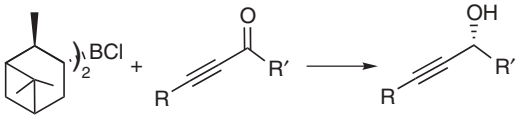
In a totally different approach, Noyori et al.[10] have used binaphthol-modified aluminum hydride reagent for enatioselective reduction of alkynyl ketones. Suitably modified boranes can be used for stereoselective reduction of ketones. Along these same lines, Midland[11] has developed Alpine borane (**1**, Scheme 21.5), which is excellent for several acetylenic ketones but has been found inefficient for hindered α,β-acetylenic ketones. To overcome this problem, Brown et al.[12] have introduced β-chlorodiisopinocamphenyl borane 2(−)-DIP-Cl (**2**, (Scheme 21.5), which reacts well with hindered ketones to provide the corresponding propargyl alcohols in 96 to ≥ 99% e.e.

It was observed that few reactions are dilution dependent, and in a few cases absence of solvent (neat borane) helped to reduce the ketone faster and with high ee. The following Table shows the results of this protocol (Scheme 21.6).



(1) alpine borane **(2)** (–)-DIP-Cl

Scheme 21.5



R = H;	R′ = Me	17% e.e.
R = Ph;	R′ = Me	21% e.e.
R = Ph;	R′ = Et	28% e.e.
R = Ph;	R′ = *i-pr*	53% e.e.
R = Ph;	R′ = *t-Bu*	99% e.e.

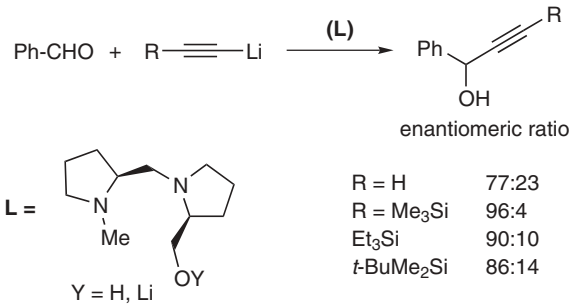
Scheme 21.6

General Procedure for Reduction of Acetylenic Ketones with (−)-DIP-Chloride An oven-dried 50-mL round-bottomed flask equipped with a side arm, magnetic stirring bar, and connecting tube was cooled to room temperature in a stream of nitrogen. (−)-DIP-chloride (3.25 g, 11 mmol) was transferred to the flask cooled to −25°C, and the ketone (10 mmol) was added. The reaction was followed by ^{11}B NMR spectrometry after aliquots were methanolyzed at −25°C at periodic intervals. When the reaction was complete (^{11}B, δ 32), the mixture was warmed to 0°C and acetaldehyde (0.73 mL, 13 mmol) was added dropwise for 3 hours when the ^{11}B NMR spectrum showed a singlet at δ 18. Sodium hydroxide (6 N, 10 mL) was added to the mixture and the organics were extracted with diethyl ether. The combined extracts were washed with brine, dried over $MgSO_4$, and distilled to separate the α-pinene and the product alcohol. The alcohol was further purified by preparative gas chromatography with appropriate columns (SE 30 or Carbowax 20M).

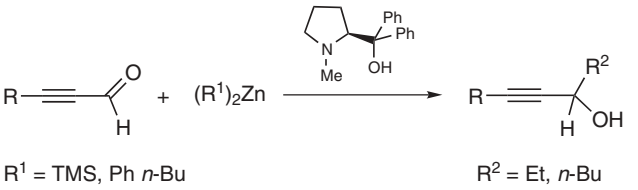
21.3 ADDITION OF ACETYLENIC ANION TO CARBONYL CARBON

One of the most practical ways to achieve chiral propargyl alcohol is to add acetylene anion to a carbonyl group in an enantiofacial manner. This art was well demonstrated by Mukayama et al. in 1979[13] using (2*S*,2[1]*S*)-2-hydroxymethyl-1-[(1-methylpyrrolidine-2-yl)methyl]pyrrolidine as a chiral ligand. The addition of lithium trimethylsilyl acetylide to benzaldehyde afforded the corresponding alkynol in over 92% optical yield. It is noted that the enantioselectivity of the present reaction depended predominantly on the trialkylsilyl group of the acetylene. Scheme 21.7 confirms these observations.

Typical Experimental Procedure To a solution of lithium acetylide [0.792 g, 4 mmol in dimethyl ether (20 mL)] was added a solution of $(CH_3)_3SiC{\equiv}CH$ (0.268 g, 2.7 mmol) in 2 mL of dimethoxymethane followed by addition of *n*-BuLi (6.7 mmol, 4.3 mL in hexane) at −35°C. After stirring for 30 minutes at the same temperature, the resulting white suspension was cooled to −123°C. A solution of benzaldehyde (0.106 g, 1 mmol) in 2 mL of dimethoxymethane was added dropwise and stirring was continued for an additional 1 hour. The reaction mixture was treated with 2 N HCl and was extracted with ether. After removal of the TMS group under basic conditions, the optical purity was measured by making the corresponding Mosher's ester.



Scheme 21.7



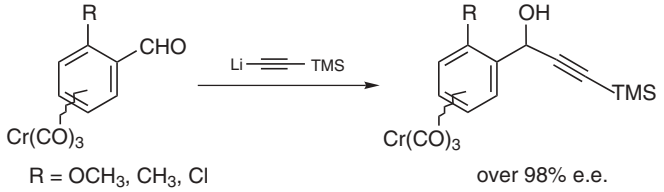
Scheme 21.8

Another very elegant approach for synthesis of this class of compounds was developed by Soai and Niwa,[13] who banked on the proline-derived aminol-catalyzed dialkyl zinc addition onto alkynals. The e.e. value of the products, however, was only in the range 64 to 78% (Scheme 21.8). Even though the e.e. was not very impressive, this method attracted several other research groups to explore the strategy by modifying the chiral catalyst.

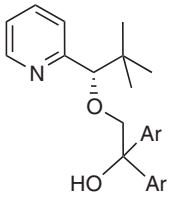
The use of chiral tricarbonyl (η^6-arene) chromium complexes in the highly stereoselective synthesis is well demonstrated.[14] Baldoli et al.[15] have amply demonstrated the application of this strategy in the synthesis of titled compounds. Known chiral *ortho*-substituted benzaldehyde tricarbonyl chromium complexes[16] were exposed to lithium acetylide in THF to furnish diastereoselective adducts in good yields (Scheme 21.9).

Typical Experimental Procedure *n*-BuLi (1.4 mmol, 0.88 mL in THF) was added to a solution of TMS acetylene (1.4 mmol in 2 mL of THF) at −78°C under an N_2 atmosphere. After 5 minutes a solution of chromium complex (1.1 mmol) in THF (3 mL) was added dropwise. The yellow mixture was stirred for 1 hour at −78°C and then quenched with saturated aqueous NH_4Cl. Workup followed by chromatography furnished the products in high yields (70 to 90%). The compounds in CH_2Cl_2 were exposed to sunlight for decomplexation to generate the chiral compounds.

Ishizaki and Hoshino[17] prepared optically active secondary alkynyl alcohols (up to 95% e.e.) by the catalytic asymmetric addition of alkyl zinc reagents to both aromatic and aliphatic aldehydes. The chiral ligands studied were based on the pyridine scaffold. Of the three aryl substitutions studied, the α-napthyl derivative was found to be superior (Scheme 21.10). Mechanistically, it was proposed that **(S)-1** would react with dialkynyl zinc alkoxide A and ethyl zinc alkoxide B. Coordination of additional dialkynyl zinc and alkynylethyl zinc with these alkoxides (A, B) would give C and D, respectively (Scheme 21.11). More bulky alkoxide (C) would have severe steric interactions with the alkynyl group and pyridine moiety, which might cause undesired conformational changes of the 1-zinc complexes. Consequently, the enatioselectivity would be decreased.

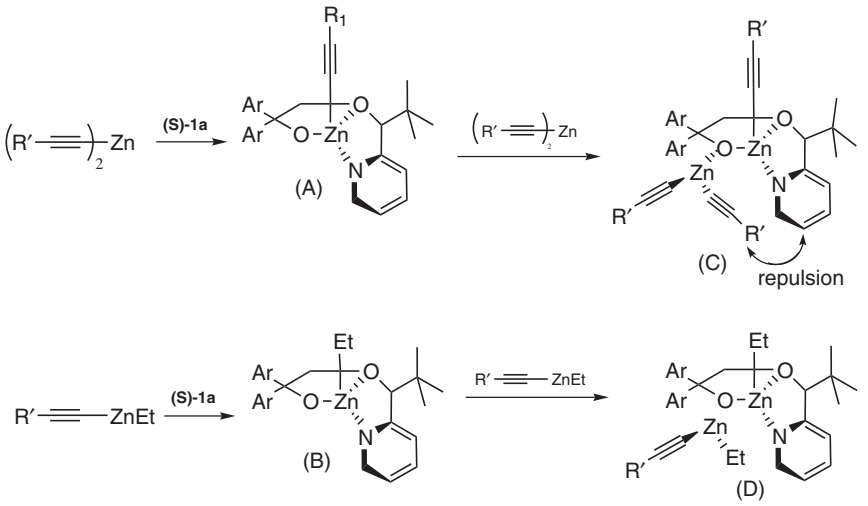


Scheme 21.9



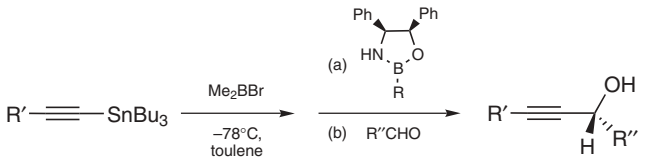
(S)-1, Ar = α-naphthyl
(S)-2, Ar = β-naphthyl
(S)-3, Ar = phenyl

Scheme 21.10



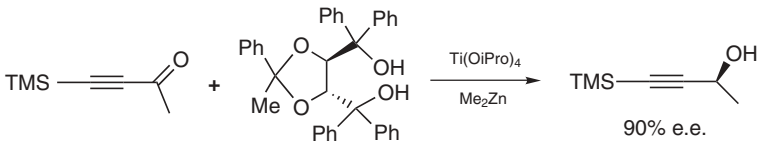
Scheme 21.11

Corey and Cimprich have used acetylenic dimethylborane as the nucleophile for the asymmetric addition of acetylenic nucleophiles to aldehydes.[18] This reagent was generated in situ from the corresponding alkynyl stannane and bromodimethylborane (Scheme 21.12). A typical procedure involved addition of a solution of bromodimethylborane[19] in methylcyclohexane to a solution of the alkynyl stannane (1.3 to 1.5) in toluene at −78°C. A solution of the oxazaborolidine (0.25 to 1 equiv in toluene) was added after 30 minutes, and after an additional 15 minutes the aldehyde was added. The chiral



85–97% e.e.

Scheme 21.12



Scheme 21.13

ligand was readily recovered as the hydrochloride salt, making the reaction practical even when a stoichiometric amount of oxazaborolidine is required.

The titanium TADDOLATE complex[20] was also used efficiently in the asymmetric addition of dimethyl zinc to 3-trimethylsilyl-2-propynal with and e.e. values over 90% (Scheme 21.13).

Typical Experimental Procedure In a Schlenk tube were placed **2** (Scheme 21.13) (151 mg, 0.2 mmol) and toluene (1.0 mL). To this solution was added Ti(*i*-OPr)$_4$ (0.06 mL, 0.02 mmol) at room temperature, the resulting solution was stirred for 16 hours, and the solvent and *i*-PrOH liberated were removed under reduced pressure. The solid residue was dissolved in Et_2O (1.0 mL) and Ti(*i*-OPr)$_4$ (0.35 mL, 1.2 mmol) was added at room temperature. Then Me_2Zn (0.12 mL, 1.8 mmol) was added to the mixture at $-20°C$, followed by addition of **1** (0.16 mL, 1.0 mmol). After stirring this solution at 0°C for 48 hours, the mixture was poured into 1 N HCl (10 mL) and stirred for 1 hour. The mixture was extracted with CH_2Cl_2 (3×15 mL), and the combined extracts were dried over Na_2SO_4. The residue after removal of solvent was chromatographed on silica gel (eluant, chloroform/hexane = 4 : 1) to give (*S*)-4-trimethylsilyl-3-butyn-2-ol (**2**) (yield 147 mg).

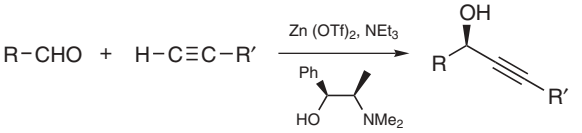
The best e.e. values reported to date were achieved by Carreira et al.[21] by direct addition of terminal alkynes to aldehydes in the presence of (+)-*N*-methylephedrine as chiral ligand. In several cases the e.e. has been 98%. This method was very general for both aliphatic and aromatic aldehydes, the alkyne component was flexible, and the reaction conditions were very short. This method involves in situ generation of zinc alkynylide by mixing alkyne, triethyl amine, and $Zn(OTf)_2$. Another very intriguing feature is that the method is very general and not substrate dependent (Table 21.4).

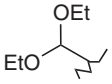
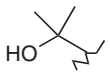
Typical Experimental Procedure Stirring a solution of an aldehyde and alkyne in toluene with 1.1 equiv $Zn(OTf)_2$ and 1.2 equiv each of Et_3N and (+)-*N*-methylephedrine, respectively, in toluene at 23°C for 2 to 20 hours furnishes adducts up to 99% e.e. (Table 21.4). Importantly, after the reaction is complete, the (+)-*N*-methylephedrine can easily be separated from the adducts by simple aqueous extraction (acid wash).

Chan et al.[22] have utilized hybrid binaphthyl amino alcohol for this transformation. The various ligands prepared by them are listed in Scheme 21.14. All these ligands were effective only in the case of aryl aldehydes in the presence of dimethyl zinc. The e.e. values varyied from 60 to 90%, and the chemical yields were found to be in the 90% range.

Toru et al.[23] have utilized the asymmetric sulfoxide precursor for the synthesis of chiral propargylic alcohol (Scheme 21.15). The e.e. values of the products was greater than 99% in a few cases. The reaction of the α-carbanion derived from (trimethylsilyl)vinyl sulfoxides with aldehydes afforded a diastereomeric mixture of products **R-4** and **S-5**. They were

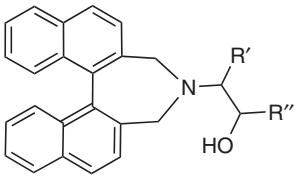
TABLE 21.4 Enantioselective Addition of RC≡CH to Aldehydes



Entry	Aldehyde (R)	Alkyne (R′)	Time (h)	yield (%)	e.e.
1	*c*-C_6H_{11}	Ph	1	99	96
2		$Ph(CH_2)_2$	4	98	99
3	*i*-Pr	$Ph(CH_2)_2$	2	90	99
4		Ph	2	95	90
5	PhCH=CH	$Ph(CH_2)_2$	20	39	80
6	*t*-Bu	$Ph(CH_2)_2$	2	84	99
7		Ph	2	99	94
8	Ph	$Ph(CH_2)_2$	20	52	96
9		Ph	20	53	94
10	*c*-C_6H_{11}	Me_3SI	2	93	98
11	Me_3CCH_3	$Ph(CH_2)_2$	2	72	99
12		Ph	2	90	97
13	*c*-C_6H_{11}	Me_3SiCH_3	4	84	98
14		$TBDMSOCH_3$	5	83	98
15			8	90	98
16			3	94	98
17	*i*-Pr		4	97	98

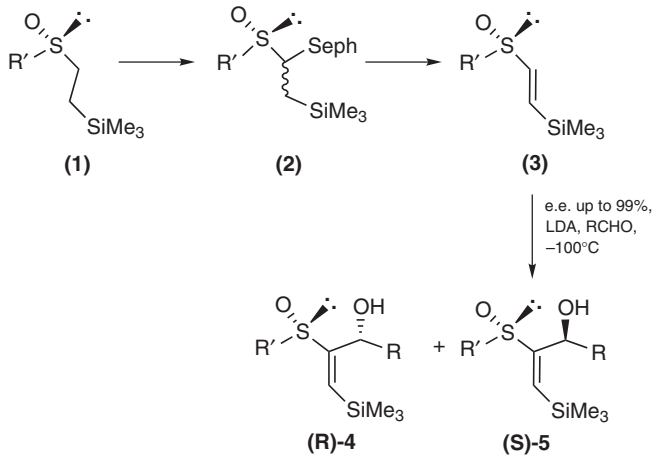
then subjected to specific elimination reactions to give optically pure propargylic, trimethylsilylated propargylic, and allylic alcohols.

Even though the purity of chiral vinyl sulfoxide was over 99%, the aldol-type vinyl anion addition onto aldehyde to generate the chiral vinyl alcohol was low in e.e. value. The diastereomers could, however, be separated by chromatography and subjected to



(1*R*,2*S*,3*R*)-1: R′ = Ph; R″ = Ph
(1*R*,2*S*,3*R*)-2: R′ = Ph; R″ = Ph
(1*R*,2*S*,3*R*)-3: R′ = Ph; R″ = Ph
(1*R*,2*S*,3*R*)-4: R′ = Ph; R″ = Ph

Scheme 21.14

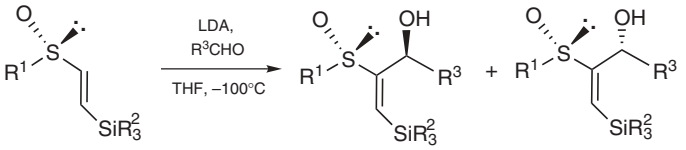


Scheme 21.15

tetrabutylammonium fluoride (TBAF) to realize corresponding chiral propargyl alcohols (Table 21.5).

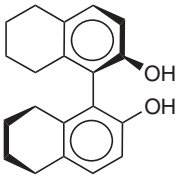
Chan et al.[24] have developed a simple and practical method to make chiral propargyl alcohols. In the presence of titanium alkoxide complex prepared in situ from titanium

TABLE 21.5



Entry		Sulfoxide R^1	Sulfoxide SiR_3^2	Aldehyde (R^3)	Product	Yield (%)	Isolated Ratio **(S)-4/(R)-4**
1	**3a**	Tol	$SiMe_3$	Ph	**4a**	88	45 : 55
2	**3a**	Tol	$SiMe_3$	Me	**4b**	82	68 : 32
3	**3a**	Tol	$SiMe_3$	n-C_5H_{11}	**4c**	98	73 : 27
4	**3a**	Tol	$SiMe_3$	i-Pr	**4d**	92	68 : 32
5	**3a**	Tol	$SiMe_3$	t-Bu	**4e**	71	76 : 24
6	**3b**	Tol	$SiPh_2Me$	n-C_5H_{11}	**4f**	77	69 : 31
7	**3c**	Tol	$SiPh_3$	n-C_5H_{11}	**4g**	70	66 : 34
8	**3c**	Tol	$SiPh_3$	t-Bu	**4h**	74	67 : 33
9	**3d**	t-Bu	$SiMe_3$	Ph	**4i**	88	34 : 66
10	**3d**	t-Bu	$SiMe_3$	n-C_5H_{11}	**4j**	82	29 : 71
11[a]	**3a**	Tol	$SiMe_3$	n-C_5H_{11}	**4e**	70	37 : 63
12[a]	**3b**	Tol	$SiPh_2Me$	n-C_5H_{11}	**4f**	35	32 : 68

[a]Hexamethylphosphoric acid triamide (HMPA) (2.0 equiv) was added.



R-H8-BINOL

Scheme 21.16

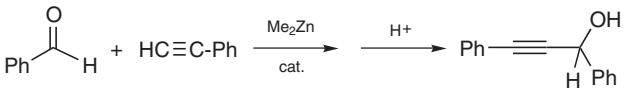
tetraisopropoxide and (*R*)-H_8-binaphthol, a variety of aromatic aldehydes were converted to the corresponding chiral propargyl alcohols with e.e. values up to 96% (Scheme 21.16 and Table 21.6).

Jiang et al.[25] utilized inexpensive chiral amino alcohol–based ligand, (1*S*,2*S*)-2,*N*,*N*-dimethylamino-1(*p*-nitrophenyl)-3-(*tert*-butyldimethylsilyloxy)propane-1-ol for asymmetric alkylation of both aliphatic and aromatic aldehydes upto 99% e.e. Other ligands were also tested for this transformation (aminols 1 to 4, Scheme 21.17).

Chiral disulfide oxazolidine ligands were developed by Braga et al.[26] more recently. The ligand was prepared from *R*-cysteine in a few steps (Scheme 21.18). The *R*,*R*-oxazolidine (**2**) was efficient in the presence of diethyl zinc to furnish chiral propargyl alcohols with moderate to good selectivity.

Other efforts in asymmetric alkynation using sterically congested BINOL were reported independently by Pu et al.[27] and Jiang et al.[28] The structures of the ligands are shown in Scheme 21.19. Again the e.e. values of the chiral propargyl alcohols were moderate to excellent.

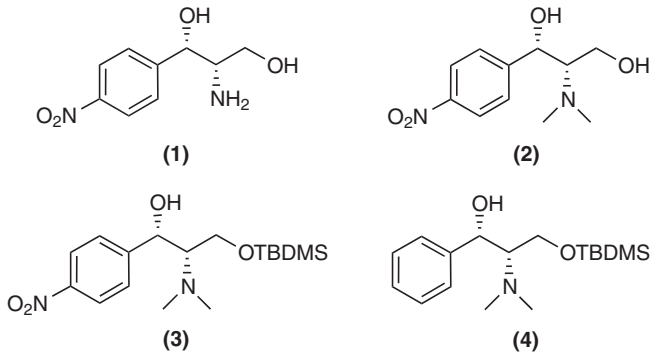
TABLE 21.6[a]



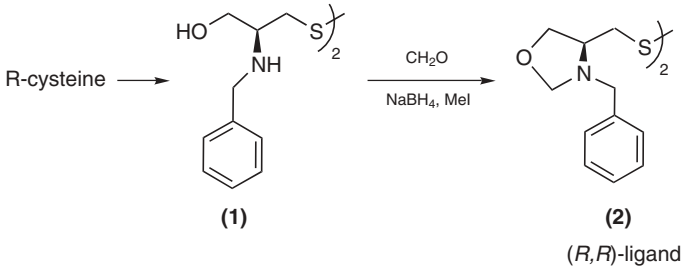
cat. = $Ti(OiPr)_4$ + ligand

Entry	Temperature (°C)	Solvent (%)	Yield Ratio (%)	e.e. (%)	Configuration
1	25	THF, 20%	86	85	(−)-(*S*)
2	0	THF, 20%	85	92	(−)-(*S*)
3	20	THF, 20%	83	92	(−)-(*S*)
4	0	Toluene, 20%	86	82	(−)-(*S*)
5	0	Et_2O, 20%	54	75	(−)-(*S*)
6	0	Hexane, 20%	85	65	(−)-(*S*)
7	0	CH_2Cl_2, 20%	84	76	(−)-(*S*)
8	0	THF, 10%	82	79	(−)-(*S*)
9	0	THF, 5%	64	48	(−)-(*S*)

[a](*R*)-H_8-BINOL as ligand; aldehyde–$Ti(OiPr)_4$–Me_2Zn = 1 : 1.5 (molar ratio). Isolated yield of the corresponding products. The configuration is based on measurement of the optical rotation comparison with the literature value.



Scheme 21.17



Scheme 21.18

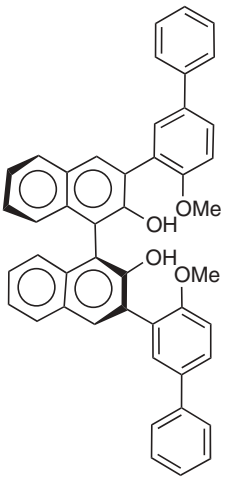
N,*O*-Ferrocene ligand was developed by Hou et al.[29] for a similar transformation with reasonable selectivities (Scheme 19.20). The selectivities of products using the ligands developed ranged from 30 to 90% e.e.

21.4 DESYMMETRIZATION AND ENZYMATIC STRATEGIES FOR CHIRAL PROPARGYL ALCOHOL SYNTHESIS

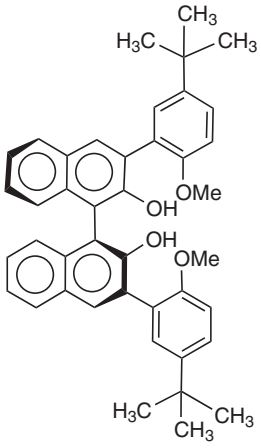
Optically pure propargyl alcohols were also obtained by reductive cleavage of chiral α,β-alkynyl acetals with organoaluminum reagent followed by removal of the chiral auxiliary by Yamamoto et al.[30] This method furnished products up to 98% e.e. Initially, the propargylic ketones were protected with chiral 2,4-pentanediol as acetal followed by cleavage of acetal with DIBAL-H. Oxidation with PCC followed by exposure to K_2CO_3 resulted in free propargyl alcohol (Scheme 21.21).

It is anticipated that the sterically less hindered alkynyl group occupies the axial position in the six-membered transition state, as shown in Scheme 21.22.

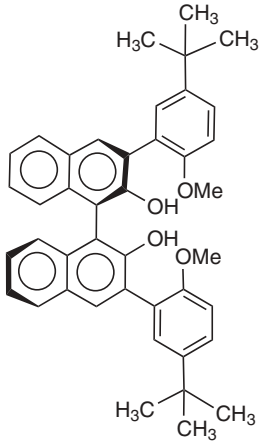
Microbial transformations also occupy a very important position in the synthesis of enantiomerically pure compounds. Mori and Akao[32] utilized the selective hydrolysis of the acetate derivatives of propargylic alcohols with *Bacillus subtilis*. However, the optical purities of the products were only up to 74% (Scheme 21.23).



(S)-13

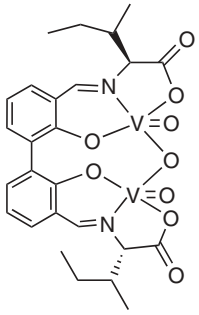


(S)-14



(R)-14

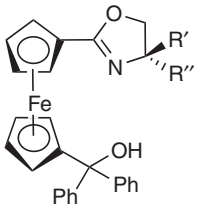
Pu's auxilliaries



Jiang's auxilliary

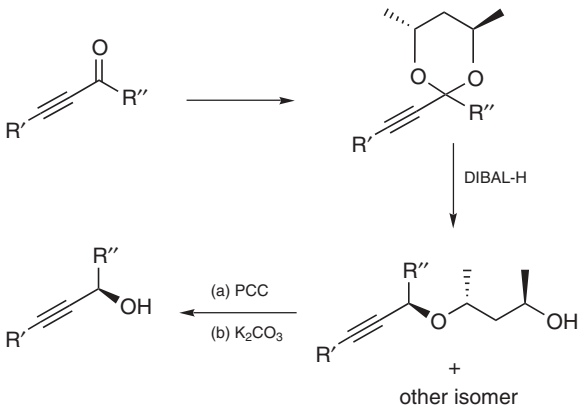
Scheme 21.19

Similar efforts were successful with lyophilized bakers' yeast[33] and e.e. values were up to 97%. Propargylic ketones were reduced to chiral alcohols under anaerobic conditions using *Geotrichum candidum* (to get both *R* and *S* isomers based on substitution), and *Aspergillus niger* (*R*-isomer) or *Mortierella isabellina* (*S*-isomer) for obtaining the

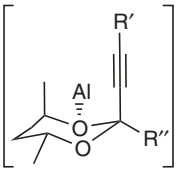


R′ = *i*-Pr, R″ = H
R′ = *t*-Bu, R″ = H
R′ = Bn, R″ = H
R′ = H, R″ = Ph

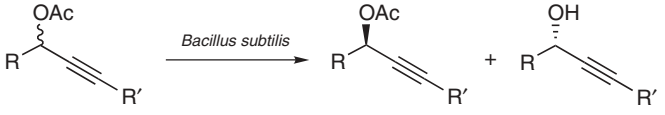
Scheme 21.20



Scheme 21.21



Scheme 21.22

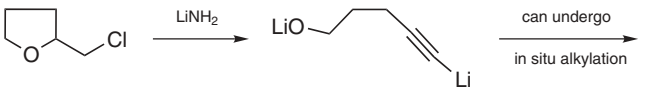


Scheme 21.23

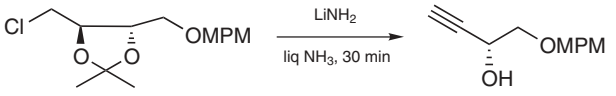
chiral propargyl alcohols up to 90% e.e.[34] This protocol was later utilized in the total synthesis of leukotriene B-4, a metabolite of arachidonic acid formed by the action of 5-lipoxygenase.[35]

21.5 β-ELIMINATION STRATEGY AND MISCELLANEOUS APPROACHES

β-Elimination is a versatile strategy for the synthesis of chiral propargyl alcohols. Our research group was the earliest to identify the potential of this approach and reported several applications in the synthesis of bioactive natural products (see also Fig. 21.1). The methodology was based on a known strategy of β-elimination of tetrahydrofurfuryl chloride in the presence of 3 equiv of $NaNH_2/LiNH_2$ to 4-pentyn-1-ol[36] (Scheme 21.24). Extending this very versatile reaction to the tartaric acid series, 1-chloro-2,3-*O*-isopropylidene-2,3,4-triol derivative (**1**, Scheme 21.25) was prepared from L(+)-tartaric acid[37] and subjected to β-elimination. This resulted in the clean formation of propargyl alcohol (**2**).

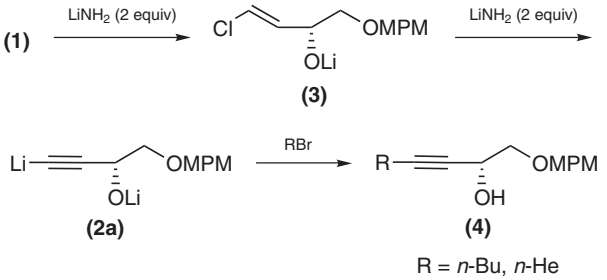


Scheme 21.24



(1) **(2)**

Scheme 21.25



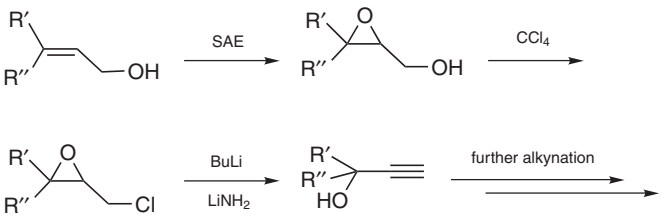
Scheme 21.26

A plausible mechanism for the formation of the product is shown in Scheme 21.26. The base $LiNH_2$ picks up the hydrogen α to the chlorine atom, followed by the elimination of a β-alkoxy group to produce a vinyl chloride (**3**). Further reaction of **3** with $LiNH_2$ results in the formulation of a dianion (**2a**). This can be trapped with various electrophiles to yield alkylated propargyl alcohols (**4**). The yields of the products were consistently over 70%.

Typical Experimental Procedure To freshly prepared $LiNH_2$ (prepared in situ by dissolving 12 mg atoms of lithium metal) in liquid NH_3 (30 mL) at −33°C was added a solution of chloride (**1**) (2 mmol) in THF (2 mL) for 3 minutes, after which solid NH_4Cl was added and ammonia was evaporated. The residue was partitioned between water and ether and the ether layer was dried and concentrated to obtain the residue, which was purified by silica-gel column chromatography to get pure **2** in 90% yield.

Inspired by these findings, Takano et al.[38] as well as our group have extended the methodology to chiral α,β-epoxy chlorides (easily accessible by Sharpless asymmetic epoxidation) and observed a similar result (Scheme 21.27).

It was also observed that controlled addition of base would allow one to isolate the vinyl chloride, which could further participate in Pd-catalyzed coupling reactions.[39] This methodology offers an advantage by providing both possible enantiomers, as the Sharpless asymmetric epoxidation works equally efficiently with (+)-DIPT and (−)-DIPT. The β-elimination strategy is also well exploited with sugar derivatives and Sharpless diols.



Scheme 21.27

21.6 CONCLUSIONS

In this chapter we have highlighted several practical approaches for the synthesis of chiral propargyl alcohols by various methods, the most practical ones being the asymmetric alkynation of carbonyl compounds in the presence of chiral auxiliaries and the β-elimination of stereochemically well-defined α,β-dioxyhalides. These two approaches have been utilized efficiently in the synthesis of several bioactive natural products.

Acknowledgments

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22

CARBOHYDRATES: FROM CHIRONS TO MIMICS

G. V. M. Sharma and Palakodety Radha Krishna

Indian Institute of Chemical Technology
Hyderabad, India

22.1 INTRODUCTION

Carbohydrates were long considered energy storage compounds and support structures because of their abundant presence as cellulose and starch. Notwithstanding their ubiquitous presence on the proteins and membranes of all eukaryotic cells, they are thought to have no significant biological function. These notions were abandoned with the discovery in 1969 that cell surface oligosaccharides are profoundly altered in cancer cells and may be related to cancer cell diffusion. This single discovery ushered in a third revolution in biology, and carbohydrates joined the ranks of proteins and nucleic acids as determinants of biological activity.[1] As the biological functions of carbohydrates continued to unravel, thanks to modern analytical tools, their stellar roles in viral and bacterial infections,[2] tumor cell metastasis,[3] and leukocyte adhesion during inflammation[4] and in blood group determinants[5] were uncovered.

With the increasing numbers of biologically active oligosaccharides being isolated and structurally characterized, their varied ways of synthesis also came into focus. However, unlike proteins and nucleic acids, carbohydrates are susceptible to biodegradation, thus limiting their therapeutic potential and use in biological studies of structure and function. Synthetic chemists have therefore begun to focus on the design of carbohydrate analogs that can tolerate the degradative forces in vivo.

Carbohydrate analogs, in which carbon atom substitutes for the glycosidic oxygen, are called *C-glycosides*.[6] During the last two decades, C-glycosides have been the subject of considerable interest in carbohydrate, enzymatic, and metabolic chemistry, as well as in organic chemistry, because (1) the discovery of naturally occurring C-nucleosides

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with important pharmacological properties[7]and the presence of C-glycosyl functionality in several naturally occurring nucleoside[8] antibiotics such as formycin and showdomycin[9] and natural products[10] gave impetus to synthetic efforts for preparing active carbohydrate analogs; (2) the requirement of C-glycoside chiral building blocks in the synthesis of biologically important macromolecules, such as palytoxin,[11] spongistatin,[12] and halichondrin,[13] has simulated the development of new synthetic methodologies; and (3) C-glycosides are potential inhibitors of carbohydrate-processing enzymes and stable analogs of glycans involved in important intra- and intercellular processes.[14]

Over the years, bioactive glycosubstances[15] have received great attention in chemical, medicinal, and pharmaceutical research. As a consequence, the design and implementation of stereoselective strategies for preparing bioactive carbohydrates by using readily available homochiral precursors constitute a prominent issue[16]. Among the various means by which a carbohydrate unit can be assembled, methods involving carbon–carbon bond formation between an enantiopure "short" precursor and a homolagative manipulable reaction are subjects of greater interest.

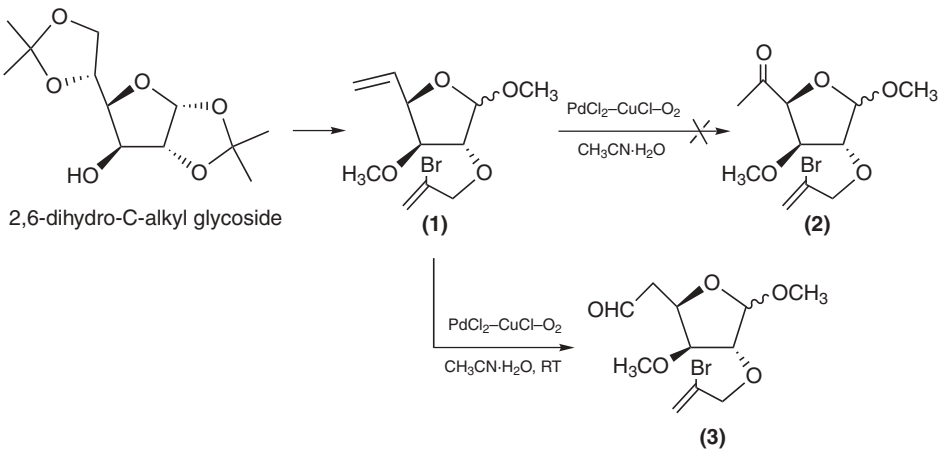
This chapter is focused on presenting of the findings of our group, such as metal-mediated routes to C-alkyl, vinyl, and aryl glycosides; de novo construction of disaccharides from furyl and propargyl sugars; [3 + 3] annulation routes to carbocycles and pseudosaccharides; 1,3-dipolar cyclo-addition to isoxazolidine sugars and saccharides; and radical routes to spiroacetal saccharides and ulosonic acid derivatives and glycosyl β-amino acids and β-peptides.

22.2 SYNTHETIC STRATEGIES FOR C-GLYCOSIDES

The discovery of several biologically active natural products having C-glycosides[17] as part structures, in addition to their use as mimics for natural counterparts in enzymatic and metabolic studies, has resulted in the development of many methods for their stereoselective construction. A novel strategy for synthesizing heterocycles under mild conditions is provided by the use of metal complex catalysts. Among these, palladium complexes undoubtedly lie in a unique position in terms of their versatility and reactivity,[18] and olefin complexes of Pd(II) are the most extensively developed, at least in part because they are both easily generated and highly reactive.

For the stereoselective synthesis of C-glycosides, deoxygenation of hemiketals, obtained from Wacker oxidation[19] of sugar-derived olefin alcohols, was envisaged as an easy and efficient protocol. In our earlier study on the synthesis of 4-epiethisolide, attempted $PdCl_2$-mediated conversion of the terminal olefin (**1**), obtained from sugar chiron into a methyl ketone (**2**) resulted in the exclusive formation of an anti-Markovnikov product (**3**), which happened to be the first report in the literature (Scheme 22.1).

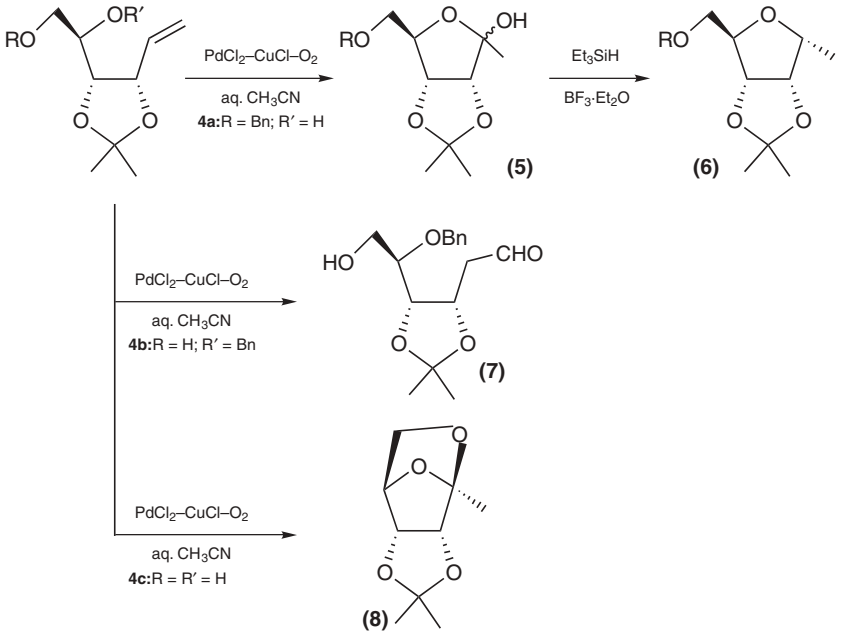
According to a literature survey,[20] the regiochemical outcome of such a reaction is dependent on the substituents that are present on the olefinic moieties, thereby leading either to ketones (Markounikou) or to aldehydes (anti-Markovnikov) exclusively or as a mixture of products. However, in the presence of an internal nucleophile,[21] the γ,δ-olefinic alcohol (**4a**; R = Bn, R′ = H) on reaction with $PdCl_2$–CuCl–O_2 in aqueous CH_3CN underwent a facile 5-exo mode of cyclization to form a five-membered ring hemiketal (**5**) by Markovnikov addition. Attempted cyclization of δ, ε-olefinic alcohol (**4b**; R = H, R′=Bn), however, underwent an anti-Markovnikov addition to result in an aldehyde (**7**), instead of forming a six-membered ring. In a similar study, an olefinic diol (**4c**; R = R′ = H), under the



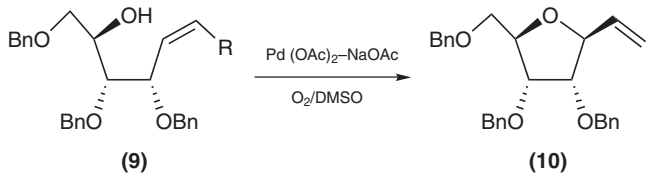
Scheme 22.1

reaction conditions above, was efficiently converted into a very useful 2,6-dihydro-C-alkyl glycoside (DAG, **8**; Scheme 22.2).

The hemiketal thus made (**5**) was deoxygenated successfully[22] with $Et_3SiH–BF_3–Et_2O$ to give the expected C-alkyl glycoside (**6**). The *cis*-stereochemical outcome of this C-glycoside was dependent on the stereochemistry of the adjacent center of hemiketal. The generality of this methodology was amply substantiated when several olefinic alcohols with a variety of stereocenters and protecting groups were subjected to oxidative



Scheme 22.2



(9) (10)

Scheme 22.3

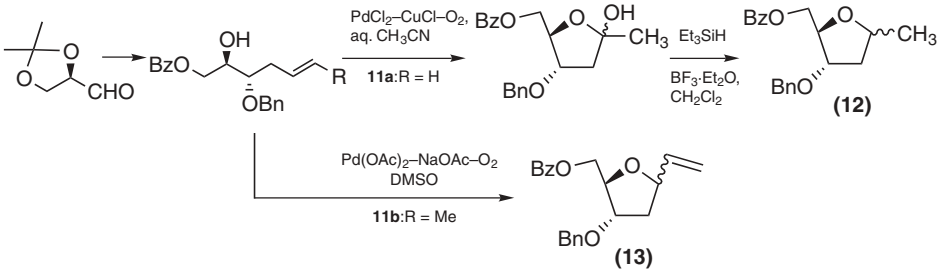
cyclization; they first resulted in hemiketals and then C-alkyl glycosides on deoxygenation with $Et_3SiH–BF_3Et_2O$ in good yields.

A literature survey[23] revealed that unlike $PdCl_2$, the oxidative cyclization of internal olefinic alcohols in the presence of $Pd(OAc)_2$ in dimethyl sulfoxide (DMSO) solvent, in the transition Pd complex, particularly of the two β-hydrogens available, elimination of terminal β-hydrogen is preferred. Such a reaction results in the formation of a double bond on the exo side, there by resulting in a vinyl group on the system. Thus, on reaction with $PdCl_2$, an internal olefinic alcohol, gives a Wacker product (hemiketal), while with Pd, $(OAc)_2$ was envisaged to give C-vinyl glycosides[24] by oxidative intramolecular cyclization reaction. Accordingly, when the olefinic alcohol (**9**) was subjected to oxidative cyclization[25] using $Pd(OAc)_2$–NaOAc in DMSO under an O_2 atmosphere, the result was, indeed, the formation of β-C-vinyl glycoside (**10**). To establish the generality, a variety of C-vinyl glycosides were prepared (Scheme 22.3).

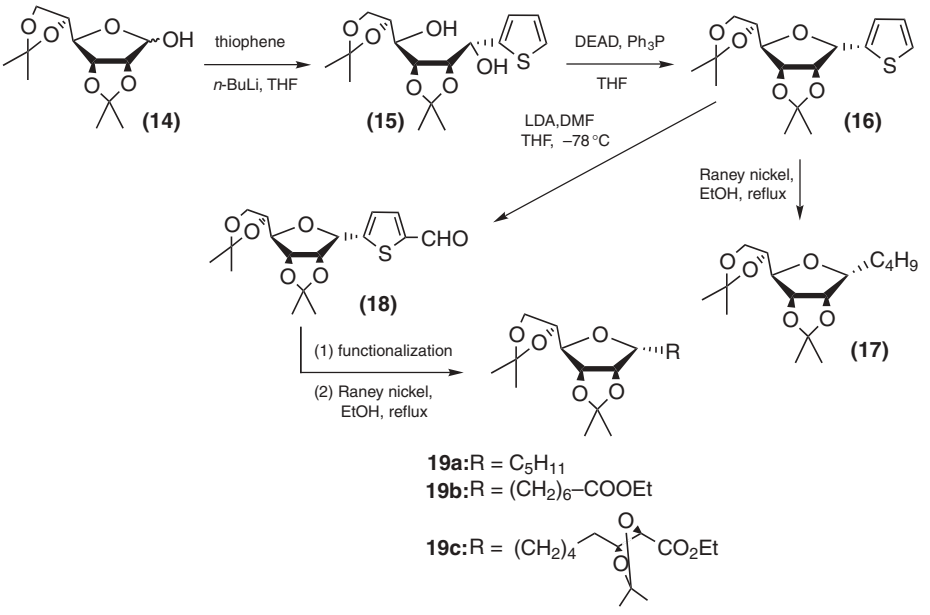
Further, the olefinic alcohols (**11a** and **11b**) derived from an (*R*)-glyceraldehyde derivative, upon $PdCl_2$- and $Pd(OAc)_2$-mediated cyclization, gave hitherto important 2-deoxy C-alkyl (**12**) and C-vinyl glycosides (**13**),[26] respectively, albeit as 1 : 1 mixtures, due to the absense of anchimeric assistance in stereodefining (Scheme 22.4).

In a further study, the strategy for the synthesis of C-alkyl and functionalized C-alkyl glycosides,[27] revolving around the utilization of thiophene as a masked C4 synthon, was planned, since the C2 and C5 carbons can be functionalized advantageously, and finally, ejection of sulfur would release a C4 carbon chain. Accordingly, mannose diacetonide (**14**) was treated with 2-lithiothiophene to give an alditol (**15**, 20 : 1), which on cyclization under Mitsunobu conditions (DEAD, Ph_3P) resulted in a C-thiophenyl mannoside (**16**). Desulfurization of the glycoside above gave the C-butyl glycosides **17**,[28] and the generality was established on other sugar lactols to give the corresponding C-butyl glycosides (Scheme 22.5).

Similarly, the C5 carbon of thiophenyl mannoside (**16**) was formylated [*N*, *N*-dimethylformamide (DMF), *n*-BuLi] to **18**, further functionalized and desulfurized to offer smoothly a variety of functionalized[28] C-alkyl glycosides (**19a to 19c**).

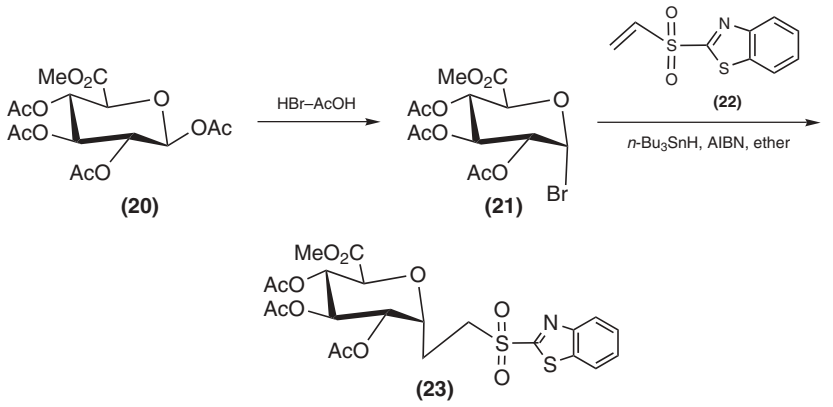


Scheme 22.4

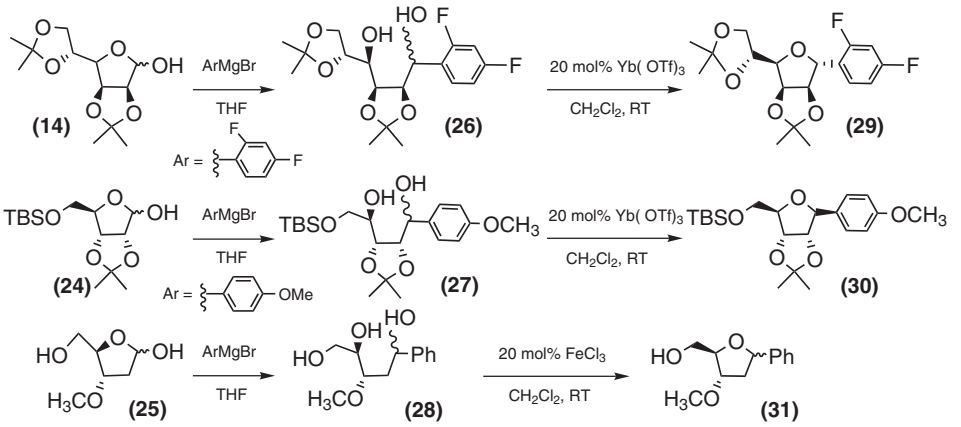


Scheme 22.5

The C-glycoside synthesis encompasses the utilization of both electrophilic and nucleophilic carbohydrates to the adoption of free-radical techniques. In a further study, on the functionalized C-alkyl glycosides, the addition of an anomeric carbon radical[29] onto the Michael acceptors was envisaged as an efficient and alternative tool. The importance of C-glycoside sulfones,[30] such as anomeric sugar phosphates as glycosyl transferase inhibitors, turned our attention to their synthesis. Accordingly, a variety of anomeric bromides prepared from protected mono- and disaccharides, such as D-glucose, D-galactose, L-arabinose, D-mannose, D-lactose, and methyl glucuronate, on reaction with *n*-Bu_3SnH and benzthiazolyl vinyl sulfone resulted in α-C-ethylsulfonylaryl glycosides[31] as exclusive products, due to the anomeric radical effect[32] (Scheme 22.6).



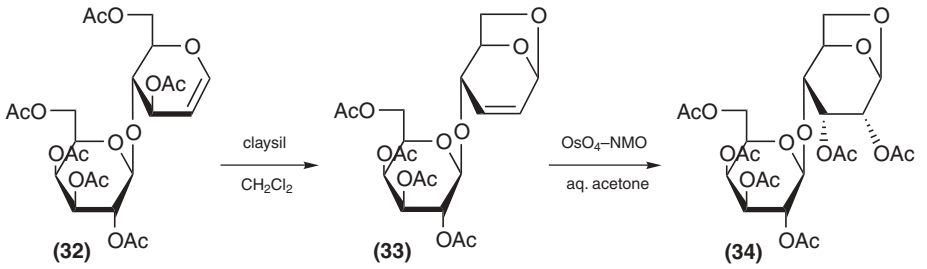
Scheme 22.6



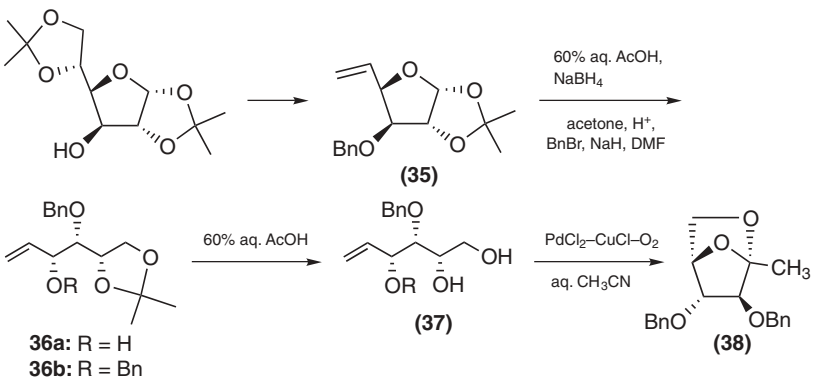
Scheme 22.7

Recent years have witnessed an upsurge in the synthesis of C-aryl and 2-deoxy C-aryl glycosides,[33] due partly to their presence as structural features in several natural products, and partly to their significant biological and pharmacological properties. A report by Kool et al.[34] on the synthesis of artificial DNA base having a difluorophenyl C-glycoside indicates the everlasting importance of C-glycosides in life processes. Cyclo-dehydration of 1,4-diols under catalytic acidic conditions was envisaged as an efficient and general protocol for the synthesis of C-aryl glycosides. Thus, a variety of 1,4-diols (**26** to **28**) having an aryl group, prepared from sugar lactols (**14, 24,** and **25**) were subjected to 20 mol% $Yb(OTf)_3$[35]-mediated intramolecular nucleophilic cyclization to afford a range of C-aryl (**29** and **30**) and 2-deoxy C-aryl (**31**) glycosides, whose stereochemical outcome is defined by the anchimeric assistance from the adjacent stereocenter (Scheme 22.7).

Rare saccharides and rare C-saccharides, in addition to C-glycosides, C-saccharides, and pseudosaccharides, find a prominent place in the arena on the synthesis of monosaccharides that are intermediates for the synthesis of natural products as well as glycosyl mimics. Glycals were converted with *claysil*, a heterogeneous catalyst developed from montmorillonite clay K-10 through an intramolecular Ferrier reaction to give the 1,6-anhydro sugars.[36] The sugars thus made gave, on dihydroxylation, the rare 1,6-anhydro saccharides (Scheme 22.8).



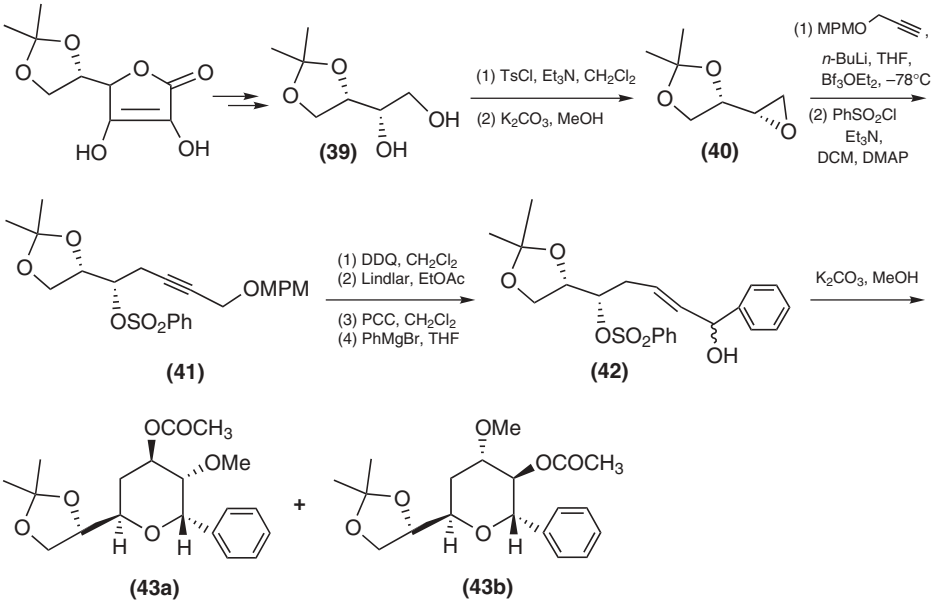
Scheme 22.8



Scheme 22.9

In a further study on rare C-saccharides, DAG was converted into 2,6-anhydro rare C-glycoside.[26] Accordingly, an olefinic alcohol (**37**), prepared from 5,6-ene (**35**) made from DAG, on $PdCl_2$-mediated oxidative cyclization afforded the rare C-saccharide, L-xylo derivative **38**. Thus, a D-sugar is efficiently converted into an L-sugar (Scheme 22.9).

Similarly, a C-aryl heptose sugar[37] is prepared for the first time from the diol obtained from ascorbic acid. The epoxide (**40**), made from the diol (**39**), on reaction with acetylenic anion and further transformations gave the olefinic alcohol (**42**). Sharpless epoxidation of **42** and cyclization furnished the C-aryl heptose sugars (**43a** and **43b**) in an efficient way (Scheme 22.10).

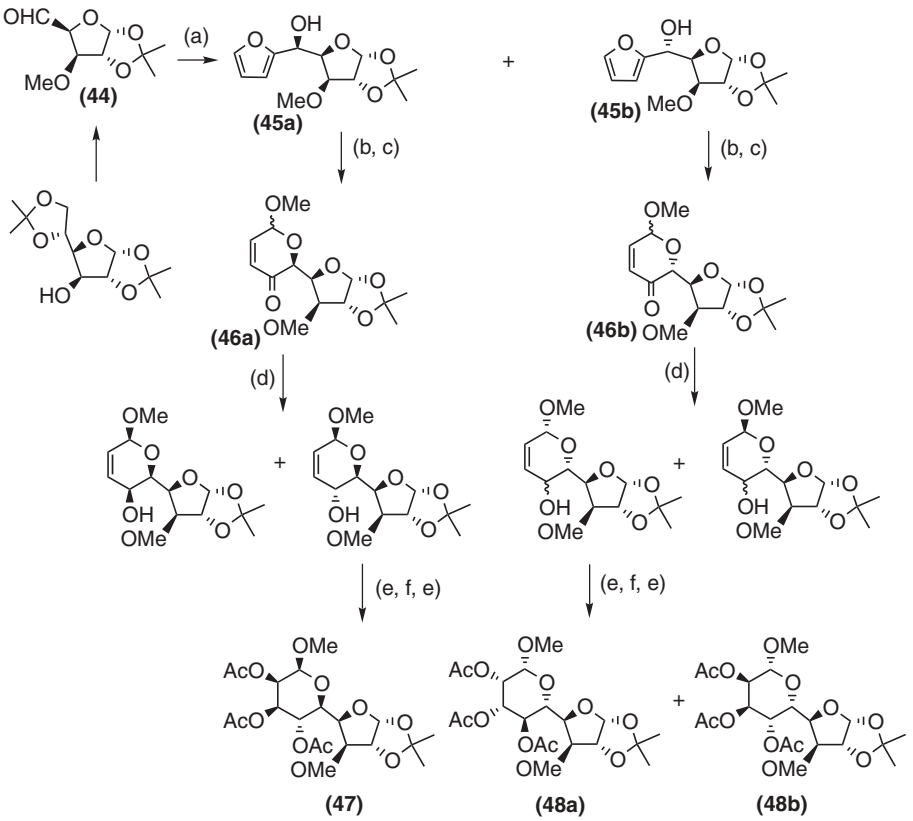


Scheme 22.10

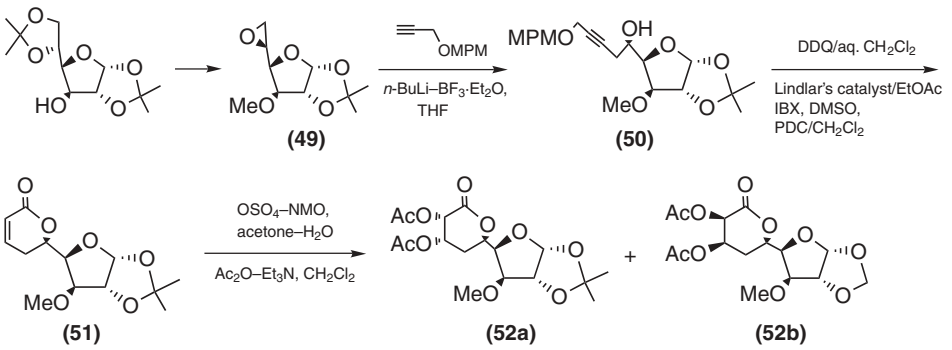
22.3 SYNTHETIC STRATEGIES FOR CARBON-LINKED DISACCHARIDES AND PSEUDOSACCHARIDES

Carbon-linked oligosaccharides are a class of nonnatural analogs of disaccharides in which a glycosyl linkage is replaced by a C—C linkage. Synthesis of this novel class of pseudosaccharides[38]–C-disaccharides in addition to the C-linked disaccharides, with[39] or without[40] spacers between sugar moieties—is of considerable importance. For the synthesis of C—C linked and spiro-carbon-linked disaccharides, the de novo construction of an additional saccharide unit by stereocontrolled manipulation of the carbon framework appended to the "chiron" derived from sugar was envisaged as a practical strategy.

Furan was identified as a masked sugar synthon for the stereoselective synthesis[41] of D- and L-C4-C5 linked disaccharides. The D- and L-furanyl saccharides (**45a** and **45b**) (1.8 : 1) obtained from the aldehyde (**44**) on oxidative unmasking gave the enone system (**46a** and **46b**). Stereoselective reduction of the enone followed by *cis*-hydroxylation offered the C—C linked D-(**47**) and L-(**48a** and **48b**) disaccharides. Thus, the de novo construction of a sugar moiety employing furan as a masked sugar synthon led to the distereospecific



Scheme 22.11 Reagents (a) 2-furyl lithium, THF,–78 ºC; (b) Br_2, pyridine,aq. acetone; (c) Ag_2O, MeI; (d) $CeCI_3{\cdot}7H_2O$ $NaBH_4$, MeOH, 0º; (e) Ac_2O, Pyridine; (f) OsO_4, NMO, *t*-BuOH:H_2O.



Scheme 22.12

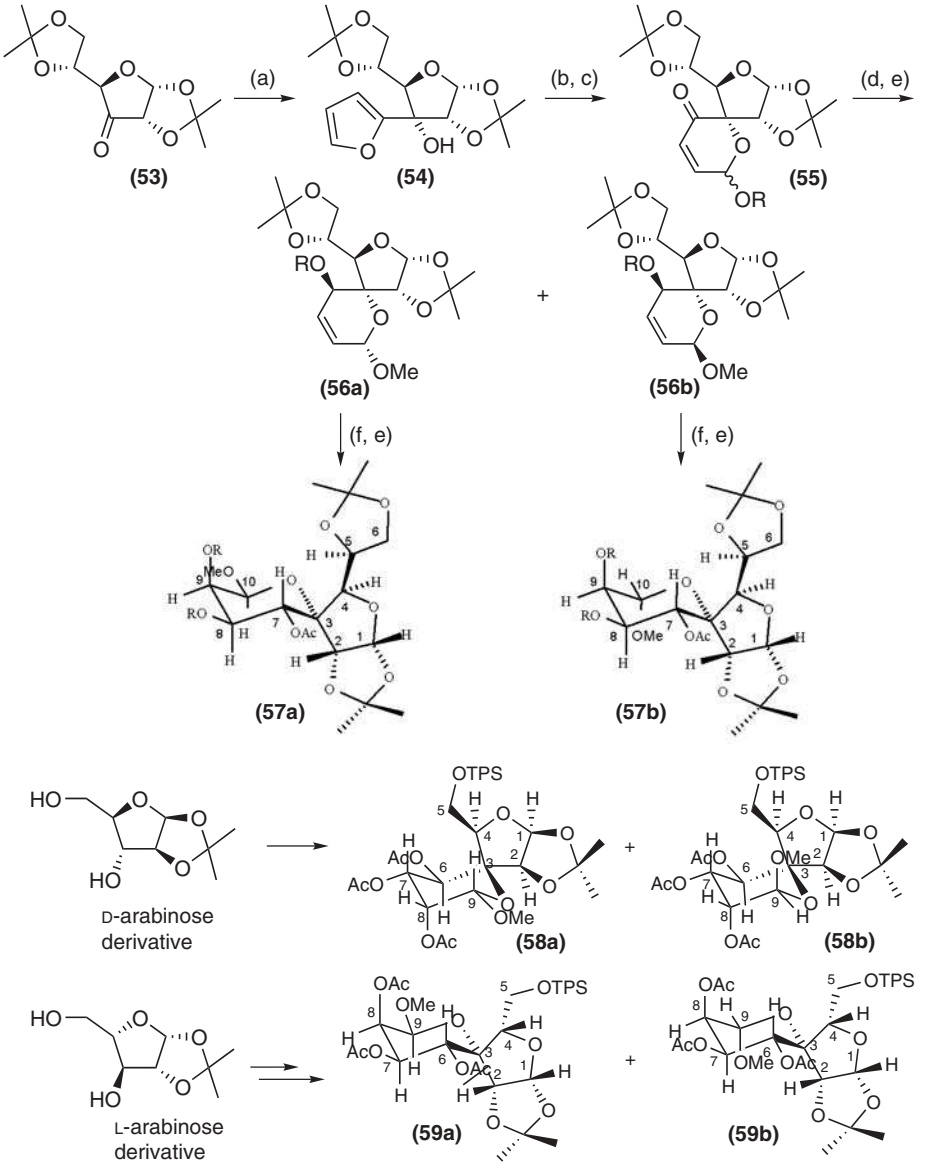
installation of the L-manno-, D-manno-, and D-gulopyranoside moieties on the C4 carbon center of D-xylofuranoside, where the chirality is transferred from the chiral sugar synthon (Scheme 22.11).

In a similar study, synthesis of deoxy C—C-linked disaccharides[42] was achieved using propyn-1-ol as homolagative reagent, both for C—C bond formation as well as for the introduction of diol group through the olefin. Thus, reaction of 5,6-epoxide (**49**) with propynol resulted in a homopropargylic system (**50**), which on reduction with Lindlar's reagent, oxidation to lactone, and finally *cis*-hydroxylation gave 6-deoxy C—C-linked disaccharides **52a** and **52b** (Scheme 22.12).

The attention focused on the synthesis of bioactive carbohydrates resulted in the synthesis of several glycosyl mimics; however, no attempt was ever made to synthesize spiro-C-disaccharides, in which the sugars are attached through a "spiro" carbon atom. Since the successful synthesis of C—C-linked disaccharides by de novo construction from furanylated sugars, the same strategy was envisaged as appropriate for the first enatioselective synthesis of spiro-carbon-linked disaccharides.[43] Accordingly, the ulose (**53**), derived from DAG, was treated with furyl lithium to give a furanyl sugar moiety (**54**). Oxidative unmasking, enantiospecific reduction[44] of the enone (**55**), and stereoselective *cis*-hydroxylation on the allylic acetates (**56a** and **56b**) paved the way for the first report on the synthesis of the spiro-C-linked disaccharides (**57a** and **57b**). Extension of the same strategy to the uloses derived from D- and L-arabinose expectedly offered the enantiomeric spiro-C-linked disaccharides (**58a** and **58b** and **59a** and **59b**, respectively; Scheme 22.13).

In continuation, on the synthesis of spiro-C-saccharides,[45] 4-yn-1-ol was envisaged as a four-carbon homolagative precursor for de novo construction of the deoxy sugar moiety. Thus, reaction of ulose derived from D-xylose with a lithiated acetylenic unit gave the propargylic carbinol **61**. Stereoselective reduction of the acetylene with LAH and Lindlar's gave *trans*- and *cis*-allylic alcohols (**62** and **63**), respectively. The *trans*-allylic alcohol (**62**) on dihydroxylation and further transformations gave 8-deoxy spiro-C-linked disaccharides (**64** and **65**). Similarly, the *cis*-allylic alcohol (**63**) on cyclization and dihydroxylation offered the targeted spiro saccharides (**68a** and **68b**) (Scheme 22.14).

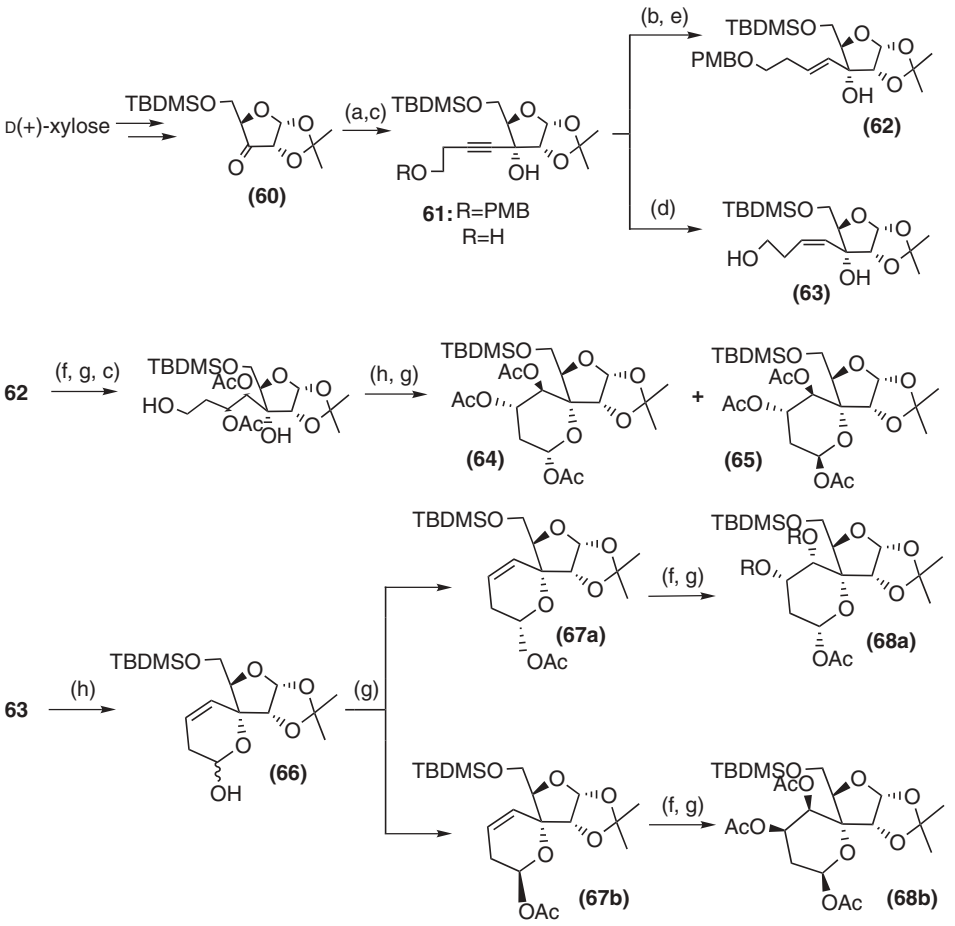
In a further study on the carbon-linked disaccharides, the Wittig ylide (**69**),[46] a C4 synthon, derived from ethyl acetoacetate, was envisaged as a homolagative precursor for the



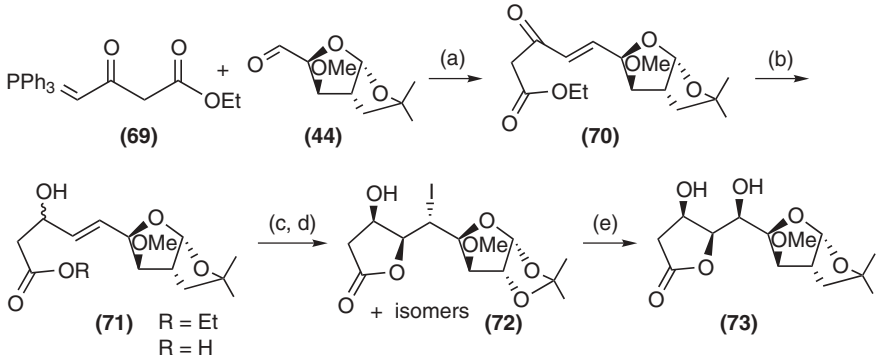
Scheme 22.13 (a) Furan, *n*-BuLi, THF, −40°C; (b) NBS, THF/H_2O (4:1), −5°C; (c) Ag_2O, MeI, CH_2CL_2, RT; (d) $CeCL_3.7H_2O$, $NaBH_4$, MeOH, 0°C; (e) Ac_2O, Et_3N, CH_2Cl_2; (f) OsO4, NMO, acetone/water (4:1).

construction of a sugar ring. Thus, reaction of the aldehyde (**44**) on Wittig olefination reduction and idolactonization and S_N2 displacement of the iodo group in **72** gave a carbon-linked disaccharide (**73**) (Scheme 22.15).

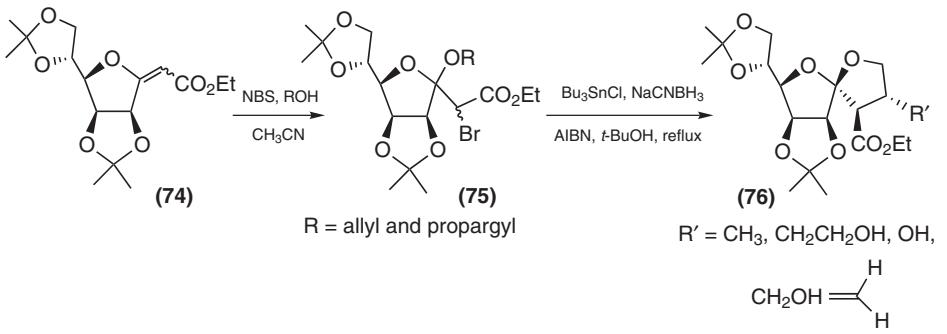
Spiroacetal[47] function is a characteristic substructure of several biologically important natural products that are produced from many sources,[48] among which papulachandrins A to D[49] represent a pyranoside-based spiroacetal class of natural products with



Scheme 22.14 Reagents (a) $HCCCH_2CH_2OPMB$, n-BuLi, THF; (c) DDQ, CH_2CI_2/H_2O (19:1); (d) H_2,Pd/$CaCO_3$, *n*-hexane, quinoline; (e) TBDMSCI, imidazole, CH_2CI_2; (f) OsO_4–NMO, acetone/water (3:1); (g) Ac_2O, Et_3N, DMAP, CH_2CI_2; (h) IBX, DMSO.



Scheme 22..15 Reagents: (a) benzene, reflux, 5h; (b) $CeCI_3{\cdot}7H_2O$, $NaBH_4$, MeOH, 0 °C to RT, 3 h; (c) 1 N aq. LiOH, DME, 0 °C to RT, 5 h; (d) I2, sat aq. $NaHCO_3$, THF, 0 °C, 5 h; (e) $NaHCO_3$, CH_3CN/H_2O (2:1), reflux.

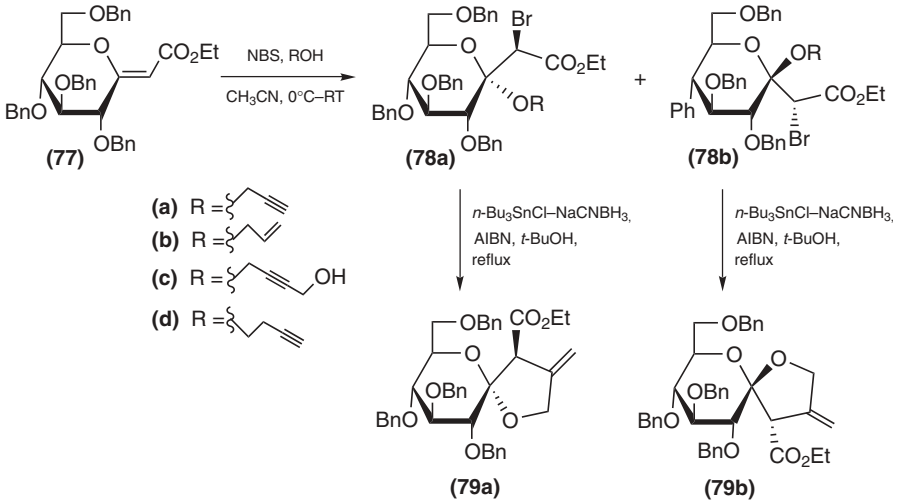


Scheme 22.16

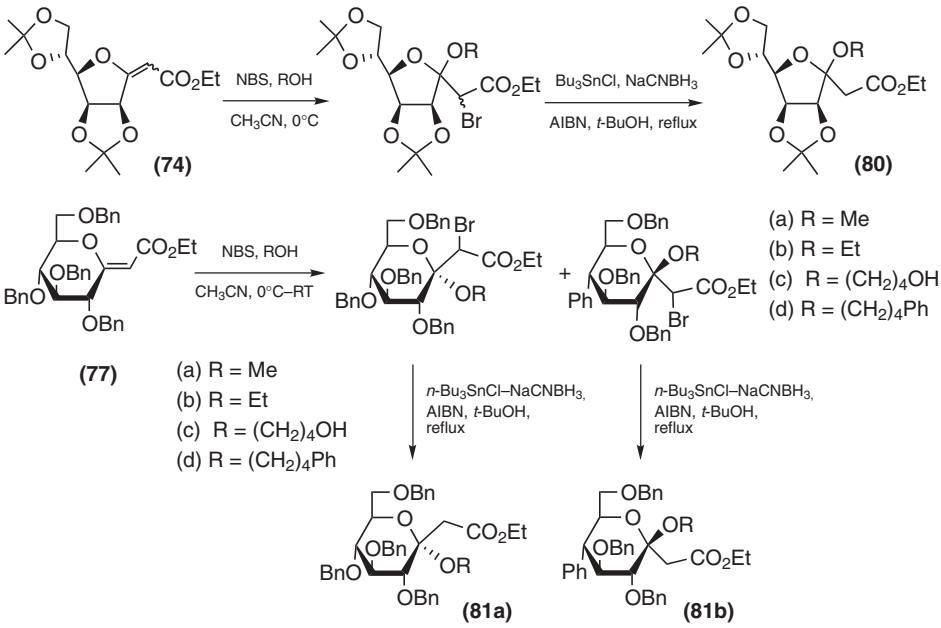
antibiotic activity. In our synthesis of spiroacetal saccharides,[50] intramolecular regio- and stereoselective radical cyclization[29] on the α-halo acetals was envisaged as an appropriate strategy starting from enol esters derived from monosaccharides. Accordingly, the enol ester (**74**), prepared from mannofuranoside, on a Michael type of bromoethification and subsequent radical reaction with *n*-Bu_3SnCl–$NaCNBH_3$ furnished α-C-spiroacetal saccharides (**76**) as exclusive products. Radical cyclization was effected on the 5-hexenyl and 5- and-6-hexynyl systems and extended to the 5-oxo systems, resulting in a variety of spiroacetal saccharides (Scheme 22.16).

In a similar radical-mediated cyclization route, unlike in the earlier case, an enol ester (**77**), derived from glucopyranoside, gave α-C-spiroacetal (**79a**) and β-C-spiroacetal (**79b**) saccharides [51] (Scheme 22.17).

Further, bromoetherification of enol esters (**74** and **77**) made from mannofuranoside and glucopyranoside and reductive debromination of α-bromoacetals furnished a very



Scheme 22.17



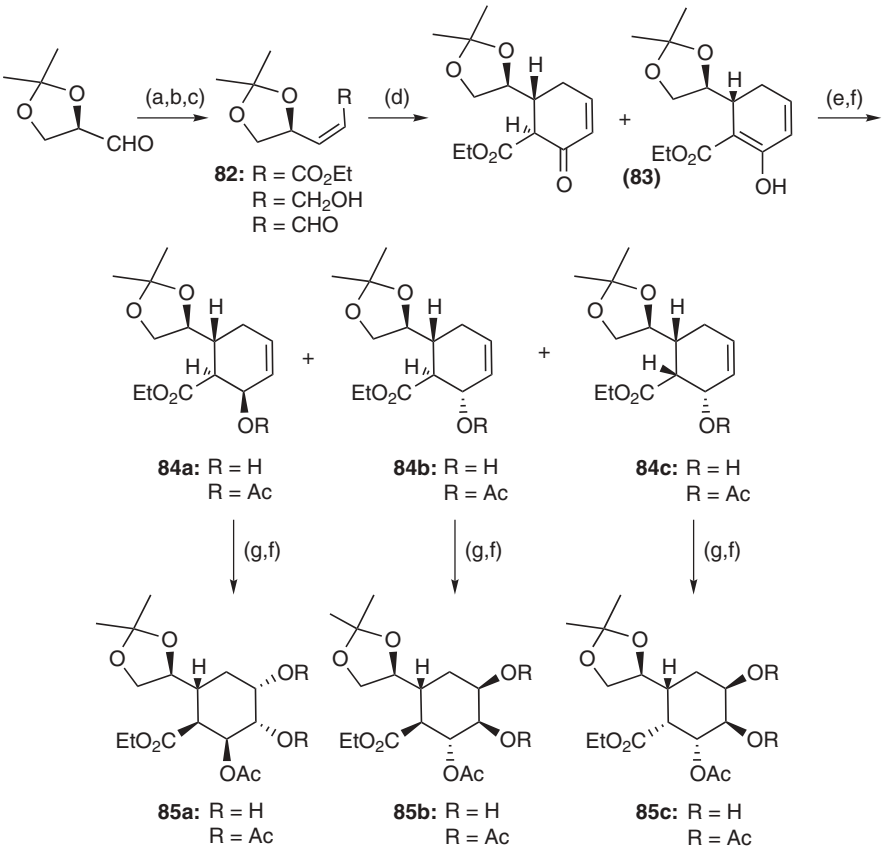
Scheme 22.18

important class of 2-deoxy-3-oct-ulsonic acid derivatives (**80**) and 2-deoxy-3-non-ulsonic acid derivatives (**81a** and **81b**),[52] respectively (Scheme 22.18).

Pseudosugars[53] are yet another class of stable carbohydrate isosteres which are resistant to both chemical and enzymatic hydrolysis at the pseudoanomeric center. Understandably, for the construction of highly functionalized carbocycle[54] the natural choice would be to take advantage of chiral templates to install the chirality endowed on their periphery. A novel [3 + 3]-annulation reaction was planned by a Michael–Wittig reaction between a chiral enal and ylide in which the chirality of the cyclohexane could be drawn from the γ-chiral hydroxy group of the enal.

A stereoselective [3 + 3] annulation by a Michael–Wittig reaction on the enal **82** derived from 2,3-*O*-isopropylidene-(*R*)-glyceraldehyde and ethyl-3-oxo-(triphenyl phosphorylidene) butanoate (**69**)[46] in one pot gave the cyclohexenone derivative **83**. Enone reduction and stereoselective dihydroxylation led to formation of highly functionalized cyclohexane systems (**85a** to **85c**)[55] (Scheme 22.19).

By employing a conceptually related strategem it was proposed to extend the synthetic methodology for more complex C—C-linked pseudosaccharide precursors with sugar-derived enal as the starting material (Scheme 22.20). Accordingly, the aldehyde **44** was converted into the enal **86**, which on further [3 + 3] annulation reaction with **69** in the presence of NaH and a drop of water in THF at 50°C for 10 minutes resulted in the formation of keto esters **87a** to **87d** as a mixture of diastereomers. The mixtures of cyclohexenone above were subjected to reduction under Luche's reaction conditions and further transformed through dihydroxylation into carbocycle moieties, to result in the formation of pseudosaccharide precursors (**88a** to **88e**).[56] Thus, by the adoption of a novel and elegant [3 + 3] annulation protocol through a Michael–Wittig reaction, a



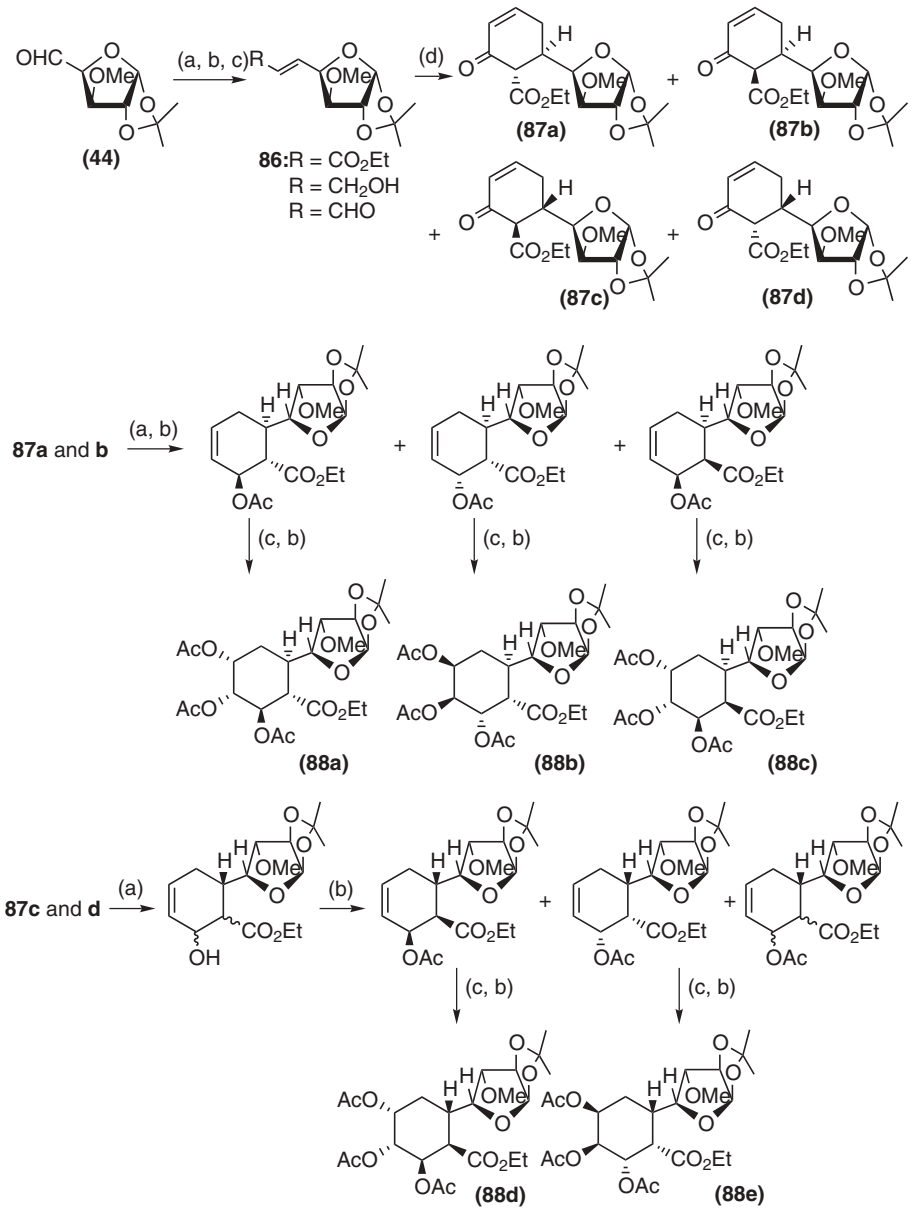
Scheme 22.19 Reagents (a) Ph_3= $CHCO_2E$ t, C_6H_6 reflux; (b) DIBAL-H, CH_2cI_2, –23 °C; (c) PDC, CH_2CI_2, reflux; (d) Ph_3=$PCHCOCH_2CO_2Et$, NaH, 2 drops of water, THF, 50°C; (e) $NaBH_4$, $CeCI_3 \cdot H_2O$, EtOH, 0 °C to RT; (f) Ac_2O–Py; (g) OSO_4-NMO, CH_3COCH_3/H_2O(3:1)

carbocycle ring system could be installed efficiently at the off-template site, C5 of the sugar synthon.

Isoxazoles and isoxazolidines[57] fused with sugar systems are found to show biological activity. In a continuation of our studies on the utilization of a variety of protocols on the sugar-derived "chirons" we embarked onto the 1,3-dipolar cycloaddition reactions. Accordingly, intramolecular oxime olefin cyclo-addition (IOOC) reactions[58] on the chiron (**91**; X=NOH) gave an isoxazolidine-fused saccharide (**92**)[59] (Scheme 22.21).

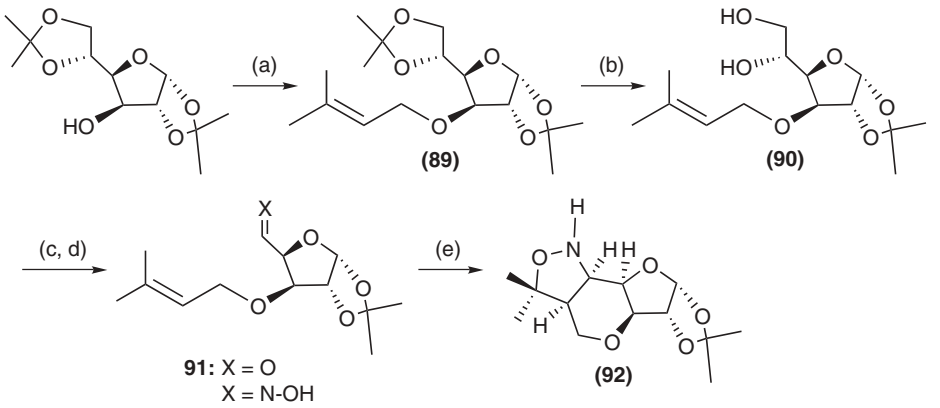
In a similar study the intramolecular nitrone cyclo-addition (INC) reaction at the off-template site afforded several isoxazolidine-fused saccharides (**93** to **98**)[60] (Scheme 22.22).

Similarly, sugar-linked iodolactones (**72**), prepared by a four-carbon elongation–idolactonization route, were converted into sugar-linked butenolides (**100a** and **100b**).[61] The butenolides thus made successfully underwent INC reaction to give bicyclic isoxazolidine saccharides (**101a** and **101b**)[62,63] (Scheme 22.23).



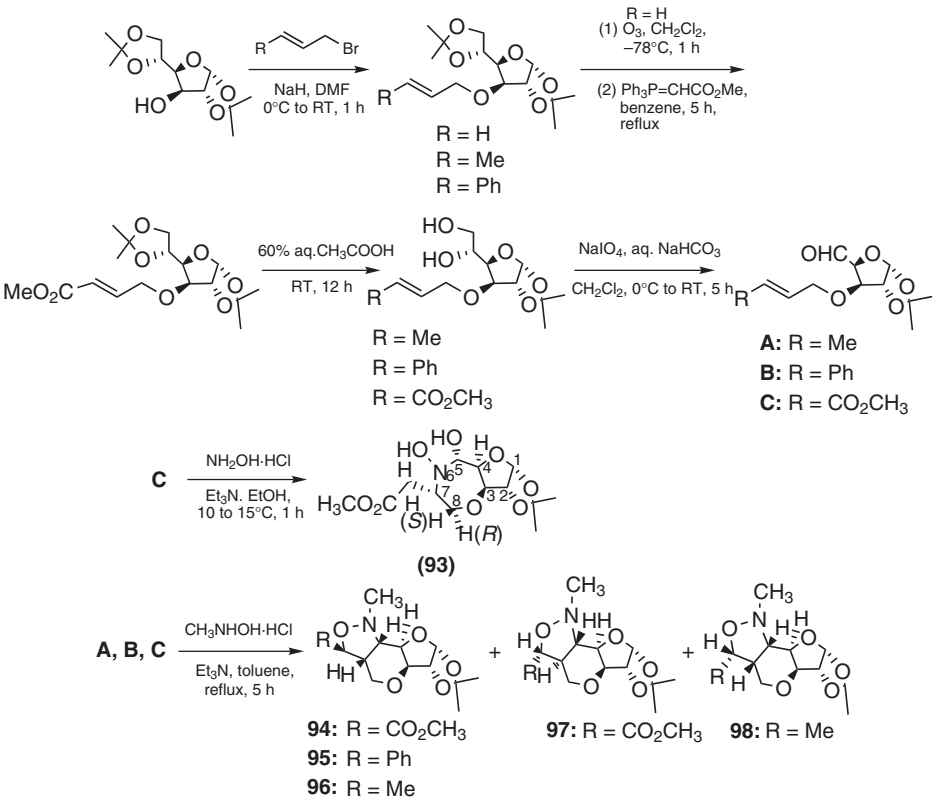
Scheme 22.20 Reagents: (a) $NaBH_4$, $CeCI_3{\cdot}H_2O$, EtOH, 0 °C to RT; (b) Ac_2O–Py; (c) OsO_4–NMO, $CH_3COCH_3/H_2O(3:1)$

It is amply revealed that the attachment of carbohydrates to peptides improves water solubility and bioavailability. Thus, glycosyl amino acids are very essential for the construction of glycopeptido mimics. β-Amino acids[64] are of premier importance as substructures of numerous biologically active natural products. Aza Michael addition onto γ-alkoxy

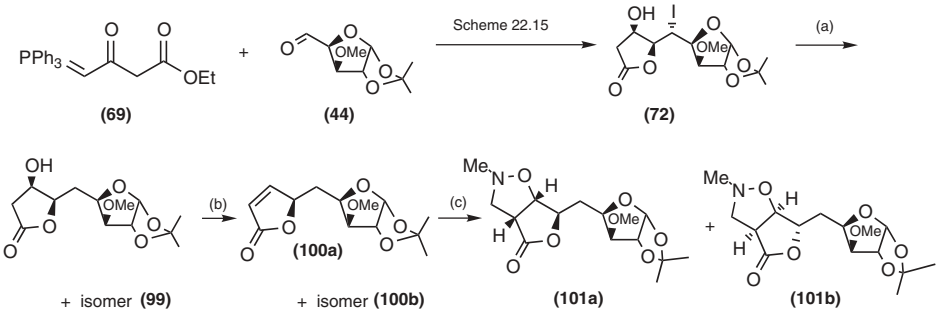


Scheme 22.21 Reagents: (a) NaH, phenyI bromide, DMF, 0 °C to RT; (b) 60% aq. AcOH, 30 °C; (c) $NaIO_4$, aq. THF, 30 °C; (d) NH_2OH HCI, Et_3N, EtOH, Heating; (e) toulene, reflux.

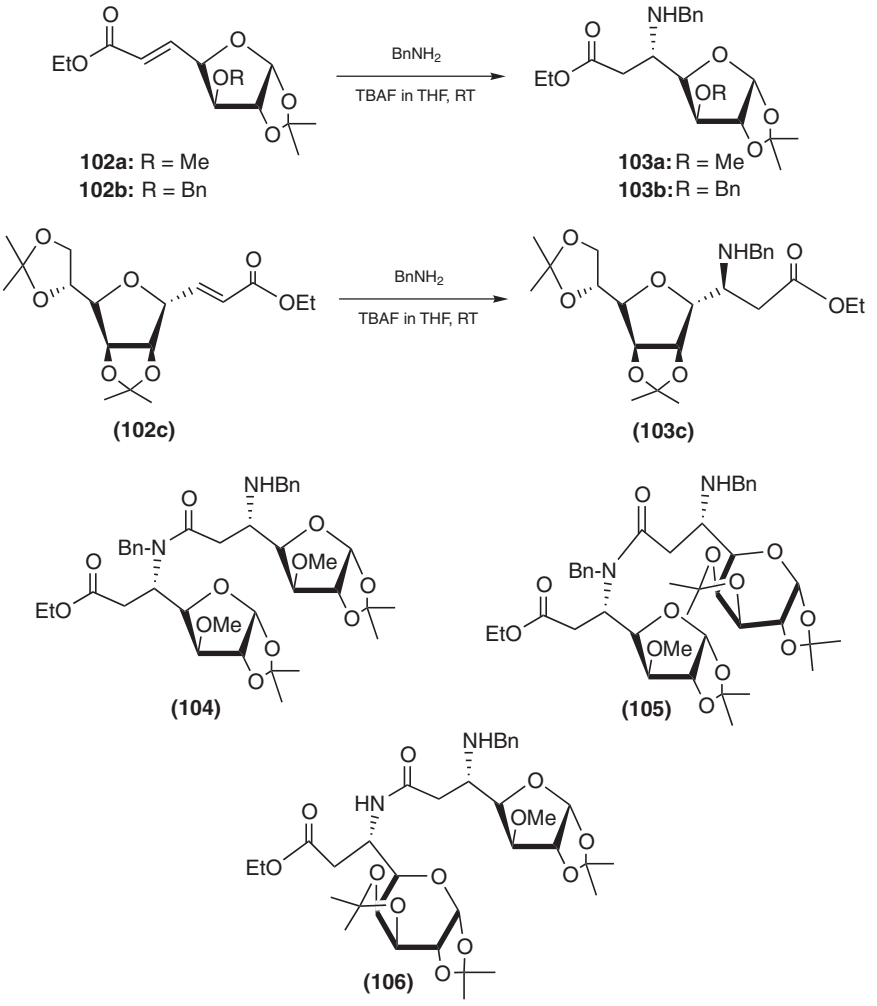
α,β-unsaturated esters (**102a** to **102c**) was envisaged as a proper protocol for the synthesis of glycosyl amino acids. TBAF was developed as an efficient base for the aza Michael addition and synthesized a variety of glycosyl β-amino acids (**103a** to **103c**),[65] which were later converted into β-peptides (**104** to **106**) (Scheme 22.24).



Scheme 22.22



Scheme 22.23 Reagents: (a) $NaCNBH_3$, cat. *n*-$BuSnCI_3$, AIBN, *t*-BuoH, reflux, 4 h; (b) Ac_2o, Et_3N, CH_2CI_2, RT, 5 h; (c) $(CH_2o)_n$ MeNHOH·HCI, C_6H_6, reflux, 4.5 h.



Scheme 22.24

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23

MEETING THE CHALLENGES OF PROCESS DEVELOPMENT AND SCALE-UP OF ACTIVE PHARMACEUTICAL INGREDIENTS

YATENDRA KUMAR
Wockhardt Research Centre
Aurangabad, Maharashtra, India

B. VIJAYARAGHAVAN
Ranbaxy, Inc.
Princeton, New Jersey

23.1 INTRODUCTION

There has been a perceptible move toward increasing the availability of generic pharmaceuticals across the world in order to contain the cost of medicines. This is especially so in the United States, which accounts for roughly 50% of world pharmaceutical sales (Table 23.1). The last two decades, beginning with the watershed Hatch–Waxman Act of 1984, have seen several legislative acts that encourage the use of generics. Although generic drugs have sometimes been called by such names as *copies*, *cheap clones*, and *imitations*, they must be comparable in quality and bioequivalent to products marketed by innovator companies, so regulatory agencies world over have strict laws governing generic drugs.

23.1.1 Drug Development in the Pharmaceutical Industry

There are two components in any medicine: the *active pharmaceutical ingredient* (API), which as the name indicates, is the component responsible for the activity of the medicine; and a component consisting of one or more inactive ingredients, commonly known as *excipients*, that help deliver the drug in the desired manner in the body so that it is effective.

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TABLE 23.1 Top 10 Generic Players in the United States, 2006

Rank	Company	Sales (Dec. 2006) (billion U.S. dollars)
1	Teva	5.551
2	Sandoz (Novartis' generic division)	2.634
3	Mylan	2.145
4	Watson	1.832
5	Greenstone (Pfizer's generic division)	1.585
6	Apotex	1.521
7	Par	1.209
8	Barr Labs (including Pliva)	0.907
9	Boehringer Ingelheim	0.906
10	Hospira	0.653

Source: IMS International.

The API is the domain of the organic chemist, and the excipients are the domain of formulation development scientists. There are several books that discuss process research and development.[1,2] In this chapter we discuss, from a generic drug development perspective, the challenges that are faced by process development chemists while developing APIs.

Generic drugs normally have the same active ingredient as the drug marketed by the innovator, but the excipients may be different. Any generic equivalent of a marketed drug must be bioequivalent to the drug (i.e., its plasma profile must be comparable to that of the marketed drug).

23.1.2 Challenges in Developing and Scaling Up Chemical Processes

The generic industry, by its very nature, is a very competitive arena. Opportunities flash in and out continuosly. Process development teams have to develop APIs that:

- Are comparable, if not better, in quality to the product being marketed by the innovator
- May have different solid-state characteristics but still are bioequivalent
- May have different related substances if the process chemistry is different, but must still meet international regulatory guidelines
- Are very cost-competitive
- Are generally tailored to fit in available facilities

By the time that generic companies start working on any given product, several process patents may have been filed, thus restricting the choice of routes. Many of the obvious and commercially feasible routes are usually patent protected, and the processes disclosed in the earliest patents normally disclose commercially unworkable processes. In addition, solid-state characteristics such as different polymorphs and solvates, particle sizes, and bulk densities of APIs and key intermediates are protected extensively by patents. Therefore, the process development chemist faces a twofold challenge: (1) identify a route that is free of any patent issue, and (2) ensure that the product obtained by using such a process is also free of any patent issues related to polymorphism, solvates, and so on.

As noted above, of late, patenting solid-state characteristics, impurities, metabolites, and so on, has been used increasingly as a product-life-cycle management strategy. Associated challenges for the process development chemists are development of alternative polymorphs,

detection of patented polymorphs, and stability issues associated with new forms and impurities that could be different from the ones present in the marketed product.

There are two main areas within process R&D:

1. *Process development*, which deals with literature search, evaluating several routes for the synthesis of a given molecule in the lab and selecting the most commercially feasible one, and optimizing the process through intensive experimentation so that it delivers consistently high-quality product in high yields.
2. *Process scale-up*, which deals with transferring the laboratory process to a semi-commercial or commercial scale facility while achieving the same cost, quality, and physicochemical characteristics as those achieved in the lab.

Despite the issues discussed above, generic companies have been successful in developing products within very tight time lines. How has this been possible? It is our experience that several factors contribute to the success, or failure, of process development work: (1) cross-functional skills and teamwork, (2) system-driven process development work, (3) planning and monitoring, and (4) nimbleness in decision making.

Despite working as part of a cross-functional team, process development chemists need to be much more than just chemists to be successful. That they must have very good knowledge of synthetic organic chemistry is a given. They also need to understand the dynamics of the generics game; must possess good process development skills, especially a clear understanding of the impact of various parameters on product quality and yield; and must be well versed in the regulatory requirements, patent issues, analytical issues, scale-up issues, safety issues, and market dynamics.

23.2 PROCESS DEVELOPMENT CYCLE

Typically, process development for an API takes about 9 to 12 months from route identification to commercial-level scale-up. The process development cycle is shown in Fig. 23.1. In the figure, the solid lines represent the normal flow of a process: from laboratory scale (10 to 100 g) to kilo-lab scale (500 g to 2 kg) to pilot-plant scale (5 to 50 kg) to full

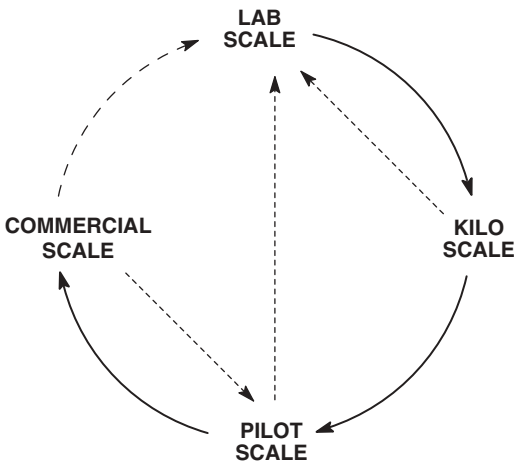


Figure 23.1

commercial scale (>50 kg). The interior dashed lines represent situations when rework or troubleshooting is required due to scale-up issues.

Process development activities are planned to run parallel to each other to the greatest extent possible. Standard operating procedures, guidelines, and protocols need to be in place for all key activities, from lab scale until commercialization. The activities are micromanaged through project management tools. In this section we discuss various key factors associated with process development and their impact on the product quality and yield. Instead of discussing the complete synthetic sequence of any particular product, we illustrate typical problems that could arise in any development process with real-life case studies from our experience.

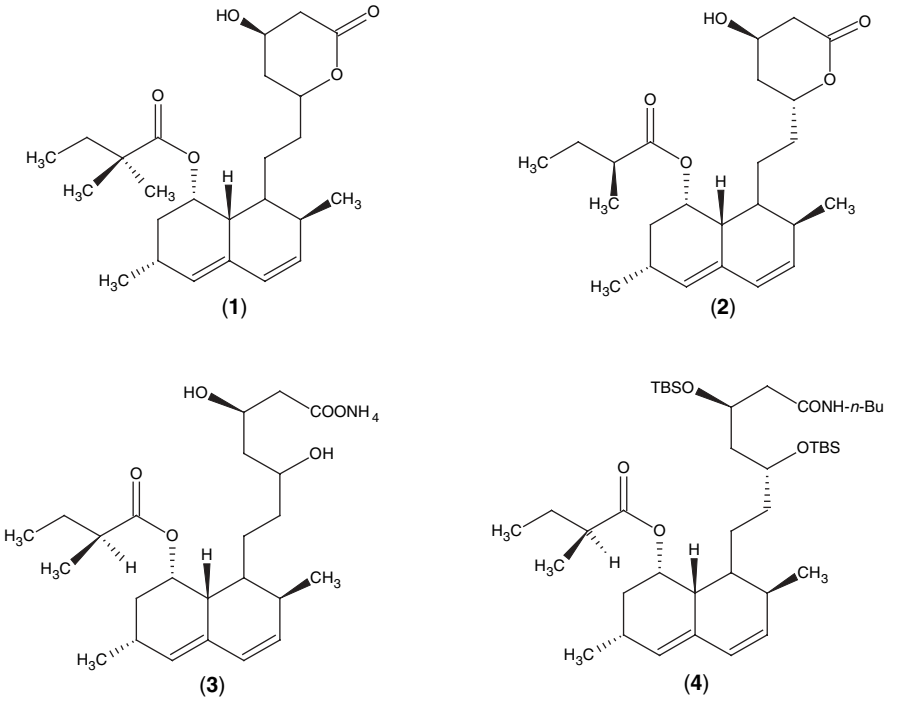
23.2.1 Stage I. Literature Survey and Analysis: Preparing the Blueprint

Process development work starts with identification of the most appropriate synthetic route. Route selection needs to consider the number of steps, quick access to key raw materials and reagents, their cost, and so on. The synthesis would preferably be convergent rather than linear, have minimum number of steps, and have a still lower number of intermediate isolations. It would also avoid column chromatographic purifications, high-vacuum distillations, and other energy-intensive operations. Safety and environmental aspects associated with the reagents, solvents, and perhaps more important, side products, are also important criteria. These criteria are relatively more tangible and thus easier to deal with. There are other parameters that are much more intangible: how robust the process would be, how the reactions will actually behave, what will the actual yields will be, and the possible impurity profile, among others. As any change in the chemistry at a later stage could affect the time lines significantly, route selection plays an important role in the overall process development program.

Case Studies 1 and 2 illustrate how the number of steps in a reaction sequence could be cut short without affecting product quality.

Case Study 1 Simvastatin (**1**, Scheme 23.1) is a high-selling HMG CoA reductase inhibitor. One of the previously disclosed processes[3] discloses the direct methylation of the natural 2-(*S*)-methylbutyryloxy side chain of mevinolin in a single chemical step using a metal alkyl amide and a methyl halide to give simvastatin. This process suffers from poor conversion rate of the C-methylation step. Additionally, many side reactions take place, due to methylation at other sites of the molecule. The C-methylation conversion rate may be improved to some extent by a second or third charge of the amide base and methyl halide. Even so, the overall yields are moderate. Also, the purity of simvastatin obtained by this process is close to borderline for use as a human health care product. The other patent[4] discloses a process wherein high-conversion C-methylation of the 2-(*S*)-methylbutyryloxy side chain of mevinolin takes place with a single charge of amide base and alkyl halide. The process described in this patent comprises six steps and is not economical, as it involves the protection and deprotection of the two hydroxy groups of the intermediate, lovastatin butylamide (**4**), using an expensive silylating agent, *t*-butyldimethylsilyl chloride.

Thus, there was a need to develop a cost-efficient synthesis while not compromising on quality. The schemes reported and patented were lengthy. Process chemists at Ranbaxy found that they could eliminate certain steps deemed necessary by the innovator and still get the quality desired. Thus, an extremely cost-efficient and practical process

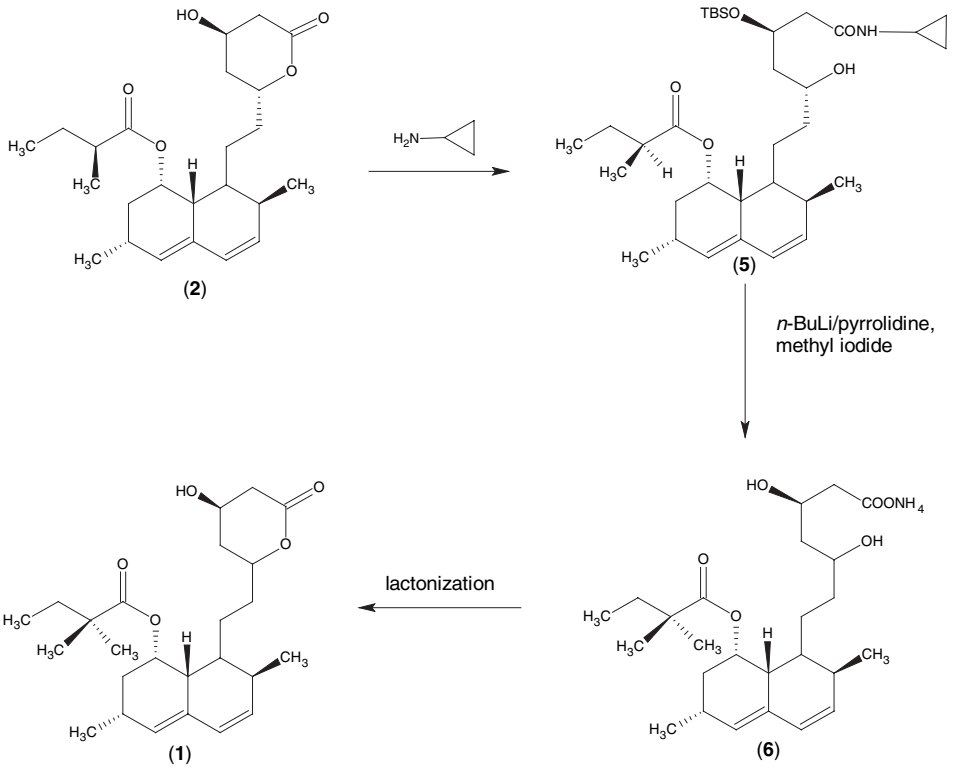


Scheme 23.1

for the large-scale preparation of simvastatin (**1**) from lovastatin (**2**) or the ammonium salt of its open dihydroxy acid form, mevinolinic acid (**3**), was developed. This process is of significance, as it involves only four chemical steps, eliminating the two expensive chemical steps of protection and deprotection of the open dihydroxy acid form of lovastatin. Simvastatin is obtained in high overall yield and pharmaceutically acceptable purity with less consumption of reagents, time, and labor. The reaction sequence is depicted in Scheme 23.2.[5,6]

Case Study 2 Repaglinide (**8**, Scheme 23.3) is the first member of a new class of oral hypoglycemic agents (meglitinides) for type II non-insulin-dependent diabetes mellitus (NIDDM). It stimulates the secretion of insulin from pancreatic beta cells, acting via calcium channels. Hypoglycemic events are fewer after the administration of repaglinide than after the administration of other antidiabetic agents, and repaglinide offers a significantly better biological profile than that of the sulfonyl urea class of hypoglycemic agents.[7–9]

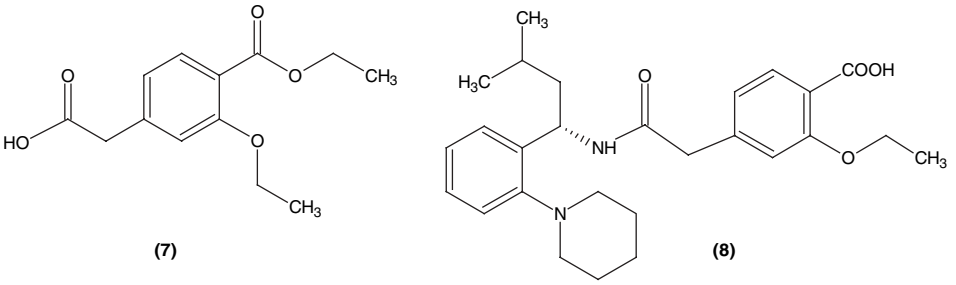
Synthesis of repaglinide involves condensation of an appropriately substituted and chirally pure benzylamine derivative with an appropriately substituted phenylacetic acid derivative (**7**) followed by saponification.[10] The reported process for the key intermediate (**7**) described a five-step process with an overall yield of about 30% of theory (Scheme 23.4).[11] We undertook development of an alternative synthetic strategy to prepare **7** and developed an efficient and commercially feasible synthesis starting from 2-hydroxy-4-methylbenzoic acid (**9**) in two steps.[12,13] Thus, **9** was first alkylated with ethyl bromide in a polar aprotic solvent and in the presence of an inorganic base to afford ethyl 2-ethoxy-4-methylbenzoate



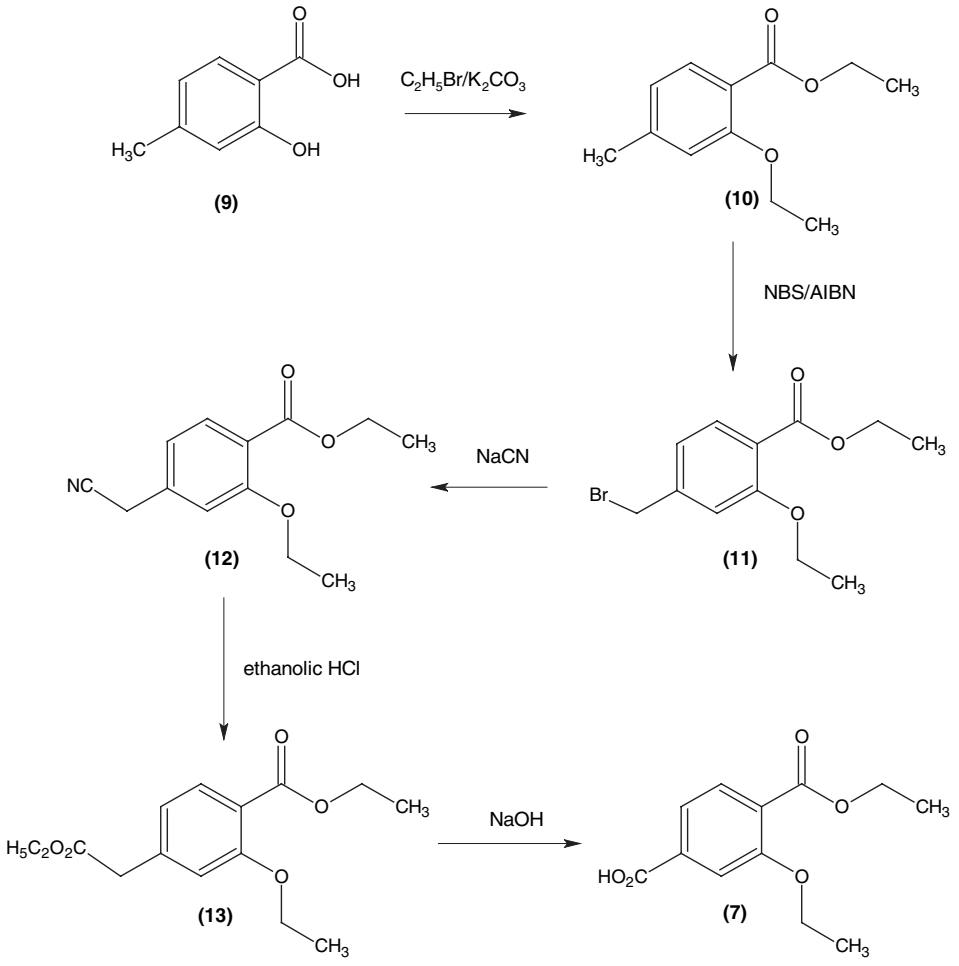
Scheme 23.2

(**10**); deprotonation with lithium diisopropylamide (LDA) and quenching the resulting carbanion with carbondioxide provided the desired compound (**7**) with improved yield and excellent purity (Scheme 23.5). This procedure is significantly better than a previously published synthesis which involves five steps and requires use of expensive and hazardous reagents.

Case Study 3 illustrates how several problems associated with a single step could be solved in one stroke simply by modifying the reaction conditions appropriately. The



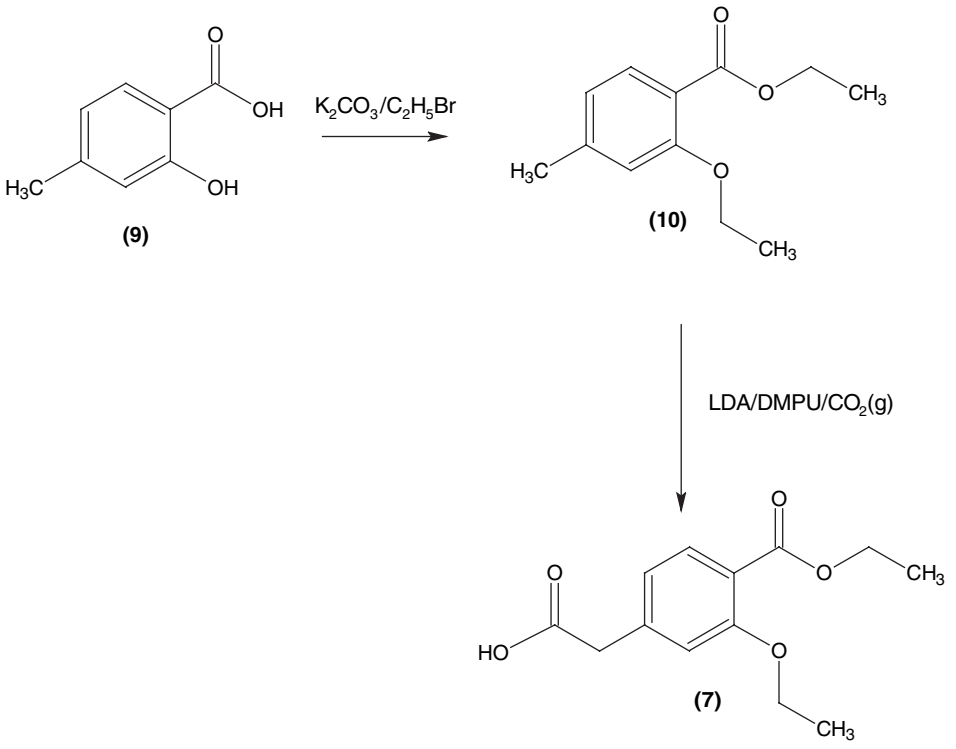
Scheme 23.3



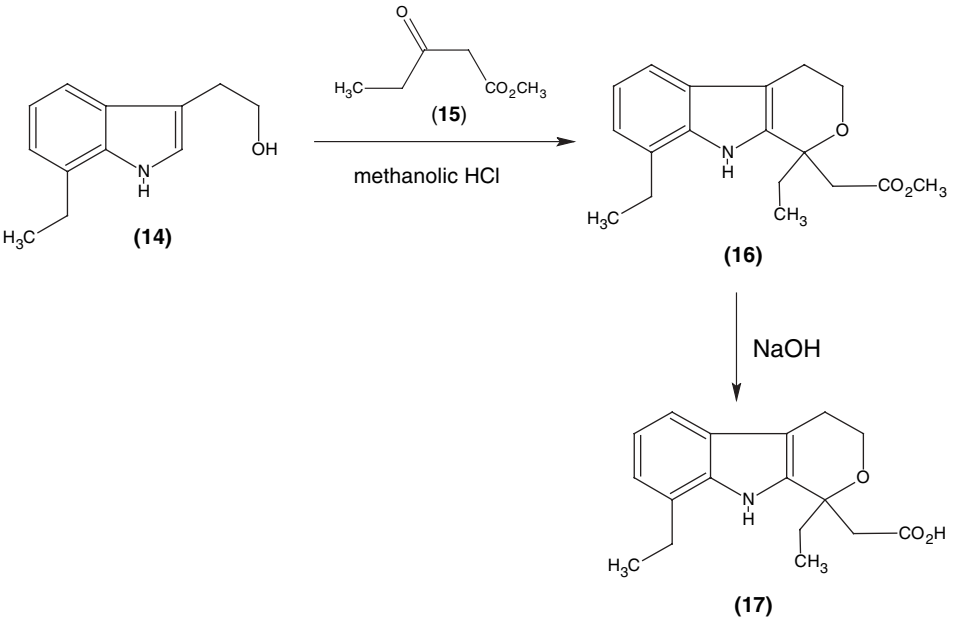
Scheme 23.4

problems to be solved here were: low-assay, semisolid starting material that was not very stable, isolation of the product from the highly impure reaction mixture, and elimination of column chromatographic purification.

Case Study 3 Etodolac (**17**) is an anti-inflammatory and an analgesic. The methyl ester of etodolac (**16**) is a key intermediate in the synthetic sequence. Several literature processes were available for the synthesis of this intermediate. Typically, the methyl ester of 3-methoxy-2-pentenoic acid (**15**) was condensed with 7-ethyltryptophol (**14**) in an aprotic solvent using an acid catalyst[14,15] (Scheme 23.6). 7-Ethyltryptophol was generally obtained through a Fischer–indole reaction and had significant levels of side products (the assay was usually around 60%). This necessitated column chromatographic purification. If crude tryptophol was used in the condensation step, the resulting product was highly impure and purification was difficult, since the starting material and product were both soluble in the reaction solvent. Moreover, use of strong acid catalysts exposed the starting material



Scheme 23.5



Scheme 23.6

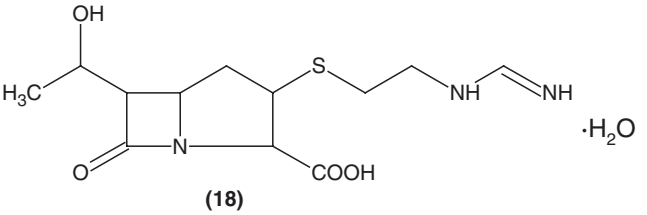
to other acid-catalyzed reactions, such as dehydration and polymerization, resulting in the formation of impurities. To minimize the impurities, the ester was either purified by column chromatography before hydrolysis, or the hydrolyzed product was purified at a cost to the yields and overall efficiency of the process, making the process very cumbersome and disadvantageous at a commercial scale because of its inefficiency and higher manufacturing costs. Therefore, there was a need to develop a simple process through which highly pure etodolac ester could be obtained directly without a need for tedious workup and column chromatographic separation. Process chemists at Ranbaxy developed a very simple and elegant solution to the problem. They found that if a protic organic solvent such as methanol is used as the reaction medium, the reaction went to completion much faster (<2 hours compared to the 6 to 7 hours in aprotic media), thus minimizing the competing acid-catalyzed dehydration and polymerization reactions. More important, the product ester crystallized out directly from the reaction medium and was very pure (>95%), eliminating the need for the use of purified 7-ethyltryptophol, tedious isolation procedures, and column chromatographic purification at any stage. This seemingly minor change resulted in a commercially feasible process for etodolac that was easily scalable.[16]

23.2.2 Stage II. Process Development: Laying the Foundation

As discussed above, the chemistry has to be such that it is cost-effective, easy to operate at a commercial scale, safe, and environmentally friendly while providing a high-quality product. At this stage the process is molded to take care of regulatory, IP, and quality issues. The solid-state characteristics of the API, including polymorphism, solvates, and particle size distribution, are studied thoroughly, including polymorphic integrity.

To develop a robust and high-yielding process in the shortest possible time, it is important that multiple options be evaluated in the beginning: not only on paper, but also experimentally. This enables the development team to have contingency plans readily available should an issue arise at a latter stage. Although this might seem a waste of resources, in our experience this could be a great time-saver. At this stage, the chemistry, analytical, and intellectual property teams work closely together. It is not uncommon for the route to change in midstream. Case study 4 illustrates the importance of evaluating an approach that is completely different from that available in the literature.

Case Study 4 Imipenem monohydrate (**18**, Scheme 23.7), which is the *N*-formimidoyl derivative of thienamycin, is the first clinically available member of a new class of β-lactam antibiotics that possess the carbapenem ring system. Imipenem exhibits an extremely broad spectrum of activity against gram-positive and gram-negative aerobic and anaerobic species, which is due partly to its high stability in the presence of β-lactamases.



(18)

Scheme 23.7

When we started working on imipenem, there were at least five processes for its preparation and isolation.[17–21] The key problem was the isolation of pure imipenem in a commercially feasible manner. Imipenem was first obtained using a lyophilization technique.[17] The product obtained by lyophilization is found to be largely amorphous and is stated to be thermodynamically unstable. The process also involves an initial purification through column chromatography using hydrophobic resins. A thermodynamically stable crystalline monohydrate form of imipenem was later reported,[18] obtained by crystallization of a lyophilized sample of imipenem. However, we found this process not to be satisfactory for commercial production, as it requires isolation of the product via column chromatography followed by lyophilization and crystallization. Moreover, the long processing time required for isolation of the final product led to degradation of the imipenem, thus diminishing product purity. Yet another procedure[19] describes a process for obtaining crystalline imipenem by purifying the crude product using column chromatography. In refs. 20 and 21, processes using lyophilization and freeze-crystallization techniques are described. All these processes were found to be tedious, cumbersome, and unsuitable for industrial use.

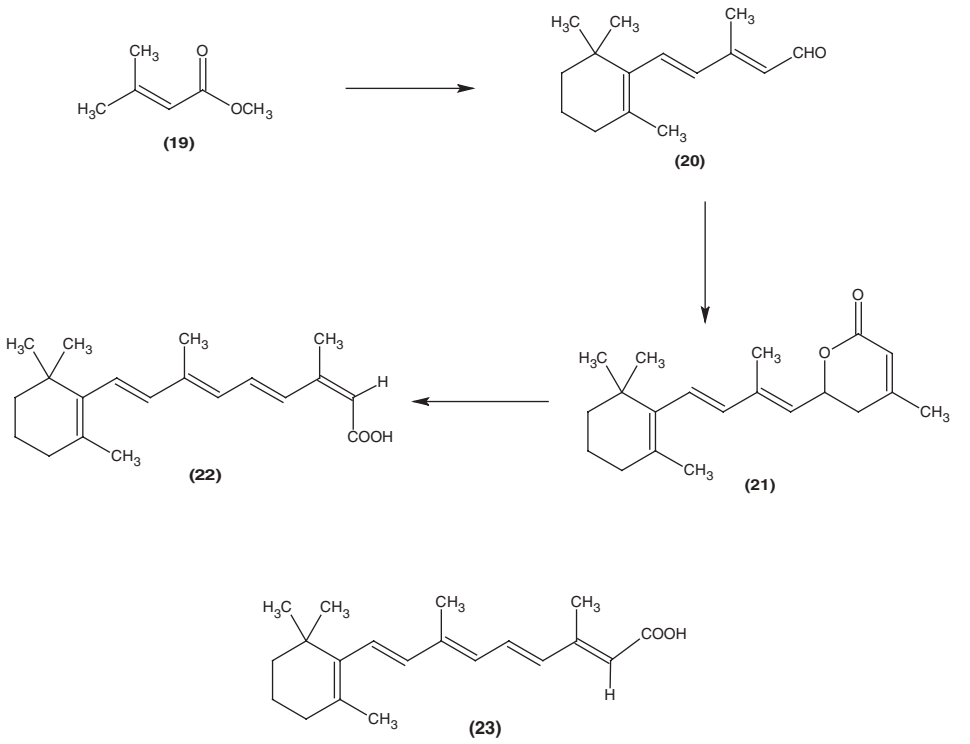
Process development work at Ranbaxy began with the aim to develop a simple, practical, and efficient method for the preparation of crystalline imipenem monohydrate, which is thermally stable, has a uniform degree of crystallinity, and is of high purity. After extensive experimentation, a process was developed for the isolation of pure crystalline imipenem monohydrate from a solution containing imipenem.[22] The process does not use capital-intensive techniques of lyophilization or freeze crystallization nor the time-consuming purification process of column chromatography using expensive hydrophobic resins.

Case Study 5 illustrates another case where several seemingly acceptable processes were evaluated simultaneously.

Case Study 5 Isotretinoin (13-*cis*-retinoic acid, **22**, Scheme 23.8) belongs to a family of vitamin A (retinol)–related compounds. It inhibits sebaceous gland function and keratinization and is used for the treatment of dermatological diseases such as acne. It is extremely effective in very severe and nodulocystic acne and prevents scarring. More recently, isotretinoin has been evaluated for its potential use in certain cancerous conditions.

Structurally, isotretinoin is a highly conjugated molecule consisting of a substituted cyclohexene moiety and a nine-carbon polyene side chain with a terminal carboxy group. All but one of the double bonds (the C13 double bond) in the side chain are *trans*, and it is the stereospecific construction of this polyene side chain that has challenged synthetic organic chemists for the past almost three decades. Commercially and readily available β-ionone has been used conveniently for the construction of the cyclohexene part of isotretinoin. Several approaches were available in the literature for the synthesis of isotretinoin.[23–29] They all suffered from one or another drawback that made them commercially unworkable.

In general, a convergent approach involving stereospecific coupling of the appropriate C_{15} (synthesized from β-ionone) and C_5 synthons, has been utilized. A linear sequence comprising seven steps, starting from β-ionone, has also been described.[23] Pattenden et al. have disclosed a procedure for the preparation of 13-*cis*-retinoic acid by reacting a C_{15}-triarylphosphonium salt (Wittig salt) and a C_5-butenolide in diethyl ether to produce an isomeric mixture (of the *cis* and *trans* isomers at the C11 double bond) of 13-*cis*-retinoic



Scheme 23.8

acid in 66 to 75% yield.[24] The desired 11-*trans*–13-*cis* content is reported to be only about 36%, the rest being the corresponding 11,13-di-*cis* isomer. Selective isomerization of the 11-*cis* double bond in the presence of 13-*cis* double bond proved extremely difficult to accomplish.

A great deal of effort has been directed to effect selective isomerization of the 11-*cis* double bond (without isomerizing the 13-*cis* double bond) in 11,13-di-*cis*-retinoic acid. The methods include photoisomerization using either iodine,[25] transition metal catalysts,[26] or photosensitizers such as erythrosin B or rose bengal.[27] These processes suffer from a number of limitations and for various reasons are not suitable for commercial production of isotretinoin. For example, the process for selective photoisomerization using iodine under diffused light is extremely difficult to accomplish without affecting the 13-*cis* double bond. This results in the generation of all-*trans*-retinoic acid (tretinoin) as a major impurity in isotretinoin produced by this process. Although U.S. patent 5,424,465[27] mentions that use of photosensitizers enhances the selectivity of photoisomerization of the C_{11}-*cis* double bond, no data are provided for the extent of tretinoin formation in this process. The use of palladium catalysts[26] could lead to the contamination of the desired isotretinoin by traces of transition metals and might lead to stability problems. In addition, the process involves an elaborate extraction procedure for the workup.

Another process[28] describes a process involving use of a phosphonate ester (as a C_{15} synthon), which is first generated in several steps starting from β-ionone. The phosphonate ester is then reacted with 5-hydroxy-4-methyl-2-(5*H*)-furanone (C5 synthon) to afford

isotretinoin. Although this approach does not involve the cumbersome photoisomerization step, it is uneconomical at a commercial manufacturing scale because of the large number of steps.

Yet another study reported the synthesis of isotretinoin by reacting a dienolate of sodium 3,3-dimethyl acrylate (C_5 synthon) with β-ionylideneacetaldeyhyde (C_{15} synthon) at −78°C for 12 hours to give a hydroxy acid intermediate. Conversion of the hydroxy acid intermediate to intermediate lactone and subsequent treatment with base afforded isotretinoin.[29] This approach suffers from the limitations that two different bases (sodium hydride and lithium diisopropylamide) are required and that generation of dienolate requires maintaining low temperatures (−78°C) for extended periods of time, which would entail very high energy costs at a commercial scale. Furthermore, the purification of the intermediate lactone by preparative high-performance liquid chromatography, as suggested, is not commercially feasible.

The challenge was to solve the problems associated with reported processes and to provide an efficient method for the high-purity synthesis of isotretinoin in a single step (stereospecific coupling of C_{15} and C_5 synthons) using conditions that are convenient in commercial operation. Such a process was developed (Scheme 23.8),[30] in which the level of tretinoin (**23**) impurity was controlled below to 0.1%.

The reaction sequence comprises the condensation of dienolate of methyl-3,3-dimethylacrylate (**19**) with β-ionylideneacetaldehyde (**20**) in a suitable solvent at −60 to −80°C for 1 to 2 hours and at −25 to −45°C for 24 hours, followed by aqueous acidic workup to give isotretinoin in a single step. The condensation reaction proceeds via the formation of the intermediate, lactone (**21**), which is not isolated. Lactonization results in the release of a methoxide ion, which in turn opens the lactone to afford isotretinoin (as carboxylate salt); the reaction of methoxide and lactone is facilitated by higher temperatures (25 to 45°C) and by carrying the reaction for a longer period. Aqueous acidic workup thus produces isotretinoin (**21**) in a single step starting from β-ionylidene acetaldehyde.

23.2.3 Stage III. Process Optimization: Constructing the Building Brick by Brick

The main purpose of the process optimization program is to identify the reaction parameters that would provide the best possible yield and quality in each step. To achieve quality and yield repeatably and reproducibly, planned optimization process parameters and strict controls on critical operating parameters are a must in each step of the process. Process optimization is done by using either the traditional one-at-a-time approach or statistically designed experiments, depending on the nature of interactions between parameters. This is ensured by studying each key parameter involved in any reaction and identifying the optimal conditions. The parameters that are always studied are:

1. Raw material quantity
2. Raw material quality
3. Temperature
4. pH
5. Dilution factors
6. Rate of addition of reagents and reactants
7. Reaction time (duration)

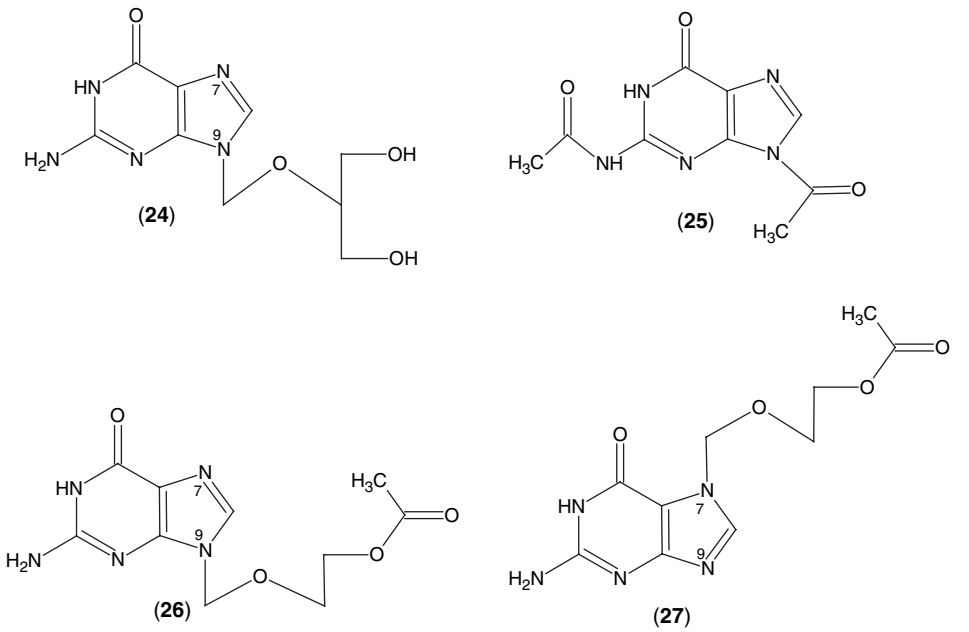
There are several other factors that are studied depending on the nature of the reaction and the outcome desired. One may sometimes need to study the effect of the presence of moisture, agitation profile, exposure to air, and similar factors.

Selection of the right parameters for in-depth study determines the success or failure of a process optimization program. If the right parameters are not studied at this stage, one may end up with problems at the scale-up stage. Therefore, key factors that could affect the reaction adversely during scale-up are studied as part of the optimization studies: for example, rate of heating and cooling, solvent recovery rates, and material of construction of the equipment, especially the reactors. Thus, the process optimization program enables the cross-functional team to understand clearly the role that each parameter plays in the process. More crucially, the team also identifies critical operating parameters: those whose control is crucial for the success of the scale-up program.

The following case studies illustrate the diverse aspects of process optimization work.

Case Study 6 Ganciclovir (**24**, Scheme 23.9), known chemically as 9-[(1,3-dihydroxy-2-propoxy)methyl] guanine, is one of the more important nucleoside analogs of biological acitivity. It exhibits a selective antiherpetic action, including clinical efficacy against cytomegalovirus. Ganciclovir is readily phosphorylated in injected cells by the herpes simplex virus thymidine kinase, and its 5′-triphosphate acts as a chain terminating nucletide analog, inhibiting the viral DNA-polymerases. Ganciclovir has also found a promising application in the "suicide gene" therapy of brain tumors, and recently, of lung tumors and preintoneal carcinomatus.

When we started working on ganciclovir, a literature search showed that several processes were available for its preparation. The simplest synthetic approach to the



Scheme 23.9

N9-substituted guanine compound involves the direct alkylation of appropriately substituted 2-aminopurines (e.g., guanine derivatives).

The first chemical syntheses of ganciclovir were reported almost simultaneously by several research groups.[31–33] These involved glycosylation reaction of N^2-monoacetyl- or 9,N^2-diacetylguanine with a chloromethyl or acetoxymethyl derivative of glycerol. All these processes suffered from practical limitations: low overall yields, lack of regioselectivity usually observed in alkylation of guanine, and difficulties in separation of the resulting 7- and 9-regioisomers of the product. Another approach used a fusion reaction between protected guanine derivatives and alkylating agents followed by conversion of the intermediates so obtained to acylic nucleosides (acyclovir and ganciclovir). Conversion of N7 isomer to N9 isomer was achieved by heating a suspension of the N7 isomer in an alkylating agent.[34,35] Extensive experimentation showed that although these methods could be used to prepare ganciclovir, they suffered from either lack of regioselectivity or low overall yields of ganciclovir (about 35%), and so were not commercially tenable.

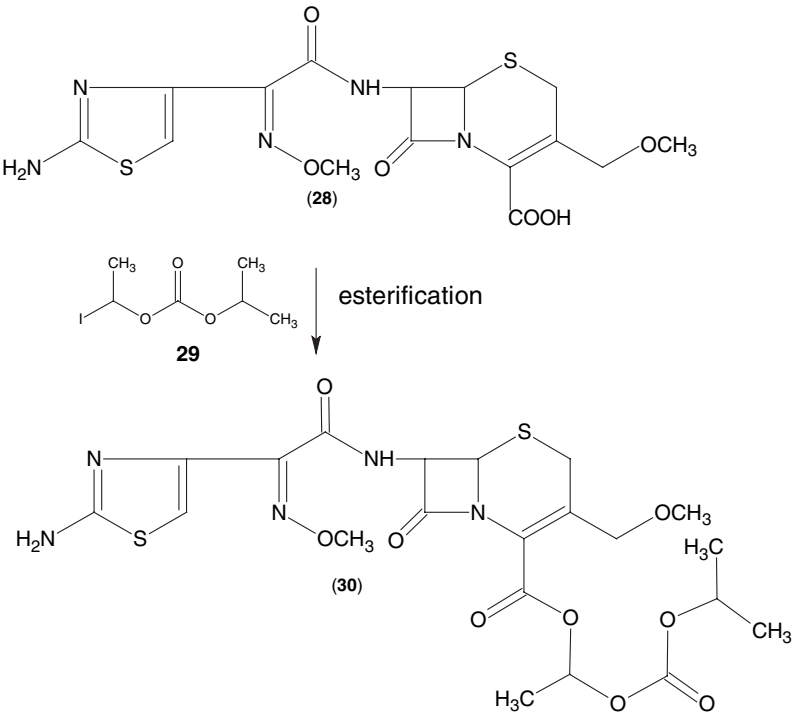
Therefore, our objective was to develop a simple and cost-effective regiospecific process that would provide the desired product in good yield from a commercial point of view and be free of by-products (e.g., undesired isomers and side products). We developed a process that was reasonably specific for the desired N9 regioisomer intermediate[36] (**26**) from an inexpensive and readily accessible guanine derivative, diacetylguanine (**25**). We found that instead of focusing on developing a method that was very regiospecific, any N7 regioisomer intermediate (**27**) that formed during the reaction could easily be recycled to give the desired N9 regioisomer.

It was observed that the recycling of the N7 regiosiomer during alkylation exclusively facilitates the formation of N9 regioisomer, and no more diacetyl guanine is converted to N7 regioisomer. This recycling of the side product along with suitable crystallization gives several advantages over any of the other methods reported, since it enhances the overall yield of ganciclovir ($>$70%).

Another example of controlling process impurity through optimization is discussed in Case Study 7.

Case Study 7 Cefpodoxime proxetil (**30**, Scheme 23.10) is a valuable antibiotic characterized by high broad-spectrum activity against gram-positive and gram-negative microorganisms. It has also been found to be highly active against susceptible and resistant streams of *Neisseria gonorrhoeae*. The proxetil ester is formed by esterification of the free acid (**28**) with iodoethyl isopropyl carbonate (**29)** in the presence of a base.

Formation of the corresponding Δ^2-isomer (Scheme 23.11) as an impurity is a major difficulty associated with the synthesis of cefpodoxime proxetil. We determined that the formation of this impurity is governed by the quantity of base that is being used. If slightly less than a mole equivalent of the base (with respect to the free acid) and the iodocarbonate (**29**) were used in the esterification, formation of the Δ^2-isomer could be controlled to less than 0.5%, but the reaction did not go beyond 90 to 92%. If the base quantity was increased to take the reaction to completion, unacceptably high levels ($>$ 5%) of the impurity were formed. The need was to develop a synthetic method in which basicity is controlled even when more of the acid-scavenging base is added to drive the reaction to completion. This clearly meant that we needed a buffer that would maintain the pH of the medium constant. We found that by using disodium hydrogen phosphate along with sodium carbonate,

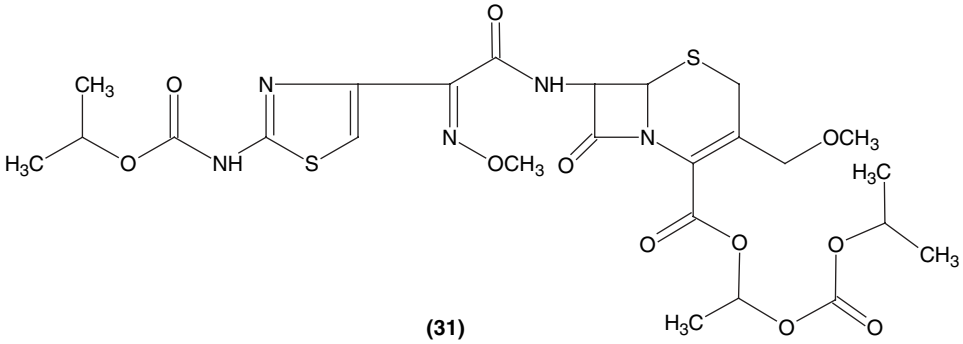


Scheme 23.10

we could take the reaction nearly to completion (>99%) while controling the Δ^2-isomer impurity level below 0.5%.[37]

23.2.4 Stage IV. Process Validation: Finishing Touches—Grinding and Polishing

Repeatability and reproducibility are two key factors in determining the success of process development and scale-up work. Failing to get the desired yield and quality consistently at a commercial scale is a humbling experience for any process development chemist. There



Scheme 23.11

is a crucial difference between not getting the desired outcome at all at commercial scale and not getting it consistently. The former case indicates that there could be a problem achieving or maintaining certain parameters. These could be rectified at the commercial scale itself. However, the latter case clearly indicates a critical parameter has not been studied thoroughly at the lab scale and would almost always necessitate rework at this scale. Therefore, validating the process developed at the lab scale at a relatively larger scale (ideally larger by a factor of 5 to 10) is necessary before finalizing the process.

Equally important, the process needs to be robust and should be able to handle the rough and tumble of scale-up work with minimal, if any, impact on yield and quality. It is important that once identified, all critical parameters that affect process performance be evaluated thoroughly for the extent of their impact on yield and quality. Tools such as cause-and-effect diagrams and failure mode effects analysis are used routinely for analyzing the criticality of parameters. When parameters with an unacceptably high degree of criticality are identified, either the process can be reworked to reduce criticality or suitable hardware can be identified that can handle the criticality appropriately. As a last measure, critical operating parameters are challenged by doing negative experiments, allowing deliberate excursions from critical values to determine the impact on quality and yield. These data are very valuable during scale-up when choosing equipment and utilities. Another important exercise that is undertaken at this stage is studying exothermic reactions using a reaction calorimeter to identify potential safety issues during scale-up. Potential runaway reaction conditions must be identified and eliminated.

The process that has been developed is validated prospectively at the end of the optimization program. This is an important exercise: If the reactions behave the way they were predicted to, and if the yield and quality of the product are as expected, the process is ready for scale-up.

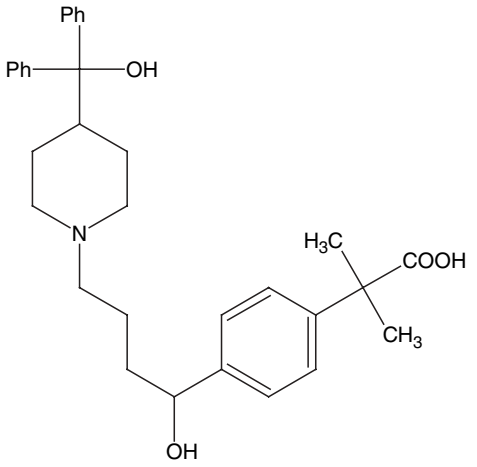
23.2.5 Stage V. Process Scale-up: The Moment of Truth

While tailoring the process to suit available equipment is a major factor during process development, once the process is developed and validated, selection of appropriate equipment that is than qualified for the process under consideration is very critical for smooth scale-up. Many a good process comes to naught due to poor equipment selection, leading to rework and avoidable waste of that most precious resource: time. Two major activities are done at this stage:

1. *Hazard operability* (HAZOP) *analysis.* The process is analyzed purely from an equipment versus safety angle. Every single operation involved in the process is discussed by the cross functional team (CFT) for potential safety issues that could arise out of operations and any unexpected equipment failure. If any such condition is identified, the CFT identifies a remedy and implements it before scale-up.
2. *Framing batch production records* (BPRs). These are the recipes that are used for production by plant personnel on a routine basis. They are entirely based on the process developed and validated in the lab. Any inconsistency could result in problems during scale-up. The CFT discusses each operation with the manufacturing and quality assurance personnel and finalizes the BPRs before beginning any scale-up activity.

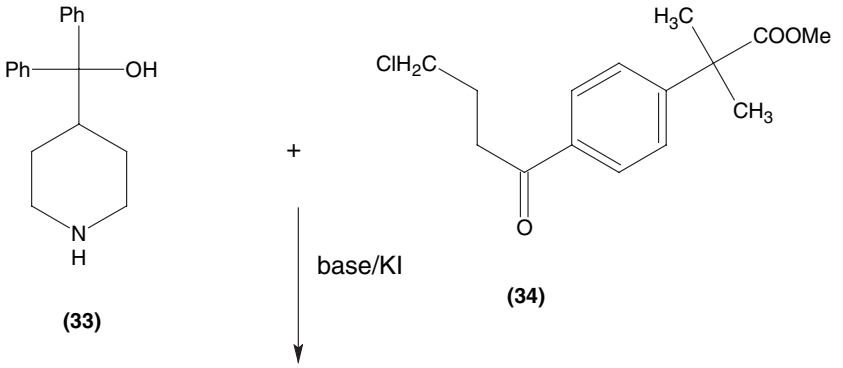
The following case study illustrates a typical problem that arises during scale-up.

Case Study 8 Fexofenadine (Scheme 23.12) is an important antihistaminic agent. The keto compound (**35**, Scheme 23.13), is a key intermediate in the synthesis of

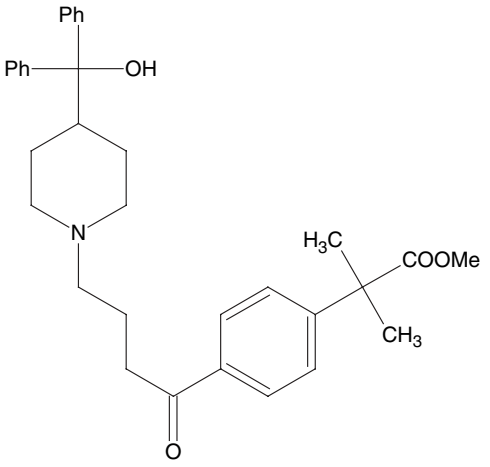


(32)

Scheme 23.12



(33) **(34)**



(35)

Scheme 23.13

fexofenadine. It is prepared by the alkylation of azacyclonol (**33**) with the alkyl chloride derivative (**34**), in a suitable reaction medium under reflux conditions in the presence of a base. This alkylation reaction requires a catalytic quantity of water in the reaction medium to facilitate neutralization of the reaction by-product, HCl, by the base. In the absence of water, the reaction does not go to completion, as azocyclonol traps the HCl and precipitates out of the reaction mixture, thus impeding further condensation. The presence of excess water was also a problem for reaction completion. The reaction conditions were thoroughly optimized in the lab. When the reaction was scaledup to commercial scale, to our astonishment we found that it did not go beyond 60%. This indicated that we were losing water from the mixture and analysis confirmed that this was indeed the case. As the reaction solvent formed an azeotrope with water, it was suspected that solvent, along with water, was being lost due to condenser inefficiency. Every possible check for problem condensation indicated that no problem. The problem was ultimately traced to the type of condenser being used. Under reflux conditions in the lab, where vertical condensers were used, there was no water loss. At the plant, however, horizontal condensers with chilled water as the cooling medium were normally used. As water was immiscible with and heavier than the reaction solvent, when solvent vapors got condensed, along with the azeotropic water vapors, the water separated out as the lower layer and settled down within the condenser as holdup liquid, so not getting back to the reactor. As the water was being used only in catalytic amounts, this holdup gradually removed the water from the medium. Once the water was completely gone, the reaction stopped. Once the problem was understood, the horizontal condensers were replaced with vertical condensers, where holdup was not possible. This solved the problem, and the lab results could be reproduced at a commercial scale.

23.3 CONCLUSIONS

Developing and scaling-up a process for an API could easily be compared to building a house. As it is important for the entire team—the architect, engineers, masons, utility personnel, and others to work in harmony for the house to be livable, so it is for process development and scale-up. The knowledge, skill, experience, and above all, teamwork of each member are critical for success. Despite being thorough, there will be problems, but the critical concerns are how quickly the team responds with solutions and how permanent these solutions are. It has been our experience that an empowered team of scientists and engineers with a system-driven process development and scale-up program is very likely to succeed in meeting project goals on the first attempt.

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24

IMPORTANCE OF POLYMORPHS AND SALTS IN THE PHARMACEUTICAL INDUSTRY

BIPIN PANDEY
Zydus Research Centre
Ahmedabad, Gujarat, India

VIDYA B. LOHRAY AND BRAJ B. LOHRAY
BHUVID Research Laboratory
Ahmedabad, Gujarat, India

24.1 INTRODUCTION

Polymorphism is the ability of a solid compound to exist in more than one crystalline form. These crystalline forms, although chemically identical, result from a different ordered arrangement of molecules in a unit cell within the crystal lattice. Polymorphism affects various kinds of physical properties depending on the nature and stability of crystal lattice. Some of these properties are heat capacity, conductivity, viscosity, surface tension, diffusivity, crystal hardness, crystal shape and color, refractive index, electrolytic conductivity, sublimation properties, latent heat of fusion, enthalpy of transition, phase diagram, and rate of reaction. Some of the other and more pertinent physical properties of polymorphs relevant to the pharmaceutical industry are solubility, dissolution rate and consequent bioavailability, chemical and physical stability, melting point, bulk density, electrostatic properties, and flow properties, including processability. Changes in the aforementioned properties of a solid substance are of considerable importance to pharmaceutical companies. A number of drugs have shown considerable differences in their physical properties due to change in their crystalline structure or polymorphic properties.[1] Naturally, the differences in solubility can affect drug efficacy, bioavailability, and safety.[2] It is reported that because of alterations in process or stor-

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age conditions, one polymorph of chloramphenicol-3-palmitate can have an eightfold-higher bioactivity[3] than another, creating a danger of fatal doses when the unwanted polymorph is administered unwittingly. Changes in polymorphic behavior may pose considerable challenges not only to scientists involved in new drug discovery but also to people involved in processing, formulation, and manufacturing research. Since different polymorphs are considered as new and patentable, they add tremendous commercial implications in terms of prolonging the patent life of a drug for the originator company, simultaneously offering opportunities to generic manufacturers for the discovery of new polymorph(s) and salts as a noninfringement strategy with a view to getting market exclusivity or market share.

Polymorph research encompasses a variety of activities and technologies. From the very outset, one should be clear about the nature of a solid drug substance either in acidic or basic salt form, or as a neutral compound, and its polymorphic nature. Suitable analytical techniques should be developed for qualitative and quantitative identification and characterization of different polymorphs. Simultaneously, process chemists should develop reproducible processes to ensure production of the same polymorphs all the time. Similarly, formulation experts should ensure the stability of polymorphs during selection of excipients and should devise appropriate technologies during granulation, compacting, wetting, and tableting.

Therefore, pharmaceutical industry scientists and technologists have to make serious chemical and engineering decisions that take into account not only safety, efficacy, and processability of the drug substance or product in a reproducible manner, but also define strategies to defend the intellectual property.

Approximately half of all the drug molecules used in medicine are administered as salts, whereas polymorphism is encountered in most of the drug substances known in solid form.[4] The issues of salts and polymorphs are at the center of new drug discovery, chemical process development, analytical chemistry, pharmaceutical sciences, pharmacokinetics, toxicity, and clinical studies, and these issues are encountered repeatedly by pharmaceutical companies. Selection of an appropriate salt form for a new chemical entity provides the pharmaceutical chemist and formulation scientist with an opportunity to modify the characteristics of the potential drug substance and develop dosage forms with good bioavailability, stability, manufacturing, and patient compliance. Salts are most commonly employed for modifying aqueous solubility, but the selection of a salt also influences a range of other properties, such as physical form, melting point, hygroscopicity, chemical stability, dissolution rate, pH of aqueous solution, crystal form, and mechanical and electrostatic properties.

24.2 DRUG DISCOVERY AND DEVELOPMENT

A modern drug discovery program is a multidisciplinary team effort involving medicinal chemistry, pharmacology, process development, cell biology, molecular biology, and drug metabolism pharmacokinetics/absorption, distribution, metabolism, excretion (DMPK/ADMET) science, along with intellectual property management under strict regulatory conditions. Usually, the initial stages of research are focused on drug target (enzyme or receptor) identification and validation. Subsequently, a *hit* is found through an appropriate screening procedure, which is further optimized to get a *lead*. After *lead optimization*, a *candidate* is selected. The issue of a suitable salt and polymorph has to be addressed at the preclinical stage (Fig. 24.1). During these studies, variations in the crystalline form of new chemical entities (NCEs) might be observed which includes variation in the melting point, bulk density, and solubility profiles. Diffraction studies, solid-state infrared (IR) and

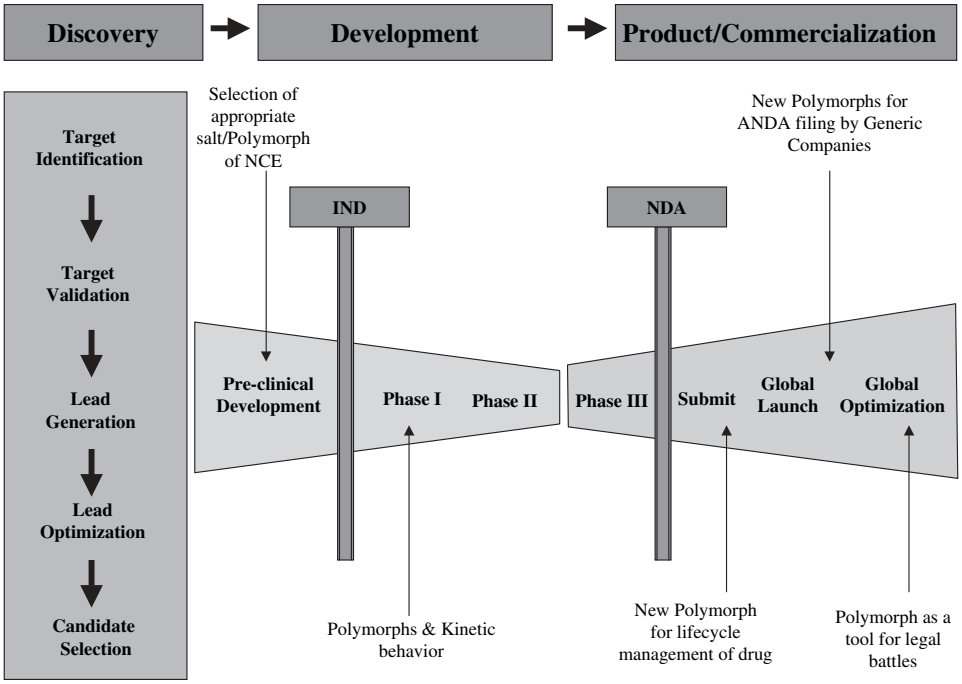


Figure 24.1 Importance of polymorphs and salts in different stages of drug development.

differential scanning calorimetry (DSC) may reasonably be considered supportive for the solid-state characterization of drug candidates at this stage.[5]

When studies of a drug candidate in humans (phase I) are planned, the U.S. Food and Drug Administration (FDA) requires that an investigational new drug application (IND) be submitted. Prudence suggests that this may be the best time to begin a search for novel polymorphs. As we know that different solvents and impurities/degradation products can trigger formation of different polymorphs, it is important at this stage to study the nature of the polymorph. Since different polymorphs may have different pharmacokinetic profiles, it is essential that various polymorphs of the drug candidate be studied before selecting a particular polymorph for the dose escalation studies in phase I and subsequently for phase II studies. At the later stage of drug development, a change of polymorph will be economically untenable. Apart from appropriate process development of NCE, one needs to reproduce in pilot-plant scale the formation of the polymorph selected, which usually comprises a batch of drug produced for phase II and III studies.

To derive maximum reliable information from phase II and III clinical trials, it becomes imperative to control the physical and chemical characteristics of the drug substance and the drug product and to ensure that the quality remains consistent throughout. Unanticipated variations in quality, such as discovering a new polymorph or a solvate, can have a major financial impact and disturb the overall development time line. During these studies one must ensure that during scale-up, parameters such as choice of solvent of crystallization, recrystallizing conditions, volatile impurities, drying conditions (temperature and vacuum), milling, grinding, storage, and transportation conditions be rigorously fixed.

At the stage of process optimization, it is best to "fix the last step first." In a multistep synthetic operation, it is advisable to fix the key reaction critical parameters of the last step very rigorously at the very beginning so that the same solid form is obtained reproducibly. Later, one may optimize the process of prior stages.

During manufacture of the drug product, the drug substance is exposed to a variety of physical and mechanical stresses. In addition to pressure, the environment needs to be controlled. Hygroscopic substances require special control to ensure same degree of solvation. A granulation step before a tablet is compacted may include a liquid, which is subsequently removed in drying, which may alter the crystalline form of the active ingredient. Awareness of the physical conditions at each step of manufacturing provides a rationale for the support of in-process or release controls (e.g., hardness dissolution). For further details, see Section 24.6. In case of interconversion of polymorphs at this stage, the kinetics of phase change should be studied to ensure that the interconversion does not change the quality of product adversely. If a phase change takes place over a period of days to months, appropriate controls during formulation manufacturing and packaging should be incorporated. Adequate statements for storage conditions should be printed on labels to assure consistency of quality. Extremely slow phase change may not have any major impact on the product, but extremely rapid changes in phase may require appropriate guidelines for release of drug substance and storage conditions.

If a new polymorph is discovered at a later stage, one needs to establish the bioequivalence of the formulation containing the new polymorph, which may require a different composition or amount of active ingredient. As the drug candidate moves to phase III clinical trials, the bulk manufacturing process should be fixed as per current good manufacturing practice, including all in-process controls and impurity profiling. All detailed studies related to polymorphic state, including issues of solvates, hydrates, and amorphousness or crystallinity, must be carried out, and any problem must be assessed to the smallest detail. Acceptance criteria should be clearly established. The manufacturing process must be validated rigorously before the drug enters phase III clinical evaluation, and enough evidence should be collected to ensure the biological profile of various polymorphs.

24.3 SALT SELECTION

Modern drug discovery technologies propelled by combichem, computer-aided drug design (CADD), and high-throughput screening (HTS) of biological targets (enzymes, receptors) generate a large number of hits, which are usually refined by further screening and selection, based on druglike properties, to a manageable number of leads. Many leads will show only weak or moderate activity, because biological assays (in vitro) are usually done in dimethyl sulfoxide (DMSO) solution, which requires further refinement and optimization of properties. These optimization protocols usually involve numerous structural modifications and reexamination through in vivo and in vitro biological assays until a small number (usually two to five) of the most highly active candidates are advanced for further study. At this stage, most candidates are usually free bases, free acids, or neutral molecules rather than salts. Due to the increasing use of DMSO solutions and solubility considerations, more lipophilic candidates are preferred. Quite frequently, several of the lead candidates are either amorphous or partially crystalline. Usually at this stage of development, little effort has been made to investigate formal

crystallization procedures. However, it is widely accepted that water-soluble candidates will have better biological profiles as drugs. Therefore, salt formation of lead candidates becomes an important objective.[6] Salts offer not only improvement in purity, crystallinity (e.g., melting point), stability, and hygroscopicity, thereby enhancing operational simplicity during bulk manufacturing and pharmaceutical formulation processing, but also improve the biological profile by enhancing the solubility and dissolution rates as well. Added to this comes the opportunity of patenting new salts, along with various polymorphs of the salt, thereby prolonging the patent term of commercially useful compounds.[6] Alternatively, as a result of recent regulatory guidelines, discovery of new salts and polymorphs offers opportunities for market exclusivity for generic manufacturers. However, complete characterization of salt form and solid state is an important criteria for drug development.

Systematic screening for salts is advantageous at an early stage of candidate selection with a view to optimize solubility, dissolution rate, solid-state stability, hygroscopicity, toxicity, and drug delivery (formulation) properties. In particular, toxicological consequences of counterions during salt formation are very important, especially during early stages of drug safety evaluation. When high-dose screening is required for toxicity studies, the concentration of counterion is much more than that actually present in the marketed drug (acute toxicity studies), and at this stage, concerns are high. Table 24.1 shows the classification of various common pharmaceutical salts as anions and cations.

Measurement of the key physiochemical properties of solid forms of lead candidates is essential during the early discovery process, not only from manufacturing and regulatory perspectives, but from a formulation viewpoint as well, where dosage form, processability, and compatibility with other excipients are the key issues. Some of the key physical, chemical, and biologically relevant properties that should be evaluated comprehensively prior to making a final decision on salts for further development are listed in Table 24.2.

TABLE 24.1 Classification of Commonly Accepted Pharmaceutical Salts

Salt Class	Examples
Anions	
Inorganic acids	Hydrochloride, hydrobromide, sulfate, hydrogen sulfate, nitrate, phosphate
Sulfonic acids	Mesylate, esylate, tosylate, napsylate, besylate
Carboxylic acids	Acetate, propionate, maleate, benzoate, salicylate, fumarate
Anionic amino acids	Glutamate, aspartate
Hydroxy acids	Citrate, lactate, succinate, tartarate, glycollate
Fatty acids	Hexanoate, octanoate, decanoate, oleate, stearate
Insoluble salts	Palmoate, polystyrene sulfonate (resinate)
Cations	
Organic amines	Triethylamine, ethanolamine, triethanolamine, ethylenediamine, choline
Insoluble salts	Procaine, benzathine
Metals	Sodium, potassium, calcium, magnesium, zinc
Cationic amino acids	Arginine, lysine, histidine

TABLE 24.2 Physiochemical Property Evaluation for Salt Selection

Property	Criteria
Melting point	Generally, higher-melting-point products are preferred
Crystallinity (crystal shape and appearance)	Crystalline nonhygroscopic material
Particle size and bulk density, surface area	Smaller particle size, bulk density, and large surface area
Polymorphism	Stable polymorph
Powder properties	Nonhygroscopic powder with a high melting point
Stability Solution stability (to acid, base, oxidation, heat) Solid-state stability according to ICH guidelines	Stable in accelerated stability condition (e.g., 40°C/75% RH 25°C/60% RH)
Electrostatic behavior	Nonelectrostatic nature
Hygroscopicity	Nonhygroscopic
Processability	Easy processability
Impurity profiling; usually, salt formation enhances purity of drug substance	Need to be fixed before phase III clinical development
Partition coefficient: c log P	Generally, lipophilic for better bioavailability
Aqueous solubility as a function of pH	Aqueous solubility at neutral pH (7.2) is preferred
Solubility at various pH values (pH 2, stomach; pH 6.8–8, mouth, esophagus, colon, intestine; and pH 9, duodenum)	Depending on the target organ, stability and solubility of the drug at various pH values need to be determined
Dissociation constants: (pK_a/pK_b)/ionization constant	
Intrinsic dissolution rate	
Permeability	High permeability is preferred
Solubility in organic solvents [e.g., ethanol, polyethylene glycol, propylene glycol, glycerol, etc. (formulation requirement)]	Nonreactivity, easy processability, and stability with formulation is desired

24.4 PSEUDOPOLYMORPHS

Pseudopolymorphs are not strictly polymorphs because they differ from each other in the solid crystalline phase, through incorporation of either solvents (solvates) or water (hydrates). The principle of hydrogen bonding and packing arrangements of the same drug substance in the crystal lattice is the guiding principle for pseudopolymorphs as well. Like polymorphs, pseudopolymorphs, exhibit different physicochemical properties and are suitable as drug substances for development and should be evaluated while selecting the final solid drug substance. There can be stoichiometric or nonstoichiometric pseudopolymorphs.

24.4.1 Hydrates

Generally, hydrates are considered appropriate psuedopolymorphs for development.[7] Many drugs are marketed as hydrates, presumably because they are either the most stable form

as hydrates during stability studies or because they offer improved physicochemical properties. The potential impact of changes in hydration state of a crystalline drug substance and excipients exists throughout the bulk drug manufacturing and development process. Substances may hydrate or dehydrate in response to changes in environmental conditions during manufacturing, drying, or processing, or over a period of time (shelf time) via a metastable state. Hydrates often, though not always, are less soluble in water than is the corresponding anhydrous form. If the hydrate is less soluble, it often crystallizes when the anhydrous form is suspended in water and allowed to equilibrate. Such hydrates are known to be formed as either stoichiometric or nonstoichiometric hydrates: namely, monohydrate, semihydrate, hemihydrate, sesquihydrate, dihydrate, trihydrate, or simply hydrates.

An important consideration for hydrates is the humidity range in which interconversion of anhydrous form occurs. Some hydrated compounds may convert to an amorphous form upon dehydration, whereas others may become chemically labile. For example, the hydrates of clopidogrel bisulfate salt are quite likely to provide carboxylic acid as a degradation product during either manufacturing or stability studies if moisture is available from environment.

The usual method used to make hydrates is to stir anhydrous drug substances in a solvent with a varying amount of water at different temperatures. Usually, the stable hydrates precipitate. Hydrates also have different physical properties (e.g., melting points, dissolution rates, hygroscopicity, chemical and physical stability and manufacturability, and of course, very important, bioavailability and bioequivalence). Suitable selection of granulation method, particle size reduction, film coating, tablet compression, excipients selection, and other manufacturing processing conditions (e.g., packaging and storage conditions) should be kept in mind when selecting the hydration state of drug substance. Since it is required to demonstrate adequate physical stability (e.g., no conversion to another form in dosage form) of the solvate, it is often better, if possible, to avoid less stable forms.

Usually, anhydrous forms that do not convert to the hydrate(s) during stability studies (ICH guidelines)[8] are likely to exhibit adequate physical and chemical stability in oral solid dosage forms. In case of a solid substance that undergoes change under accelerated stability conditions (e.g., 40°C/75% RH), adequate manufacturing and packaging precautions are taken to protect solid dosage forms. Conversely, hydrates that do not convert to anhydrous forms up to relative humidities below 20% are also likely to exhibit adequate physical stability in solid dosage forms. Since the physical and chemical properties of the drug substance can vary considerably from anhydrous to various hydrated forms, each of these forms is usually considered as a separate novel drug substance, and hence the drug regulatory authorities usually seek to establish their pharmacokinetic behavior in human subjects.[9] From legal and commercial points of view, these novel forms may be considered as novel drug entities and therefore help in extending the life cycle of the drug substance in the market (such as extended patent protection, filing of abbreviated new drug application (ANDA), etc.; see Fig. 24.1).

24.4.2 Solvates

Generally, solvates are undesirable as drug substances especially if the solvents used are not class III solvents (see per ICH guidelines for organic volatile impurities). However, the formation of solvates does occur frequently during drug synthesis and manufacturing. Solvates, as inclusion complexes, can retain their structure, even after the solvent is removed, washed, and dried under vacuum. Solvates also exhibit distinct physicochemical

properties [e.g., melting point, dissolution rate, solid state, IR, x-ray diffraction (XRD), DSC, thermogravimetric analysis (TGA)] and the percentage of solvent can be quantified. However, one must rigorously establish their desolvation characteristics during stability studies, manufacturing, and formulation processing. Solvates, like hydrates, are considered to be novel pharmaceutical materials with characteristic physicochemical properties; they have patentability status as well (see section 24.9). For example, recently, Teva has patented several hydrates and solvates of sertraline hydrochloride[10] and clopidogrel hydrogen sulfate[11] as novel drug substances which become very useful while filing ANDA and break the ever-expanding exclusive marketing rights of innovator companies. Discovery of novel solid-state forms as polymorphs, hydrates, solvates, and so on, offer opportunities to generic manufacturers of the drug substance to file ANDA under paragraph IV certification and launch the product earlier than the expiry of patent belonging to the innovator company (for detailed strategy, see the orange book guidelines).[12]

24.4.3 Amorphous Solids

Although crystalline solids are usually preferred for formulation studies, which offer unique advantages, amorphous solids are also used occasionally. Amorphous substances may be prepared by solvent evaporation, freeze-drying, spray-drying, or coprecipitation with an antisolvent, growth in the presence of additives, sublimation or crystallization from melts, and so on. A recent study has reported the formation of stable amorphous forms from crystalline forms of four drugs: ketoprofen, indomethacin, naproxane, and progesterone, by milling with Neusilin (amorphous magnesium aluminosilicate).[13] It has also been shown that milling without Neusilin for 48 hours did not result in the formation of an amorphous substance.

Amorphous forms do not exhibit any characteristic XRD patterns, although other physicochemical properties (e.g., melting point, solid state, IR, TGA, DSC) are quite distinct from their crystalline forms. Because of their higher energy state, amorphous solids are used to improve solubility or dissolution rate. Amorphous solids are generally hygroscopic, chemically less stable than crystalline forms, and difficult to handle. Similar to solvates and hydrates, amorphous forms of the drug substance are also considered novel drug substances and can be used to extend and manage the life cycle of the drug substance in the marketplace. However, great care must be taken during formulation and stability (accelerated and extended) studies so that an amorphous form of the drug substance does not get converted to crystalline form, which might infringe a competitor's patent and create unwarranted legal challenges by the competitor company.[12]

During the last decade, several generic drug manufacturers have taken advantage of various hydrates, solvates, and amorphous forms of drug substance to penetrate the highly regulated drug market with successful legal challenges to innovator companies and have reaped enormous benefits therefrom.

24.5 ANALYTICAL TOOLS

Polymorphism in drug substances has indeed opened up an interesting field for analytical scientists. Drug companies need to monitor the polymorphs in drug substances and drug products to ensure that they persist (remain the same) during manufacture and shelf life. Qualitative and quantitative methods are required to analyze solid mixtures as well

as various types of formulations (e.g., tablets, microspheres, transdermal patches, stints, slurries, lyophile cakes). During stability studies of either drug substance or drug product, one must confirm that polymorphic purity is retained throughout.

All the solid-state properties of the various polymorphic modifications of a compound are different, but the difference is only marginal in nature, sometimes beyond the limit of detection of a particular analytical method. Therefore, to avoid erroneous conclusions it is important to look at potential polymorphic systems using a variety of analytical techniques.[14] By relying on too few analytical techniques, one may fail to discover a polymorph. It may require substantial effort for complete elucidation of substances with multiple polymorphic forms, especially when previous studies have characterized the forms inadequately.

The analytical techniques that are routinely used for characterization of polymorphs are mentioned in group A category below, whereas additional information is gathered via employing additional analytical methods discussed in group B. Researchers, however, are continuously examining other methods mentioned in group C to examine complete specification and characterization of polymorphs thereby avoiding any litigation, possibly arising due to inadequate analytical supports.

A. Polymorphs are usually characterized using:
   - Melting point
   - Solid-state IR
   - X-ray powder diffraction (XRD)
   - Thermal analysis (DSC, TGA)
   - Solubility measurements

B. Additional analytical methods used for characterization are:
   - X-ray single-crystal diffraction
   - Solid-state nuclear magnetic resource (NMR) (proton and carbon)
   - Hot stage microscopy (cross-polarized)
   - Diffuse reflectance IR spectroscopy
   - Near IR spectroscopy

C. *Other characterization methods*. Several newer methods are appearing in the literature for characterization of polymorphs:
   - Decomposition and evolution of gas(es) in silicon oil
   - Lattice energy content
   - Density measurements (flotation, pyknometry, dilatometry)
   - Hot stage IR spectroscopy
   - Chemical shift tensors (^{13}C NMR)[15]
   - Scanning tunneling microscopy (STM)
   - Atomic force microscopy (AFM)
   - Video recording on microscopes
   - Electron diffraction
   - Electron microscopy
   - IR microscopy

- Thermobarometric measurements
- Area detectors on diffractometers
- Crystal etching
- Thermobarometric measurements

24.6 PROCESS DEVELOPMENT

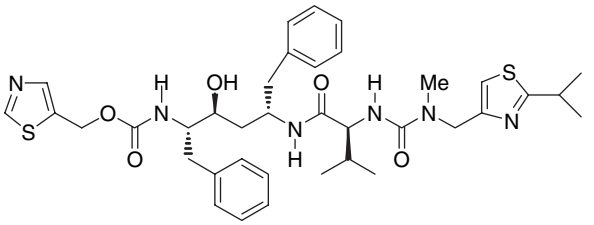
Formation of a particular polymorph depends on the crystallization conditions applied, especially when the solid can exist in various polymorphic forms. This warrants development of well-tuned optimized conditions during process development which can be reproduced in a manufacturing plant. Hydrogen bonding, as suggested by Lepinski et al.,[16] during the design of molecules for drug discovery plays an important role during crystallization also. This is why the presence of moisture or impurities or the addition of polar or nonpolar solvent alters the formation of polymorphs, which is critical in process development. Crystalline products are usually the easiest to isolate, purify, filter, dry, handle, and store in a batch bulk drug manufacturing process. These advantages continue during formulation to get drug products as well. However, the process of crystallization and the factors governing the creation of individual polymorphs are poorly understood. Crystals are usually in equilibrium with saturated solutions, but efficient crystallization requires supersaturation. The crystallization of desired polymorphs depends on several key reaction parameters:

- Quality of raw materials (including tight specifications for impurities) and reagents
- Quality and amount of solvents, cosolvents, and antisolvents
- Degree of saturation and supersaturation at the onset of crystallization
- Stirring, speed, and type of stirrer
- Reaction temperature for crystallization
- Speed of nucleation
- Role of moisture and its content
- Rate of cooling
- Degree of cooling (lowest temperature reached)
- Duration of cooling and crystallization
- Seeding (when, how much, and which temperature?)
- Initiate crystallization by scratching
- Splash monitoring
- Drying and temperature of drying and vacuum
- Grinding, milling, and any mechanical disturbance during transfer
- Storage conditions

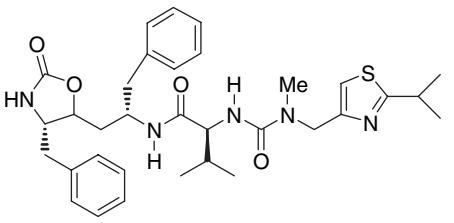
Seeding The technique for seeding to get a desired polymorph during crystallization is one of the powerful tools for controlling polymorphism.[17] This requires a seeding strategy where the choice, characterization, and preparation of seed and the various process parameters (e.g., temperature, stirring, cooling, rate of cooling, and degree of cooling, and various scale-up issues from lab scale to plant) are taken into consideration.

Leaching,[18] stirring of one polymorphic form (solvates, anhydrous, hydrates, amorphous form) in a particular solvent (polar or nonpolar) at a varied length of time and temperature, is also a common technique used to discover a new polymorph or to get a desired polymorph. Although the solid does not dissolve, the crystalline features of solids do change, simply by leaching.

Seed crystals are often helpful in obtaining the polymorphs desired. Manufacturing processes and process development to get the desired polymorph seem to have been worked out by trial and error, aided by serendipity. However, once the desired polymorph starts forming, rigorous process controls of the key reaction parameters are introduced, reproduced, and finally, validated to get a rugged process. Despite all this, a new polymorph may suddenly be encountered, and the earlier crystalline form being produced regularly may disappear totally in certain cases. Interestingly, a story of a disappearing polymorph by Dunitz and Bernstein[19] is worth reading. In this context, a recent case of the appearance of a new polymorph for Abbott's ritonavir, marketed for AIDS treatment as Norvir, needs special mention.[20] Although comprehensive studies to get alternative polymorphic forms were carried out prior to the launch of the drug and during drug development, only one crystalline form was obtained repeatedly. Two years after the launch and after 240 batches of Norvir production, a new, less soluble (and perhaps more stable) form appeared which had different physical properties. Thus, the new formulation started failing in dissolution tests and a large portion of the drug substance was precipitated out of the final (semisolid) suspended formulation product. Several batches were recalled and the incidence had major social, commercial, and regulatory ramifications. Subsequently, two polymorphic forms (I and II) of Norvir were identified.[20,21] The key information was that Norvir exhibits conformational polymorphism with two unique crystal lattices, with significant solubility profile differences. The most important finding was that heterogeneous nucleation for supersaturated solution was triggered by an impurity from a degradation product (cyclic carbamate, **2**, Scheme 24.1) that had a rigid conformation.

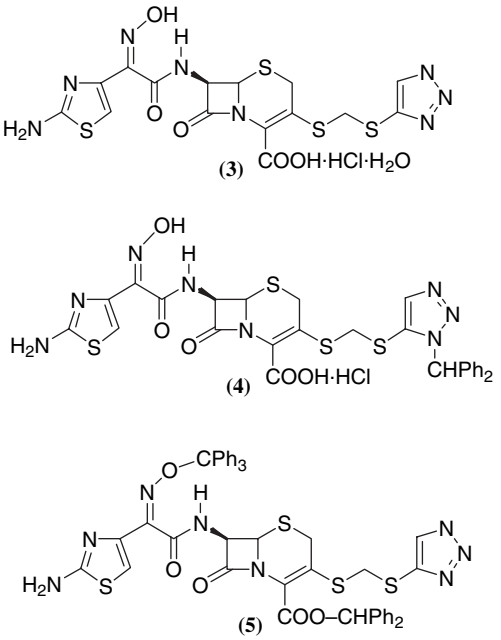


(1)
ritonavir base



(2)
cyclic carbamate impurity

Scheme 24.1



Scheme 24.2

Another example of control of crystal habit and size of cefmatilen hydrochloride hydrate (**3**, Scheme 24.2)[22] with a habit modifier, an impurity, has been reported recently. It has been demonstrated that concentration of a related substance, a diphenyl methyl substitution at the N3 position in the triazole (**4**) (formed during deprotection of trityl and diphenyl methyl group (**5**) with $AlCl_3$ and anisole in methylene chloride at elevated temperature) is critical for formation of the particular crystal habit.

In a detailed study, the role of $AlCl_3$ concentration, the temperature of addition to **5** and subsequent formation of **4** has been found to be critical for polymorph prediction. Subsequently, very small amounts of habit modifiers (0.05 to 0.2 wt%) have been added to an amorphous suspension to change polymorphic nature and to ensure stability of the solid form. Some of the habit modifiers employed are hydroxy propyl cellulose, poly(vinylpyrrolidone), poly(vinyl alcohol), methyl cellulose, and ethyl cellulose in varying concentrations (0.05 to 0.1 wt%). Interestingly, these habit modifiers are commonly used as excipients during formulation studies and are generally regarded as safe (GRAS) materials. Thus, the insights above for controlling the limits of impurity **4** and the addition of polymorphic additives has been used to develop a robust process to get the same crystal habit of **3** reproducibly. A recent study has used glycerol, mannitol, sorbitol, inositol, and xylitol as additives to control the polymorphism of sertraline HCl.[23]

24.7 FORMULATION DEVELOPMENT

The interface of formulation development, dosage forms, and polymorphism is extremely important in the pharmaceutical industry. A tablet can turn into either a powder or a rock, due to polymorphic instability triggered by either interconversion of polymorphs or by

hygroscopicity or its reaction with excipients. Naturally, such changes will affect the dissolution profile and consequent bioavailability. The issue of polymorph integrity is also important in oral suspensions, creams, ointments, and solids dispersed in liquid and their suggested transit and storage conditions. The various mechanical devices used in formulation during milling, granulation, compacting, wetting, tableting, and packaging may affect the polymorph status of a bulk drug. For hydrates, there is a likelihood of desolvation during drying, thereby introducing enhanced hardness in tablets. Alternatively, anhydrous materials can gain a molecule of water from high-humidity exposures during transit. Therefore, extreme care is required to ensure the polymorphic integrity and stability of a drug substance at every stage of formulation development, to maintain safety, efficacy, processability profile, and recommended storage in a reproducible manner. The issues of identity, strength, purity, quality, stability, bioavailability, and bioequivalence are extremely important to the pharmaceutical industry.[24]

Recently, Yamaoka[25] has reported capping like cracking in tablets of anhydrous crystalline carbochromen hydrochloride upon storage under high-humidity conditions. This was found to be due to transformation of the anhydrous form into a dihydrate. Similarly, the amorphous form of calcium pantothenate is known to revert to a stable crystal form when wetted with a variety of solvents used as granulating solvents.[24] Even during formulation as aqueous suspension for novobiocin, a metastable crystalline form is observed which is biologically inactive. However, this slow transformation can be retarded by the addition of methyl cellulose.[26] Anhydrous theophylline converts to metastable anhydrate in less than 10 days when stored at either 33 or 52% relative humidity.[27] Scanning electron microscopy has been used for visual evidence of recrystallization.[27] Similarly, solid dispersions of carbamazepine, formulated by supercritical carbon dioxide, and subsequent evaporation altered the morphology and led to a faster dissolution rate.[28]

24.8 REGULATORY CONCERNS

The major regulatory concerns for a drug substance and drug product during manufacturing are identity, strength, purity, quality, stability, safety, efficacy, and bioequivalence. Since polymorphs have different dissolution profile and stability, their reproducible characterization to ensure the foregoing properties is of considerable regulatory concern. Therefore, one should use the most stable polymorphs and control particle size, which are critical for the evaluation of equivalence. During ANDA filing, generic companies are required to establish bioequivalence for the same dosage forms, strength, route of administration, quality, and intended use with respect to the innovator's drug. Ideally, one should use previously commercialized polymorph as the drug substance. However, if the current marketed drug is under patent, one can use new forms (e.g., amorphous, solvates, hydrates, anhydrous forms) and establish bioequivalence. Adequate acceptance criteria must be established at every level of drug development to ensure that there is no interconversion of polymorphs and that there is a systemic level of equivalence (i.e., clinical trials with other polymorphs), despite interconversion.[9]

24.9 PATENT IMPLICATIONS

Polymorphs are different crystalline forms of the same pure substance or its salts. Hydrates, hemi- and semihydrates, mono- and dihydrates, anhydrous, solvates, and amorphous forms

with varied degrees of crystallinity are included broadly in this area. Since every new solid-state form is regarded as a new and novel material, no polymorph patent has yet been found to be "obvious", due to the difficulty of proving "anticipation" of a new solid-state form (polymorph). Due to the absence of any well-defined theoretical insight for polymorph prediction (see Section 24.10), all new polymorphs are always nonobvious from a patent point of view. Thus, new polymorphs have patent protection implications. Some polymorphs show a better stability and dissolution profile, which offers an opportunity for justification of a patent, thereby extending patent protection. Drug companies usually patent a product without much mention of the polymorphic forms, and subsequently, patent its polymorph to extend patent life for the product.

Polymorphs and various solid-state forms mentioned above have been central to a number of legal cases between innovator and generic drug companies.[29] For example, efforts by GlaxoSmithKline (GSK) to protect the antidepressant Paxil (paroxetine hydrochloride) from generic competition have been based in part on separate patents claiming anhydrous and hemihydrate forms of drug substance.[12] Similarly, GSK has claimed extended patent protection of the antiacidity drug Zantac (ranitidine hydrochloride) on the basis of a new crystal form patented eight years after the drug substance patent was issued.[30,31] In another example, Bristol-Myers Squibb sued a generic company marketing a hemihydrate of the antibiotic cefadroxil.[32] Recently, GSK sued Ranbaxy, an Indian multinational company, for infringement of its patent on cefuroxime axetil,[33] which was patented as "substantially amorphous". Thus, degree of crystallinity and amorphous nature became an important issue. Another Indian multinational, Dr. Reddy's Laboratories, has been sued by Pfizer for a salt-related issue: for patent violations of amlodipine maleate versus amlodipine besylate.[29,34] Thus, there is a great need for proper patent protection of salts and polymorphs to avoid generic competition and consequent litigation.

Polymorphic purity is yet another issue that attracts litigation by innovator companies. With advances in analytical chemistry, polymorphic impurities up to 0.5% can be detected not only in a drug substance (bulk drug), but even in a formulated drug product. For some products where more than five different polymorphs are already patented, it becomes a great synthetic and analytical challenge to ensure that these patented polymorphs are not present or not going to be formed during the shelf life of the drug product.

A biopharmaceutics classification system strictly delineates drug substances based on their aqueous solubility at various pH and intestinal permeability values into class I, II, III, and IV categories, and these properties are in some or another way intertwined with polymorphism. Polymorph form and particle size are considered to be critical by regulatory authorities for evaluation of equivalence. Rigorous dissolution testing results at every stage of formulation development can ensure polymorphic integrity of a drug substance.

24.10 PREDICTIONS AND UNCERTAINTIES

Despite the availability of knowledge in the field of polymorphism, the saga of uncertainty continues. Since different polymorphs have different rates of uptake in the body, it may lead to lower or higher biological activity than desired. In extreme situations, an undesired polymorph can even be fatal. From the drug manufacturing perspective, regulatory compliance requires that the polymorph for which drug application was submitted is manufactured consistently. Some of the recent polymorph issues (e.g., Abbott's ritonavir process-related

news[20,21] or several litigation cases between originator and generic companies for extending or grabbing market rights) are examples.[29–34] Therefore, it is essential that various polymorphs of a potential drug are known right from the beginning. This knowledge can help in fine-tuning a manufacturing process to get the desired polymorph reproducibility and can also ensure better patent protection for the drug.

Although considerable effort has been directed toward understanding various theories and origins of polymorphism via thermodynamics, enantiotrophy, monotropy, kinetics, nucleation, and phase rule at the solid–liquid interface and solid–liquid–vapor interface and through an understanding of various intermolecular forces (e.g., hydrogen bonding, conformational energetics, molecular recognition forces), it is still fair to presume that no method exists to predict polymorphs with a considerable degree of certainty. Molecular Simulation Inc. has introduced the Polymorph Predictor software, which employs a Monte Carlo simulated annealing approach to generate thousands of possible crystal packing alternatives.[3] Lattice energies are calculated and subjected to a lattice energy minimization program with respect to all inter- and intermolecular degrees of freedom, with subsequent selection of the lowest-lattice-energy packing arrangements as potential polymorphs. Although there are few successes in predicting new polymorphs of old drugs, a lot remains to be understood. In the polymorph discovery world, one author has stated that "every compound has different polymorphic forms—and the number of forms known for a given compound is proportional to the time and effort spent in research on that compound.[35] Thus, it is not surprising that sertraline hydrochoride is already known and patented as having 16 polymorphs to date.[10]

The polymorph discovery and prediction world is filled with hits trial and error, and serendipities. There are late-appearing polymorphs, metastable polymorphs, and pseudo-polymorphs, and disappearing polymorphs. The world of solvates, hydrates, hemi- and semihydrates, anhydrous and amorphous forms, and degree of crystallinity keeps throwing up new challenges to scientists.[36–39] Experimentalists do not have a good handle on crystallization and crystal engineering. Problems of particle engineering, crystal shape, size, and critical process parameters for reproducibility keep confronting our understanding of solid formation processes. We do not know why some molecules are solids and some are not. We do not know the factors governing the electrostatics and relationships between crystalline forms and the compressibility of powders and excipients. The mechanisms involved in crystallization are not properly understood. Knowledge of crystals and powders (excipients) is scattered among various disciplines, such as crystallogenesis, crystallography, thermodynamics, physical chemistry of multisphere systems, powder flow characteristics and mechanics, piezoelectrostatics, the physics of complex uniceller systems, solid-state molecular recognition processes, and chemical properties of crystalline surfaces at subatomic levels. There is a great need to collect all these understandings and to systematize the knowledge to face the challenges of polymorph prediction, discovery, and production.

24.11 CONCLUSIONS

The uncertainties involved in the prediction of polymorphism tells us something about the solid state. Investigations of polymorphic systems, especially those with a large number of forms, can help in understanding solid-state phenomenon, molecular properties, intermolecular interactions, molecular recognition and various forces involved at the interface of

supersaturated solution and solid.[36–39] The relationship between crystal structure, crystal growth, and crystal habit (form) and their influence on bulk properties requires further exploration. Apart from knowledge for its own sake, this understanding will have clear applications in the development of drug substances and drug products with the most desirable biological profiles, development of organic electronics, organic semiconductors, nanoparticles, liquid crystals, other speciality products, and a superior understanding of function of biological membranes. How to fine-tune the borderline between the degree of amorphous and crystalline substances needs further study. The relationship between chirality, one aspect of molecular dissymmetry, and crystallography opens up a fascinating frontier. The science of salts and polymorphism has to grow further for us to understand the subtle intermolecular forces making polymorph-type solid forms of two, three, or perhaps many relevant partners (e.g., ligands, enzymes, receptors, and their permutations and combinations.

Despite considerable interest in pharmaceutical companies and the large volume of information available on polymorphism, problems (e.g., out-of-control crystallization, changes in product stability, migration of ingredients within structured tablets, polymorph interconversion during manufacturing and storage) crop up. Very sophisticated techniques are required for troubleshooting. Spectroscopic and XRD mapping are particularly useful in providing two- or three-dimensional representation of the polymorph content within a selected area of product.

Polymorphs are the lowest-energy structural snapshots of actual molecular recognition processes between identical molecules or between a molecule and water (hydrates) or a solvent (solvates) in their most stable conformation. Ultimately, our exact understanding of polymorphism will help to unravel the mysteries of the formation of diverse shapes and appearances of organs, human beings, and the natural biological world—the world that we see with our eyes as solids—through genomics, proteomics, and system biology.

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25

ROLE OF OUTSOURCING IN DRUG MANUFACTURE

PETER POLLAK
Wachtweg 3
Reinach, Switzerland

25.1 INTRODUCTION

Since the beginning of the industrial age, division of labor has been used as an important means to increase productivity and profitability. Whereas the "one person/one task" principle was implemented originally only within the perimeter of an enterprise, the concept of outsourcing is of more recent date. Outsourcing differs from purchasing insofar as it relates to an activity that previously had been performed in-house and is therefore associated with a knowledge transfer. Outsourcing is defined as transferring to a third party through a contractual arrangement an industrial activity that so far has been carried out in-house. If outsourcing is done with an overseas partner, it is often referred to as *offshoring*.

Outsourcing is chosen for a variety of reasons:

- To free-up capital and other resources
- To gain access to third-party expertise
- To lower unit costs of products or services
- To focus on core competencies
- To obtain faster response times
- To avoid safety, health, and environment exposure

Outsourcing was pioneered by the automotive industry. The predominant part of the production process, working with steel, originally was backward-integrated all the way from the final assembly of the car to ferrous ore mining. In a first phase, the industry abandoned the ore-to-steel segment in the manufacturing chain, and later more ancillary

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TABLE 25.1 Outsourcing in the Automotive Industry: Seats

Phase	Outsourcing Activity	Remarks
I	Upholstery	All components (steel frame, foam, textile fabric) supplied
II	Assembly	Design in-house, steel structure supplied
III	Construction	Only design in-house
IV	Design and construction	

activities were outsourced. An example of this are the seats, where first the upholstery work was entrusted to a third party, and now entire seats are designed, developed, and produced by specialist contractors (Table 25.1).

For a pharmaceutical company, the main advantage of outsourcing is the possibility to concentrate its resources on the core competency: drug discovery and commercialization. A disadvantage of outsourcing is the loss of *corporate memory*, the knowledge pool within the company that is shared within the organizational members in general and direct control over the outsourced activity in particular. The closer the latter is to the core business of a company, the more relevant the associated corporate memory loss becomes.

25.2 OUTSOURCING IN THE PHARMACEUTICAL INDUSTRY

Outsourcing has become an important business process for the pharmaceutical industry since the 1970s. The development of a new drug all the way from conception to commercialization is one of the most demanding processes in the entire industrial world. This is confirmed by the R&D expenditure of the pharmaceutical companies, which is the highest of all industries. It amounted to $32 billion in absolute terms in 2002 (+50% vs. 1998; +100% vs. 1994): more than 7% of sales for all the companies. For "big pharma" alone

TABLE 25.2 Structure of the Drug Industry

Type, Number, and Sales	Characteristics	Examples
Big <20 $>$\$5 billion/yr	Global companies with large in-house capabilities (R&D, marketing, manufacturing); ethical drugs	1. Pfizer, USA 2. GlaxoSmithKline, USA/UK 3. Merck, USA 4. AstraZeneca, UK
Medium 50–100 \$1–5 billion/yr	Regional reach, moderate in-house capabilities; mainly generics	Altana (Germany); Boehringer-Ingelheim (Germany), Dr. Reddy's (India), Forest Lab (USA); Fujisawa (Japan), Lundbeck (Denmark)
Virtual >500 $<$\$1 billion/yr	R&D focused, one or two developmental drugs, no production, no marketing; venture capital funded	Actelion (Switzerland), Antigenics (USA), Avanir Pharmaceuticals (USA), Cell Therapeutics (USA), Cubist Pharma (USA)

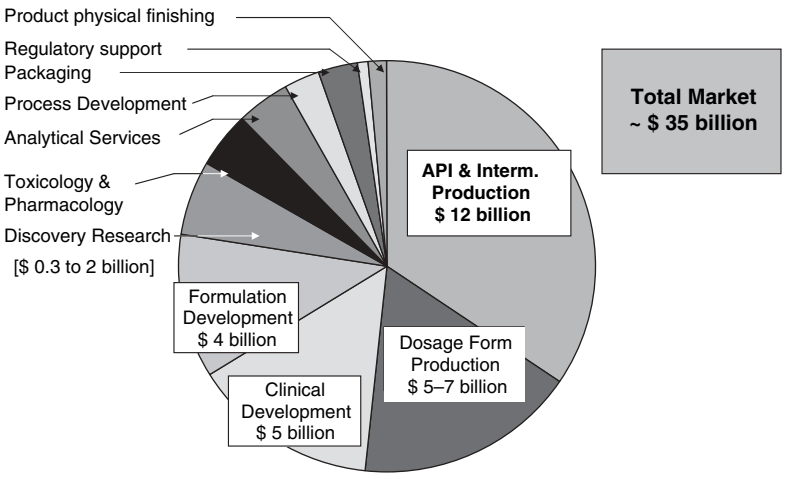

Figure 25.1 Outsourcing in the pharmaceutical industry. (Adapted from Arthur D. Little.)

(Table 25.2), the percentage is in the range 15 to 20%. The development of one successful new drug now costs $900 million, almost twice as much as in the early 1990s. Despite this tremendous innovative effort, there is a distinct downward trend in new drug launches: Whereas the U.S. Food and Drug Administration (FDA) approved 51 new chemical entities (NCEs) for commercialization in 1997, the number dropped to 17 in 2002! Big pharma is particularly hard hit by this development, as innovation is shifting more and more to small pharma. Actually, the FIPCO (fully integrated pharmaceutical companies) business model, which has to foster two completely different cultures, is coming of age. Whereas in research, creativity, innovation, and flexibility à la Hollywood dream factories is needed, manufacturing requires a production efficiency à la Detroit. Managing these two opposite cultures under the same umbrella is no longer the strategy of choice for an effective and efficient business. It can be expected that big pharma, like virtual and biotech companies, will concentrate on innovation in the future. The increasing pressure on the industry will force managers to rely much more on outsourcing and finally, "break up their cozy empires."

Within the new drug development process, about a dozen activities are eligible for outsourcing. They represent a total value of approximately $35 billion (Fig. 25.1). The lion's share, $12 billion, is constituted by chemical manufacturing, followed by dosage-form production, $5 to 7 billion, and clinical development, $5 billion. Given the outstanding importance of chemical outsourcing, it is treated in particular detail in this chapter.

For the outsourcing process as such, three business models exist, ranging from a purely opportunistic search for the lowest unit price to a strategic partnership that yields the best long-term benefit (Fig. 25.2). Nowadays, the *preferred supplier model* is the most frequently used. For the pharmaceutical partner it combines the freedom of choice for each project and the advantage of doing business with fine chemical companies with a proven track record of successfully completed outsourcing and custom manufacturing projects, thus eliminating a lengthy selection and qualification process. For the custom manufacturing partner it avoids reinventing the wheel and ascertains a fair chance of success.

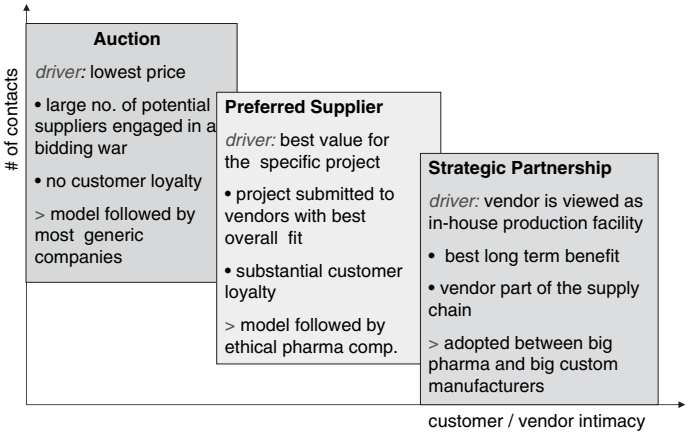

Figure 25.2 Pharmaceutical outsourcing options.

25.2.1 Outsourcing of Chemical Manufacturing

Pharmaceutical Industry Outsourcing of chemical manufacturing ranges about midway in a scale extending from irrelevant to the core business competency, such as catering or office building maintenance, all the way to very close to the core competency, such as finance and R&D. The three main categories of pharmaceutical companies (Table 25.2) differ fundamentally in their level of outsourcing. Big pharma ranks highest regarding the total outsourcing dollar value but lowest with respect to the ratio outsourcing to in-house manufacture. But even within big pharma the policies are not uniform. U.S. pharmaceutical companies, which originate from drugstores and have more of a marketing culture than a production culture, generally are more inclined to outsourcing. An example in case is Wyeth Pharmaceuticals, which has no chemical manufacturing capabilities at all and outsources all its pharmaceutical fine chemical needs. Leading European pharmaceutical companies which have their roots in chemicals and chemical manufacturing, such as AstraZeneca, Aventis, GlaxoSmithKline, and Novartis, on the other hand, possess large manufacturing facilities of their own. They start their API manufacture from readily available standard organic intermediates and typically do all the synthesis steps that require current good manufacturing practice (cGMP) compliance in-house. All in all, among big and medium-sized pharmaceutical companies a tactical approach to the business prevails. Outsourcing chemical manufacturing is still a means of gaining additional capacity, or freeing up internal capacity as opposed to a shift to have all (or most) active pharmaceutical ingredients (APIs) or drug product manufactured externally.

A particular form of outsourcing chemical manufacturing is the sale of a plant by a pharmaceutical company to a fine chemical company linked with an outsourcing contract. The best known examples are GlaxoSmithKline's sales of its sites in Greenville, North Carolina to DSM and in Annan, Scotland to Rhodia-Chirex. For virtual pharmaceutical companies the situation is exactly opposite. They have in-house capabilities, at best, for kilogram quantities of APIs and depend totally on outsourcing for commercial quantities. The total value of the outsourcing contracts, however, is still very small (Table 25.3).

Selection of a suitable fine chemical company as a partner in an outsourcing project is a demanding and time-consuming task. It involves benchmarking of a sizable number of strategic, technical, commercial, and financial performance criteria (Table 25.4). They

TABLE 25.3 Levels of Chemical Manufacturing Outsourcing

	Level of Outsourcing		
Type of Company	Relative	Absolute	Remarks
Big pharma	Small	Large	Only about 40% of fine chemical requirements are outsourced, but overall, by far the largest business volume
Medium pharma	Medium	Medium	70 to 75% outsourcing (most generic houses only formulate)
Virtual pharma	Large	Small	Almost 100% outsourcing, but overall, very limited business volume

can be only partially quantified. The selection process usually involves several steps, beginning with a visit to one of the big fine chemical trade shows, such as ChemSpec, CPhI, or Informex. This allows the pharmaceutical company to gain an overview of the players and to draw up a preliminary list of candidate companies. On the basis of

TABLE 25.4 Selection Criteria for Outsourcing Partners

Criteria	Subcriteria	Importance
Innovation and technology	Plant and facilities	****
	Technology toolbox	
	Process development capability	
	Analytical development capability	
	Project management	
Quality	FDA record	****
	Quality systems	
	Performance measures	
	Change control	
Risk and security of supply	Financial stability	***
	Backup capacity	
	Approach to inventory	
	Safety, health, and environment approach	
	Global resource management audit	
	Political situation	
Business attitude	Secrecy	****
	Reliability (on time/on budget)	
	Continuous improvement	
	Risk sharing	
	Capacity and lead time	
	Flexibility	
	Communication	
Strategic Fit	Conformity with tax optimization policies	***
	Conformity with market access strategies	
	Culture match	
Price	Total cost	**
	Price reduction performance	
	Cost breakdown availability	

responses to a questionnaire sent to the latter, a short list of prime candidates is established. These are then audited on site by in-house specialists. Based on the results of the audit on the one hand, and the specific requirements for the project in question on the other hand, detailed business and technical discussions with one to three potential partners are initiated.

Views on how many partners a pharmaceutical company should have for any given outsourcing project are divided. The possibility to take advantage of economies of size, the reduced resources tied up in the procurement department or supply chain management in managing the relationship, and confidentiality concerns favor a one customer/one supplier policy. Considerations of security of supply and competitive challenge favor the selection of more than one partner. Those partners with the best overall performance on the project obviously are candidates for becoming preferred suppliers. Once a pharmaceutical company has defined a list of preferred suppliers, it becomes very difficult for a newcomer to be considered for an outsourcing project. It would have to be submitted to the entire selection process, whereby certain criteria, such as flexibility and reliability, are almost impossible to determine in advance. Therefore, the entry barriers to big pharma are high for fine new chemical companies.

Fine Chemicals Industry Pharmaceutical fine chemicals represent about 70% or $50 billion of the total production value of this product segment (Fig. 25.3). Fine chemicals are chemical substances:

- Produced in relatively small quantities (typically, less than 1000 tons per year) by multistep synthesis (more than five steps) in multipurpose plants
- Priced above $10 per kilogram (as distinguished from commodities)
- Sold on the basis of specifications (as distinguished from specialities)

The share of the pharmaceutical fine chemicals within the universe of fine chemicals is so important that many fine chemical companies operate only in this area. About 80% of pharmaceutical fine chemicals are used for manufacturing commercial drugs, and 20% for drugs in various phases of clinical development. Actual business transactions account for about 40%, or $20 billion, of the $50 billion; the balance of 60% represents the value of the pharmaceutical industry's in-house production. The share of custom manufacturing (i.e., the counterpart to outsourcing chemical manufacturing) is about $12 billion. The balance is made up by standard or catalog products, including APIs for generics. Overall, the $20 billion pharmaceutical fine chemicals trade is very fragmented, comprising a large

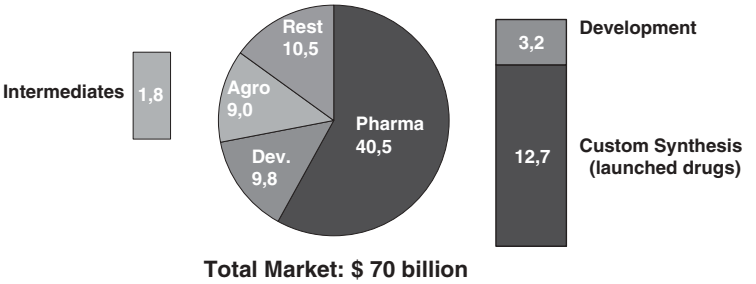

Figure 25.3 Structure of the fine chemicals market. (Courtesy of Prochemics.)

TABLE 25.5 Structure of the Fine Chemical Industry

Type, Number, and Sales	Characteristics	Examples
Big ≈ 10 > $250 million	Global companies with large in-house capabilities (R&D, marketing, manufacturing); typically divisions of large chemical companies	Archimica (UK), Degussa FineChem (Germany), Dowpharma (USA), DSM Pharma Products (The Netherlands), Group Novasep (France), Lonza (Switzerland), Matrix (India), Saltigo (Germany)
Medium ≈ 50 $100–250 million	Adequate technology toolbox, one or two sites (in home country), limited global marketing organization; divisions of large chemical companies or privately owned	Bachem (Switzerland), Borregaard (Norway), Dottikon Exclusive Synthesis (Switzerland), F.I.S. (Italy), KemFine (Finland), Hovione (Portugal), Isochem (France), Matrix (India), Minakem (France), PCAS (France), Siegfried (Switzerland)
Small > 500 < $100 million	Focused on niche technologies (azide chemistry, bromination, fluorination, nitration, peptide synthesis, phosgenation); typically privately owned	Chemada (Israel), Dipharma (Italy), Divi's Lab's (India), Flamma (Italy), Helsinn (Switzerland), Hikal (India), Medichem (Spain), Nippoh Chemical (Japan), SIMS (Italy), Synthetech (USA)

number of products and companies. The structure of the latter reflects the structure of the pharmaceutical industry, albeit with much smaller turnovers (Table 25.5): Whereas the largest company, Pfizer, has sales in excess of $30 billion, the largest fine chemical company, Lonza, had sales of about $650 million in 2002. The two main reasons for the huge difference in size are (1) that APIs account for only about 10% of the sales of a pharma company, and (2) there is little economy of scale in custom manufacturing (see also "Plant Design" below) so that smaller companies can exist in their own right.

Since the early 1990s, the fine chemicals industry has expanded very rapidly. It is estimated that the total capacity of the industry in terms of reactor volume reached 26,000 m^3 in 2002 (Fig. 25.4). This represents 33% of the total global pharmaceutical fine chemical manufacturing capacity of close to 80,000 m^3, more than 35% of which is not utilized. In the fine chemical industry, a variety of products are being manufactured in multipurpose plants during the course of a year. It is therefore, not possible, to determine the production capacity of a plant in terms of tons per year. Instead, m^3 of reactor volume has been chosen as the reference unit.

Apart from capacity expansions, the main reason is reduced demand due to a substantial reduction of new drug launches over the past five years. Fully integrated pharmaceutical companies themselves have also invested heavily in new primary manufacturing plants, particularly in Singapore (e.g., GlaxoSmithKline, Merck, and Schering-Plough) and Ireland (Bristol-Myers Squibb and Novartis) over the last years. The term *primary manufacture* refers to the chemical manufacturing of the API, whereas *secondary manufacture* includes the formulation and packaging of the finished drug.

The pharmaceutical industry, which holds 60% of the 80,000 m^3 reactor volume, has an occupancy of about 60%; the fine chemicals industry, which holds the remaining 40%, fares slightly better, with an occupancy of about 70%. Taking the actually used capacity as

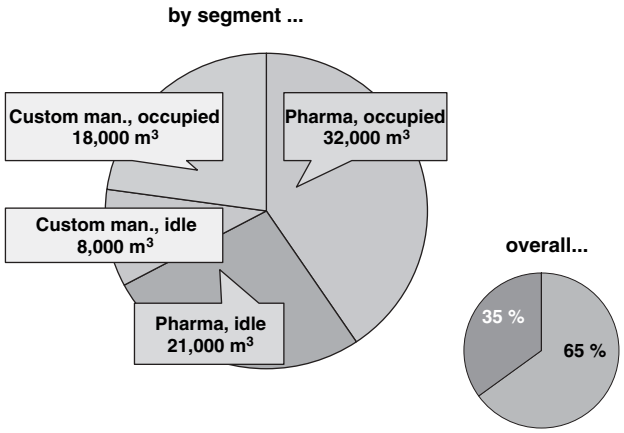

Figure 25.4 Global pharmaceutical fine chemical manufacturing capacity utilization. (From Rabobank, NL, December 2002.)

the guide, one arrives at a ratio of 36%/64% between in-house and outsourced pharmaceutical fine chemical manufacturing.

During the life cycle of a drug, there are four windows of opportunity for fine chemical companies (Fig. 25.5), but they do not all present the same risk/reward profile. At the beginning of a drug development cycle, the emphasis is on producing small-scale samples quickly without taking into account economic or ecological considerations. The contract manufacturing organizations (CROs) are the principal players in this segment. For the fine chemical/custom manufacturing companies, phases II and III of clinical development deserve particular attention. At this point the first-generation manufacturing process for the new API is determined and fixed. At the same time, chances for a successful launch become tangible. The price at which the daily dosage can be sold is a pivotal element in the new drug project. Also, the third gateway, located at the peak level of the drug production volume,

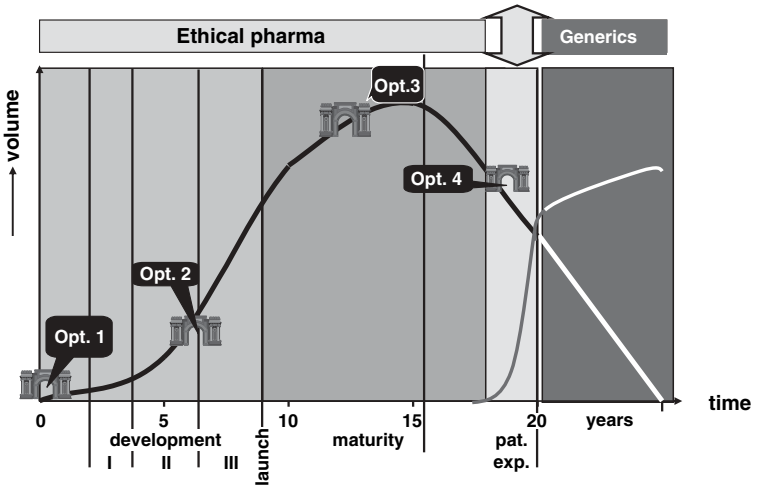

Figure 25.5 Custom manufacturing gateways along the drug life cycle.

represents an interesting opportunity. Drugs that are introduced in the same therapeutic class by competitors start to impact the market share negatively at this point. The cost of goods sold becomes a major concern, and therefore more cost-effective second-generation processes are wanted urgently. The fourth gateway is linked to the decline phase of the life cycle, when patent expiration is approaching. To fend off generic competition, it now becomes a question of survival for the originator to develop *the* most economical process. At the same time, the forthcoming generic competitors are struggling for market share and are looking for new or improved processes that do not infringe existing patents.

During the 2003–2007 period, $50 billion worth of blockbuster drugs lost patent protection in terms of 2002 sales.[1] The APIs for these generics are often made on an exclusive basis by custom manufacturing arrangements. They represent an attractive business opportunity for the fine chemicals industry. In contrast, the APIs for the established generics are standard products. Both categories combined constitute the second-largest outlet for pharmaceutical fine chemicals (first is custom manufacturing). The market is expected to grow at a low-double-digit annual rate from about $8 billion in 2002 to some $17 billion in 2007. The main drivers for this growth are (1) the forthcoming loss of patent protection for a large number of drugs and (2) government pressure on health care cost reduction in many countries, which encourages a switch from ethical to generic drugs. In both the United States and Germany, more than 50% of packages sold now are generics, and other countries are catching up. As most generic companies are only formulators and marketers, their share of outsourcing chemical manufacturing is higher than that of ethical pharmaceutical companies, probably 70 to 75% versus about 40 to 50%, which is also good news for API suppliers. All this, plus the fact that APIs for generics do not require any different technology for their manufacture than APIs for patented drugs, are attractive aspects of this option. There are, however, also major disadvantages:

- The competition in generics in general, and for their active substances in particular, is fiercer than in custom manufacturing, primarily because low prices are the raison *d'être* for generics and also because the entry barriers are low for Far Eastern companies. Confidentiality considerations are much less relevant. Consequently, Chinese, Indian, and South Korean fine chemical producers have been penetrating this market aggressively. The price decrease is particularly dramatic in the first year after patent expiration, when prices drop to between 45% for small-sales-volume drugs and 75% for blockbuster drugs. As the customer base is different, existing customer relations are of no value—there is no customer loyalty. Agents are frequently used as intermediaries.
- To be successful, an intimate knowledge of the patent situation for both product and process patents is a *conditio sine qua non*.
- To be able to start large-scale industrial production of a new API for generics, evaluation of the patent situation and R&D work on the process have to start five to eight (!) years prior to patent expiration, requiring a significant up-front capital investment.

Plant Design The key element of each fine chemicals plant, be it for the pharmaceutical or fine chemicals industry, is the reaction vessel. That's where the actual chemical reaction between the various components of a reaction mixture takes place. On the one hand, it has to have a minimum size or volume to enable economical production. On the other hand, the capacity requirements for individual pharmaceutical fine chemicals do not allow full utilization throughout the year. Furthermore, the product portfolio is changing at a fast

pace. This set of circumstances leads to the concept of the multipurpose plant, as opposed to a dedicated plant for large-volume commodity-type chemicals. Every production train of a multipurpose plant is capable of handling several types of chemical reactions and performing a series of unit operations. It consists basically of a jacketed vessel made from stainless or glass-lined steel, equipped with an agitator, manhole, and pipe connections. Cooling water (or brine) or steam is circulated in the jacket. In more sophisticated plants, a heat exchange fluid is circulated in the jacket. By means of external heat exchangers, the fluid is either heated or cooled.

Solid raw materials are charged through the manhole, and liquids (raw materials or solvents) are fed through a manifold system installed above the reaction vessel and connected to one of the inlet nozzles. If solvents evaporate during the reaction, they are liquefied in the overhead condenser and either redirected to the reaction vessel (reflux) or collected in a distillate receiving tank. After completion, the reaction mixture, typically a solid–liquid slurry, is either cooled down directly in the reaction vessel or in an intermediate crystallization vessel, and discharged through the bottom valve to a solid–liquid separator such as filter or centrifuge (Fig. 25.6). The wet cake from the filter or centrifuge is subsequently transferred to the finishing part, where the product is dried, milled, sieved, and packaged.

A layout of a multipurpose plant is shown in Fig. 25.7. The wet section comprises three production trains, a distillation unit for solvent recovery or liquid product purification, and a scrubbing system for effluent gas. The dry section consists of a paddle dryer, a silo, a sieve, a mill, a mixer–homogenizer, and the filling and packaging station.

The volumes of reactor and crystallization vessels vary widely, between 1000 and 10,000 L, or in rare cases, 16,000 L. Reactors with standard sizes of 4000 and 6300 L are used most commonly. Multipurpose plants also differ with regard to the degree of sophistication. Simple plants are equipped with jacketed reactors that operate at limited ranges of temperature (-10 to $+120$°C) and pressure (20 mbar to 5 bar). Also, the filters and centrifuges are discharged manually, and the dry section is not contained. Process control is manual.

The basic layout of plants for the manufacture of biopharmaceuticals is similar. A production train also consists of feed tanks, a reactor or fermentor, and workup equipment. In most cases, all apparatus is installed in the wet section. The main difference lies in the substantial and demanding provisions that have to be taken to avoid contamination in any part of the production unit. The process water or water for injection has to fulfill particularly demanding requirements.

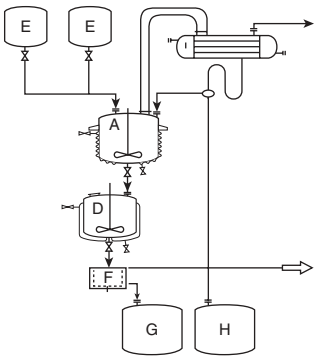

Figure 25.6 Basic design of a fine chemicals production train. (From ref. 2.)

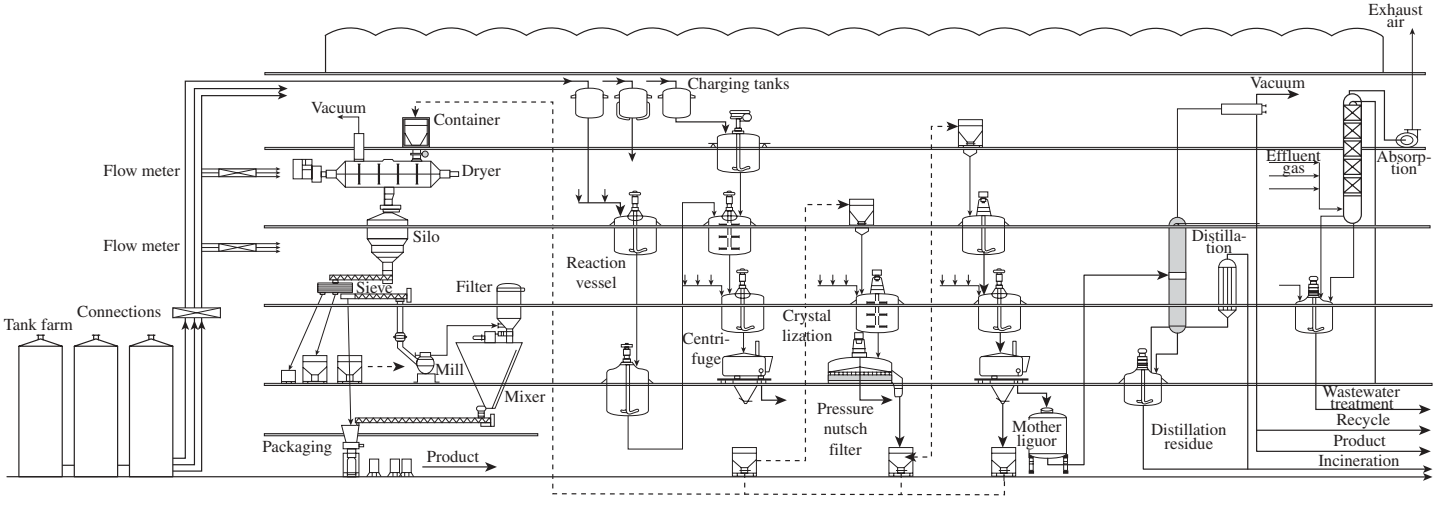

Figure 25.7 Schematic of a multipurpose fine chemicals plant. (From ref. 2.)

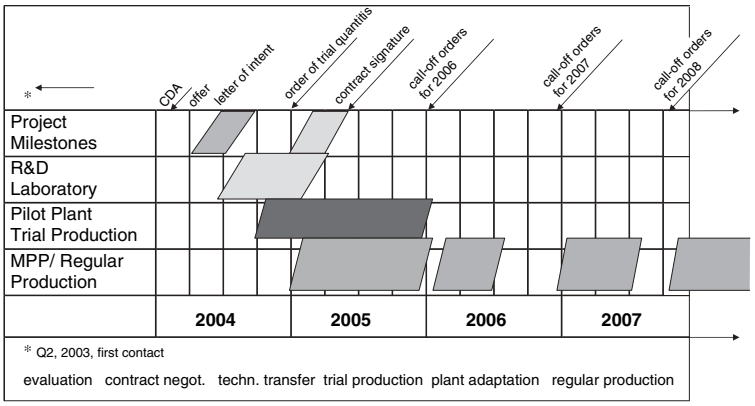

Figure 25.8 Project schedule.

Project Schedule Professional project management is essential for the successful completion of an outsourcing project. This is particularly the case for the vendor, the custom manufacturing company, which is responsible for the bulk of the project. However, the customer, the pharmaceutical company, is also challenged. A mandatory prerequisite for success is the nomination of a contact person by each partner. Whereas information can flow through various channels, such as R&D, commercial, or regulatory functions, all instances that cause project changes have to be channeled exclusively through the contact persons, who typically are also the project managers. Cases in point are changes in volume requirements or specifications on the customers' side, and process and analytical method modifications on the vendors' side.

A simplified project schedule is shown in Fig. 25.8. Whereas it is the responsibility of the project manager to monitor project milestones, the workload shifts from R&D to the pilot plant and engineering and manufacturing as the project progresses. Once regular production is initiated (in the example, three years after the first contact!), the project organization is dissolved and the business is transferred to the commercial functions of the partnering companies.

Contractual Arrangements From a lawyer's perspective, outsourcing involves passing to a third party the operational responsibility for one or more services. Outsourcing the chemical manufacturing of a pharmaceutical fine chemical entails a considerable financial exposure, sometimes hundreds of millions of dollars; covers an extended period of time, typically three to 10 years; and has to take into account a number of imponderable elements of the cooperation. It is mandatory, therefore, to hedge against unpredictable events by concluding an outsourcing or supply agreement. Prior to entering into contract negotiations, the partners should clearly define the scope and objectives that they wish to achieve. It should have the necessary provisions to cope with such "what if's" as delays in drug approval or startup of production, a substantial increase or decrease in demand (in the worst case, withdrawal of the drug), failure to meet the agreed-upon yield and throughput figures, unsolicited offers from third parties, subcontracting, and takeover of one partner by a competitor. The two main elements of a contract are the technical clauses, the specification or description of the services that the custom manufacturer is to provide; and the legal clauses, warranties, indemnities, and other boiler plate clauses that lay out the

TABLE 25.6 Key Elements of Outsourcing Contracts

Commercial	Technical and Regulatory	Legal
Product	Product specifications	Duration (extension/cancellation)
Quantities	Process description	Investment guarantees
Prices (price/quantity)	Process improvements	*Force majeure*
Forecasts	Plant description	Insurance coverage
Provision of starting materials	Quality control and assurance	Confidentiality
	Batch records	Intellectual property rights
Call-off orders	Audits and inspections	Liabilities[a]
Shipments	Drug master file	Compliance with laws
Packaging and labeling	Safety, health, and environment	Applicable law
Backup capacity		

[a]liabilities for consequential damage, such as claims of patients against the drug companies, are usually excluded.

parties' responsibilities to each other. The technical clauses are drafted by specialists from the activities involved. Key commercial, technical, and legal elements of an outsourcing contract are listed in Table 25.6. Independent of the degree of elaboration of the contract, a successful outsourcing arrangement will rely on the mutual trust of the parties. This also means that arbitration rather than litigation is the usual method used to settle a dispute.

25.2.2 Outsourcing of Research and Development

After the discovery of a new lead compound, a pharmaceutical company needs samples (typically, in the gram to kilogram range) of the development drug for the first clinical tests, and shortly afterward, an economical and environmentally acceptable method of synthesis for the production of larger quantities. The corresponding activity is indicated as "Lead Dev." in the drug development added-value chain (see the upper line in Fig. 25.9). It precedes manufacture of the NCE for clinical trials.

As such modern tools of pharmaceutical R&D as combinatorial chemistry and rapid-throughput screening have increased the production rate of lead compounds dramatically, there is a rapidly growing demand for the preparation of samples for NCEs and process

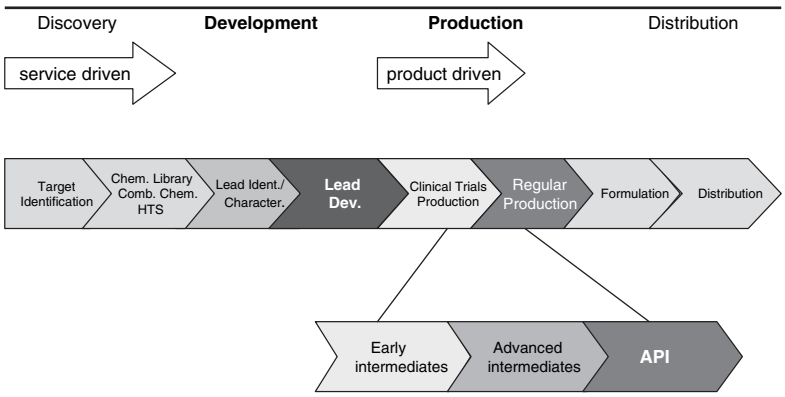

Figure 25.9 Pharmaceutical outsourcing along the value-added chain.

development services. Two types of companies are active in this business: small independent contract research organizations (CROs) and development centers of established fine chemical companies. The strategic rationale for the latter is to provide services throughout the life cycle of a new drug, dubbed a *one-stop shop*. In addition to the volume differences, a CRO differs from regular custom manufacturing in being service driven rather than product driven. The respective positions of contract research and custom manufacturing on the pharmaceutical supply chain are shown in Fig. 25.9. The calculation basis for custom manufacturing is the unit manufacturing cost (raw materials + transformation cost), or cost of goods sold. CROs base their pricing on labor hours spent on a project. The standard unit is the full-time equivalent, which comprises one year's work of a Ph.D. chemist plus ancillary services (mainly analytical work) in the laboratory. In terms of profitability, by and large a CRO is able "to make money, but not get rich." Given the inherent problems of CROs (e.g., moderate profitability, large number of nonrecurring small projects), it is not surprising that most CROs have been acquired by larger fine chemical companies: for example, Torcan/Avecia; ChemShop/DSM; PharmEco/Johnson Matthew; Oxford Asymmetry/Evotec; Lancaster Synthesis/Clariant; Palmer Research/Great Lakes Chemicals; Carbogen/Solutia.

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26

REGULATION-DRIVEN PROCESS CHEMISTRY

SHRIKANT V. KULKARNI
KulTech Incorporated
Cary, North Carolina

26.1 INTRODUCTION

A casual review of the offerings by various chemistry departments in U.S. universities indicates a general absence of training courses in process chemistry or industrial chemistry. Process chemistry is probably not recognized as a separate curriculum because it is truly an interdisciplinary chemical science. It needs the support of various chemical disciplines, such as synthetic chemistry (organic or inorganic), analytical chemistry, material science, physical chemistry (involving kinetics and thermodynamics), and environmental chemistry (including the effects of the product, its impurities, and its emissions into the environment). It also requires some knowledge of chemical engineering, such as the cost impacts of the choice of chemicals and process equipment, and of environmental engineering, waste treatment technology, and mechanical engineering which are needed for plant design and equipment selection as well as cost calculation. Nonchemical factors such as regulatory compliance requirements to reduce pollution also affect process development.

26.2 CHEMICAL INDUSTRY REGULATORY GUIDELINES

Traditionally, most industrial chemical processes were targeted toward meeting required product specifications at competitive prices without much regard to emissions from hazardous chemicals and their by-products. Recently, however, these considerations, especially process emissions such as solid waste, aqueous discharges, and air emissions, have dominated process development because of the regulatory and permit requirements of the

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U.S. Environmental Protection Agency (EPA). Secondary considerations include the cost of manufacture of product and the cost of disposal of waste.

With the increasing awareness of human exposure risk factors, the EPA has promulgated several regulations that apply to air and water emissions from process activities, storage of bulk chemicals, and treatment and disposal of solid wastes in a given manufacturing facility:

- Clean Air Act
- Clean Water Act
- Safe Drinking Water Act
- Resource Conservation and Recovery Act
- Comprehensive Environmental Response, Compensation and Liability Act
- Emergency Planning and Community Right-to-Know Act
- Pollution Prevention Act
- Toxic Substances Control Act
- Federal Insecticide, Fungicide, and Rodenticide Act

It is essential that process chemists, who have to design a synthesis using environmentally friendly chemicals and processes, have general background information on these requirements. For the purposes of this discussion, other EPA and federal acts, such as the Federal Food, Drug, and Cosmetic Act, the National Environmental Policy Act, and the Superfund Amendments and Reauthorization Act, are not emphasized here since they do not have a direct impact on process chemistry thinking involved in the example process discussed here. Updated summary information on the prevailing major environmental laws that form the basis for the programs of EPA may be obtained from http://www.epa.gov/epahome/laws.htm.

The chemical industry has paid a high price for retrofitting and installing sophisticated pollution control equipment to tackle wastewater, solid waste, and air emissions, and for financing soil and groundwater cleanups of National Pollution or Superfund sites. Like the rest of the chemical manufacturing industry, pharmaceutical products or intermediates manufacturing is subject to emission standards for hazardous air pollutants (HAPs) under the Clean Air Act Amendments. All pharmaceutical manufacturing plant sites must comply with the national emission standards for HAPs (NESHAPs) for the pharmaceutical industry, and EPA has promulgated maximum achievable control technology (MACT) standards for the industry. The MACT analysis requires that a production facility determine if it contains processes to manufacture a pharmaceutical product, precursor, or intermediate, according to EPA definitions, that may be subject to MACT provisions and other regulations. MACT looks basically at compliance with the best "end of pipe" treatment available, and the added cost for pollution prevention or pollution abatement and treatment gets absorbed in the cost of manufacturing.

The Clean Air Act (CAA) regulates the release of HAPs from a manufacturing facility and a region such as a state. Currently, the CAA list of regulated compounds contains 188 substances and categories of substances that may be considered HAPs, and 139 extremely hazardous substances, as well as ozone-depleting substances such as chlorofluorocarbons (CFCs), halons, hydrochlorofluorocarbons (HCFCs), carbon tetrachloride, methyl chloroform, and methyl bromide. Depending on the health risk assessment reviews and available information, more and more compounds or classes of compounds will be regulated. The candidate list (see Table 26.1) does not mean that the compounds or compound categories

TABLE 26.1 Hazardous Air Pollutants

CAS Number	Chemical Name[a]	CAS Number	Chemical Name[a]
75070	Acetaldehyde	98828	Cumene
60355	Acetamide	94757	2,4-D, salts and esters
75058	Acetonitrile	354704	4DDE
98862	Acetophenone	334883	Diazomethane
539632	Acetylaminofluorene	132649	Dibenzofurans
107028	Acrolein	96128	1,2-Dibromo-3-chloropropane
79061	Acrylamide	84742	Dibutylphthalate
79107	Acrylic acid	106467	1,4-Dichlorobenzene(p)
107131	Acrylonitrile	91941	3,3-Dichlorobenzidene
107051	Allyl chloride	111444	Dichloroethyl ether [bis (2-chloroethyl) ether]
926714	Aminobiphenyl		
62533	Aniline	542756	1,3-Dichloropropene
90040	*o*-Anisidine	62737	Dichlorvos
1332214	Asbestos	111422	Diethanolamine
71432	Benzene, in gasoline	121697	*N*, *N*-Diethyl aniline (*N*, *N*-dimethylaniline)
71432	Benzene		
92875	Benzidine	64675	Diethyl sulfate
98077	Benzotrichloride	119904	3,3-Dimethoxybenzidine
100447	Benzyl chloride	60117	Dimethyl aminoazobenzene
92524	Biphenyl	119937	3,3′-Dimethyl benzidine
117817	Bis(2-ethylhexyl)phthalate (DEHP)	79447	Dimethyl carbamoyl chloride
542881	Bis(chloromethyl) ether	68122	Dimethyl formamide
75252	Bromoform	57147	1,1-Dimethyl hydrazine
106990	1,3-Butadiene	131113	Dimethyl phthalate
156627	Calcium cyanamide	77781	Dimethyl sulfate
105602	Caprolactam (see modification)	534521	4,6-Dinitro-*o*-cresol, and salts
133062	Captan	51285	2,4-Dinitrophenol
63252	Carbaryl	121142	2,4-Dinitrotoluene
75150	Carbon disulfide	123911	1,4-Dioxane (1,4-diethyleneoxide)
56235	Carbon tetrachloride	122667	1,2-Diphenylhydrazine
463581	Carbonyl sulfide	106898	Epichlorohydrin (l-chloro-2,3-epoxypropane)
120809	Catechol		
133904	Chloramben	106887	1,2-Epoxybutane
57749	Chlordane	140885	Ethyl acrylate
7782505	Chlorine	100414	Ethyl benzene
79118	Chloroacetic acid	51796	Ethyl carbamate (urethane)
532274	2-Chloroacetophenone	75003	Ethyl chloride (chloroethane)
108907	Chlorobenzene	106934	Ethylene dibromide (dibromoethane)
510156	Chlorobenzilate		
67663	Chloroform	107062	Ethylene dichloride (1,2-dichloroethane)
107302	Chloromethyl methyl ether		
126998	Chloroprene	107211	Ethylene glycol
1319773	Cresols/cresylic acid (isomers and mixture)	151564	Ethylene imine (aziridine)
		75218	Ethylene oxide
95487	*o*-Cresol	96457	Ethylene thiourea
108394	*m*-Cresol	75343	Ethylidene dichloride (1,1-dichloroethane)
106445	*p*-Cresol		

(*Continued*)

TABLE 26.1 (*Continued*)

CAS Number	Chemical Name[a]	CAS Number	Chemical Name[a]
50000	Formaldehyde	56382	Parathion
76448	Heptachlor	82688	Pentachloronitrobenzene (quintobenzene)
118741	Hexachlorobenzene		
87683	Hexachlorobutadiene	87865	Pentachlorophenol
77474	Hexachlorocyclopentadiene	108952	Phenol
67721	Hexachloroethane	106503	*p*-Phenylenediamine
822060	Hexamethylene-1,6-diisocyanate	75445	Phosgene
680319	Hexamethylphosphoramide	7803512	Phosphine
110543	Hexane	7723140	Phosphorus
302012	Hydrazine	85449	Phthalic anhydride
7647010	Hydrochloric acid	1336363	Polychlorinated biphenyls (Aroclor)
7664393	Hydrogen fluoride (hydrofluoric acid)	1120714	1,3-Propane sultone
		57578	β-Propiolactone
7783064	Hydrogen sulfide (see modification)	123386	Propionaldehyde
		114261	Propoxur (Baygon)
123319	Hydroquinone	78875	Propylene dichloride (1,2-dichloropropane)
78591	Isophorone		
58899	Lindane (all isomers)	75569	Propylene oxide
108316	Maleic anhydride	75558	1,2-Propylenimine (2-methyl aziridine)
67561	Methanol		
72435	Methoxychlor	91225	Quinoline
74839	Methyl bromide (bromomethane)	106514	Quinone
74873	Methyl chloride (chloromethane)	100425	Styrene
71556	Methyl chloroform (1,1,1-trichloroethane)	96093	Styrene oxide
		1746016	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
78933	Methyl ethyl ketone (2-butanone)	79345	1,1,2,2-Tetrachloroethane
60344	Methyl hydrazine	127184	Tetrachloroethylene (perchloroethylene)
74884	Methyl iodide (iodomethane)		
108101	Methyl isobutyl ketone (hexone)	7550450	Titanium tetrachloride
624839	Methyl isocyanate	108883	Toluene
80626	Methyl methacrylate	95807	2,4-Toluene diamine
163404	Methyl tert butyl ether	584849	2,4-Toluene diisocyanate
101144	4,4-Methylene bis(2-chloroaniline)	95534	*o*-Toluidine
75092	Methylene chloride (dichloromethane)	8001352	Toxaphene (chlorinated camphene)
101688	Methylene diphenyl diisocyanate (MDI)	120821	1,2,4-Trichlorobenzene
		79005	1,1,2-Trichloroethane
101779	4,4-Methylenedianiline	79016	Trichloroethylene
91203	Naphthalene	95954	2,4,5-Trichlorophenol
98953	Nitrobenzene	88062	2,4,6-Trichlorophenol
92933	4-Nitrobiphenyl	121448	Triethylamine
100027	4-Nitrophenol	1582098	Trifluralin
79469	2-Nitropropane	540841	2,2,4-Trimethylpentane
684935	*N*-Nitroso-*N*-methylurea	108054	Vinyl acetate
62759	*N*-Nitrosodimethylamine	593602	Vinyl bromide
59892	*N*-Nitrosomorpholine	75014	Vinyl chloride

TABLE 26.1 (*Continued*)

CAS Number	Chemical Name[a]	CAS Number	Chemical Name[a]
75354	Vinylidene chloride (1,1-Dichloroethylene)		Cobalt compounds
			Coke oven emissions
1330207	Xylenes (isomers and mixture)		Cyanide compounds
95476	*o*-Xylenes		Glycol ethers[b]
108383	*m*-Xylenes		Lead compounds
106423	*p*-Xylenes		Manganese compounds
	Antimony compounds		Mercury compounds
	Arsenic compounds (inorganic and arsine)		Fine mineral fibers[c]
			Nickel compounds
	Beryllium compounds		Polycyclic organic matter[d]
	Cadmium compounds		Radionuclides (including radon)[e]
	Chromium compounds		Selenium compounds

[a]All listings that contain the word *compounds* and for glycol ethers, unless otherwise specified, are defined as including any unique chemical substance that contains the named chemical (i.e., antimony, arsenic, etc.) as part of that chemical's infrastructure. X′CN, where X 5 = H′ or any other group where a formal dissociation may occur [e.g., KCN or $Ca(CN)_2$].

[b]Includes mono- and diethers of ethylene glycol, diethylene glycol, and triethylene glycol $R—(OCH_2CH_2)_n—OR'$ where $n = 1, 2, \text{or } 3$; R = alkyl or aryl groups; R′ = R, H, or groups that when removed yield glycol ethers with the structure $R—(OCH_2CH)_n—OH$. Polymers are excluded from the glycol category (see modification).

[c]Includes mineral fiber emissions from facilities manufacturing or processing glass, rock, or slag fibers (or other mineral-derived fibers) of average diameter 1 μm or less.

[d]Includes organic compounds with more than one benzene ring that have a boiling point greater than or equal to 100°C.

[e]A type of atom that undergoes spontaneous radioactive decay.

on the list cannot be used but that these are merely candidates for regulation. In the future, many new compounds may be added to the list. Obviously, a process chemist designing a synthesis should avoid or limit use of the chemicals listed or provide for strict control of their release.

26.3 MANUFACTURING TECHNIQUES IN PROCESS CHEMISTRY

The traditional approach in process development is to focus on manufacturing a product with a process technology based on laboratory synthesis to meet the required specifications. When several synthetic pathways are options, the ready availability of raw materials, as well as their procurement and storage costs, plays a significant role in the selection of a process. Almost as an afterthought, emission control considerations are applied to treat the wastes generated so that the plant meets the regulatory and emission permit requirements. This approach, of treating waste to control pollution, is aptly described as *end-of-pipe treatment*. It is a commonly used approach that includes adding pollution control devices such as scrubbers, filters, wastewater treatment units, carbon adsorption, or off-site disposal at

the end of the process train without regard to selective use of starting materials or process conditions. Of course, as discussed earlier, implementation of MACT adds considerably to the cost of manufacturing, and unfortunately, these pollution control devices also lead to their own waste streams.

A second approach to emission control involves recycling materials so that the quantity of the emissions is reduced. For example, recycling of solvents using an efficient solvent recovery system reduces air emissions and the use of raw materials. Even though the cost of energy required for solvent recovery may not justify recycling over using virgin material, the reduction in cost of waste treatment may compensate for the difference. Stringent requirements for air emissions need expensive equipment with considerable capital outlay. Pollution prevention (P2) describes this second approach, in which the object is to recycle as much material as possible. Recycling may involve solvent recovery or recovery and purification of unused starting materials that may make the process cost-effective after allowances for labor, energy, and waste treatment. A number of practical examples of payback using P2 are available in several P2 databases from EPA and state agencies. EPA also makes available a wide range of information, such as P2 publications and advice from P2 experts, and several guides are available on the Web pages of the Michigan Department of Environmental Quality (http://www.michigan.gov/deq/0,1607,7-135-3585—,00.html).

As a next stage beyond P2, EPA is currently promoting a P3 concept that highlights people, prosperity, and the planet: three pillars of sustainability (http://es.epa.gov/ncer/p3/index.html). In this program, EPA considers challenges from a wide range of categories. These include agriculture (irrigation practices, storage and handling of food products, etc.); built environments (green buildings, transportation and mobility, smart growth, etc.); ecosystems (protection of ecosystem health, protection of biodiversity, etc.); materials and chemicals (materials conservation; inherently benign materials and chemicals through green engineering, green chemistry, and biotechnology; recovery and reuse of materials through product, process, or system design; renewable, bio-based feedstocks; etc.); energy (energy production; energy distribution; energy conservation; inherently benign energy through green chemistry, green engineering, and biotechnology; etc.); resources (delivery of and access to educational and medical information, etc.); water (water quality, quantity, conservation, availability, and access, etc.). This highly interrelated concept complicates the life of a process chemist but also offers opportunities for innovation and development of fresh approaches to product manufacturing.

A third approach, which forms the basis of *green chemistry*, is to adopt a synthetic pathway to manufacture a product that uses a process and materials that reduce or eliminate the use and generation of hazardous substances. EPA's concept of green chemistry in manufacturing processes shifts chemical thinking from control and remediation to sustainability and reduction of the environmental impact. In its description of green chemistry, EPA includes the "design, manufacture, and use of environmentally benign chemical products and processes that prevent pollution and reduce environmental and human health risks." EPA's Green Chemistry Program works with many partners to promote pollution prevention through green chemistry. Partnering organizations represent academia, industry, other government agencies, scientific societies, trade organizations, national laboratories, and research centers. Information on green chemistry and a list of partnering organizations are available online at http://www.epa.gov/opptintr/greenchemistry/whats_gc.html. This approach differs markedly from the prevailing approach of abating pollution through an

end-of-pipe treatment or recycling of materials (P2). The goal of process chemistry in the environment should be to select a chemical pathway and design a process to make a product economically, safely, and without adverse environmental impact. These requirements severely restrict the number of acceptable chemical pathways and processes but also allow for innovative ideas.

26.4 EFFECTS OF PESTICIDE INDUSTRY REGULATION

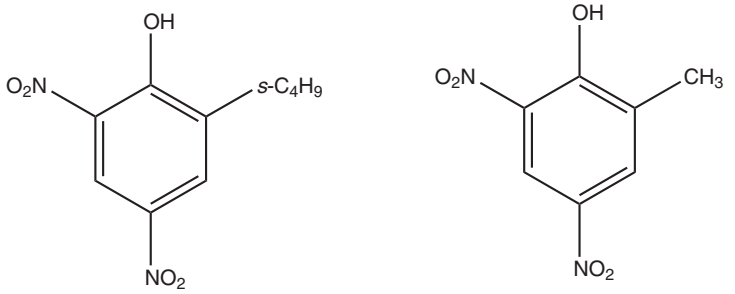
In the late 1970s, a process group led by the author when he worked as a process chemist with a contract manufacturer of pesticides and intermediates encountered an interesting example of the impact of regulations on the plant process and the changes that had to be implemented to comply with regulations as more and more regulations came into being. In the late 1960s and early 1970s, the plant was involved in the manufacture of various herbicides, insecticides, and fungicides used in the chemical control of weeds and insects to boost agricultural production. One of them was a popular preemergent herbicide Dinoseb (4,6-dinitro-2-*sec*-butylphenol). It was first described as an herbicide in 1945 (R1) and later marketed as Premerge by Dow Chemical Company.

One of the first regulatory actions of the EPA was to regulate the pesticide industry under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1976. FIFRA required that all pesticides used in the United States be registered or licensed by EPA to ensure that they do not cause unreasonable adverse effects on humans or the environment. FIFRA's definition of unreasonable adverse effects took into account the economic, social, and environmental costs and benefits of the use of pesticides. Pesticides were expected to be prime candidates for posing some risk because they were meant to kill or control insects, weeds, rodents, and other pests. Regulations were developed based on pesticide use site, such as crops (e.g., corn, soybeans, tomatoes, strawberries) and noncrops (agricultural/industrial/commercial) and the pests (e.g., insects, weeds, rodents) controlled through their application.

Even though pesticides presented some risks, they provided substantial benefits by increasing the food supply at reasonable cost. The risk assessment consisted of hazard identification, dose–response assessment to explore the relationship between the level of exposure and the occurrence of any adverse effect, and exposure assessment. Since epidemiological studies with reliable quantitative data on exposure were not readily available, the regulations changed as the tools for such evaluations became more sophisticated and acceptable. Another main regulatory concern was based on the fate of the chemicals in soil and water and their effect on life forms in those media. Because of severe restrictions on application and use, pesticide regulations and the marketplace kept changing as more and more reliable exposure information became available. In 1982, production of Dinoseb was estimated to be 6.2 million pounds. The use of Dinoseb in the United States was discontinued in 1986 as information became available on its acute toxicity, chronic toxicity, reproductive effects, teratogenic effects, mutagenic effects, carcinogenic effects, organ toxicity, and effects on birds, aquatic organisms, and bees. Environmental fate studies, including its breakdown in soil and groundwater, led to the application of other important environmental acts and regulations, such as the National Pollutant Discharge Elimination System (NPDES). These regulations centered on the discharge of pollutants in air, wastewater, and solid media and affected the manufacturing process considerably.

The process concepts and ideas implemented in the nitration process discussed in this chapter have universal applicability because of the importance of nitrated aromatic chemicals and because of the common nature of the discharge from the process. The nitro-aromatics are industrially important because the reduction of these compounds leads to aromatic amines, which form the backbone of pharmaceutical, pesticide, and textile chemicals. This process presented the author's process group with opportunities to work on nitration processes that used some of these pollution prevention concepts. This was before labels such as *green chemistry* and *P2* were articulated. The company was involved in the manufacture of several widely used large-volume pesticides for different pesticide companies and distributors under contract. As a custom manufacturer, the company specialized in adopting client processes in the company's own midsized plants to develop data needed to build larger manufacturing facilities. This approach provided a means for large manufacturers or clients to check out their laboratory and pilot-plant processes, or to meet their marketing needs when their products were being introduced in the market and their plants were being built. This contract manufacturing environment offered unique opportunities to learn about then-current manufacturing processes and to assist in their implementation in manufacturing plants. Also, the company's flexibility in operations supported innovations that resulted in capital cost savings and time for implementation. Most of the processes for the major pesticide products used classic late-1970s approaches such as end-of-pipe waste treatment. As the country and EPA became increasingly aware of the problems of human exposure to pesticides and related operations, including application, manufacturing, and waste disposal, pesticide use was increasingly regulated and restricted. Consequently, end-of-pipe treatment options and waste disposal options were under severe scrutiny.

Because of the expertise and available facilities for manufacturing 4,6-dinitro-2-*sec*-butylphenol, the company had an opportunity to manufacture a product with an approximately 22 to 23% solution of 4,6-dinitro-*o*-cresol as a monomer stabilizer for styrene (Scheme 26.1). The opportunity to introduce this new product posed an interesting problem: whether the company should employ the proven nitration process used for the manufacture of DNBP or explore alternatives. The plant had been manufacturing DNBP and selling it as a preemergence herbicide under its trade name and pesticide registration during the early 1970s. The process was a typical scale-up of laboratory synthesis



4,6-dinitro-2-*sec*-butylphenol (DNBP)

4,6-dinitro-*o*-cresol (DNOC)

Scheme 26.1

using an old facility that was regulated under a grandfather clause. In the period when environmental concerns were just emerging and regulations were being formulated, manufacturing facilities that operated under a grandfather clause were not as stringently regulated and were allowed to use a patchwork of pollution measures. The plant was a typical scaled-up process with add-on pollution control measures without consideration for integrated P2 technologies.

The nitration of *o-sec*-butylphenol (OSBP) followed a classic nitration reaction used by synthetic organic chemists over a period of time. OSBP was reacted with concentrated sulfuric acid until it was essentially converted to a sulfonic acid derivative. This reaction product was then dissolved in water and sufficient nitric acid was gradually added to the solution with agitation to convert the sulfonic acid derivative to the desired dinitrophenol product. In this nitration process, a sulfonated alkylphenol adduct intermediate in acid solution was treated with 17% nitric acid to convert it into dinitrated *sec*-butylphenol. The product of nitration formed a separate phase from the aqueous mixed acids, so the mass transfer was involved simultaneously with the chemical reaction, thereby reducing the conditions for secondary reactions.[1] The progress of the nitration reaction was monitored by tracking the loss of the alkylphenol from its initial amount at the beginning of the nitration process. The end of the reaction was determined by the amount of unreacted *sec*-butylphenol in the in-process samples. Another indication of the end of the reaction was the reduction of nitric acid content in the reaction mixture.

The *sec*-butyldinitrophenol compounds could be prepared in 95 to 98% purity after product wash. The large volume of wastewater—approximately 0.63 gallon of wastewater generated per pound of product formed—contained a mixture of sulfuric acid, nitric acid, trace amounts of phenol, nitrated product, and other process impurities. The residual mixture of sulfuric and nitric acids at the end of the reaction could not be separated and reused because of the significant energy expense. Recovering fuming sulfuric acid was out of the question, and recovering the nitric acid, which could have been recovered by distillation, would require a large amount of energy. The nitric acid produced at the plant site was a lot cheaper. The large volume of wastewater containing the mixture of sulfates and nitrates and trace amounts of nitrated organics, as indicated by its yellow color, was neutralized and treated with activated carbon to remove organics. Disposal of that wastewater, which met all regulatory permit requirements, was difficult because of environmental awareness and the "not in my backyard" attitudes of communities where the waste disposal operations were located. Eventually, the EPA banned this pesticide and destroyed the stocks of it by incineration. However, the explosive characteristics of these compounds, which structurally resemble trinitrotoluene (TNT), in turn, created another interesting process problem for disposal of the pesticide stocks.

26.5 EFFORTS AT DENITRIFICATION

An attempt was made to reduce the amount of wastewater generated in the reactions for manufacture of DNBP at the source by controlling the molar ratios of OSBP and fuming sulfuric acid. Instead of using 2 mol of sulfuric acid only 1 mol was used. This change resulted in the same yield (94 to 97%) and purity (97 to 98.5%) of the product. Since the sulfuric acid use was reduced by half, the volume of wastewater per pound of product fell from 0.63 gallon/pound of product to 0.45 gallon/pound of product. This attempt to reduce

pollution still did not solve the problems of on- and off-site waste disposal. The difficulties in waste treatment and disposal suggested the need for an alternative approach to the dinitration of similar alkylated phenol. To develop a successful process, we realized that the process must meet the following five targets:

1. The product must meet user specifications.
2. The reaction must be safe and able to be controlled properly.
3. The reaction rate must be adequate to meet the throughput requirements.
4. Waste streams must be minimal and manageable.
5. Management support must be obtained to implement changes and new ideas, with emphasis on cost savings, safety, and ease of operation.

With these criteria in mind, we investigated various alternative approaches for the preparation of DNOC (Scheme 26.1). Nearly every textbook on organic chemistry deals with the reactions to synthesize aromatic nitro compounds. In addition to their commercial uses as explosive compounds, the reduction of nitro groups leads to a variety of functional groups, the most important being the amino group. Since the Sandmeyer and related reactions permit replacement of amino groups with many other groups, a synthesis of virtually any polysubstituted aromatic compound can be based on nitration as an early step. As is well known in the history of chemistry, in 1771 Woulfe prepared picric acid by reacting nitric acid on indigo and showed that it dyed silk in bright yellow shades. In 1842, Laurent converted phenol into picric acid. The first synthetic dye to be manufactured, mauve, was discovered in 1856 by Perkin and used in practical dyeing. Faraday's discovery of benzene in 1825 was followed by Hofmann's recovery of benzene from coal tar. Kekulé's structure determination of benzene paved the way for the systematic study of aromatic compounds. Since then there has been an explosion of manufactured chemicals accompanied by the realization that exposure to certain chemicals can be very harmful for the environment and human health. Modern pharmaceuticals and related biologically active compounds have their own set of problems associated with their production, manufacture, and processing.

Virtually all nitration reactions involve electrophillic attack by nitronium[2] ions, NO_2^+. Consequently, reactions can be regulated by controlling the concentration of nitronium ions in solution. The conditions required for the nitration reaction vary greatly with the reactivity of the aromatic substrate. The nitration mixture required for introduction of the second nitro group into benzene to prepare dinitrobenzene and concentrated nitric and sulfuric acids at 95°C is unsuitable for dinitration of alkylated phenol because it provides the conditions for an uncontrollable exothermic reaction.[3] The DNBP process described above was based on controlling the nitration conditions and has the advantage of having very few side reactions because it is a two-phase system with nitration reactivity based on mass transfer between phases.

Literature review in the late 1970s suggested that industrial nitration reactions had been studied extensively because of the use of nitrated aromatic compounds in dyes, pesticides, and the preparation of explosives such as TNT. American Chemical Symposium Series 22, *Industrial and Laboratory Nitrations*, by Albright and Hanson,[4] provided a very broad review of the activities up to that point. Since small amounts of NO_2^+ are present in concentrated nitric acid, the study of homogeneous, single-phase,

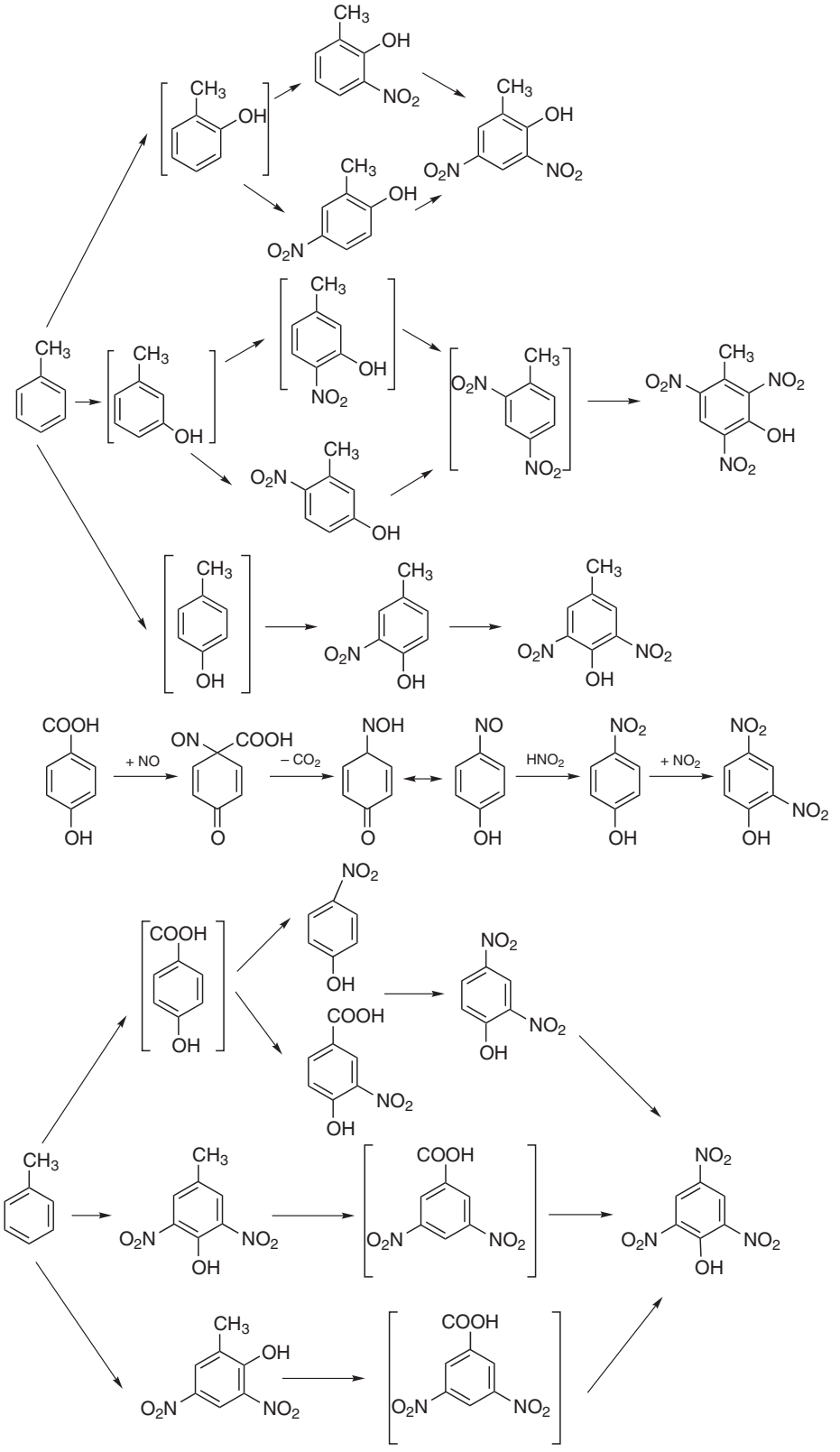
or direct nitration using concentrated nitric acid has been of great interest. It was widely reported that the rates and kinetics of nitration are very dependent on the strength of the acid.[5] Unfortunately, the concentrations at which one gets a satisfactory rate of nitration are also conducive to oxidizing the organic compounds.[6] The result is that this type of nitration gives rise to considerable oxidative degeneration of impurities, thereby affecting the purity of the final product. A good understanding of physical chemistry, kinetics, and mechanisms of reaction was found to be essential to develop this process.

In addition to reducing the purity of the product, the concentrated acids react with the substrates, producing reddish-brown fumes of nitrogen oxides (NO_x), which need to be controlled carefully as NO_x emissions are a subject of regulatory concern. Some of the side products may also get nitrated and produce thermally unstable compounds that may lead to explosions if the reaction conditions are not suitably manipulated. There are several anecdotal references on explosions during industrial nitration processes.[7] Because the senior managers had heard a lot of stories of explosions and reactions "running wild," they were resistant to the idea that a direct nitration should be considered instead of the proven sulfonation–nitration approach. "If it ain't broke, don't fix it" was the argument used most, even when disposal of waste from similar processes made the manufacture of DNOC virtually impossible.

During the process development phase, we conducted experiments to determine the sequence of addition of the reagents nitric acid and *o*-cresol, the optimal concentrations of nitric acid, and the optimal operating conditions. The direct nitrations are associated with induction periods during which no apparent reaction takes place and temperatures remain unchanged. After the induction period, there is a rapid rise in temperature and copious evolution of reddish-brown nitrogen oxide (NO_x) fumes, indicating the initiation of nitration. Once initiated, the nitration reaction rate can be controlled by the rate of addition of reagents. We let the reactions become vigorous and uncontrollable, depending on the amounts of cresol added to the reactor (Scheme 26.2). The reaction profile of an uncontrolled reaction is shown in Fig. 26.1.

Starting from ambient temperatures and letting the reaction continue after the induction period, we observed that the product purity at the completion of the reaction was approximately 79 to 80%. We hypothesized that in the induction period the oxidative reactions continued until a minimum temperature was reached to initiate nitration. Based on this concept, we decided to eliminate the nitration induction period and add alkylphenol to the 15% nitric acid preheated to 75 to 85°C. The product purity increased considerably, up to 92 to 94%. We also found that when more concentrated nitric acid was used, such as 25 to 30%, fuming resulted, giving lower yields and purity of DNOC. This increased fuming presumably produces more oxidation and subsequently more decomposition of the organic moieties. Thus, the yield and purity of the final product are reduced.

The Dow Chemical Company patent[8] titled "Direct Nitration of Alkylphenols with Nitric Acid" recommends the use of oxidation inhibitors such as a secondary or tertiary alcohol, a secondary alkyl nitrate, an aldehyde, a ketone, or a mixture thereof. The inventors suggest that the antioxidants reduce the amounts of undesirable oxidation by-products, especially quinone, and increase the conversion to the desired nitrated product. Since the 90 to 94% purity for DNOC achieved in direct nitration was acceptable and the antioxidant additives did not appear to increase the product purity significantly, we decided not to pursue incorporation of antioxidants.



Scheme 26.2 Mechanism for cresol oxidation reactions. (From ref. 6.)

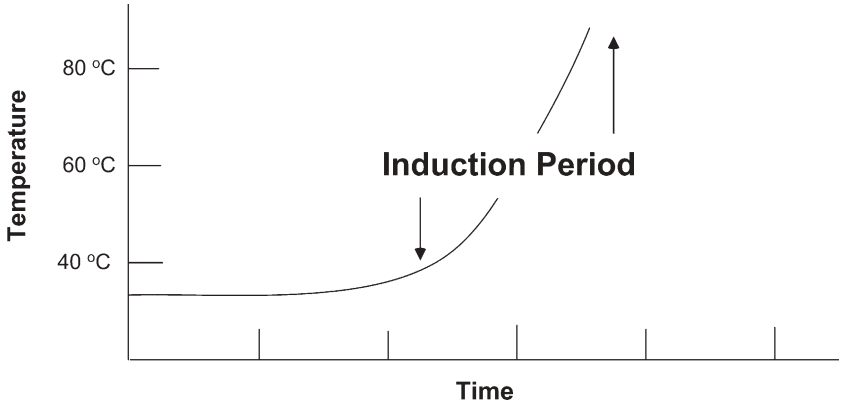


Figure 26.1 Reaction profile for nitration reaction.

Our laboratory investigations indicated that the purity of the dinitro compounds prepared by homogeneous or direct nitration would not exceed 90 ± 2%. On the other hand, achieving product purity between 95 and 98% using the traditional sulfonation process followed by nitration was no problem. Since the pesticide DNBP was required to be at least 95% pure, the direct nitration process could not be adopted for that product. In this particular case, the client needed approximately a 22% solution of DNOC in styrene. Product testing by the user indicated that the DNOC purity requirement for the styrene monomer stabilizer solution was not critical as long as it exceeded 90%. The final formulation contained 22 to 24% DNOC in styrene. The product met the specifications, was tested by the user, and performed well in field tests. The product also had no chlorinated compounds present even in trace amounts as specified by the user.

We emphasized that for the process to be successful, we had to provide controlled conditions, achieve the desired product purity, and reduce pollution control costs. One approach was to carry out direct nitration in a temperature-limiting solvent such as ethylene dichloride (boiling point 83 to 84°C) with 40% nitric acid (Fig. 26.2). In the laboratory, the reaction was conducted by preheating 40% nitric acid to 75°C and adding a 30% solution of *o*-cresol in ethylene dichloride (EDC) to the stirred acid after heating was discontinued. The reaction is exothermic. A vigorous reflux was observed at 76°C. The rate of reaction was controlled by the rate of addition of *o*-cresol solution. Reddish-brown fumes were observed at the beginning of the reaction. The rate of generation of the NO_x fumes subsided considerably after the addition of approximately 20% of the EDC solution of *o*-cresol. After the addition was complete, the reaction was heated to reflux (75 to 76°C) while stirring. A sample removed at the end of 2 hours and analyzed by gas chromatography showed more than 97% conversion, and the reaction was assumed to be complete. The end of the reaction was achieved in 2 to 3 hours and had better than 97% dinitro-*o*-cresol and less than 3% mononitro-*o*-cresol and *o*-cresol. The stirring was stopped and layers were allowed to separate. The separation was observed to have a clear interphase. The lower layer of DNOC in EDC was removed. This step was followed by solvent exchange to remove ethylene chloride, which was vacuum stripped from the product and replaced by a solvent of the client's choice. The possibilities of recycling the ethylene chloride and the spent acid were explored. Approximately 4% spent nitric acid was brought up to 17% nitric acid using the plant-manufactured 55% nitric acid

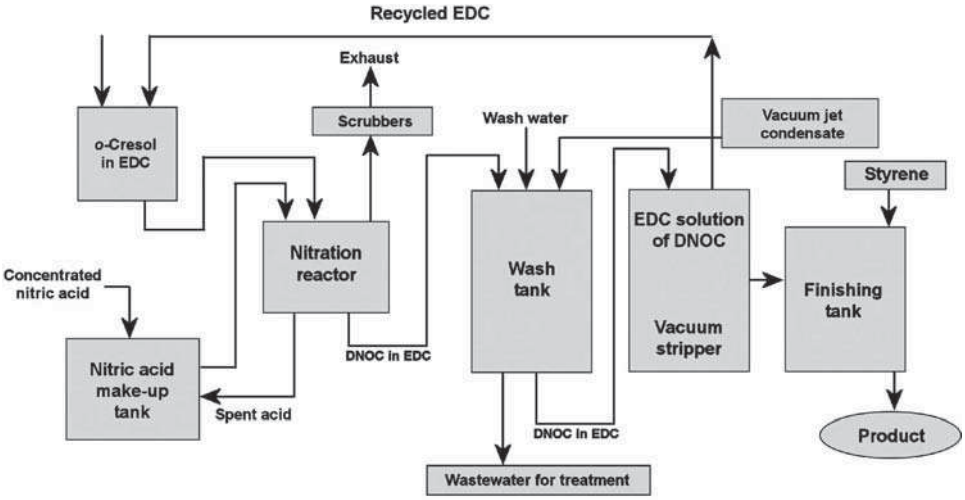


Figure 26.2 Process schematic for direct nitration using solvent and reconstituted nitric acid.

and was reused for nitration. The excess spent acid that was not used in the subsequent recycling could easily be disposed of either by neutralization of acid, by vacuum stripping, or in the plant waste treatment facilities. In the laboratory, recycling proved to be feasible in seven sequential batches using the ethylene dichloride recovered and the acid reconstituted.

Although the product indicated satisfactory removal of the chlorinated solvent, the product specification proposed by the client excluded the use or presence of chlorinated compounds. Solvent use would have provided us with more controlled conditions, allowed us to recycle most of the material, and provided reasonable throughput. Very few solvents have a boiling-point range of 75 to 85°C and are inert to nitric acid. The search for an inert substitute for EDC suitable in this boiling-point range was not successful. An alternative solution would be to run the reaction without the use of solvent.

The process of choice for nitration thus turned out to be direct nitration at 75 to 85°C with preheated 15% nitric acid, which provided gentle and controllable nitration conditions for manufacture of the product while keeping oxidation reactions to a minimum. At the completion of the reaction, the product could be separated, washed with a minimum amount of water for complete phase separation, and then dissolved in styrene to prepare a 22% solution for customer use.

The process of direct nitration of alkylated phenols was developed and implemented in the manufacturing plant. Later, the process was extended to nitration of phenol and several other alkylated phenols, substituted aniline compounds, and so on, by McDaniel and Gross.[9] The process was implemented successfully to meet customer demand because of considerable reduction of in-process pollution streams, which could be treated in the plant treatment facility with minimal plant modifications and ease of operation. The problems of treating and disposing of process wastes off-site were eliminated, making the process feasible compared to the DNBP process.

26.6 EVOLUTION TO GREEN CHEMISTRY

Many of the innovations we implemented are based on common sense and are included in EPA's definitions of green chemistry. The EPA issued 12 principles of green chemistry,[10] which explain how green chemistry is used in practice. The principles cover such concepts as:

- Design of processes to maximize the amount of raw material converted to product
- Use of safe, environmentally benign solvents where possible
- Design of energy-efficient processes
- Use of the best form of waste disposal: aiming not to create it in the first place

The 12 principles of green chemistry are as follows:

1. *Prevention*. It is better to prevent waste than to treat or clean up waste after it has been created.
2. *Atom economy*. Synthetic methods should be designed to maximize the incorporation into the final product of all materials used in the process.
3. *Less hazardous chemical synthesis*. Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to people or the environment.
4. *Designing safer chemicals*. Chemical products should be designed to effect their desired function while minimizing their toxicity.
5. *Safer solvents and auxiliaries*. The use of auxiliary substances (e.g., solvents or separation agents) should be made unnecessary whenever possible and innocuous when used.
6. *Designing for energy efficiency*. Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. *Use of renewable feedstock*. A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. *Reduction of derivatives*. Unnecessary derivatization (use of blocking groups, protection/de-protection, and temporary modification of physical and chemical processes) should be minimized or avoided if possible because such steps require additional reagents and can generate waste.
9. *Catalysis*. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. *Designing for degradation*. Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. *Real-time analysis for pollution prevention*. Analytical methodologies need to be developed further to allow for real-time in-process monitoring and control prior to the formation of hazardous substances.
12. *Inherently safer chemistry for accident prevention*. Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

Additionally, under the Europa Environmental Directive 96/82/EC called Saveso II Directive[11] on the control of major accident hazard involving dangerous substances, information on dangerous substances is inventoried. Of the several interesting literature databases, a process chemist would find the literature references from www.rxeforum.com very useful for the kinetic models and calorimetric data for product development and validation very useful while considering the thermal stability of the reactions. The article "The Identification of Decomposition Products in as Industrial Nitration Process under Thermal Runaway Conditions"[12] would have been very useful in developing the direct nitration process.

Another tool for the process chemist is EPA's SMART[13] (Synthetic Methodology Assessment for Reduction Techniques), a nonregulatory way of using chemistry to achieve pollution prevention. The SMART review operates in parallel with the New Chemicals Program. The general methodology of the SMART review is applicable to any chemical process. EPA uses the SMART review to assess the manufacturing methods described in new chemical submissions. The EPA may eventually recommend green chemistry approaches to reducing pollution at the source prior to commercial production of new chemical substances. A process developer can use his own SMART assessment to find a solution to pollution problems by conducting technical and economic studies of the manufacturing process. A Green Chemistry Expert System can be found at EPA's Web site: http://www.epa.gov/opptintr/greenchemistry/smart.html.

The plant was able to produce the required dinitrated *o*-cresol as long as the emissions and wastewater discharges were controlled carefully and met all the regulatory emission requirements. The two-step nitration process was abandoned because of pollution control problems. Even after meeting the wastewater discharge guidelines, the disposal of wastewater was a difficult proposition. Moreover, the difficulties in the off-site disposal of treated wastewater were enormous because of public perception of the product. The production of the compound for noncrop and nonagricultural use was made possible by using the nonconventional approach to normally accepted reaction and process chemistry. Basically, the waste treatment problems were tackled by eliminating the generation of untreatable waste.

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27

CHEMICAL PROCESS SCALE-UP TOOLS: MIXING CALCULATIONS, STATISTICAL DESIGN OF EXPERIMENTS, AND AUTOMATED LABORATORY REACTORS

ANDREI A. ZLOTA
The Zlota Company, LLC
Sharon, Massachusetts

27.1 CHEMICAL PROCESS SCALE-UP CHALLENGES

After medicinal chemists nominate a compound as a developmental target, process chemists initiate process research and development activities. As described elsewhere in the book, process research chemists carefully analyze the medicinal chemistry route and make suitable modifications. Chemical process development converts a bench synthetic procedure into a safe, robust, and economically viable process suitable for manufacturing.[1] The concept of *process robustness* is described as follows[2]:

"A rugged process is one that is understood well enough to produce reproducible quality and yields without resorting to unexpected problem solving… [for a robust process] *critical process parameters* have been identified. Plateau-type conditions, where yield and quality are not affected by deviating from the optimal conditions, are preferred."

An excellent summary of some of the challenges encountered in chemical process scale-up[3] activities was published recently[4]; some of the most important differences between plant equipment and laboratory glassware that can be the source of such challenges are described below:

1. Typical plant reactors do not allow for good *visual monitoring* because limited observation is possible from the top of the reactor. Recent techniques, such as the Mettler Toledo-PVM (Particle Vision and Measurement),[5] enable in-process imaging.

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Additional in-process analytical tools are used increasingly in plants for better process monitoring and control, aligned with the newly developed PAT (process analytical technology) concepts introduced by the U.S. Food and Drug Administration.[6] Such in-process control provisions remove another possible challenge of plant processing: sampling.

2. The *mixing conditions* in the plant reactor are different from those in a small-scale laboratory (see detailed discussion of this topic below). Different mixing conditions mean different mass and heat transfer capabilities of the equipment. Such differences must be understood and used to benefit the process. Some processes, such as reactive crystallizations and fast reactions, are much more sensitive to mixing than other processes.
3. *Operational times* are usually greater in the plant than in the laboratory. In some cases, process robustness can be challenged by greater than expected hold times of reaction masses and slurries.
4. *Volume measurement* is likely to be less accurate at plant scale, either because of the use of simple devices such as calibrated dipsticks, or because more sophisticated instruments such as scales or ultrasound-based devices cannot be used reliably.

Chemical process development is a highly interactive activity involving synthetic chemists, chemical engineers, analytical chemists, formulation scientists, and so on. Effective teamwork is crucial for the success of such cross-functional teams. The following is a useful summary describing the role of chemical engineers in pharmaceutical process research and development:[7] "Chemical Engineers augment the extensive organic chemistry creativity of the [process] chemists, with insights in scale-up concerns related to reaction kinetics, heat and mass transfer, mixing and separations."

There are several definitions of scale-up, the one in a classical scale-up textbook being[8]: "Scale-up is defined as the successful startup and operation of a commercial size unit whose design and operating procedures are in part based upon experimentation and demonstration at a smaller scale of operation."

For the pharmaceutical industry, scale-up activities often refer to the scale-up of processes in pilot plants. More important, pharmaceutical process development frequently involves fitting a process to available plant equipment rather than designing an optimal plant for the manufacture of every drug substance in the pipeline. Only for the manufacture of commercially successful drugs is a dedicated plant designed and built.

Although process scientists develop scale-up strategies aimed at reproducing process results obtained at a small scale, we must remember that:[9]

"Safe scale-up or scalability means that one can ensure that the final plant or unit can meet all of the product and process specifications on the basis of data available [at the time of design]. It does not mean that the plant can exactly duplicate laboratory or pilot-plant operation. It may not even duplicate a large pilot plant."

The objective of scale-up and process development in the pharmaceutical industry is[10]: "… designing to operate a process safely and cost effectively with predictable results at the scale of choice, by making best use of data and knowledge available at a certain time."

In addition to the business pressure for success from the first scale-up experiment, development engineers are frequently reminded that a surprise in research is called discovery, whereas a surprise in development is often called a disaster. A recent analysis of

70 cases of poor scale-up performance revealed that in more than half the cases, failure was attributed to the unexpected impact of physical issues, such as longer processing times or heat/mass transfer and mixing effects.[11]

The two examples below describe aspects of the chemical engineers' contributions to the development of a process to produce a drug substance.

27.2 CASE STUDY: DEVELOPMENT OF AN ACTIVE PHARMACEUTICAL INGREDIENT CRYSTALLIZATION PROCESS[12]

Bench chemists developed a preliminary process for the production and purification by crystallization of an active pharmaceutical ingredient (API) as follows:

1. Dissolve 0.2 mol of drug precursor as a free base in 1 L of an isopropanol–water (95/5% v/v) solvent mixture upon heating to 35°C.
2. Add an equivalent amount of a chiral acid as a 3 M aqueous solution to the stirred solution of the free base prepared above. Crystallization is observed immediately (such fast crystallizations are often referred to as *precipitations*). The solids are filtered, washed, and dried to afford a good yield (>90%) of the API as a chiral salt.

Such experiments are typically executed in round-bottomed flasks, sometimes jacketed, other times heated or cooled using a bath. The temperatures reported in such procedures are sometimes batch temperatures; other times they refer to the temperature set point in the heat transfer device. Such laboratory flasks are mixed using magnetic stir bars or overhead agitators fitted with half-moon Teflon blades. As mentioned above, plant mixing, and hence heat and mass transfer, are different from laboratory mixing. "Good mixing" as reported by the bench chemist is often difficult to translate immediately into good mixing at a large scale. Mixing and scale-up calculations as described below are some of the tools required to execute such a translation of mixing conditions.

The approach used in bench experimentation can be explained by the focus of the process chemist to identify a synthetic route or crystallization conditions without paying too much attention to the operational details as they will occur on large scale. These are some of the apparently minor challenges of technology transfer. Technology transfer is one of the most important activities in pharmaceutical development and will not be discussed further here, as it is addressed well in many publications and courses.[14]

We must note that the examples below address the heart of the chemical process: the reactions occurring therein. Every chemical process includes various unit operations, such as distillation, filtration, and drying. There are scale-up challenges associated with each such unit operation (distinct process step) but they are not addressed in this chapter.[15]

When the process described above was scaled up to the kilo-laboratory, the API failed specifications for residual organic volatile impurity (OVI)/isopropanol (IPA) content. The measured levels were about 1%, whereas the specification is "no more than 0.5%." Additional drying did not lower the OVI–IPA value to acceptable levels. More data were gathered as additional bench experiments were conducted. In the meantime two important project developments occurred:

1. The project team requested that 100 kg of API be produced within three months.
2. A polymorph[16] screening alerted the development team to the fact that in addition to the polymorph desired, an additional form can be obtained under the process conditions practiced at the time.

When the speed of process development and implementation to manufacturing is very high, process development using only fundamental knowledge is impractical. One of the very helpful tools used to solve complex technical problems is statistics. The most important benefit of statistically designed experiments is that they provide a strict mathematical framework for changing all pertinent factors simultaneously, and that they achieve this in a relatively small number of experiments.

The guidelines for a design of experiment (DoE) were summarized by Montgomery[17]:

1. Recognition and statement of the problem
2. Choice of factors, levels, and ranges
3. Selection of the response variable
4. Choice of experimental design
5. Performance of the experiment
6. Statistical analysis of the data
7. Conclusions and recommendations

In this case the problem could be stated as follows: Develop a reproducible crystallization process that produces an API of acceptable quality attributes, including OVI–IPA and solid-state form. A brainstorming session with the team produced a list of 13 such factors, four of which were deemed very likely to be critical to the process outcome, and were incorporated in the design. These parameters were:

- Solvent composition: IPA, methyl *tert*-butyl ether (MTBE), water
- Temperature of chiral acid solution addition

Solubility measurements guided the selection of the concentration of the substrate, and bench-gained experience helped determine the ranges for factors to be investigated. In addition to OVI–IPA and polymorph type, yield and optical and chemical purity were included as response variables.

When performing such DoEs, the use of automated laboratory reactors is highly recommended. In addition to having recipe capabilities and agitation and temperature controls, they also have data acquisition and analysis modules.[18] For example, the Mettler Toledo RC1 (reactor calorimeter) is an automated laboratory reactor capable of measuring heats of reaction. Such measurements are important in mechanistic investigations and safety evaluations.[19] We also believe that after the development chemist, reaction calorimetry is the development engineer's best friend.

The exact operating parameters to be used in the design, such as batch size and agitation speed, are determined by prior experience and mixing and scale-up calculations. In the second example below, on the Bourne III reactive system (Section 27.3), we discuss such calculations in more detail.

TABLE 27.1 Experimental Matrix with Design and Factor Ranges[a]

	Input				Output		
	Temp., T	Solvent Composition (% wt/wt)			OVI–IPA	Yield	
Exp.	(°C)	IPA	H_2O	MTBE	(% wt/wt)	(%)	Comments
1	35	97	3	0	0.64	97	Preliminary process
2	50	97	3	0	0.17	96	
3	50	87	13	0	0.18	66	
4	35	89	3	8	0.66	97	
5	35	80	13	7	0.15	72	
6	50	89	3	8	0.18	97	
7	35	87.5	8.5	4	0.20	87	Center point[b]
8	35	97	3	0	0.61	92	Replica of experiment 1
9	50	89	3	8	0.19	97	Replica of experiment 6, new process
10	50	89	3	8	0.14	97	Replica of experiment 6, new process

[a]IPA, isopropanol; MTBE, methyl *tert*-butyl ether; OVI, organic volatile impurity.
[b]Because temperature was considered a categorical factor, the temperature 35°C was used instead of a "true" middle point of 42.5°C.

The experimental matrix, including the results for OVI–IPA and yield, is depicted in Table 27.1. The data analysis was executed using the DoE FusionPro package from S-Matrix Co.[20] Additional statistical information useful for chemical experiments is available.[21]

The analysis of such statistically designed experiments tries to answer the following questions:

1. Which of the factors investigated affects the process results?
2. What equation best describes the dependence observed?
3. What levels of the factors investigated are necessary to obtain optimized process results?

In statistically designed experiments, a broader screening set of experiments frequently answers the first question, a more focused experimental matrix generates the model for the second, and that matrix delivers the optimization to answer the third question. It is important to put statistical methods for chemical process optimization in a suitable context[9]: "When one designs a new jet passenger plane or a fighter, one has to spend a lot of money and time in obtaining reliable model information…."

An analysis of this matrix showed that for yield, the most important factors are the water and isopropanol levels in the crystallization solvent. The corresponding model developed has a very good correlation coefficient (r^2 adj. = 0.98). For OVI–IPA, isopropanol–temperature and isopropanol–temperature–water interactions are the most important. The quality of the model developed for OVI–IPA was acceptable (r^2 adj. = 0.85).

When executing an optimization analysis, the white region depicted in Fig. 27.1 describes the allowable operating space. The factor with the most restricted range is water, at 3 to 5%. To confirm the quality of the optimization analysis, confirmatory runs were

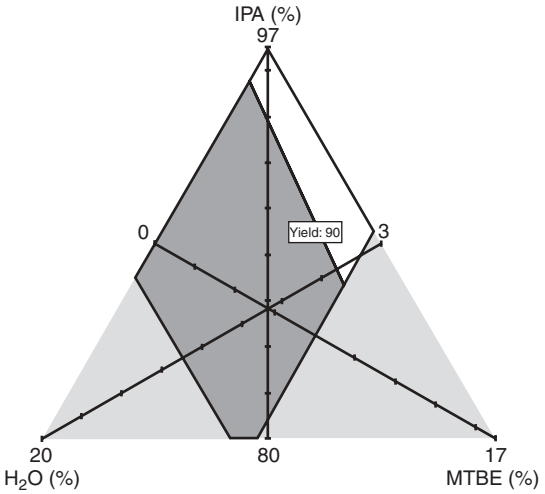

Figure 27.1 Visualization of the optimization analysis using DoE FusionPro. The white area is the desirable operational space.

executed under 89:8:3 conditions (IPA/MTBE/water) at 50°C. In three experiments, the yield and the OVI–IPA values were 96.5 ± 0.5% and 0.17 ± 0.02%, respectively, as predicted. The new process designed uses those conditions and was scaled up successfully to the kilo-laboratory and pilot-plant scales, producing an excellent-quality API of the desired polymorph. In conclusion, in this case study we showed how we use statistical design of experiments to optimize the purification by crystallization of an active pharmaceutical ingredient. "Statistical techniques are widely used in process development… Analysis of the results permits identification of the most important variables, and of the optimum process conditions. Acceptance of the need for the technique admits that the variables contributing to process performance are insufficiently understood to permit a process design from first principles…."[22]

27.3 CASE STUDY: DETERMINATION OF A SCALE-UP FACTOR FOR THE BOURNE III REACTIVE SYSTEM[23]

After the experimental conditions are optimized but before scale-up to a larger reactor is begun, the development engineer must assess the impact of the equipment on process results. The type of agitator, agitator speed, and batch size must be determined. Frequently, this is done by running suitable mixing calculations and executing the scale-up in a kilo-laboratory reactor by increasing the reaction mass by a factor of about 50. In rare cases, the reduced complexity of the process allows direct scale-up to the pilot plant. In many cases**,** because processes are mixing sensitive, scale-up must be executed with caution.

The Bourne III reactive system has been investigated extensively in the past 10 years and used to design pilot-plant equipment.[7,24b] This is a reactive system comprised of two

reactions, one extremely fast ($k_1 = 1.3 \times 10^8$ m^3/mol · s) acid–base neutralization, the other a slower basic ester hydrolysis ($k_2 = 0.03$ m^3/mol · s). Under ideal mixing conditions, no ester hydrolysis should occur. Under typical nonideal mixing conditions, some ethanol formation can be observed.

Reactive systems such as Bourne III are often encountered in the pharmaceutical industry, as in the case of pH adjustment in the presence of labile substrates. For cases where the kinetic information available is limited, a similar approach to the one described below can be used, albeit with a more likely need for refinement upon scale-up.

$$HCl + NaOH \xrightarrow{k_1} H_2O + NaCl$$

$$CH_2ClCOOC_2H_5 + NaOH \xrightarrow{k_2} C_2H_5OH + CH_2ClCOONa$$

For those cases where no kinetic information is available, generating kinetic data is recommended, thus reducing the risk upon scale-up; for example:

- Determine the impact of concentration, stoichiometry, and reagent ratios on the outcome of individual reactions as well as on the complete reactive system; frequently, most of this information is provided by process research chemists. When resources are available, determining reaction rates and Arrhenius constants (temperature sensitivity for the selectivity of the system) is very valuable for scale-up.
- Establish the relative reaction rates (i.e., how much faster one reaction is compared to the second). Even for the slower reaction, it is useful (as reported here) to estimate its kinetics (e.g., time to completion under representative conditions). If both reactions are very fast, different approaches must be employed on scale-up.

As you may have guessed by now, the effectiveness of such kinetic work can be increased by using statistical design of experiments. In addition, as illustrated in Fig. 27.2, assessing whether the process can be under mesomixing control is important (evaluate the impact of the addition rate and addition point on process results).

The goal of this work was to establish a method that allowed the selection of batch size and agitation speed during scale-up, with the objective of reproducing the selectivity of the system as measured by the amount of ethanol formed. It is important to remember that in the pharmaceutical industry, scale-up often occurs using existing multipurpose equipment. In some cases, when economical factors allow, specialized pilot-plant equipment is designed.

This study[23] focused on determination of a scale-up factor: that is, determining which process parameter must be held constant or changed in a prescribed way to reproduce the selectivity upon scale-up. Published results of investigations of the Bourne III system related to concentration effects and addition times were implemented in the experimental protocol. Operationally, the experiments were conducted as follows:

1. Charge the reactor (in our case, the reactor calorimeter, RC1) with a suitable amount of deionized water, and start the RC1 imposing a constant temperature in the reactor (21°C).

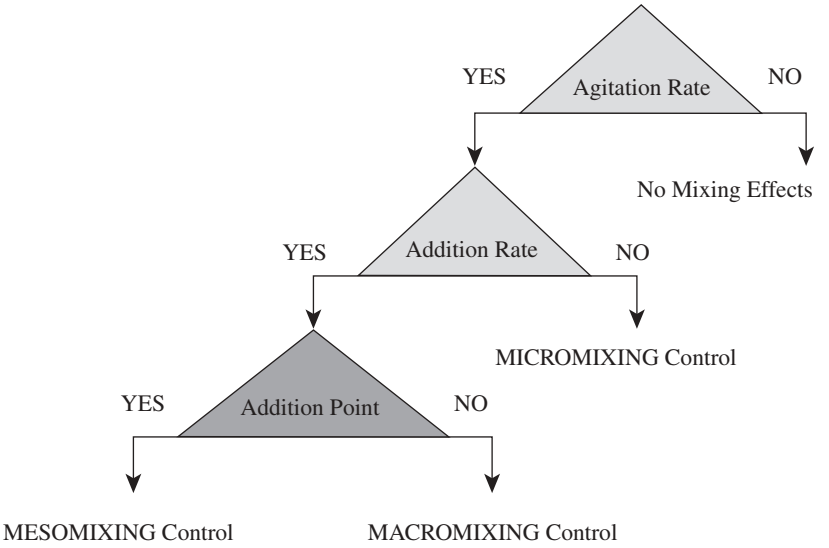

Figure 27.2 Proposed laboratory protocol to assess the sensitivity to mixing of a chemical process. (Based on ref. 24b.)

2. Charge the RC1 with suitable amounts of ethyl chloroacetate and concentrated aqueous HCl; homogenize (at high agitation speed, if needed).
3. Set the RC1 agitation speed as required.
4. Start adding concentrated sodium hydroxide at the addition rate prescribed (by surface addition).
5. Sample and rapidly analyze (using gas chromatography) the amount of ethanol present in the reaction mixture.

Mixing calculations executed using VisiMix[25] have allowed the design of the six-experiment matrix presented in Table 27.2.

Mixing calculations are a key component of scale-up strategies. Such calculations can be executed with a variety of tools, from simple spreadsheets to advanced modeling. The models used by spreadsheets are often developed within an organization over years of experience with certain processes and equipment. Advanced models such as those used by VisiMix were developed using much broader databases of processes and equipment, enhanced with research focused on mixing and scale-up.

There are a few, albeit not many, commercially available options, similar to VisiMix, such as those provided to the members of the fluid mixing processes consortium at British Hydrodynamics Research Group. In addition, computerized fluid dynamics codes are increasingly popular, allowing for very effective visualization of the fluid mixing in tanks of various configurations. Depending on the resources available and on the criticality of scale-up activities, engineers use one or more of those tools, and a few lucky engineers use them all.

TABLE 27.2 Experimental Design To Be executed in the Reactor Calorimeter RC1

	Input[a]		Process Parameters Calculated Using VisiMix				
Exp.	Batch Size (mL)	Agitator Speed (rpm)	Reynolds Flow[b]	$(P/m)^c_{max}$ (W/kg)	$(P/M)^c_{av}$ (W/kg)	Macromixing Time[d] (s)	Micromixing Time[e] (s)
1	900	180	6,050	1.1	0.010	6.3	21.4
2	900	360	12,300	8.5	0.090	3.1	7.6
3	900	420	14,400	13.5	0.150	2.6	6.1
4	900	520	17,900	25.5	0.285	2.1	4.4
5	1800	350	8,960	11.8	0.065	6.5	5.7
6	1800	400	10,300	17.6	0.097	5.8	4.9

[a]The input for VisiMix calculations includes all the geometric information related to the reactor (its diameter, height), the agitator (type, size, position in the reactor), and the baffles (type, size, position in the reactor). In addition, the physical properties of the media (e.g., density, viscosity) are included in the input. Some of the VisiMix calculated process parameters are potential scale-up factors.

[b]Reynolds number for flow is a dimensionless number characterizing the turbulence in the reactor. When the Reynolds number is below 2500, the flow regime is described as laminar, and suitable scale-up approaches are used. The Reynolds number range, 2500 to 10,000, is considered by many as transitional, and engineers are very careful not to operate in that regime because of its unique characteristics. Reynolds numbers above 10,000 describe fully developed turbulence.

[c]*P*/*m*, power per mass (W/kg). The maximum value for this parameter is calculated for the zone with the highest degree of turbulence. In most cases it takes place in the vortices formed behind the impeller blades. The most important microscale phenomena, such as drop breakup, breaking of crystals, nucleation, and efficient micromixing, take place in these zones. The ratio $(P/m)_{max}/(P/m)_{av}$ is one of the reactor's fingerprints.

[d]Macromixing time is a parameter characterizing the time required for the distribution of a compound (added instantly) throughout the entire volume of the tank. It is calculated as the time required to reduce the maximum difference of local concentrations of the added substance to approximately 1% of its final average value (under batch mixing conditions).

[e]Micromixing time is an estimate for the time required to accomplish uniform distribution of an added compound up to molecular level.

Note that experiments 5 and 6 in Table 27.2 can be deemed to be internal scale-up experiments: experiment 5 was designed such that the macromixing time was comparable to the macromixing time of experiment 1; experiment 6 was designed to reproduce the average power/mass in experiment 2. The corresponding results are depicted in Table 27.3.

TABLE 27.3 RC1 Experimental Results

Exp.	Batch Size (mL)	Agitator Speed (rpm)	Reynolds Flow	$(P/m)_{max}$ (W/kg)	$(P/m)_{av}$ (W/kg)	Macromixing Time (s)	Micromixing Time (s)	EtOH (g/L)	X_{EtOH}[a] (%)
1	900	180	6,050	1.1	0.010	**6.3**	21.4	1.86	24.7
2	900	360	12,300	8.5	**0.090**	3.1	7.6	2.10	27.8
3	900	420	14,400	13.5	0.150	2.6	6.1	1.65	21.9
4	*900*	*520*	*17,900*	*25.5*	*0.285*	*2.1*	*4.4*	*1.50*	*17.9*
5	1800	350	8,960	11.8	0.065	**6.5**	5.7	1.40	18.6
6	1800	400	10,300	17.6	**0.097**	5.8	4.9	1.35	17.9

[a]The standard error for the ethanol concentration measured was approximately 2%.

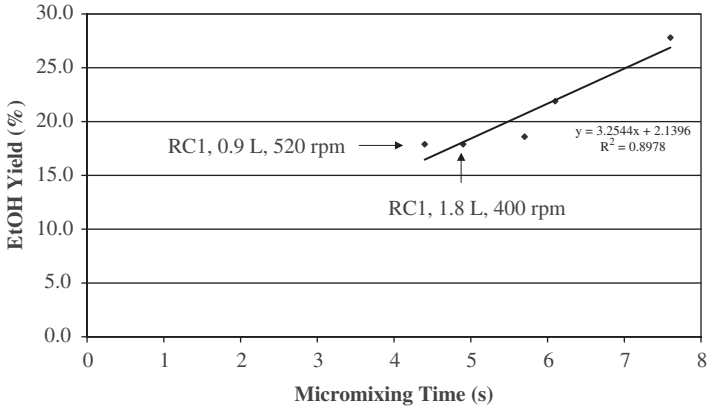

Figure 27.3 The amount of ethanol obtained correlates well with the micromixing time calculated using VisiMix.

From the data analysis one can see that neither macromixing time nor power/mass are good candidates as scale-up factors in this study.

It is interesting to note that when conducting a control experiment (no HCl), the extent of ester hydrolysis was quite significant, producing ethanol in 92% yield. When the data were analyzed in detail, the ethanol amount was found to correlate well with the micromixing time (Fig. 27.3). Based on this finding, and using VisiMix calculations for the kilo-laboratory reactor, the scale-up scenarios listed in Table 27.4 were identified. Careful consideration was given to turbulence at the addition point and an estimation of the reaction zone was also executed. When the experiments were executed in the 70-L reactor (in the kilo-laboratory, so called because it is often used to produce kilogram quantities of pharmaceutical intermediates or ingredients), the results in Table 27.5 were obtained. These confirm that micromixing time is indeed a scale-up factor, as 4 seconds of micromixing time produced approximately 18% ethanol at both scales, the RC1 and the kilo-lab. The experiment at 320 rpm confirms one of the limitations of the test system as described by Bourne: above 1 W/kg power per mass, the sensitivity to mixing is more difficult to detect. Of note is the fact that the

TABLE 27.4 Scale-up Scenarios for the Kilo-Laboratory

		Variable Manipulated:	Parameters Calculated			
	Reaction Mass (kg)	Agitator Speed (rpm)	$(P/m)_{max}$ (W/Kg)	$(P/m)_{av}$ (W/Kg)	Macromixing Time (s)	Micromixing Time (s)
RC1	1.0	180	1.1	0.01	6.3	21.4
		520	25.5	0.29	2.1	4.4
Kilo-laboratory	35.0	115	12.0	0.30	8.6	5.0
		130	17.3	0.42	7.6	4.2
		320	258.0	6.3	3.1	1.1
		460	765.0	18.8	2.1	0.6

TABLE 27.5 Kilo-Lab Results

Exp.	Batch Size (L)	Agitator Speed (rpm)	Reynolds Flow	$(P/m)_{max}$ (W/kg)	$(P/m)_{av}$ (W/kg)	Macromixing Time (s)	Micromixing Time (s)	EtOH (g/L)	X_{EtOH} (%)
1	35.0	130	57,400	17.3	0.420	7.6	4.2	1.43	**17.3**
2	35.0	130	57,400	17.3	0.420	7.6	4.2	1.67	**20.2**
RC1	0.9	520	17,900	25.5	0.285	2.1	4.4	1.50	17.9
3	35.0	320	142,000	258	6.300	3.1	1.1	1.34	**16.2**
RC1	0.9	360	12,300	8.5	0.090	3.1	7.6	2.10	27.8

Bourne data, submitted to the same analysis, conformed micromixing time as a scale-up factor.

In conclusion, in this case study we showed how the use of mixing and scale-up calculations with VisiMix, combined with experimentation in automated laboratory reactors such as the reactor calorimeter RC1, assisted us in scaling up the Bourne III system, a typical reactive chemical system.

27.4 CONCLUSIONS

The transfer of chemical technology from the bench to the pilot or manufacturing plant is a complex process. Its complexity depends on many elements, which, besides human factors, includes equipment performance and availability, and on the type of chemical process to be scaled-up. Active pharmaceutical ingredients' crystallization steps are often challenging because of the heterogeneous nature of the process, and because of the possible sensitivity to processing conditions of the solid form obtained. Similarly, the selectivity of mixing sensitive reactive systems, such as those involving very fast reactions, can be challenging to scale-up. The use of mixing and scale-up calculations, statistically designed experiments executed in automated laboratory reactors, and suitable fundamental understanding of the process physics and chemistry were shown to be useful scale-up tools in such challenging cases.

Acknowledgments

The author is grateful to Stephen A. Wald and Roger P. Bakale for continued support and encouragement in the pursuit of novel developments in scale-up science at Sepracor. Thanks are expressed to Kostas E. Saranteas and Robert J. Prytko for numerous challenging and stimulating discussions on many scale-up topics in the pharmaceutical industry. John R. Bourne (ETH, retired) is thanked for his extensive review of the manuscript. Leonid N. Braginski (VisiMix) is acknowledged for his aid in clarifying certain output parameters in VisiMix. Richard Verseput of S-Matrix Co. is thanked for explaining complex statistical concepts required for the powerful use of DoE FusionPro. Last but not least, I appreciate very much the kind support provided by Olivier Ubrich from Mettler-Toledo AutoChem in the advanced use of the RC1.

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28

LIBRARY QUALITY METRICS

RICHARD L. WIFE
SORD BV
Malden, The Netherlands

JOHAN TIJHUIS
Specs BV
Delft, The Netherlands

28.1 DRUG DISCOVERY AND DEVELOPMENT

The real costs to discover, develop, and bring a new drug to market are now approaching $800 million. Over the last 10 years, the R&D costs have tripled while the number of new chemical entities (NCEs) has remained the same. It is clear that some significant problems remain despite these massive investments. What these problems are today, and how they can be solved, is now becoming clearer. The largest investments have been in biology, notably in genomics and proteomics, as well as in developing the technology platforms designed to speed up the drug discovery process. What these investments did not solve were problems such as the time it takes to bring a drug to market, the novelty and patent issues, and the horrendous attrition rate of drugs in clinical trials. It is now time for chemistry to address these problems.

In this chapter, the focus is on chemistry. This focus is not confined to ways to make compounds in the screening libraries; it encompasses broader issues, such as the quality of the library, its biological relevance, and such crucial practical matters as compound purity, stability, and solubility. The pivotal role of the compound library in every pharmaceutical company is illustrated in Fig. 28.1, where the drug discovery and development process is flanked by the two key disciplines, biology and chemistry.

Genomics will produce many more targets. High-throughput screening (HTS) will continue to generate more and more leads, and much better decision tools must be developed if project teams are going to be able to make intelligent lead selections. Integrated chemical

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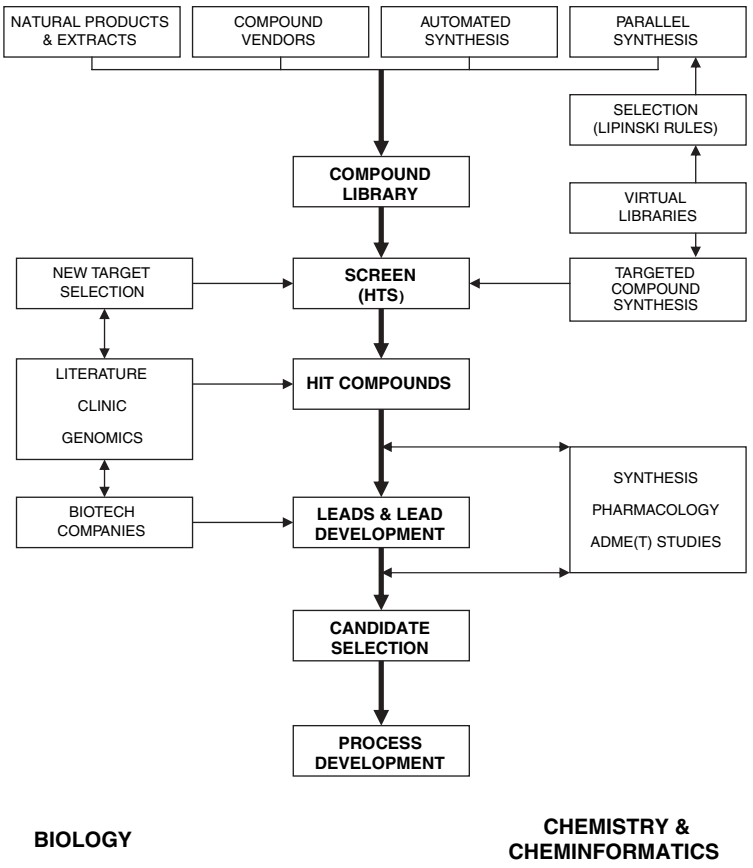

Figure 28.1 Drug discovery and development.

data, reliable data, will be the basis of these tools, with a heavy accent on the word *reliable*. Bad compounds produce not just bad but also very expensive data. The quality of the compounds screened deserves much closer scrutiny.

The main challenges for pharmaceutical companies in the years ahead is to develop drugs faster and at lower costs. Development time can probably be shortened significantly only in the preclinical research phase (lead discovery and lead optimization). The duration of the clinical trial is influenced heavily by federal requirements to ensure the development and launch of safe drugs. However, the requirements are getting tougher and tougher, so it is not very likely that the development time will be reduced in clinical trials (provided that federal regulations will not change dramatically). The main advantages of the reduction in development time will be twofold: simply reduction of costs, and probably a longer patent protection time once the drug has been approved.

At this moment the attrition rate in clinical trials is far too high. If, better-quality candidates are developed in preclinical research, the likelihood that they will pass the clinical trials will be increased. The cost savings in such a, case will be enormous. A recent study has revealed that more than 40% of the candidates in clinical trials fail due to poor (bio)pharmaceutical properties and more than 20% due to toxicity. These reasons depend heavily on the chemistry of the candidate and probably could be identified in preclinical research. The rate-limiting aspect of drug discovery is not in finding the lead; it is turning the

lead into a drug. Solving the problems of absorption, specificity, metabolism, and safety is only slightly, if any, easier today than it was 10 years ago. The conclusion therefore is that the overall quality of the leads must be increased as well as making the entire process more efficient in all stages. Speed really matters, and precious time (and thus money!) will be saved.

The chemical pool in screening libraries must be enriched. As companies concentrate on similar targets, with screening libraries acquired from the same commercial vendors or prepared in-house with automated synthesis from common building blocks, the novelty and patent problem increases. Compound novelty is a neglected issue that deserves urgent attention.

The success rate of drugs in clinical trials is no better than 1 in 12. This tells us that designing a drug with the right pharmacological profile needs much more attention. The important features are absorption, distribution, metabolism, excretion, and toxicology (ADMET), all of which are associated with the molecular structure. A much better understanding of this structure–performance relationship is needed to develop predictive algorithms that will increase the survival rate in clinical trials.

Biologists initiated a revolution in the drug discovery process during the 1990s. It is now time for chemists to tackle the crucial issues that cannot be solved by biology; it is time for a revolution in chemistry. Better drugs reaching the market faster, with reduced costs—that is what chemistry must deliver. The library quality metrics are at the center of deciding what this chemistry will become.

28.2 COMPOUND LIBRARIES

Typically, screening libraries in the larger pharmaceutical companies will contain several million compounds. These libraries are not static. They are in a continuous state of flux, with new additions and deletions aimed at biological relevance to the targets that are important. Building and maintaining a good compound library is as much an art as a science. Although this chapter is focused on library metrics, the compound sources are as relevant to efficient drug discovery as are quality metrics.

Basically, compounds are either prepared in-house or acquired from third parties:

In-House	Third Parties
Handcrafted by medicinal chemists	Academic compound collections
Combinatorial chemistry	Combinatorial chemistry
Automated (parallel) synthesis	Automated (parallel) synthesis
Natural-product derivatives	Natural products (extracted/synthesized)

The cost and value of these compounds in the drug discovery and development process are discussed later in the chapter. What is worth remembering is that the compound sources are no more than those outlined above. Chemistry is done by people, by machines programmed by people, or by nature—and that is it.

Although the crucial metric for a compound library is its biological relevance, there are other decision components in building and maintaining a good library. Almost all of these relate back to the chemical structure of the compounds that are added to, or deleted from, the library. More recently, the chemical structure of *virtual* compounds has become important in library analysis. The building of compound libraries is no longer limited to what has been done, or more accurately, to what has been reported in the chemical literature.

In-silico screening is one way to expand the chemical space beyond what has been reported after some 200 years of chemistry. While Chemical Abstracts and Beilstein might contain millions of chemical structures, there is another real chemical world that has yet to be mined and that is *lost chemistry*, the chemistry that was done but never reported. Current estimates are that on the order of 80% of the chemistry performed over the past years has not been published. It is to be found in files and lab books around the world, on dusty shelves, or destroyed long ago and lost forever. How some of the lost chemistry can be retrieved and evaluated is not our subject here. The visible chemistry that is real, the actual "compounds in vials," is the center of the current focus.

28.3 LIBRARY METRICS

A good compound library is a corporate asset, it deserves a place on the balance sheet of any company engaged in drug discovery and development. There are two more global statements that can be made about compound libraries before looking at the individual metrics that really matter:

- *Quality saves money.* The rubbish-in/rubbish-out maxim is especially true in drug discovery. Not only is screening expensive, but also the processing of screening data and building accurate structure–activity relationships, and "bad" compounds must be removed.
- *Control saves time.* Instant access to archive amounts of hit and lead compounds is essential for rapidfollow-up and evaluation studies. Time is money, and control means having the compounds in-house, not on the other side of the world.

Money and time are inseparable. Getting a billion-dollar drug into the market even just one month earlier can achieve an extra $100 million in revenue! Library metrics are not confined to making the drug discovery process more effective; they are as relevant in the downstream activities of drug development, process development, and clinical trials, where the big money is spent.

So what makes a compound library good or bad, or indifferent? What is going through the minds of the people who decide what compounds to add to the library, to delete or to screen, and what are the equally relevant but practical issues that need more full appreciation?

28.3.1 Chemical Structure

Chemists have their own very special *shorthand* depiction of a chemical compound, the chemical structure. A two-dimensional molecular structure drawing contains a wealth of information from which chemists and biologists can form opinions based on their experience and intuition. Much of the time, their thought processes will be the same, sometimes not. It depends on what particular aspect of the discovery/development process concerns them most.

Just *looking* at a chemical structure with trained (experienced) eyes can be enough to make a decision or draw a conclusion. Many chemists engaged in the acquisition of compounds from third-party vendors rely on their experience and intuition in using this visual inspection of the chemical structures to make their compound selections. The chemical structure in machine-readable format enhances the selection process, where a wide variety of computational tools can identify key structures that fulfill required

criteria. Computational chemistry improves at a startling pace, but it, too, relies on solid reliable data that are linked directly to the purity and integrity of each compound.

Increasingly, the chemical structure is used by medicinal chemists, even in the library selection process, with anticipation of the question: What chemistry can I do on this molecule if it turns out to be a lead? In other words, are there sufficient "handles" in the structure to enable a trained chemist to prepare analog compounds and develop the lead? Even thinking deeper beyond lead development, can such compounds be serious candidates for effective process development, survive clinical trials, and eventually be manufactured? It may be dangerous to base these decisions on the chemical structure alone, but as better tools are developed, this basic information will increase in importance. Not to be forgotten is the fact that most of the time, the only information presented to the people who acquire compounds from third-party vendors is what the chemical structure is and how much (in weight) of the compound is available. The current trend in the better vendor companies is to add information and to help companies make better compound selections.

28.3.2 Druglikeness

Over the years, a consensus developed concerning what a drug looks like. The preferred relationships and combinations between a core structure and the surrounding functionality became better understood. It would have been nice if we were now at a stage where druglikeness could be applied as a prime metric in the entire discovery and development process. It cannot. What the consensus now tells us is what the current drugs look like, it provides a picture of medicines over the years, but it does not help us predict the future with absolute certainty. Druglikeness is a metric, but it should not be exaggerated in its importance.

The Lipinski rules are probably one of the more explicit forms of the consensus as to what can, or cannot, become a drug. The rules describe some easily visualized criteria derived from a careful analysis of successful drugs and the behavior of compounds under biological conditions. They provide a "feel" for what a drug might look like, but indiscriminate analysis of the current listings of successful drugs in the market shows that some 20% do not meet the Lipinski criteria. Nevertheless, what the Lipinski rules do provide us with is a tool with which all organic compounds, simply on the basis of their chemical structure, can be divided into two pools: one that meets and one that does not meet the "rules of five." What happens after that depends largely on the other metrics.

Basically, it is all about balance between risk and certainty. If 11 of the 12 compounds that enter clinical trials are going to fail, it makes sense to find ways to reduce this attrition rate. This message in-house is no longer new, and more and more outside companies are developing commercial tools to make better predictions for compounds based on the ADMET parameters. The mood inside pharmaceutical companies is serious in taking a close look at compounds in the library that could not be considered druglike and removing them from the file.

As stated previously, more than 40% of the compounds in clinical trial fail due to poor (bio)pharmaceutical properties (solubility, log P, log D, chemical stability, permeability, metabolism, protein binding, plasma stability, etc.). A lot of efforts are now made to calculate or predict these properties in an early stage of preclinical research to prevent disappointment and the loss of a lot of money farther downstream in the drug discovery process.

Solubility was probably the most underestimated property of a molecule several years ago. At this moment, however, there is a lot a research to try to predict the solubility of compounds in water, simply because an active compound has to be dissolved before it can be absorbed. Several methods for predicting aqueous solubility based on molecular structure have been reported. A significant challenge that remains, however, is the ability to predict solubility accurately within a narrower but more pharmaceutically relevant range of up to 100 μg/mL.

Absorption The effect of physicochemical properties on transport processes is complex and interrelated. Consequently, drugs must possess an effective balance between water solubility and lipophicilicity. Once dissolved, drug compounds must pass through the intestinal wall to reach the bloodstream. The lipophicility of a compound can be "represented" by *c* log *P*, which is the calculated negative logarithm of the *n*-octanol/water partition. Many algorithms are now available to calculate *c* log *P*. The general aim is not to exceed a *c* log *P* value of 5 for lead molecules. Furthermore, the Caco-2 cell line is used widely as a model for intestinal membrame permeability. There is a correlation between the polar surface area of a molecule (which can be calculated) and either the Caco-2 permeability or the intestinal absorption. Compounds with a PSA value greater than 140 $Å^2$ have a much lower probability of diffusing through the intestinal membrame.

Distribution After the drug has passed through the intestinal wall, it will be distributed by the bloodstream over the human body. Little is know about the uptake of compounds in various organs and tissues, but it is obvious that this will be very important for the efficacy of a compound in the patient.

Metabolism Also after the uptake in the bloodstream, the drug will face a multitude of metabolizing enzymes in the liver. Several attempts have been made to develop models predictive of metabolic stability. Still a lot of work has to be done in this field. Many in vitro screens have been set up to measure the metabolic stability of compounds in different cell types.

Excretion As soon as a compound has been excreted by a patient, it will no longer be able to perform its medicinal purpose. It is obvious that modeling of the excretion process is not trivial. So far, no good models have been developed with practical applications.

Toxicology For general prediction of toxicity of compounds, software packages are available that uses linear discriminate analysis and multiple linear regression models with two-dimensional descriptors to generate toxicology profiles for organic compounds. Unfortunately, knowledge of the toxicity of compounds is poor with respect to translation into computational algorithms.

28.3.3 Novelty

What is the real value of novelty when it comes to looking at a compound library? Novelty as such says little more than "these compounds have not been seen before"; it says nothing about the potential to develop them into successful drugs. Other metrics apply here. One of

the driving forces in favor of novelty is having compounds that other companies will not have. It is a competitive edge instrument and is still applied in obtaining exclusivity from a compound vendor for particular classes of compounds.

Novelty relates to uncommon, or previously unknown, combinations of core structures and functional groups. A patent position will focus on the character of the core structure, so novelty measurement can be one of the tools to strengthen the compound library in anticipation of a promising lead compound. But is the real value of novelty based on measuring a compound against what compounds are already successful drugs or on what compounds are entirely new, regardless of their medicinal qualities? Or, to put it more simply, "how much chemistry have we already seen?"

The virtual chemical world is frighteningly big. One simple calculation showing how big this world is was based on a maximum of 30 atoms (which could be carbon, oxygen, nitrogen, or sulfur), which arrived at 10^{62} possible core structures. If by whatever computational procedure were applied it took just 1 second to evaluate each of these virtual structures, it would take more than 10^{56} years to complete this analysis. Clearly, such numbers teach only one lesson: Virtual chemistry must be tamed before it can be applied usefully. There are clever ways to reduce the virtual chemical world to one that can be navigated in days, but we do not cover these here. The virtual chemical world as it is today is useful in helping a chemist look at structures that have never been seen before, to stimulate a new way of thinking about molecules.

When the comparison is with the real chemical world, novelty concerns much smaller numbers. In March 2002, the Chemical Abstracts Registry file contained more than 37 million chemical substance registrations, which contained almost 19.5 million organic and inorganic substances. The CrossFire Beilstein database contained data on over 8 million organic compounds on the same search date. It is a tedious exercise, but very much worth the effort, to take databases such as these based on Beilstein and remove trivial small molecules ("non-druglike") and see how many compounds remain. The result is less than half a million.

There is another very useful analysis, and that is to compare the compounds in Beilstein to the around 6000 compounds in the CMC (Current Medicinal Compounds) database. The Bemis and Murcko analysis of the CMC database in 1996 drew attention to the fact that 94% of the compounds contained at least one ring system. The results from a comparative analysis based on ring sizes is depicted in Fig. 28.2, and it is both simple and relevant. It is derived from the size of the largest ring system present in the molecular structures of the compounds and the occurrence of this maximum ring size from rings with three or more atoms.

The almost exact overlay for the compounds from Beilstein and those from the CMC database is quite frightening. What it tells us is that almost no medicines have been developed that fall outside the pattern of ring sizes contained in Beistein. In other words, in terms of ring sizes, the medicines that have been developed are an exact mirror of the chemistry developed in the last 200 years.

Besides the pleasant advantage brought about by developing a drug with a novel structure and the stronger patent position, there is another aspect of novelty that is much more biological than legal. Microorganisms evolve at truly remarkable rates, so fast that drug resistance has become a serious problem, especially in areas such as antibiotics and antimicrobials. This is a powerful incentive to come up with a succession of novel core structures that can compete with the evolutionary prowess of the target microorganisms!

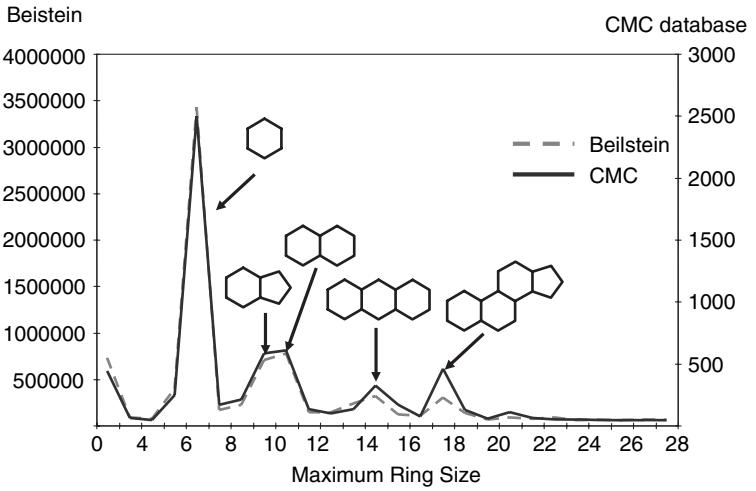

Figure 28.2 Maximum ring size comparison (Beilstein and CMC database).

Novelty is a library metric, and it can be measured. The only point to debate is: Novel compared to what? The next metric is much more difficult to pin down and remains highly subjective. It is called *diversity*.

28.3.4 Diversity

At the beginning of the 1990s, when HTS was in its early development, the average compound collection in a pharmaceutical company was little more than a compilation of in-house and handcrafted compounds from focused medicinal chemistry programs. These were not compound libraries but a series of collections based on then-relevant core structures, such as β-lactams, bendiazapines, and tetracyclines, each with its place in chemical space but certainly not filling it.

The term *molecular diversity* was introduced around this time as an expression of what people wanted to achieve in what later would become the compound library. The concept of chemical space was synchronous since molecular diversity was best illustrated by having representative structures that filled most chemical space, the degree of which depended on the axes that described this mysterious chemical space. In the years that followed, software was developed internally and commercially to quantify diversity in such a way that libraries could be improved by adding compounds that increased molecular diversity.

Is molecular diversity relevant today, when we know more about the meaning of druglikeness? The answer is a resounding "yes," not necessarily because of the almost equally illusory concept of druglikeness but because of the progress made in biology (and notably in genomics) in the ensuing time, which has generated so many more targets. This large number of new targets is just one challenge; the other is that for many of the new targets, absolutely nothing is known about the sort of compounds that have any probability of being active. What is still needed is a "nicely" diverse compound library that contains as many different types of structural types, such that one or more of the compounds will generate some leads for the medicinal chemist to follow.

It may seem contradictory, but if molecular diversity is a worthy goal, this should not mean that compound analogs are to be minimized. Not that diversity is improved by the presence of analogs, the reason for keeping them is because of the time problem. One representative structure is referred to as a *singleton*, and when a singleton becomes a hit molecule, any follow-up will require the making of analogs, and this takes time. Better to have a little less diversity and not remove the analogs.

After 10 years and many publications, it would still be difficult to imagine a group of chemists agreeing on the concept of molecular diversity. For a short while, the terms *diversity* and *combinatorial chemistry* were linked, but this is, understandably, no longer so. Probably the only one (practical) means to ensure good diversity is to collect compounds from as many different sources and as complete a geographical diversity as possible, since the type of chemistry practices varies from location to location. This *global diversity* is a reflection of the educational system and its influence on the chemists, the natural and other resources at a given place, and exposure to what is happening in the world. In the years during which the former USSR was closed to the rest of the world, chemists there developed chemistry that was quite different from that of their Western colleagues. Sadly, this is no longer the case, although apart from the diversity issue, conditions have improved.

There is one more diversity term that was introduced in the 1990s: *biodiversity*. Biodiversity was applied in many arguments, not the least of which concerned plant species in exotic parts of the world and the need for their protection. In more scientific terms, other arguments pointed toward living systems as being rich sources of unusual and complex molecules (true) with potentially many candidate structures that could become drugs. Supporting this argument was the (then) fact that 50% of the commercial drugs were either natural products or their direct derivatives. But biodiversity is as elusive a concept as molecular diversity, and the contention that a natural molecule that plays an important role in some living system has a better chance of becoming a new drug is blurred thinking. What biodiversity can teach us is what a difference there is between synthetic chemistry and natural products, and that is all.

In summary, diversity is a good thing, but it is difficult to quantify it in such a way that we then know exactly how to improve it. It is a metric for compound libraries, of that there is no doubt, but it is still very subjective. Molecular diversity should always be weighed against biological relevance; it is not a goal in isolation.

28.3.5 Numbers and Costs

When compound libraries were small and contained mainly islands of traditional medicinal chemistry research (compound collections), the size of these collections was in the low hundreds of thousands. Today, well-run company libraries will contain several millions of compounds from in-house efforts, supplemented by external acquisitions from third parties and vendors. Building libraries of this size does cost money but is an investment. A properly maintained library will last for years, due largely to the continuing trend toward better automation technologies and more sensitive assays.

With millions of compounds to assay, the actual cost of an assay becomes a large part of the consumables budget. With assays now costing anywhere between $0.1 and $10, depending on its complexity, it no longer makes sense to run the entire library against one assay unless this is absolutely necessary. These accumulated running costs are always going to be much larger than those for the building and maintenance of the compound library.

Returning to the origin of the compounds in the library and the in-house/third-party sources, the approximate comprative costs associated with preparing sufficient material to perform assays and retain archive amounts are:

	In-House	Third Parties
Handcrafted by medicinal chemists	$1000–5000	$25–100
Combinatorial chemistry	25–50	25–250
Automated (parallel) synthesis	250–1000	25–250
Natural products and derivatives	1000 +	1000 +

The handcrafted compounds from third parties are generally compounds that have been collected by vendors from academic sources which meet the selection criteria set by pharmaceutical companies for their compound acquisition programs. It is not our purpose to make a case for or against any particular source of compounds in the library; it is to arrive at an approximate cost for a library of useful size. A nicely balanced library with 1 million compounds will cost between $25 million and $50 million to purchase. The costs of proper storage, retrieval, and maintenance are not included (see below).

Even at the high end of the scale, the cost of assembling the compound library is only 6.5% of the cost to bring one successful drug to the market, and the library will be there for years to come and result in more commercial drugs. Put in terms of the total R&D budget for a decent-sized pharmaceutical company, a library will account for no more than 0.25% of the annual R&D expenditure. In these terms, a compound library may appear to be so small in the total budget that it deserves little attention. Nothing could be further from the truth. Without the compound library, drug discovery is back in the dark ages. Properly applied metrics make the library the cornerstone for future drug discovery and development, despite its apparent low cost.

28.3.6 Analogs

The value of analogs in the compound library has already received some attention. It is not so much the actual cost of preparing analog compounds to develop a lead that matters, it is the time that it takes. But what exactly is an analog compound? It would be nice if it were based on what the receptor "sees" in terms of pharmacophores, but it is not. The traditional way of describing a series of analogs is that it is a common core structure with variations in the functional groups and perhaps also their points of attachment to the core structure. Easy to visualize, but far away from the concept of analogous pharmacophores.

Besides saving precious time in following up and developing leads, having analogs in a compound library has yet another upside. Small changes in a molecule can result in dramatic changes in biological activity and even quite different modes of action. Low or moderate activity in one analog does not mean that the entire analog set is going to perform as badly; the set could still contain a significant hit molecule. With singletons, this opportunity will certainly be missed.

A controlled number of analogs does therefore seem wise. But how many, and with what structural variations? A series of chain homologs would, without thinking about it too much, seem not a good way to go about selecting analogs for the library. What difference does a methylene group make in a hydrocrabon chain? The answer is that it can make a

considerable difference. Even an alkyl substituent "walking" around an aromatic ring can exert an enormous influence on the rest of the molecule and its shape and interaction with receptors.

With the advent of combinatorial chemistry in the early 1990s, the opportunity arose to make compound "families" based on combinations of core structures and functional groups. Indeed, one of the earlier claims was that combinatorial chemistry was drug discovery and lead optimization in one single experiment! This myth lived for some years before the downside of this approach was recognized. Excessive numbers of analogs are costly, not just to make and to purify, but also to screen. If an acceptable number of analogs is around 10 per core structure, combinatorial chemistry is a very expensive tool. Today, more controlled but still automated synthetic methods such as parallel synthesis are widely accepted in lead development, but are seldom applied in building a compound library. It is just too expensive.

28.3.7 Resupply

Ten milligrams of a compound in the library is an acceptable amount to support years of HTS and even for spectral characterization in the event of a hit. But more of the compound will be required if the hit is to be developed into a lead and beyond. Even today, as the minimum amount required for HTS and characterization continues to fall, some companies prefer to play it safe and have as much as 100-mg amounts in the library where possible.

There is an unfortunate correlation between the weight of an available compound and its intrinsic complexity, as any synthetic chemist knows. The more chemistry that is done to a core structure, the smaller the amount in simple weight terms. If the compounds originate from academic research programs, here too the advance in synthetic techniques and analytical instrumentation has reduced the hitherto gram amounts in synthetic pathways to milligrams.

Resupply is an issue and certainly a library metric. One of the criteria applied in buying compounds from third-party vendors is whether more material can be delivered in a short time. In other words, can the risk of buying a small amount of a compound be reduced by the certainty of resupply? The last years have seen a rapid growth in small synthetic chemistry laboratories that perform exactly this service to pharmaceutical companies, the resupply of interesting compounds regardless of the original source. The trend will continue.

28.3.8 Compound Purity

The schools of thought on compound purity are many and various. They range from the "I do not care" through to 90 % minimum purity. The average requirement would be somewhere in excess of 80 % for purchases from compound vendors. Here it is a cost factor that applies: The purer the compound must be, the higher the price. Interestingly, the purity standard expected from a compound vendor is often higher than the company internal standard, especially when the compounds are from combinatorial chemistry.

Compound purity is a real issue for structure–activity correlations, which become more and more important in discovering drugs. For this reason alone, purity is something to invest in because *quality saves time*. The label on the bottle should say something meaningful about the compound inside. If the compound is more than 90 % pure, it should also be the right compound! The combination of ^{1}H NMR and LC–MS is now an accepted standard in quality control for compound libraries. Also of note is the current trend for many

pharmaceutical companies to monitor compound purity in the library at intervals, most often with statistical subsets, to be sure that the structure–activity relationship is meaningful and to delete compounds that are impure. Purity is an important library metric.

28.3.9 Compound Stability

Very few solid crystalline compounds decompose at room temperature in storage. Although there are instances where decomposition has taken place but the crystalline form has not changed, these are extremely rare. Most of the time, the decomposition of solids is visually apparent. So where is the problem? It lies in the way that companies store their compound libraries, not as solids but in dimethyl sulfoxide (DMSO) solution.

DMSO is a very good solvent. Around 90 % of the compounds that end up in a library will dissolve in DMSO. In chloroform or acetone, the figure is only 70 %. Although it is easy to understand the choice for DMSO, not until quite recently was there any concern as to what DMSO might actually do to the compounds. It is not an inert solvent. DMSO is not only mildly reactive but absorbs large amounts of water when exposed to normal air. This became a problem when aware of the problems associated with stability in DMSO solution, companies started to store daughter plates at low temperatures (down to −20°C) only to find large increases in the water content from repeated freeze–thaw cycles.

Water and the possibility of compound hydrolysis is a relatively simple issue to understand. Esters, for example, could be expected to hydrolyze in DSMO solution, and measures could be taken to prevent this. But what of the other functionalities, or groups of functionalities, and their behavior in DMSO solution? Some estimates as late as 1999 put the decomposition of normal compound libraries as high as 30 %, and this triggered programs to quantify the real damage and plans to minimize risks.

The immediate danger concerns compounds that are sensitive to oxidation and hydrolysis. As the studies continue, a much better understanding will be developed regarding how to improve compound stability during storage and to identify compound types that require special storage conditions. It seems that DMSO is here to stay, but now we know what the risks can be.

28.3.10 Compound Solubility

That 90 % of library compounds dissolve in DMSO has been proven experimentally. But when the compounds are to be assayed, as much as 90 % of water is added, and what happens then? Many compound will precipitate out of solution, but this is only now becoming an issue for serious consideration. How it will be tackled is an interesting question. It is not too difficult to devise high-throughput systems to quantify the real solubility after dilution in experiments. It might be as efficient to develop algorithms that predict solubility for assay media in the same way that the log *P* predictions are used to estimate octanol–water partitions for lipophilicity. A precipitated solid will not be doing much that is meaningful to a biological or biochemical system during the assay. It is rather difficult to explain why compound solubility has received so little attention until now, but it is nevertheless a library metric.

28.3.11 Serendipity

In this chapter we focused on metrics designed to improve the quality of a compound library. Each metric can be rationalized; most can be measured. Serendipity is not necessarily

a library metric—more an awareness that despite all of the practical measures taken to improve quality, luck can be as important a part of the discovery process as rigorous science.

One of the celebrated illustrations of serendipity in drug discovery has to be the *cis*-platin drugs. Turning off the current did not kill the interesting therapeutic effect on tumor cells, and some alert scientists found the reason why. Penicillin, barbiturates, and more recently Viagra definitely underline the case for serendipity as a metric in drug discovery.

There will be many more fascinating illustrations buried in the scientific literature and masked by rational arguments instead of the story of what actually happened. There has to be almost no chemist in the world who will not acknowledge, perhaps privately, that luck is one of the nicest surprises in synthesis—that there must be room for serendipity. It is just that no one really wants to write about it!

Library metrics lead us to believe that only certain classes of compounds can ever be seriously considered as potentially becoming drugs. Druglikeness, novelty, and diversity can become such awful mantras that we will miss the "blue sky" quantum leaps into new drug types. Even today, there is still room in the screening programs of some companies for compounds that are quite remote from the metric-designed library.

28.4 CONCLUSIONS

Most of the library quality metrics are just commonsense. This is the power of retrospective thinking. Ten years ago, with the surge toward HTS and the rush to build a compound library, too little attention was given to the chemistry. What it was and how it was managed and preserved was less prestigious than what was going on in biology and automated technology platforms. Even today, chemistry might not be neglected, but it has yet to receive proper acknowledgment despite its invaluable contribution to drug discovery and development.

The next 20 years will be different. Economic and regulatory constraints direct efforts to get good drugs on the market faster. Even the R&D-rich pharmaceutical industry cannot afford to let the $800 million price tag for a successful drug escalate further. With the biological revolution behind us, chemists take up the challenge to remove some of the remaining bottlenecks. There will be new chemistries, better ways to design and construct the molecules that we care about, and there will be better drugs as a result.

29

TYING A GABA FROM COPENHAGEN TO CHICAGO: THE CHEMISTRY OF TIAGABINE

MUKUND S. CHORGHADE
Chorghade Enterprises
Natick, Massachusetts

MAHENDRA N. DESHPANDE
OrgSyn Laboratory, Inc.
Chicago, Illinois

RICHARD J. PARIZA
Cedarburg Pharmaceuticals
Grafton, Wisconsin

29.1 INTRODUCTION

Epilepsy is a disorder characterized by recurrent spontaneous electrical discharges within the brain which are manifested by clinical seizures. Four million patients in the United States are afflicted with this ailment; 20% of these have seizures that cannot be controlled sufficiently with existing medications to permit normal activities of everyday life. The market for anticonvulsants is substantial; a historical growth rate of 10 to 12% is expected to be sustained thereafter. Epileptic seizures can be classified as primary epilepsies (35%) or partial epilepsies (65%). Primary epilepsies (generalized convulsions) can be controlled with valproate (Depakote), carbamazepine (Tegretol), phenytoin (Dilantin), or phenobarbitol. Most partial epilepsies have resisted control with chemotherapeutic agents.

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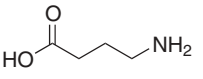


(I)

Scheme 29.1

Several drugs marketed currently have exhibited problems with toxicity or tetratogenicity. Hepatotoxicity, aplastic anemia, Stevens–Johnson syndrome, and neurotoxicity have been concerns cited frequently. Idiosyncratic reactions involving hematologic and dermatologic systems have proved fatal in a few cases. Considerable research effort has been directed toward identifying novel pharmaceutical moieties with a broad spectrum of efficacy and fewer side effects.

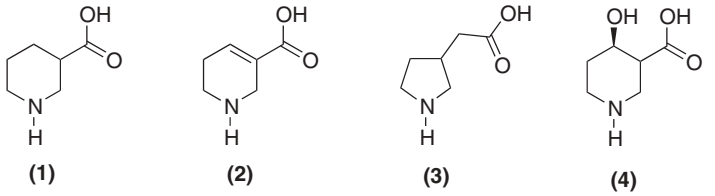
Anticonvulsing agents were originally developed empirically using crude in vivo assays at a time of limited understanding of the mechanisms of synaptic transmission and its regulation. The commonly prescribed anticonvulsants cited above share this heritage. Current targets for therapeutic intervention with respect to epilepsy are (1) blocking of receptors of excitatory amino acids, (2) modulating membrane ion channels (sodium and calcium) involved in neuronal membrane excitability, and (3) enhancing the neurotransmittory effect of gamma-aminobutyric acid (GABA) (**I**)[1,2] (Scheme 29.1)

I is the principal inhibitory neurotransmitter in the mammalian central nervous system (CNS).[3] It may inhibit mammalian CNS activity in numerous ways[4]:

1. Binding to GABAA receptors on the GABA–benzodiazepine receptor complex at the somatic level of hippocampal pyramidal neurons increases the chloride conductance of the membrane. This causes hyperpolarization and shunting of excitatory synaptic currents.
2. At the dendritic level, GABA response is depolarizing because the chloride equilibrium potential is more positive than the membrane potential.[5] Chloride ions and sodium or calcium ions may be involved in the response.[6] The associated conductance increase is so large that the net result is inhibition because the membrane potential is clamped below the spike threshold.[7]
3. Binding to GABAp receptors causes an increase in the potassium conductance of the membrane, thereby hyperpolarizing the neuron.[5] Disfunctioning of GABA-ergic synapses has been invoked for Parkinson's disease,[8–10] epilepsy,[11,12] and some forms of schizophrenia.[13,14]

Various approaches to the enhancement of GABA-ergic function in humans include:

1. Direct agonism of GABA receptors.[15,16] This has met with limited success due to tachyphlaxis.
2. Inhibition of enzymatic breakdown of GABA[17,18] GABA transaminase catalyzes pyridoxal-dependent transamination of GABA to succinic semialdehyde. The enzyme can be inhibited by a variety of compounds [e.g., γ-vinyl GABA (Vigabatrin)].
3. Inhibition of GABA uptake into neuronal and glial cell bodies[19,20] by designing appropriate substrates. This has proved to be the most promising strategy.



Scheme 29.2

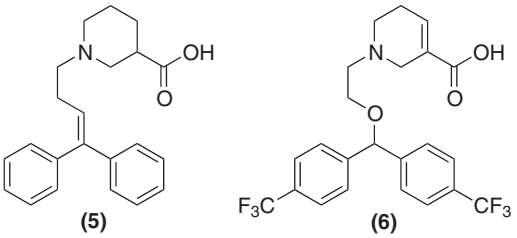
Some years ago a number of cyclic amino acids (Scheme 29.2), such as nipecotic acid (**1**), guvacine (**2**), homo-6-proline (**3**), and *cis*-4-hydroxynipecotic acid (**4**)[21,22] (which can be considered as conformationally restricted GABA analogs[23]) were found to display in vitro activity as inhibitors of [^{3}H]GABA uptake. However, detailed investigation revealed that these cyclic amino acids do not readily cross the blood–brain barrier.[21,24,25] Preparing more lipophilic prodrug esters[15] of **1** and **2** provided compounds that were protective in various seizure models.[21,26] Despite this, their cholinergic effects[27] had a negative influence on their in vivo utility.

In the early 1980s, attention was focused on preparing lipophilic derivatives of **1** to **4**, wherein the lipophilic moiety was attached directly to the nitrogen of the cyclic amino acids.[28,29] These compounds successfully crossed the blood–brain barrier following peripheral administration[28] and exhibited excellent seizure protection [29,30] in animal models predictive of anticonvulsant activity.[31]

The studies cited above resulted in a few preliminary generalizations[1]:

1. The R-enantiomer was able to inhibit GABA uptake in a more pronounced manner than was its S-counterpart. Moreover, nipecotic acid derivatives were more potent than guvacine derivatives.
2. Inhibitory potency was not influenced dramatically by the flexibility of the GABA analogs.
3. Reduction of inhibitory potency was more pronounced in the case of neuronal GABA carriers than in the case of the astroglial carriers.
4. Inhibitory potency was modulated most effectively by the relative distance between the charged centers of the molecule. Analogs with the shortest distance between the charged centers [e.g., nipecotic acid (**1**), guvacine (**2**)] were potent inhibitors of GABA uptake.
5. Lengthening or shortening the alkenyl chain linking the piperidine ring to the aromatic groups as well as saturation of the double bond in the side chain led to loss of activity.
6. Substituting nipecotic acid with a methyl or phenyl group at the 2-position led to inactive products. Based on this information, a general pharmacophore activity model was developed.[2]

The compounds also revealed lower CNS depressant effects than those of products such as diazepam.[32] Examples of such compounds are products **5** and **6**, which were first synthesized at Smith-Kline-French and Parke-Davis/Warner-Lambert,[33] respectively (Scheme 29.3).



Scheme 29.3

Clinical results on **5** and **6** were disappointing because the compounds were not transported in a saturable fashion by GABA carriers.[34]

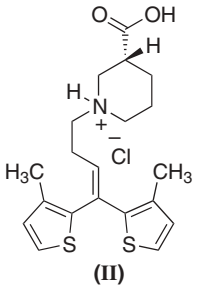
An extensive program to synthesize a series of novel and selective GABA uptake inhibitors was undertaken initially by a team of researchers at Novo-Nordisk in Copenhagen, Denmark.[35] These researchers synthesized numerous 4,4-disubstituted-3-butenyl GABA uptake inhibitors containing both aryl and heteroaryl groups. Of particular interest were compounds that incorporated alkyl substituents that limited, by steric repulsion, the coplanarity of the two aryl moieties.

Table 29.1 depicts the in vivo anticonvulsant effects in mice of some of the most potent compounds studied. The convulsion model was based on observing the inhibition of clonic seizures induced by a 15 mg/kg intraperitoneal dose of methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM). This compound is an inverse benzodiazepine receptor agonist. Fjalland's procedure[36] was used for these studies. Table 29.1 shows some of the compounds that showed promise.

One compound from the series, (*R*)-l-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidine carboxylic acid (tiagabine, **II**, Scheme 29.4), was selected for further study as the most promising candidate.

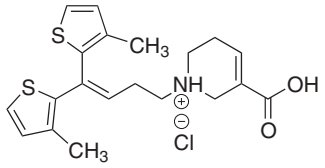
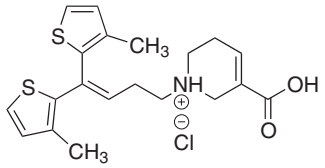
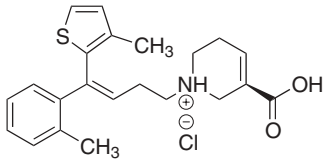
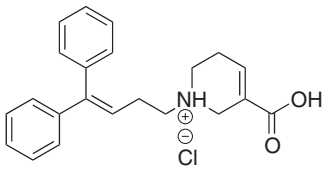
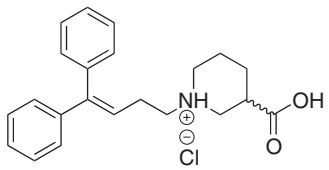
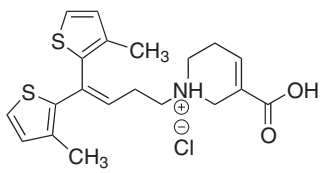
Tiagabine (**II**) is a noncompetitive mixed-type inhibitor of GABA. The tissue homogenate used in these studies contained both neuronal and glial elements; GABA uptake in both was completely inhibited. Tiagabine was not taken up into synaptosomes by a sodium- or temperature-dependent process. The product was tested on the DBA/2 strain of mice. These respond to high-intensity sound by developing clonic seizures and tonic extensor spasms, usually followed by death. Tiagabine was extremely effective against bicuculline- and pentylene tetrazol–induced seizures in rats.

The pharmacology[37,38] in laboratory animals and biochemistry[4,39] provide evidence of the compound's potency and selective mode of action. Tiagabine also possesses some



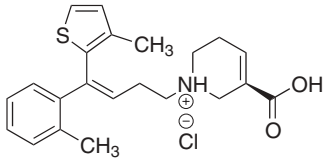
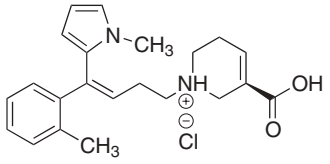
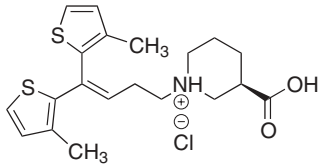
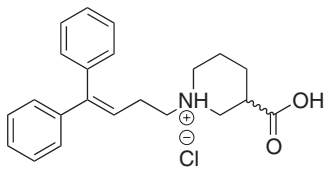
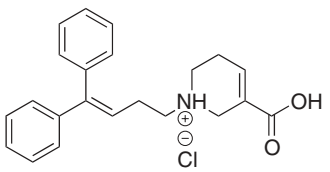
Scheme 29.4

TABLE 29.1 In Vivo Anticonvulsant Effect in Mice

Compound	[^{3}H] GABA Uptake IC_{50} (nM)	Inhibition of DMCM-Induced Seizures in Mice; ED_{50} (mg/kg) After Intraperitoneal Administration
	60	2.5
	67	1.5
	78	1.2
	82	1.3
 (racemic)	87	2.6
	112	2.1

(*Continued*)

TABLE 29.1 (***Continued***)

Compound	[^{3}H] GABA Uptake IC_{50} (nM)	Inhibition of DMCM-Induced Seizures in Mice; ED_{50} (mg/kg) After Intraperitoneal Administration
	130	1.9
	130	1.8
	138	1.8
 (racemic)	330	3.1
	341	3.6

Source: Ref. 35.

analgesic[40] and anxiolytic[41] activity. No tolerance has been developed for the anticonvulsant activity, and the drug has a suitable lipophilicity for availability in the central nervous system.

The innovator company, Novo-Nordisk, was looking for a U.S. partner to codevelop a new drug with them. Abbott was a potential collaborator since they already had a presence in the anticonvulsant field. Each group had excellent internal scientists and systems, so each partner decided to play to their strengths for joint development of this drug. "Cost sharing by task sharing" became the byline. As the following delineation of the chemical challenges and mutual solutions should elucidate, this approach was enormously successful, leading to efficient manufacturing chemistry, identification and synthesis of metabolites and degradates, and even the development of new chemistry for further work in medicinal chemistry. Tiagabine (**II**) is now marketed as Gabitril for treatment of epilepsy. The results of human clinical trials of this material have been reported.[42]

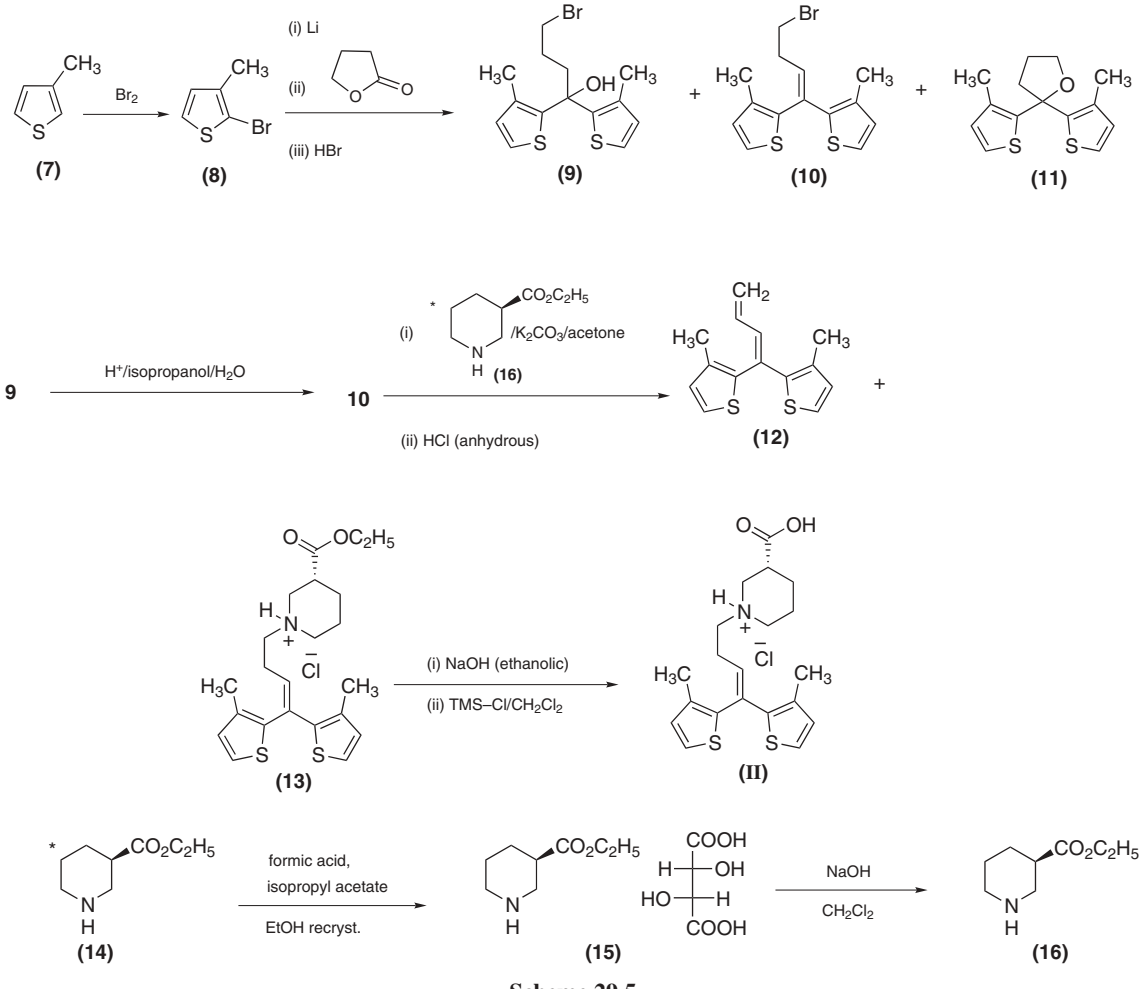
29.2 SYNTHESIS OF SYMMETRICAL ANALOGS: TIAGABINE

Synthesis of symmetrical analogs in best exemplified by the synthesis of tiagabine. In an earlier procedure (Scheme 29.5), 3-methyl thiophene (**7**) was brominated with bromine to yield the 2-bromo-3-methyl derivative (**8**). This reaction was not regiospecific: products arising out of bromination at the 4,5 and the methyl group were formed in varying yield. Formation of the lithio derivative occurred with considerable scrambling of the bromine substituent. Subsequent condensation with γ-butyrolactone followed by workup with 48% HBr yielded a complex mixture comprising predominantly three products, **9** to **11**. These could be separated by chromatography and **9** and **11** could be converted to **10** by treatment with HBr.

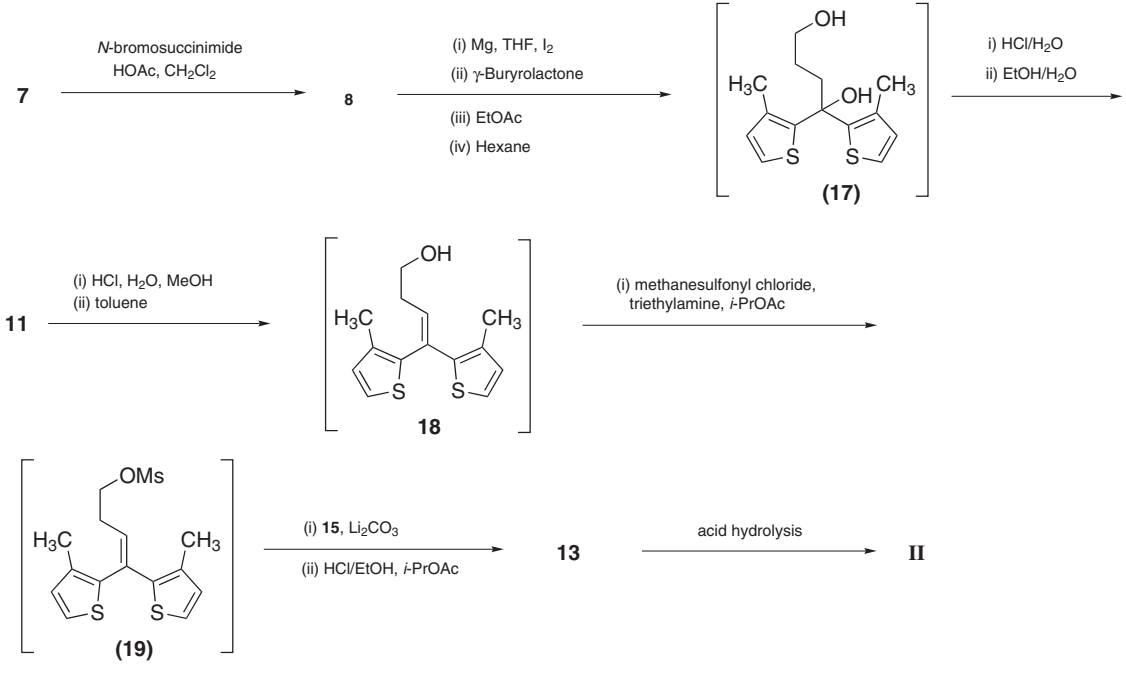
Ethyl (*R*,*S*)-piperidine-3-carboxylate (**16**) was resolved by literature procedures[43] to furnish the *R*-enantiomer as the L-(+)-tartrate (**15**). Conversion of the ethyl *R*-(−)-piperidine-3-carboxylate tartaric acid salt (**15**) to the base (**16**) was a cumbersome and low-yielding process. The base (**16**) was significantly soluble in water and necessitated extraction with copious volumes of methylene chloride. Condensation of ethyl *R*-(−)-piperidine-3-carboxylate (**16**) with the "ene-bromide" (**10**) was accompanied by formation of large amounts of the diene (**12**). Spirotetrahydrofuran (**11**) was separated from the product mixture by precipitation of the hydrochloride salt, but appreciable quantities of the salt were retained in the solvent mixture.

The ethyl ester (**13**) was saponified with ethanolic sodium hydroxide; no racemization of the nipecotic acid moiety accompanied this transformation. The product was extracted with methylene chloride, the solvent was evaporated and residue was redissolved in toluene. Treatment with stoichiometric quantities of methanol followed by chlorotrimethylsilane yielded the anhydrous product. This method was demonstrated to be generally useful for the isolation of hydrochloride salts under anhydrous conditions.[35]

The synthesis was suitable for providing multigram quantities of product for research and toxicology but was not amenable to scale-up. Among the contentious issues were (1) low yields of intermediates, (2) capriciousness of the synthetic reactions, and (3) large volumes of methylene chloride required for efficient extraction of methylene products. Elegant work by Mahendra Deshpande and Subhash Patel was instrumental in defining an efficient, reproducible process that was useful in the production of large-scale quantities of tiagabine (Scheme 29.6). Considerable work was done to optimize the reactions and


(i) Li
(ii)
(iii) HBr
Br2
(7)
(8)
(9)
(10)
(11)
9
H+/isopropanol/H2O
10
(i)
/K2CO3/acetone
(16)
(ii) HCl (anhydrous)
(12)
(13)
(i) NaOH (ethanolic)
(ii) TMS–Cl/CH2Cl2
(II)
(14)
formic acid,
isopropyl acetate
EtOH recryst.
(15)
NaOH
CH2Cl2
(16)

Scheme 29.5


7
N-bromosuccinimide
HOAc, CH2Cl2
8
(i) Mg, THF, I2
(ii) γ-Buryrolactone
(iii) EtOAc
(iv) Hexane
OH
H3C
OH
CH3
S
S
(17)
i) HCl/H2O
ii) EtOH/H2O
11
(i) HCl, H2O, MeOH
(ii) toluene
OH
H3C
CH3
S
S
18
(i) methanesulfonyl chloride,
triethylamine, i-PrOAc
OMs
H3C
CH3
S
S
(19)
(i) 15, Li2CO3
(ii) HCl/EtOH, i-PrOAc
13
acid hydrolysis
II

Scheme 29.6

generate the product in optimum yield and purity. The anhydrous form of tiagabine·HCl was used in formulations of the bulk drug; it was deemed technically more expedient to crystallize the product as the hydrate. The symmetrical structure makes the synthesis more facile than the unsymmetrical congeners.

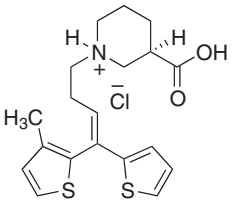
29.3 SYNTHESIS OF UNSYMMETRICAL ANALOGS: DESMETHYLTIAGABINE[44]

Desmethyltiagabine (**III**, Scheme 29.7) is an example of an unsymmetrical analog that shows interesting biological activity. Our initial encounter with an unsymmetrical analog such as desmethyltiagabine was under unusual circumstances. We had completed delivery of a multikilogram lot of tiagabine after a five-week campaign and were eagerly contemplating a relaxing weekend. Then our colleagues in the department of analytical chemistry provided us with the most unwelcome news that our sample was contaminated with a hitherto unseen "impurity." The concentration of the impurity was high enough to cause rejection of the lot. Extensive liquid chromatography/mass spectrometry (LC–MS) work revealed the structure of the impurity as the desmethyl congener of tiagabine.

We could not envision any scenario wherein a methyl group could be eliminated from the thiophene moiety during any of the synthetic operations outlined in Scheme 29.6. We checked all earlier bulk drug lots under diverse high-performance liquid chromatographic conditions. Not a trace of the new impurity was discernible. Frantic activity during the course of the month established the desmethyl derivative to be present in every intermediate of the contaminated bulk drug lot as far back as the starting material, 2-bromo-3-methylthiophene. When confronted with "People's exhibits A, B, C,…" (our chromatograms and spectra), our supplier advised us that they had conducted bromination of thiophene for another client. The operators did not clean the reactors thoroughly or sufficiently and charged the vessels with 3-methylthiophene. Thus, the mystery of how our supply of 2-bromo-3-methylthiophene contained the 2-bromo derivative was solved!

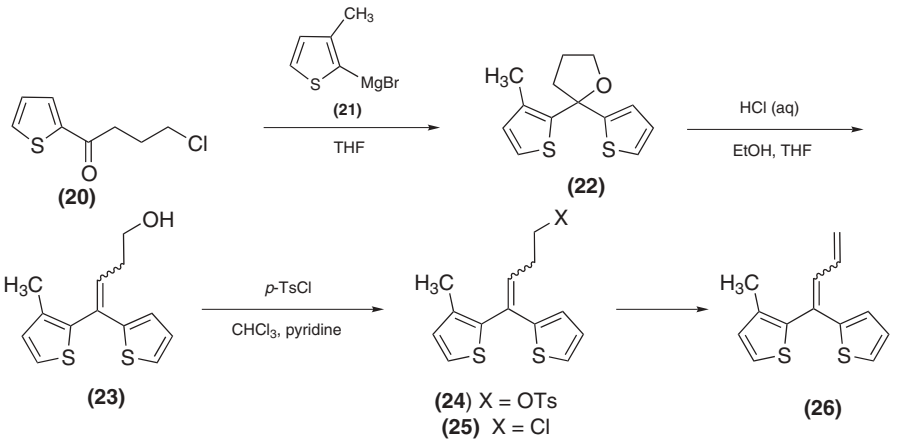
While all the detective work was in progress, we formulated synthetic schemes designed to achieve high-yielding synthesis of desmethyltiagabine. This material had been prepared earlier (Scheme 29.8 and 29.9); the present authors participated in a de-novo synthesis (Scheme 29.10). Retrosynthetic analysis of the target molecule would suggest a strategy of alkylating a nipecotic acid residue (protected as the ester derivative) with a 4-halo- or 4-tosyl-l,1-diaryl-l-butene. Such an approach is shown in Scheme 29.8.

Reaction of 4-chloro-l-(2-thienyl)-l-butanone (**20**) with the Grignard reagent (**21**) provided the 2,2-disubstituted tetrahydrofuran (**22**) in good yield. The tetrahydrofuran ring could be opened with concomitant dehydration to provide an



(III)

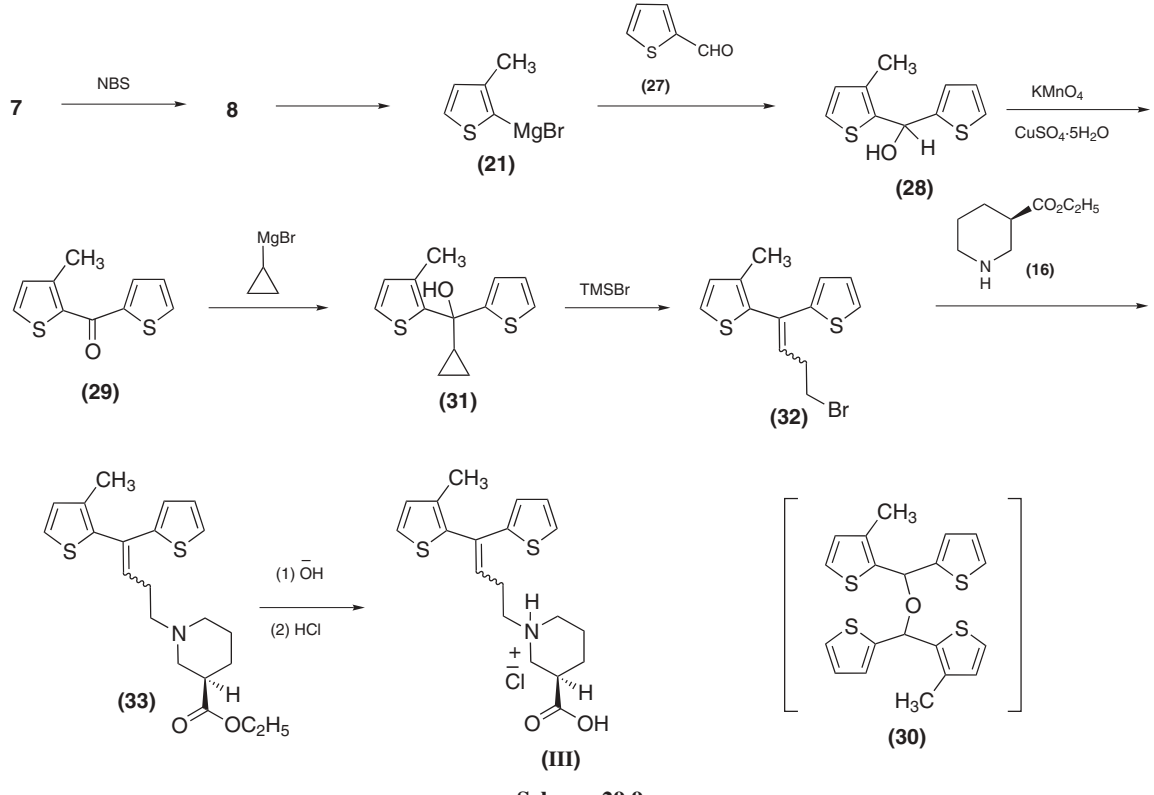
Scheme 29.7



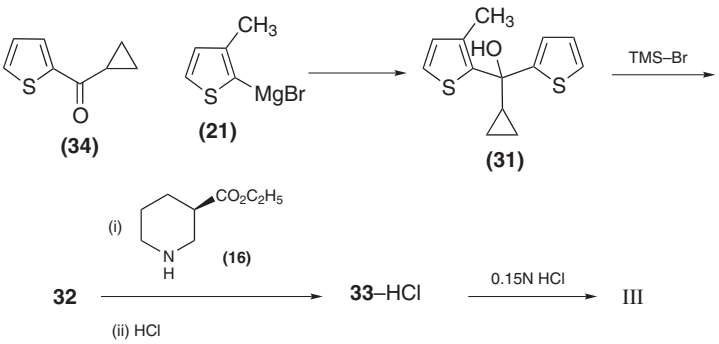
Scheme 29.8

unsymmetrical butenol (**23**). Conversion to the *p*-toluenesulfonate derivative (**24**) with *p*-toluenesulfonyl chloride in pyridine–chloroform at 45°C was a facile process; at reflux temperature, the corresponding chloride derivative (**25**) was obtained. These derivatives could be used to alkylate nipecotic acid esters, but the yields were variable. The chloride displacement in particular was not susceptible to catalysis by crown ethers, dimethylaminopyridine (DMAP), and so on. Elimination to the diene (**26**) was also a frequent problem.

We were able to design technically superior syntheses that were amenable to scale-up. Scheme 29.9 depicts the first of two methods that appeared to be particularly successful. Again, 3-methylthiophene (**7**) was brominated with *N*-bromosuccinimide by standard procedures[45] to yield 2-bromo-3-methylthiophene (**8**). No product arising from the bromination of the methyl group was discernible. Grignard reagent (**21**) was prepared in high yield[45] and condensed with 2-formylthiophene (**27**) to yield the carbinol (**28**). This carbinol is unstable to storage and is readily converted to the corresponding ether dimer (**30**) after standing even at −10°C or on contact with acids. The ether dimer (**30**) is a crystalline compound; its structure was confirmed by nuclear magnetic resonance (NMR) and mass spectra. The Grignard reagent was superior to the aryl lithium reagent; with the latter, more isomeric products were obtained. Carbinol (**28**) was oxidized with a mixture of potassium permanganate and copper sulfate pentahydrate to the ketone (**29**).[46] The reaction was slow and necessitated the addition of fresh portions of the reagent after 24-hour periods. Only a small amount of **30** was formed in this reaction. Oxidation with numerous other reagents, such as MnO_2, pyridinium chlorochromate on alumina, or oxidation under phase transfer conditions, yielded the ketone in much lower yields which were difficult to reproduce. The ketone (**29**) could not be prepared directly by reaction of the Grignard reagent (**21**) with 2-cyanothiophene or the acid chloride of thiophene-2-carboxylic acid.[47] The carbonyl carbon of the dithienyl ketone (**29**) was refractory to attack by Grignard reagents designed to introduce the straight-chain three-carbon fragment. Reaction with cyclopropyl magnesium bromide furnished the carbinol (**31**) in high yields.[48] The cyclopropyl ring was unraveled with simultaneous dehydration and bromination with hydrobromic acid in acetic acid or with bromotrimethylsilane to yield the 4-bromo-l, l-diaryl-l-butene.


7
NBS
8
CH3
MgBr
(21)
CHO
(27)
CH3
HO
H
(28)
KMnO4
CuSO4·5H2O
CH3
(29)
MgBr
CH3
HO
(31)
TMSBr
CH3
Br
(32)
CO2C2H5
(16)
CH3
(33)
(1) OH
(2) HCl
CH3
Cl
OH
(III)
CH3
H3C
(30)

Scheme 29.9



Scheme 29.10

Condensation of **32** with ethyl *R*-(−)-piperidine-3-carboxylate (**16**) in acetone yielded **33**. Hydrolysis of the ester residue with base followed by extraction and acidic workup yielded the hydrochloride salt of desmethyltiagabine (**III**). It is important to note the crystallization of the product as a methylene chloride solvate. The material revealed slow loss of methylene chloride on standing and was shown to be a 90 : 10 mixture of the Z and E isomers, respectively, by NMR.

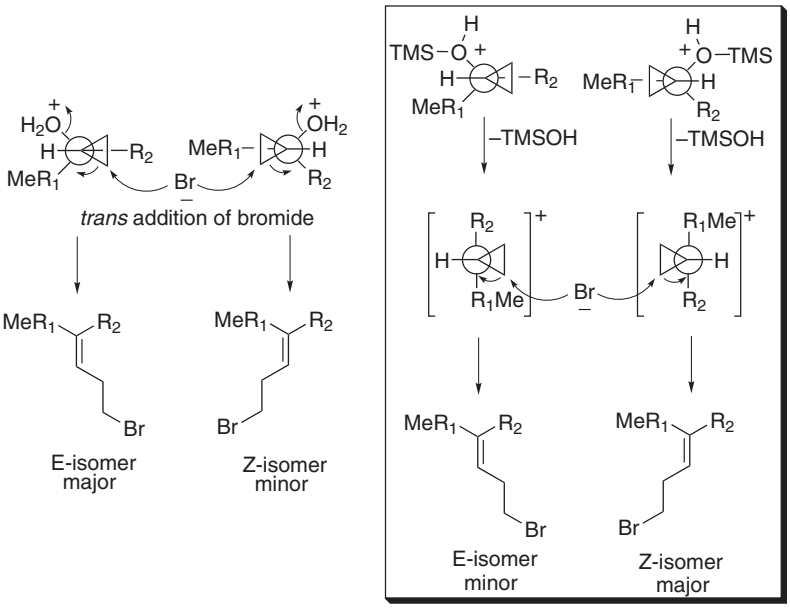
Further improvements in the synthesis are shown in Scheme 29.10.

Cyclopropyl thien-2-yl ketone (**34**), available commercially, was subjected to a Grignard reaction with **21** to yield a carbinol (**31**) in excellent yield. Bromotrimethylsilane-mediated opening of the cyclopropyl ring led to the 4-bromo derivative **32**. Condensation with ethyl *R*-(−)-piperidine-3-carboxylate (**16**) was conducted in isopropyl acetate as a solvent with anhydrous lithium carbonate as the base. The transformation was cleaner and did not provide any of the diene (**26**) arising from elimination of hydrogen bromide. The alkylation of the nipecotate residue could also be directly effected with a mixture of the tartrate salt of **16**, lithium carbonate, and isopropyl acetate. Compound **33** was readily isolated as the hydrochloride; isopropyl acetate was superior to all other solvents used in this reaction. Since it is not itself prone to hydrolysis, it is preferable to ethyl acetate. Moreover, it appears to crystallize hydrochlorides much better.

Acid-catalyzed hydrolysis with aqueous hydrochloric acid was a facile process; the hydrochloride salt of the product could be crystallized out of the same solution. 0.15 N hydrochloric acid was found to be optimally effective in cleaving the ester without racemizing the chiral center. The methods developed here were general ones that could be adapted to the synthesis of several analogs of tiagabine.

29.3.1 Syntheses of Regioisomers of Tiagabine

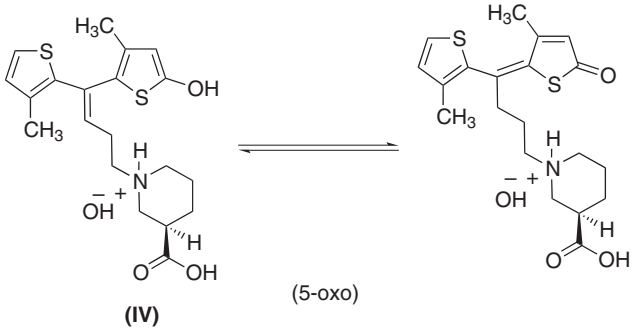
Unraveling of the cyclopropyl ring would necessitate S_N2 *trans* addition of bromide. This would lead to a preponderance of the E-isomer over the Z-derivative. We believe that the reaction on our substrate proceeds by an S_N1 carbonium ion mechanism, yielding the Z-isomer as the major product (Scheme 29.11).



Scheme 29.11

29.3.2 Human Metabolite of Tiagabine: 5-Hydroxytiagabine[50]

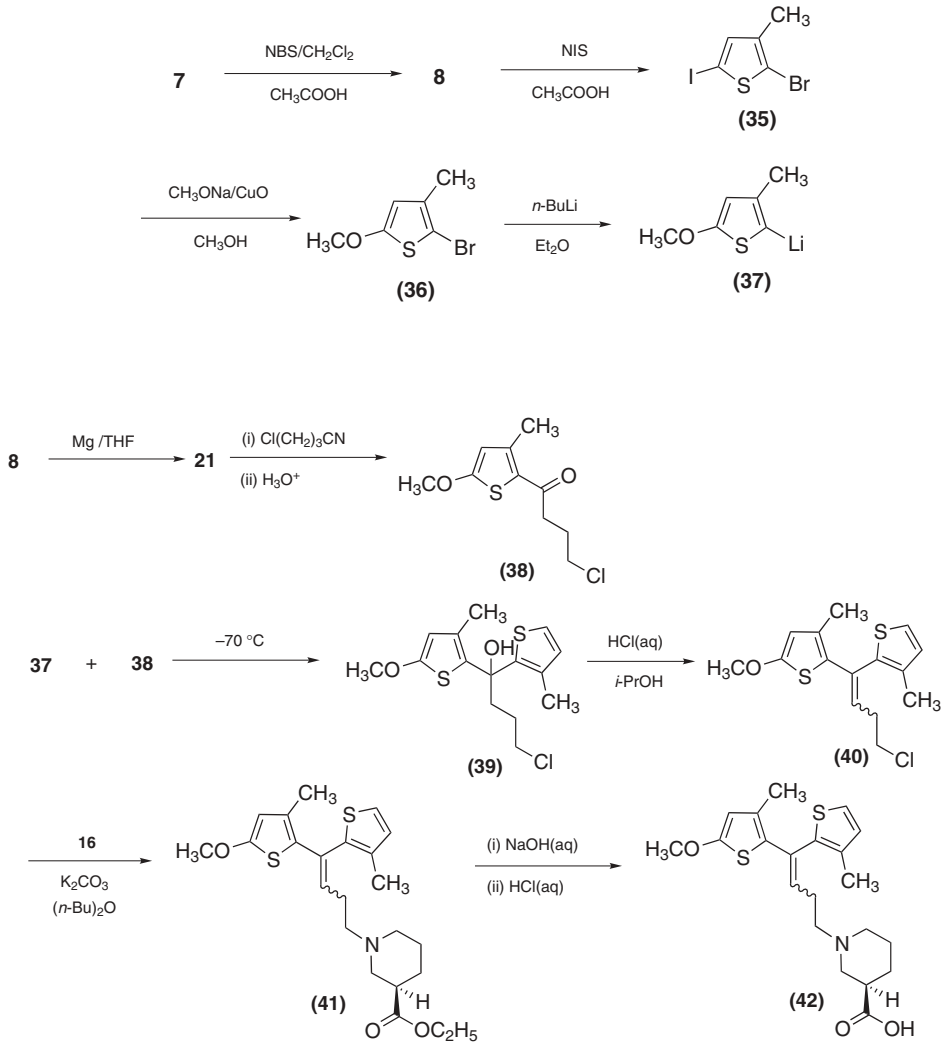
After oral or intravenous administration of [^{14}C]tiagabine (30 mg/kg) to rats, the metabolic profile in urine was characterized by two major peaks, which together accounted for about 90% of the urinary radioactivity or 10 to 15% of the [^{14}C]dose. Upon isolation, each peak was shown to convert to the other and eventually equilibrate to an approximate 1 : 1 mixture that showed identical protonated molecular ions ($m/z = 392$). The ion fragmentation patterns suggested thiophene ring oxidation (+16 amu). The ^{1}H NMR spectrum of an equilibrium mixture of the two metabolites revealed a pair of thiophene ring proton singlets (6.42 and 6.44) and absence of the olefinic proton present in the tiagabine spectrum (5.98). Based on these data and literature precedents for the existence of hydroxythiophenes as keto tautomers, it was postulated that oxidation in one of the thiophene rings of tiagabine (**II**) formed 5-hydroxytiagabine (**IV**) (Scheme 29.12). This was believed to be present entirely



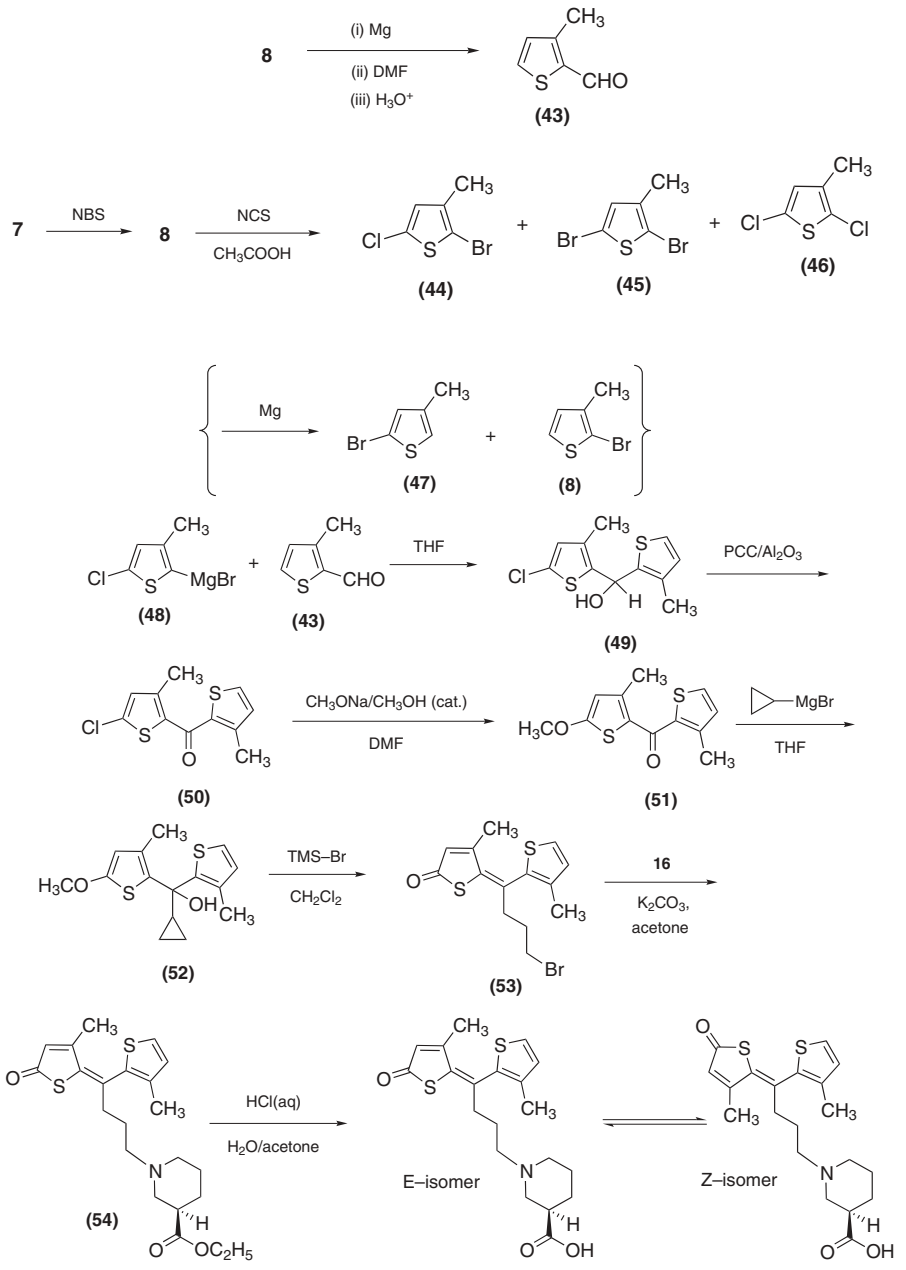
Scheme 29.12

as E and Z isomers of the tautomeric 5-oxo form. The urinary metabolites in rats were also identified as being identical to the human metabolites, following oral administration of [^{14}C]tiagabine to adult male subjects.

To ensure the position of oxidation in tiagabine, a total synthesis starting from well-characterized starting materials was performed. Two major approaches for the preparation of 5-hydroxytiagabine (**IV**) shown in Schemes 29.13 and 29.14 have been studied. The method shown in Scheme 29.13 was the least efficient of the methods attempted. The intermediate (**36**) was attainable in only 27% yield and necessitated extensive chromatography. This intermediate was also unstable and difficult to handle. The reaction of the Grignard reagent (**21**) with 4-chlorobutyronitrile proceeded in a meager 7% yield and could not be optimized. Subsequent steps proceeded in acceptable yield to furnish the target. The structure was confirmed by double quantum filtered COSY and ROESY NMR two-dimensional spectra and ^{1}H-^{13}C HMQC and HMBC NMR spectra.



Scheme 29.13



Scheme 29.14

Compound **7** was brominated with *N*-bromosuccinimide by standard procedures[45] to 2-bromo-3-methylthiophene (**8**), which was then chlorinated to give 2-bromo-5-chloro-3-methylthiophene (**44**). Initial attempts to chlorinate selectively at the 5-position failed. Chlorination of **8** with *N*-chlorosuccinimide[51] or sulfuryl chloride[52] has been reported to give **44** in 62 to 90% yield. In our hands, these procedures gave mixtures of **44**, 2,5-dibromo-3-methylthiophene (**45**), and 2,5-dichloro-3-methylthiophene (**46**). The two side

products that probably formed by halogen exchange can be observed in high field by ^{1}H NMR spectra. A similar mixture was obtained using chlorine in dichloromethane in the presence of mercury oxide.

2-Bromo-5-chloro-3-methylthiophene (**44**) was converted to 5-chloro-3-methylthien-2-ylmagnesium bromide (**48**), which was added to 2-formyl-3-methylthiophene (**43**), producing bis(thien-2-yl) methanol (**49**). 2-Formyl-3-methylthiophene (**43**), in its turn, was prepared by formylation of 3-methylthien-2-yl magnesium bromide (**21**) with *N*,*N*-dimethylformamide. Bis(thien-2-yl) methanol (**49**) was sensitive to acid but could be oxidized to the corresponding ketone (**50**) under controlled conditions. Manganese dioxide and pyridinium chlorochromate supported on basic aluminum oxide were used, the latter providing the best yield.

The chlorine in bis(thien-2-yl) ketone (**50**) was replaced with a methoxy group to give compound **51** by treatment with sodium methoxide in *N*,*N*-dimethylformamide(DMF)–methanol (9 : 1). These conditions seem particularly effective for substitution of the weakly reactive chlorine with the methoxide functionality. Sodium methoxide in dimethylformamide in the absence of methanol gave slow conversion to **51** and formation of several unidentified by-products. While displacement of the chlorine in compound **50** could be achieved by this procedure, reaction on the corresponding bromide and iodide afforded only dehalogenation.

5-Methoxyketone (**51**) was treated with cyclopropylmagnesium bromide to give cyclopropylmethanol (**52**). Treatment of this compound with bromotrimethysilane resulted both in demethylation and opening of the cyclopropyl ring with elimination of trimethylsilanoxide, and migration of the double bond thus formed to give the conjugated system **53**. The aliphatic bromine atom of compound **53** was displaced with ethyl (*R*)-3-piperidine carboxylate (**16**) and the resulting ester (**54**) was hydrolyzed to yield 5-hydroxytiagabine (**IV**). Acidic hydrolysis was found superior to basic hydrolysis, providing acid **IV** in virtually quantitative yield.

It was increasingly evident that the lengthy sequences involved allied with the low yields obtained would make this synthesis untenable. Our hopes of efficiently preparing large quantities of the metabolite had subsided to low ebb. We reflected on the old maxim:

"Necessity is the mother of invention" and expanded it to a new adage (with a fond hope that it will find a place in our lexicon!): "Frustration is the mother of innovation." Our frustration with the synthesis was rapidly mounting. Clearly, finding an innovative solution was the order of the day. The solution taxed our powers of concentration and recall of literature to the utmost.

At this juncture, we had to fly to Switzerland for consultations with our collaborators at a leading custom synthesis company. Our flight from O'Hare Airport was scheduled to leave at 4:30 P.M. A 1-hour delay was announced; we cheerfully imbibed the excellent wines provided in the departure lounge. Finally, we were strapped in our seats and awaited takeoff—when suddenly, King Winter decided to grant us an audience. A blinding blizzard descended on the area. Our plane was deiced, and deiced, and deiced. We pulled back at 9:30 P.M. and were forty-fifth in line for takeoff. The plane in front skidded off the runway and we could move neither forward nor back. The airline refused to allow us to deplane and seek the sanctuary of a hotel. Time went by, the food was gone, passengers had secured firm experimental evidence of their capacity (or lack thereof) for alcohol, and horror of horrors, the coffee supply was exhausted! We squirmed in our seats, sheep held captive by a modern conveyance! We did land safely at our destination after 20 hours ensconced on the plane, despite having sworn (under several oaths) never to fly this airline again!

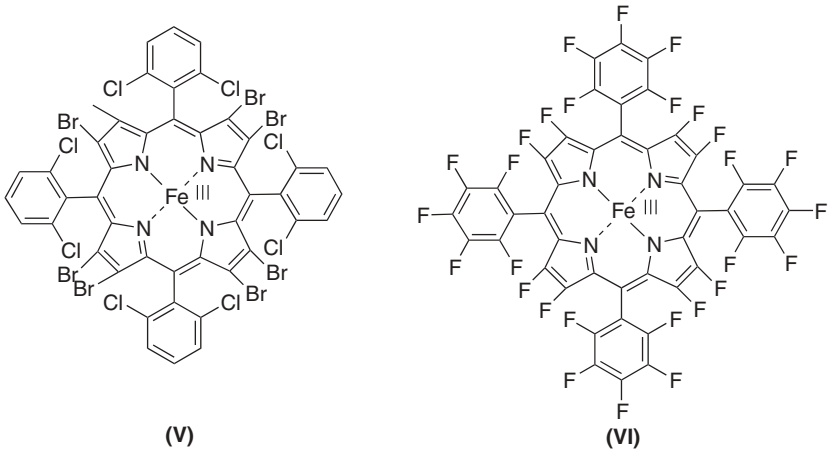
We were resigned not to get much sleep when an inspiration dawned on us: "Try a metalloporphyrin. It catalyzes regiospecific hydroxylation." Preliminary experiments were planned on one of the few available unused napkins. We landed in Zurich after the exhausting sojourn. We were weary but had the nucleus of an idea that was initially unsuccessful but was to play a very important role in our future research programs.

29.4 ATTEMPTED BIOMIMETIC SYNTHESIS OF 5-HYDROXYTIAGABINE

The synthetic problems associated with the method described above prompted us to look for a novel method of preparation. 5-Hydroxytiagabine is formed in vivo by a regiospecific cytochrome P450 monooxygenase-mediated hydroxylation; extensive work has established the intermediacy of high-valent iron porphyrin intermediates in similar transformations.[50] Sterically protected and electronically activated metalloporphyrins have been studied as synthetic models for cytochrome P450–mediated epoxidations and hydroxylations. These catalysts are robust, not destroyed under strongly oxidizing conditions, and effect catalytic oxidations with high turnover numbers.[54–61]

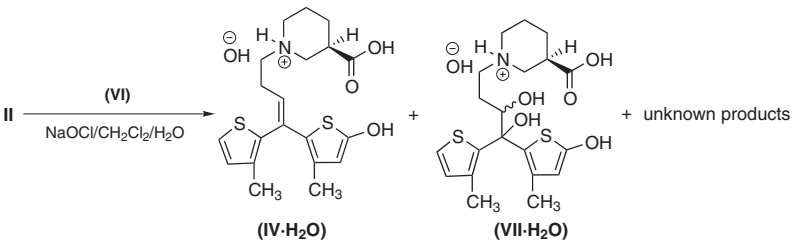
The central double bond in tiagabine is hindered and relatively inert to epoxidation under a wide variety of reaction conditions. Treatment with hydrogen peroxide, sodium hypochlorite, or *m*-chloroperbenzoic acid did not lead to significant amounts of epoxide formation. Porphyrin-assisted oxidation was therefore anticipated to be directed toward hydroxylation of the thiophene ring. We attempted such envisaged hydroxylation with octabromotetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl [abbreviated as $C_{l8}Br_8Fe(III)TPP$] (**V**) and octafluorotetrakis(pentafluorophenyl)porphyrin Fe(III)Cl [abbreviated as perfluoro Fe(III)TPP] (**VI**) (Scheme 29.15).

The hemins were synthesized by the methods of Traylor, Dolphin, and Tsuchiya.[61–63] Condensation of the appropriate aldehydes (2,6-dichlorobenzaldehyde and pentafluorobenzaldehyde, respectively) with pyrrole in the the presence of anhydrous zinc chloride in refluxing lutidine yielded the zinc complexes of the porphyrins after chromatographic



(V) **(VI)**

Scheme 29.15

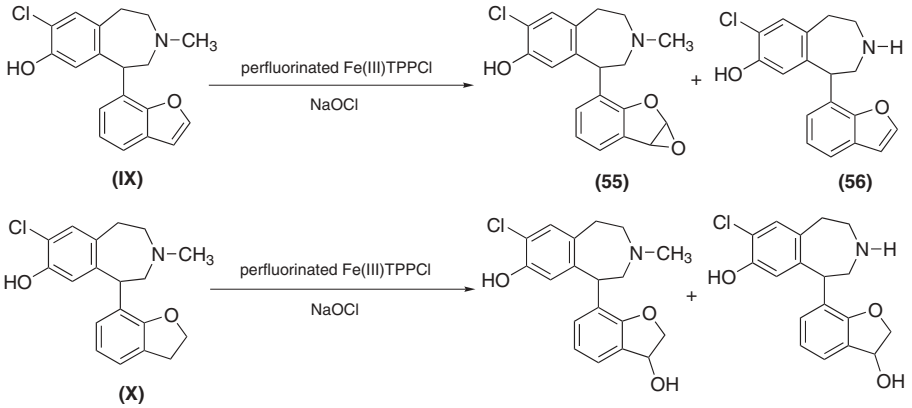


Scheme 29.16

purification. Halogenation of the pyrrole moiety with *N*-bromosuccinimide in refluxing CCl_4 or with anhydrous Ag(l)F in refluxing CH_2Cl_2 furnished material approximating the perhalogenated materials. Demetallation with trifluoroacetic acid followed by conversion to the hemin ($Fe^{II}Cl_2$/DMF at reflux) by the method of Adler[64] and Kobayashi[65] provided the hemins after alumina chromatography.

Treatment of tiagabine (**II**) with the synthesized hemins in a methylene chloride–water biphasic system with NaOCl or 30% H_2O_2 as the exogenous oxygen donor gave a product that was initially incorrectly identified as the 5-hydroxytiagabine–H_2O (**IV**·H_2O) in 62 to 74% yield. Phase transfer catalysts were not required. Conversion of tiagabine to the base allowed reaction with the hemin and *t*-butyl hydroperoxide in methylene chloride. The yields were lower (40%) and more side products were discernible. Spectroscopic and chromatographic comparison with the authentic sample of the human metabolite was disappointing in that the requisite product had not formed. Dihydroxytiagabine (**VII**), arising presumably via epoxidation of the central double bond followed by epoxide ring opening, was the major product of the reaction. This product was confirmed to be the dihydroxy material by independently subjecting **II** to Sharpless asymmetric dihydroxylation.[66,67] Other polar and apolar side products of the metalloporphyrin-assisted hydroxylation were not identified conclusively (Scheme 29.16).

The technology was rapidly adapted to the preparation of metabolites of other sophisticated pharmaceutical entities. Scheme 29.17 shows the products obtained from two other compounds (**IX** and **X**) under investigation for the treatment of psychosis.[68] The hydroxy and desmethyl derivatives shown were difficult to synthesize by conventional organic chemistry methods.



Scheme 29.17

Availability of larger amounts of metabolites would facilitate biochemical, pharmacological, and toxicological studies. Additional details of our work with metalloporphyrins are presented at the end of the chapter.

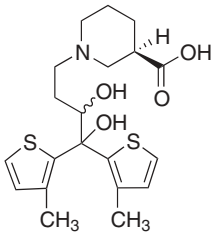
29.5 OXIDATIVE DEGRADATION PRODUCTS OF TIAGABINE[67]

Initial liquid and tablet formulations of tiagabine had a short shelf life of one to two years and revealed the presence of two major and other minor oxidative degradation products. The degradation products could be suppressed by addition of antioxidants such as β-tocopherol to the formulation. This indicated the degradation process to be of oxidative origin. The products could also be seen in stressed solutions of bulk drug exposed to ultraviolet light in the presence of air. The major products were dihydroxytiagabine (**VII**) and ketotiagabine (**VIII**) (Scheme 29.18).

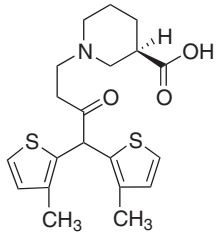
29.5.1 Dihydroxytiagabine

A solution of tiagabine was placed in a light chamber and exposed to 6000 lux for 1 week. The degradation product was separated by high-performance liquid chromatography(HPLC) with ethanol/trifluoroacetic acid as the eluant. Neutralization of the eluates followed by desalting and freeze-drying provided the pure product. The ^{1}H NMR spectrum revealed a doublet of a doublet and two additional doublets (2H) in the aromatic region (7.10 and 6.70 ppm) compared to the four clearly separated doublets (1H) in the tiagabine spectrum.[35] The aliphatic portion of the spectrum was difficult to interpret, due to numerous conformational possibilities. The resonance (triplet) in the vinylic hydrogen in tiagabine was replaced by a new resonance at 450 (m, $CDCI_3$). This was suggestive of the functionalization of the double bond.

The ^{13}C NMR spectrum revealed resonances of eight carbons in the aromatic region and two additional carbons at 73 and 78 ppm indicative of carbons bound to −OH groups. Mass spectrometry (thermospray interface) revealed an (M + 1) quasimolecular ion at $m/z = 410$. This was in agreement with two extra oxygen atoms having been introduced. The peak at $m/z = 392$ (p-H_2O) suggested an aliphatic positioning of the hydroxy functionality. This was also seen in an LC–MS/MS experiment, where the (M + l) ion at $m/z = 410$ was collision activated to further fragmentation. An $m/z =$ 142 ion confirmed the nipecotic acid portion of the molecule to be intact. The structure was assigned as R(−)l-(4,4-bis(3-methylthien-2-yl)-3, 4-dihydroxy-l-butyl)piperidine-3-carboxylic acid (**VII**).

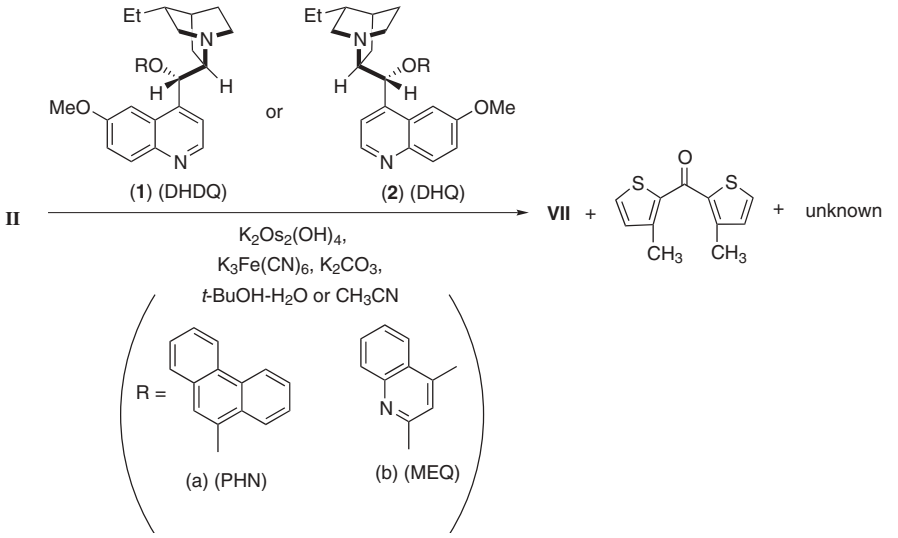


(VII)



(VIII)

Scheme 29.18



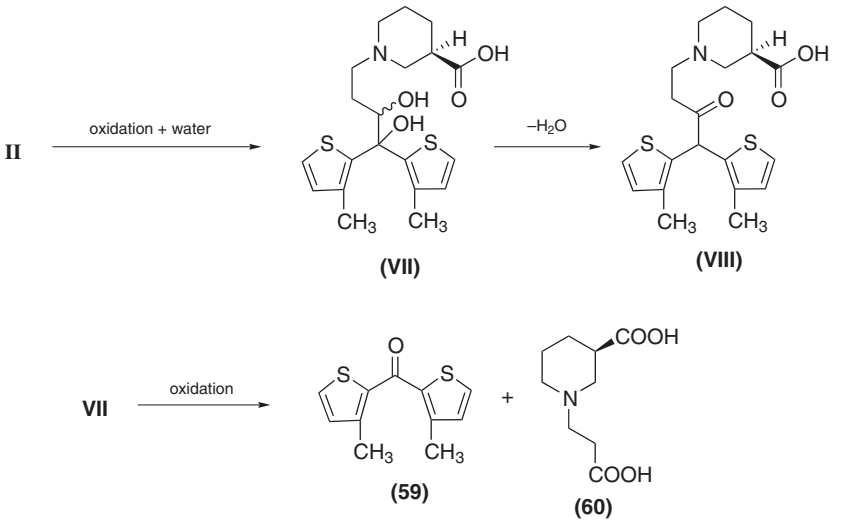
Scheme 29.19

Preparation of dihydroxytiagabine (**VII**) was accomplished by the method shown in Scheme 29.19. Synthesis of the 9-*O*-(4′-Methyl-2′-quinolyl) ether of dihydroquinidinol proved difficult in that the central double bond is hindered and proved to be refractory to attack by reagents such as *m*-chloroperbenzoic acid and hydrogen peroxide. The putative epoxide was not detected under a variety of reaction conditions; a complex mixture of products was always obtained. Reaction with osmium tetroxide/pyridine/*N*-methylmorpholine-*N*-oxide was slow and yielded the requisite diol in low yield, but extraction of the product from water proved to be a problem.

The Sharpless procedure[66] for effecting osmium-catalyzed ligand-accelerated asymmetric dihydroxylation was utilized successfully; the reaction could also be scaled up. Bis(3-methylthien-2-yl) ketone (**59**) was also a product in these reactions and was accompanied by an impurity whose structure has not been elucidated. Optimum yields of the dihydroxy material were obtained in dioxane–water/*t*-butanol–water mixtures. Use of osmium tetroxide instead of potassium osmate led to a slower reaction and increased the formation of undesired products. The material derived from synthesis revealed complete identity with the tablet degradates: Any diastereomers that formed were not resolved under our chromatographic conditions. Attempted functionalization of the vicinal dihydroxy groups (acetate, acetonide, trflate) was unsuccessful and led to complex mixtures of products.

29.5.2 Ketotiagabine

The abundance of this product in the tablet degradates increased with the length of storage. When dihydroxytiagabine (**VII**) was stored under acidic conditions, it was slowly converted to **VIII**. The degradates identified hitherto are summarized in Scheme 29.20. A 70-eV solid-probe mass spectrum showed no parent ion peak. The peak at $m/z = 207$ gave evidence of benzylic cleavage. An $m/z = 142$ peak indicated the nipecotic acid portion of the molecule to be intact. A thermospray mass spectrum showed an (M + 1)



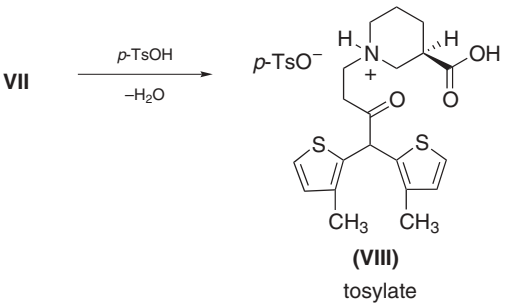
Scheme 29.20

ion at $m/z = 392$, suggesting the product to be generated by loss of water from dihydroxytiagabine. Further, loss of water gave a dominant peak at $m/z = 374$. A daughter ion spectrum was obtained by collision activation of the $m/z = 392$ ion with argon at 10 eV. This spectrum had $m/z = 142$ and 130 ion peaks characteristic of the nipecotic acid moiety.

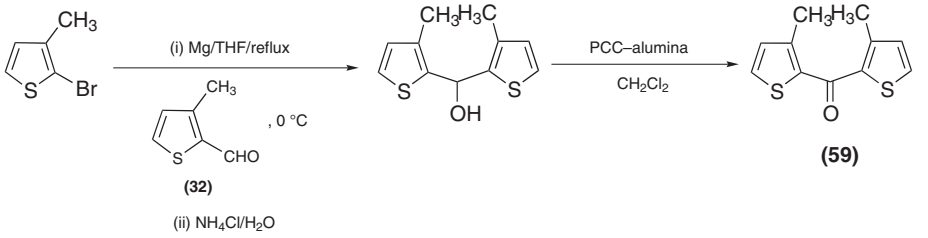
The ^{1}H NMR spectrum showed the presence of two closely spaced doublets at 6.80 ppm and a doublet at 7.15 ppm in the aromatic region arising from the four protons in the 4- and 5-positions in the two thiophene rings. The singlet (1H) at 5.60 ppm disappeared when the sample was mixed with deuterated water. No other peaks were present in the unsaturated part of the spectrum. In the aliphatic portion of the spectrum, the two methyl groups on the thiophenes were present as two singlets at 2.20 ppm. Further, a complex pattern from the other aliphatic hydrogens was observed from 1.30 to 3.70 ppm (1H).

^{13}C NMR showed the presence of an aliphatic carbonyl carbon at 202 ppm and a carboxy carbon at 173 ppm. In the aromatic region of the spectrum, four signals were present. These are due to the four aromatic carbons in the thiophene rings. Signals at 162 and 110 to 135 ppm indicated the isolated substance to be a trifluoroacetic acid salt. In the aliphatic part of the spectrum, two methyl carbons were at 14 ppm, and eight other aliphatic carbons were seen.

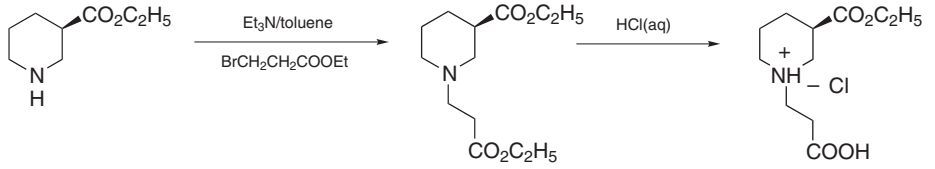
It was concluded that the carbonyl group was adjacent to the carbon benzylic to the two thiophenes, because the hydrogen on this carbon is a singlet. Enolization of the carbonyl will cause this hydrogen to be exchanged with deuterated water. Hence, the structure can be assigned as l-(4,4-bis(3-methylthien-2-yl)-3-oxo-l-butyl)piperidine-3-carboxylic acid (**VIII**). Increased conjugation with an aromatic system provided the driving force for the elimination of water. If a base is used in the extraction medium, the ketotiagabine (**VIII**) is replaced with other unknown impurities with longer retention times. Syntheses of **VIII**, **59**, and **60** were achieved as shown in Schemes 29.21, 29.22, and 29.23, respectively.



Scheme 29.21



Scheme 29.22

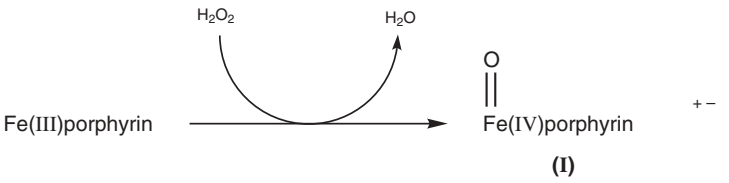


Scheme 29.23

From this account it will be clear that the chemistry of antiepileptic drugs presents a fascinating area of research. Our involvement in the diverse aspects of research and development encountered in the progress of a drug from discovery to manufacture has been an extremely rewarding and intellectually enriching experience. The success of our teams bears eloquent tribute to the globalization of science, need for interdisciplinary cooperation, and the spirit of friendship that existed between our research groups on two continents.

29.6 METALLOPORPHYRINS AS CHEMICAL MIMICS OF CYTOCHROME P450 SYSTEMS

In humans and other animals, most drugs are metabolized in the liver. Many drug metabolites are formed by oxidative mechanisms catalyzed primarily by heme- and cytochrome-containing enzymes.[69] Most biological oxidations involve primary catalysis provided by the cytochrome P450 monooxygenase enzymes. All heme proteins that are activated by hydrogen peroxide, including catalases, peroxidases, and ligninases, function via two-electron oxidation of the ferric resting state to an oxoferryl porphyrin cation radical (**I**).[70]



(I)

Although this oxidation state has yet to be characterized for the cytochromes P450, most of their reactions and those of the biomimetic analogs can be accounted for by oxygen transfer from **I** to a variety of substrates to give characteristic reactions, such as hydroxylation, epoxidation, and heteroatom oxidation.[71] Other products resulting from hydroxyl and hydroperoxyl radicals have also been detected. The metabolic processes in vivo contribute in substantial measure to the efficacy, side effects, and toxicity of a pharmaceutical entity. These factors are responsible for the success or failure of a clinical candidate. Metabolic processes of drugs are always the subject of intense scrutiny in pharmaceutical companies.

Pharmacologists have traditionally been involved with isolation and identification of the metabolites of a drug. It is imperative to conduct such studies early in the drug development process. Toxicological and pharmacological studies on the metabolites form a crucial segment in the identification of a clinical candidate.

Several problems are currently associated with the use of biological systems in studying drug metabolism:

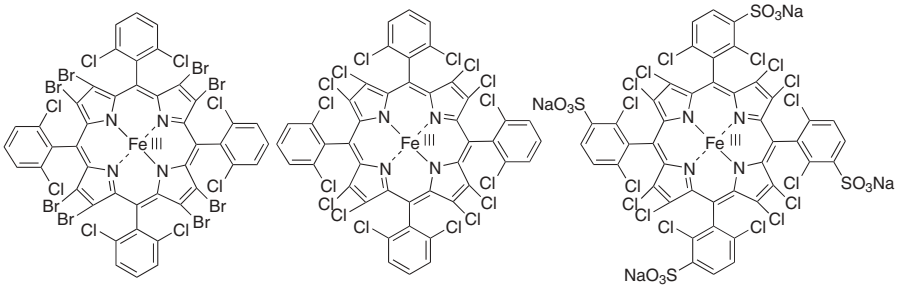
- In vitro studies produce very small quantities of a product. Primary metabolites are often hydrophilic and difficult to isolate.
- Animal studies necessitate the sacrifice of animals and are extremely expensive to conduct. Liver slice preparations are of variable potency; it is difficult to quantitate the precise stoichiometry of the oxidant.
- Pharmacologists do not know, in advance, the structure of the metabolites they should seek.
- Many of the metabolites are not amenable to organic synthesis by conventional routes.

We therefore turned to the metalloporphyrins as mimics of the in vivo metabolic processes.

29.6.1 Oxometalloporphyrins

Synthetic metalloporphyrins have received a lot of recent attention as mimics of numerous enzymes. In addition, 10 models have been developed for peroxidases and particularly, ligninases. Metalloporphyrins have also found utility as model systems for studies of the oxidative metabolism of drugs.[72–74] A detailed study of the metabolism of lidocaine has been reported,[75] as have preliminary studies on the use of metalloporphyrins as chemical mimics of cytochrome P450 systems (Scheme 29.24).[76]

The first synthetic metalloporphyrins were found to be oxidatively labile. Few catalytic turnovers were seen, due to the rapid destruction of the porphyrin macrocycle. It has been shown that introduction of halogens onto the aryl groups (of mesotetraarylporphyrins) and



Scheme 29.24 Metalloporphyrins used for our studies.

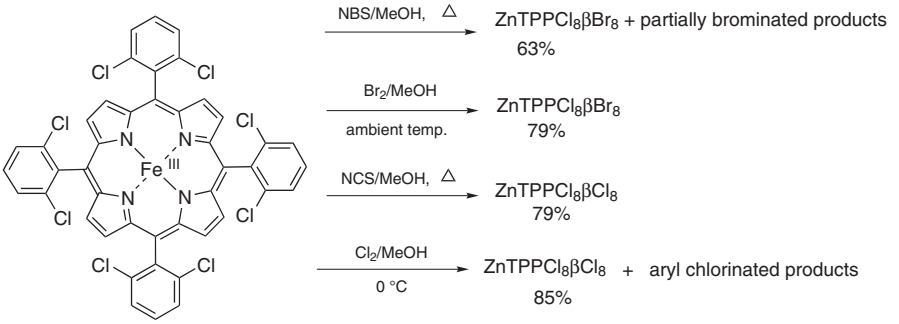
on the β-pyrrolic positions of the porphyrins increases the turnover of catalytic reactions by decreasing the rate of porphyrin destruction.[77] In addition, the combined electronegativities of the halogen substituents are transmitted to the metal atom, making the corresponding oxo-complexes more electron deficient and thus, more effective oxidation catalysts.[78]

29.6.2 Synthesis of the Sterically Protected and Electronically Activated Metalloporphyrins

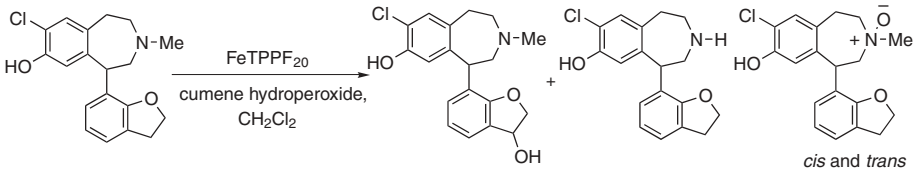
The past decade has seen considerable advances in the development of synthetic metalloporphyrin complexes.[79–86] We have now designed numerous practical and efficacious methods of synthesizing porphyrins with halogens at the *o*-aryl and also the β-pyrrole positions.[87] The methodology provides facile access to a large number of porphyrins in optimum yields and purity (Scheme 29.25). Demetallation with trifluoroacetic acid followed by insertion of iron yielded the corresponding hemins.[88]

29.6.3 Application of the Methodology to Selected Drugs

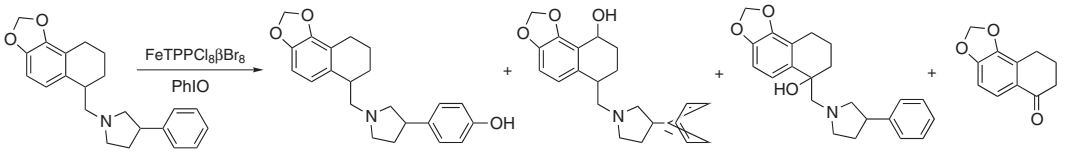
We have recently applied this technique to a study of the oxidative metabolism of several drugs (Schemes 29.26 to 29.29). Reactions with tetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl, octachlorotetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl, and octabromotetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl were conducted in methylene chloride–water or water–acetone to nitrile (80 : 20 v/v) at ambient temperatures. Iodosobenzene, cumene hydroperoxide, hydrogen peroxide, or sodium hypochlorite was used as the source of



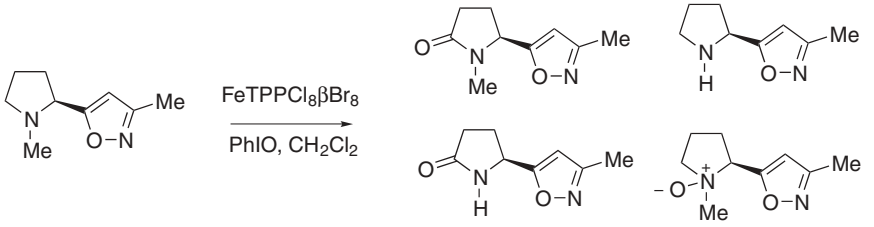
Scheme 29.25 Improved methods for the halogenation of sterically hindered metalloporphyrins.



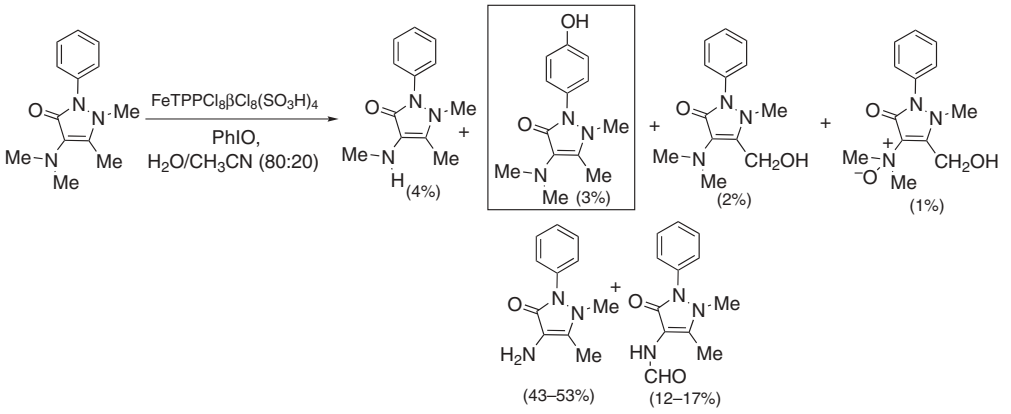
Scheme 29.26 Oxidation of odapipam (antipsychosis drug).



Scheme 29.27 Oxidation of ABT-200 (an antidepressant drug).



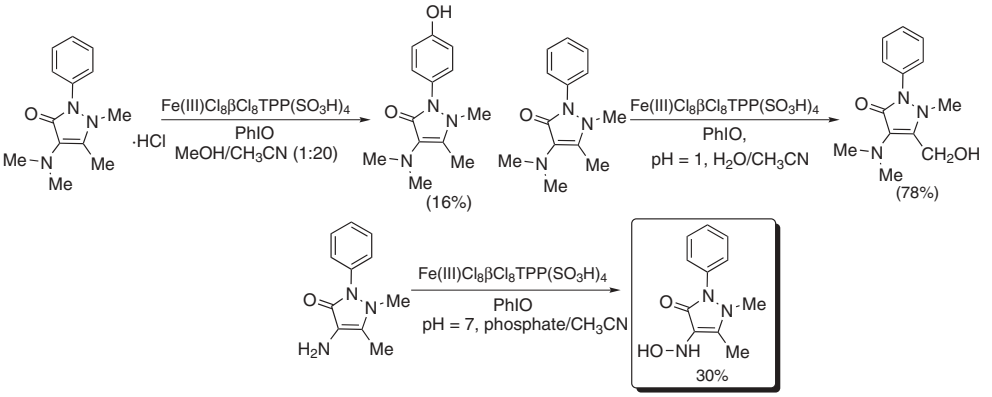
Scheme 29.28 Oxidation of cholinergic channel activator for the treatment of Alzheimer's disease



Scheme 29.29 Selective oxidation of aminopyrine.

exogenous oxygen. Products were separated by HPLC and revealed identity with authentic reference samples of the major metabolites of these drugs, previously isolated from the urine of rats or characterized from rat liver microsomal incubations.

The oxidation of aminopyrene was particularly interesting in that new, hitherto-unobserved metabolites were detected. Variation of the reaction conditions then led to the

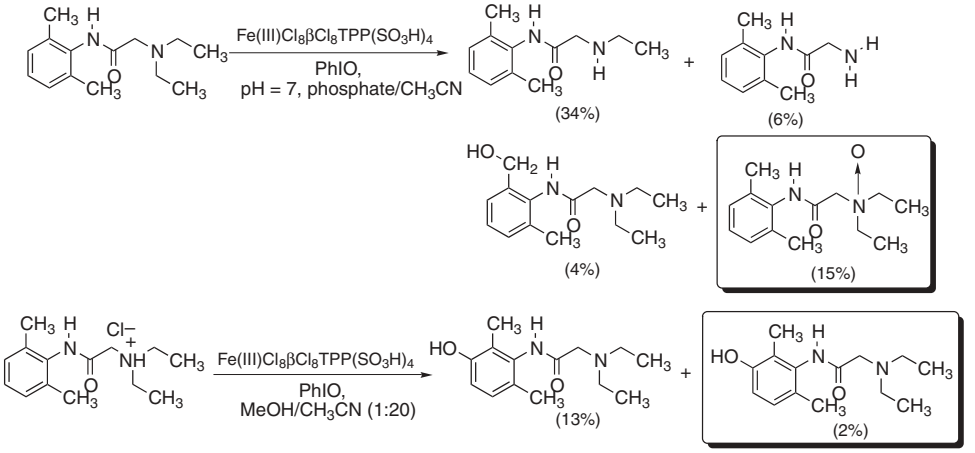


Scheme 29.30 Selective oxidation of aminopyrine.

synthesis of selected metabolites in increased yields (Scheme 29.30). This was also exemplified by the oxidation of lidocaine (Scheme 29.31); two new oxidation products were observed. These had escaped detection in all previous studies.

It is useful to note that these are rare examples of porphyrin-mediated oxidation of sophisticated pharmaceutical entities. The reactions described above are generally applicable and have been used in our laboratories to achieve hydroxylation and N-demethylation on numerous other substrates. The hydroxy metabolite and the desmethyl derivative were usually difficult to synthesize by conventional organic chemistry methods.

This approach affords an efficient method for the systematic preparation and identification of the entire spectrum of metabolites from a chosen drug. We can envision the screening of a series of compounds by reacting them with various permutations of a metalloporphyrin, cooxidant, and a suitable solvent. Reaction conditions that produce the maximum number of metabolites can be defined. This logically leads to subsequent scaled-up optimal processes. The oxidation products can then be separated and subjected to toxicologic, pathologic, histopathalogic, or genotoxic testing.



Scheme 29.31 Oxidation of lidocaine.

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30

BUILDING CONTRACT RESEARCH BUSINESSES BASED ON INTEGRATION OF BASIC AND APPLIED RESEARCH

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30.1 INTRODUCTION

The pharmaceutical sector has traditionally been a vibrant, innovation driven, and highly successful component of industry at large.[1] In recent years, a confluence of spectacular advances in chemistry, molecular biology, genomic and chemical technology, and the cognate fields of spectroscopy, chromatography, and crystallography has led to the discovery and development of numerous novel therapeutic agents for the treatment of a wide spectrum of diseases. To facilitate this process, there has been a significant and noticeable effort aimed at improving the integration of discovery technologies, chemical outsourcing for route selection/delivery of active pharmaceutical ingredients, drug product formulations, clinical trials, and refined deployment of information technologies. Multidisciplinary and multifunctional teams focusing on lead generation and optimization have replaced the traditional

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specialized research groups. To develop a drug from conception to commercialization, the biotechnology/biopharmaceutical industry (which has been highly entrepreneurial) has reached out and established global strategic partnerships with numerous companies.

The pharmaceutical industry primarily in the United States and Europe has undergone unprecedented changes in recent years due to mergers and acquisitions. The rapidly increasing pace of regulatory reform, allied with the necessity of effecting drastic reductions in the price of bulk drugs, have also resulted in marked shifts in the strategic paradigms in this industry. Numerous corporations are seeking strategic partnerships overseas to enhance their global capabilities for drug discovery and development. Prerequisites such as a highly trained and motivated workforce, political stability, and the formidable process research skills of the chemists make for a winning combination. The recent signing of the GATT and WTO accords has also paved the way for collaboration in the area of new drugs, biotechnology, and agricultural products. As expected, significant strategic outsourcing is on the increase, particularly to India and China.

India, the home of one of the world's oldest civilizations and several of the greatest religions, arouses a mixture of amazement, awe, and fascination among many foreigners. It occupies the seventh-largest landmass in the world; nearly 1 billion people living in its boundaries make it the second-most-populous country. India is a melting pot of a myriad of races, languages, and religions; has the most striking disparities in wealth and poverty; and operates with primitive as well as sophisticated technologies. These dichotomies in the world's largest democracy have lent an exotic flavor to India.[1]

The country was ruled for nearly a millennium by a disparate succession of local and colonial rulers; the economy was largely feudal and agrarian. The growth of science and technology in India in the postindependence (1947–) era has been impressive. The country possesses a well- developed Western-style legal/administrative structure, a proficient and competent civil service administration, and considerable support and enthusiasm among the populace for betterment of life through science. The academic and publicly funded institutions have provided a steady stream of multilingual, mainly English-speaking, educated workers. The scientific labor pool is the second-largest in the world; many of the country's technocrats, scientists, and engineers have been trained in the finest laboratories of Europe and the United States. Several researchers have won international awards and have published and lectured abroad. Chemical research has increased in breadth, sophistication, and finesse. Modern instrumentation is readily available; numerous laboratories, pilot plants, and manufacturing facilities conforming to stringent specifications of good laboratory practice (GLP) and current good manufacturing practice (cGMP) have been established. These facilities have received regulatory approvals from international bodies such as the U.S. Food and Drug Administration (FDA), MCA in the UK, TGA in Australia, MCC in South Africa, and the World Health Organization (WHO). Total quality management programs are in place in the leading professionally managed institutions, with noteworthy improvements in product quality and reliability. The industrial base is therefore strong and technologically sophisticated. The Indian government's economic liberalization program has resulted in abandonment of the stifling protectionism of "Faubian and Nehruvian socialism." Trade barriers have been lowered, taxes cut, and bottlenecks for foreign investment removed. The government of India has relaxed drug price controls and provided fiscal incentives to promote collaboration with the Western world.

The Pharmaceutical Sector in India India is emerging as one of the largest and cheapest producers of therapeutics in the world, accounting in volume for nearly 8.5% of the world's drug requirements. The Indian pharmaceutical sector has achieved global recognition as a low-cost producer of bulk chemicals and formulation products.

In the initial years of independence, the industry was monopolized by a few multinationals. A decade later, the industry showed signs of doing away with multinational dominance with the emergence of Indian companies with a capacity for production of formulations based on imported bulk drugs. The 1970s saw the emergence of bulk drug manufacturing based on imported as well as indigenous technologies. In the 1980s the Indian R&D contributions became significant, and imports of bulk drug technologies reduced drastically. The industry today manufactures practically the entire range of therapeutic groups, is nearly self-sufficient in raw materials, and its level of operation is on par with international standards in production, technology, and quality.

The Council of Scientific and Industrial Research is an umbrella organization for a network of 40 laboratories around the country. The titular head is the Prime Minister of India. On the scientific front, the Director General (CSIR) and Secretary to the Ministry of Science and Technology heads the organization. This organization is gifted with 10,000 highly trained scientists and has rendered yeomen service to the cause of scientific research and training in India. These scientists have provided globally competitive R&D and high-quality science-based technical services. The R&D thrust in the chemical–pharmaceutical sector is focused on the development of new drugs, innovative and indigenous processes for known drugs (with special emphasis on drugs for tropical and other diseases endemic to the country), and development of plant-based drugs through investigation of leads from the traditional systems of ayurvedic medicine. Technologies developed invariably involve indigenous substitutes for expensive imported raw materials, innovative modifications to optimization of conventional process routes, and application of novel techniques for product quality and purity.

The National Chemical Laboratory, Pune and the Indian Institute of Chemical Technology, Hyderabad are the "flagships" of the government of India chemical laboratories and operate under the auspices of the Council for Scientific and Industrial Research.[1] The labs have access to supercomputing facilities and can be used for collaboration in process research and optimization, process engineering, analytical, and other technical services. The R&D strengths encompass world-class expertise for organic synthesis and facilities for isolation and structure elucidation and biological screening. The essential complement of expertise in chemistry and infrastructure facilities allied with strong institutional linkages built up with various universities and the pharmaceutical industry ensures successful scale-up, seamless technology transfer, and implementation of technology.

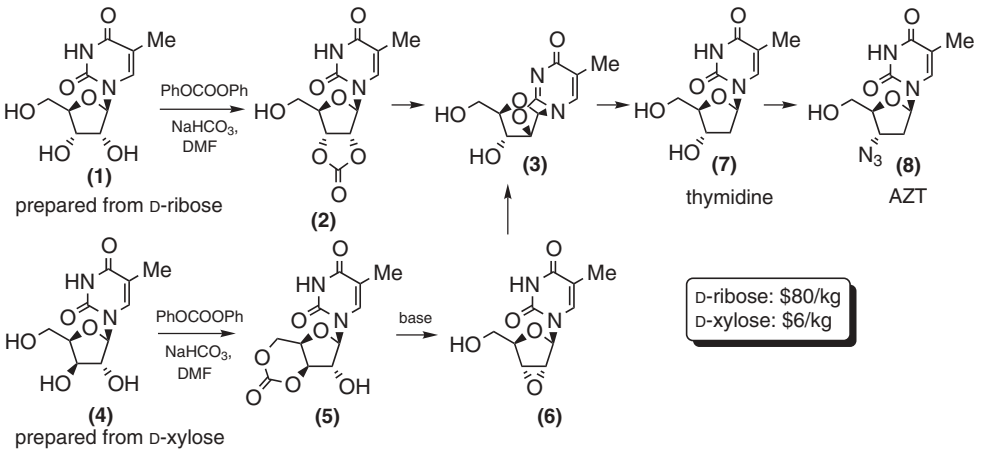
The avenues of cooperation that have been exploited by various multinational pharmaceutical and biopharmaceutical companies are listed as illustrative examples of the enormous benefits that could accrue worldwide.

1. Synthesis of analogs for broad-spectrum and high-throughput screening
2. Lead optimization and analog design
3. Designed organic synthesis, scaffolds, and building blocks for lead generation and development of synthetic methodologies
4. Route selection, process chemistry: preparation of 1 to 5 kg of drug candidates for preclinical and phase I evaluation
5. Technology development and transfer to contract manufacturing of sunset molecules at the end of the patent production period
6. Strategic licensing of compounds discovered in India (several groups have advanced programs in the areas of antiinfective, antihistamine, central nervous system (CNS) drugs, cardiovascular, and natural products–based drug discovery)

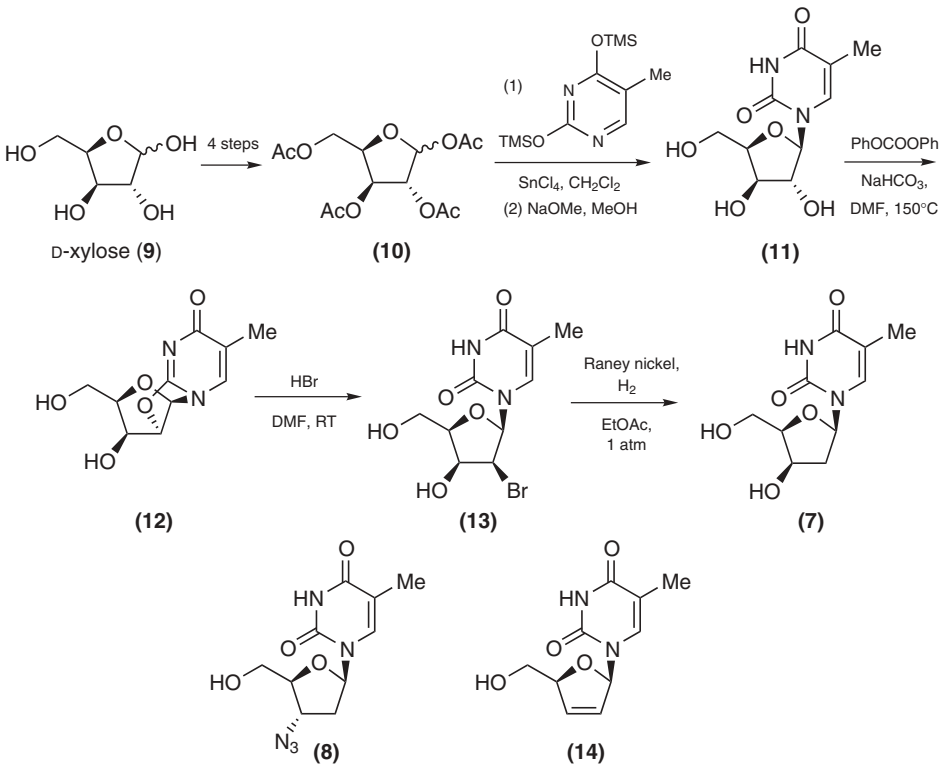
30.2 SOLVING REAL-WORLD PROBLEMS

The scientists' mission to "to advance knowledge and to apply chemical sciences for the good of people" is best exemplified by the following examples, in which world-class expertise in synthetic organic chemistry was applied for solution of real-world problems. The knowledge and wisdom gleaned from basic research programs focused on asymmetric synthesis have been creatively extrapolated and translated on several occasions to applied research work. We have developed processes for several anti-AIDS drugs to increase their affordability for less affluent patients.[1] Drugs such as zidovudine or stavudine are prohibitively expensive for the average consumer. We realized that the high cost of the raw material β-thymidine precludes cost-effective availability of the drug. Manufacturing of β-thymidine (**7**) currently proceeds from naturally occurring but expensive D-ribose. We reasoned that D-xylose (**9**), an inexpensive naturally occurring pentose, could be an ideal precursor for cost-effective production of β-thymidine, despite a lack of stereochemical correlation of ring carbon at C3′ of β-thymidine with that of D-xylose, coupled with deoxy functionality at C2′. We envisaged that deoxygenation at C2 of D-xylose and epimerization at C3 could be induced simultaneously. We had discovered, by serendipity, a new rearrangement in nucleoside chemistry to prepare 2,2′-anhydronucleosides with concomitant epimerization at C3′ (Scheme 30.1). Application of this rearrangement to the synthesis of β-thymidine from D-xylosyl-β-thymine (**11**, Scheme 30.2) paid rich dividends.[2] Successful scale-up at Cipla led to the commercial indigenous and overseas production and manufacture of the popular anti-HIV drug AZT at a much lower cost. Further, successful collaboration with Cipla resulted in cost-effective processes for stamuvidine and lamuvudine, which are part of the "anti-AIDS cocktail." This process was used to deliver AIDS remedies to the populace at an affordable price; several government and international organizations and private foundations are actively involved in this enterprise.

Among the various chiral pools available in nature, carbohydrates have occupied a special niche in the armamentarium of organic chemists: The allure of an abundantly available, enantiomerically pure, and inexpensive material has been irresistible. Our group has developed carbohydrate based approaches for the stereoselective synthesis of several



Scheme 30.1 Key rearrangement in the preparation of thymidine–AZT from D-xylose.

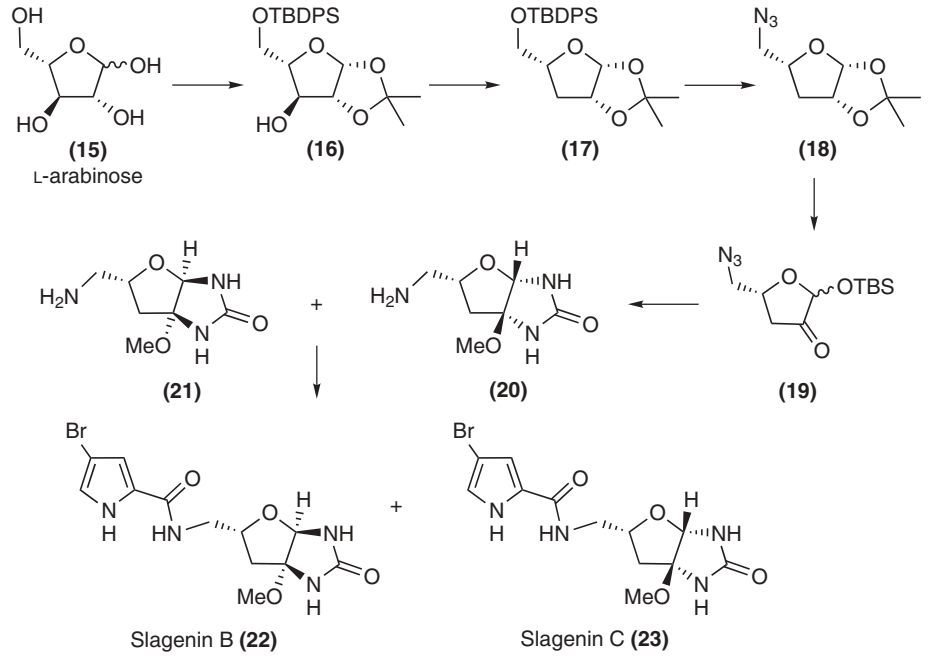


Scheme 30.2 Route for β-thymidine.

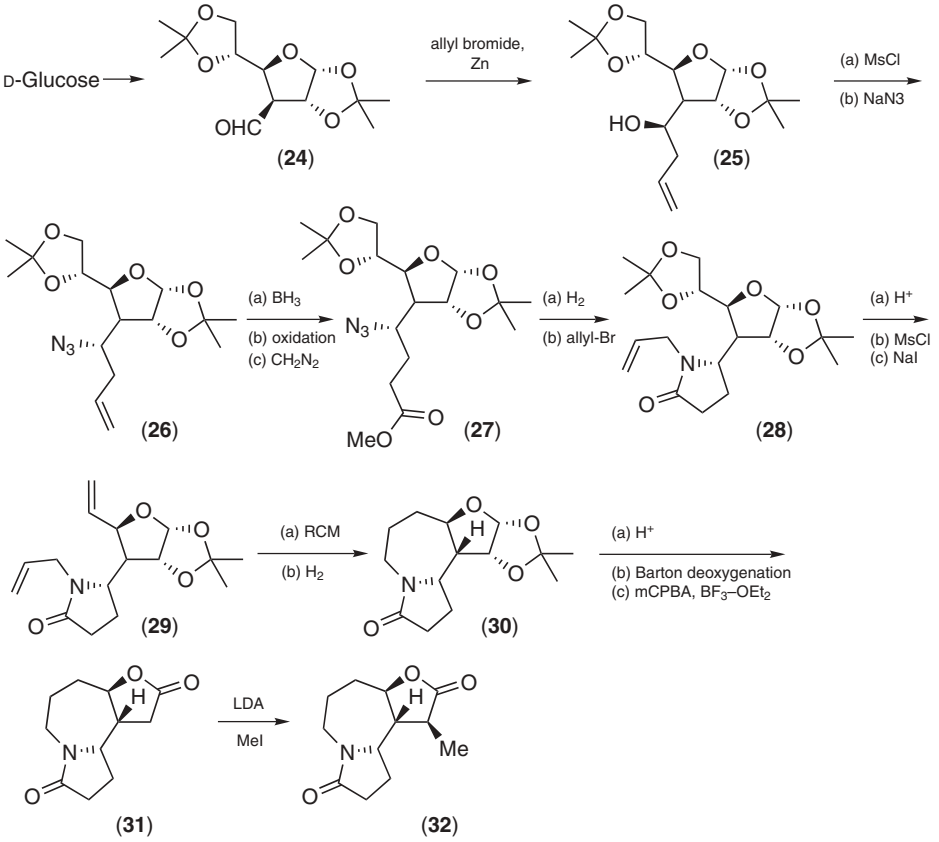
biologically active natural products, such as slagenin B, slagenin C, and microcarpalide. The L-sugar L-arabinose has been exploited as a suitable chiron in the first total synthesis of naturally occurring slagenins B and C (Scheme 30.3).

Asymmetric synthesis of complex biologically active compounds has been a topic of continuous interest in our laboratory, primarily for investigating new synthetic methodologies, designing analogs with better therapeutic properties, and more important, using new chemistry developed above for process research and technologies. Stemoamide (**32**), a member of the Stemona class of alkaloids, was isolated in 1992 from *Stemona tuberosa* by Lin et al.[3] The extracts of this plant species (both Stemona and the closely related Croominaceae species) have long been employed as anthelmintics and as antitussives in traditional folk medicine of China and Japan.[4] Several of these polycyclic alkaloids, because of their powerful insecticidal activity have attracted considerable attention of synthetic chemists, resulting in elegant partial and total syntheses of the targets. We have reported[5] a carbohydrate-based synthesis of stemoamide (**32**) from D-glucose. The basic strategy is founded on the stereocontrolled synthesis of the 2-pyrrolidinone derivative at the C3 position of D-glucose followed by installation of the azepine ring structure using a ring-closing metathesis (RCM)[6] approach (Scheme 30.4).

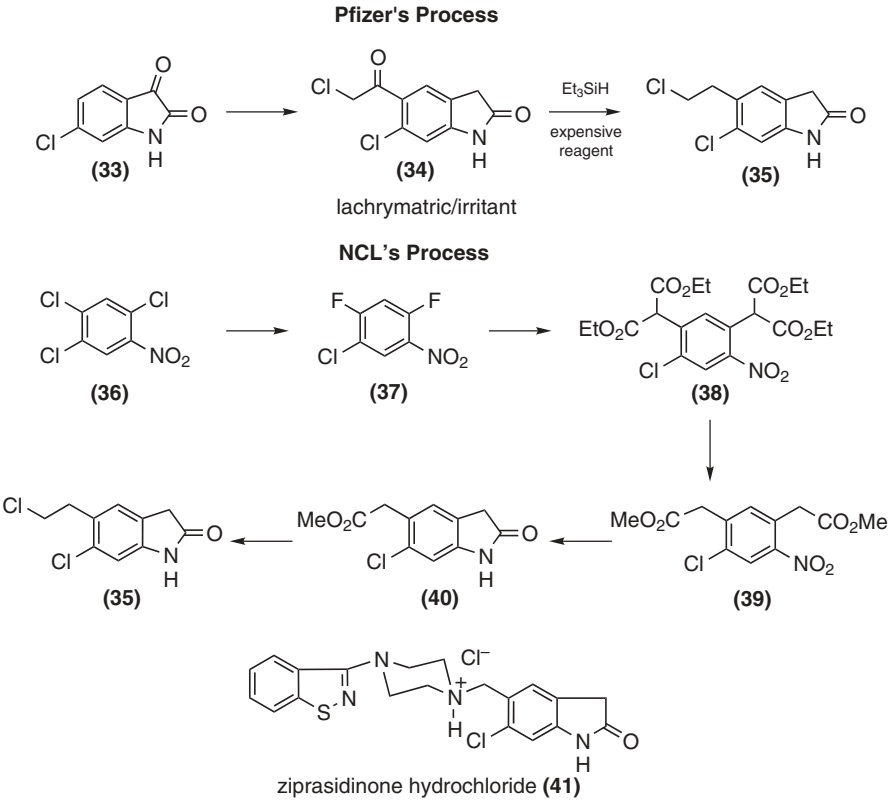
The laboratories are transformed performance-driven organizations funded by the export of knowledge and globally competitive technologies. During the renaissance period of the past decade, intensive efforts have led to process technologies for etoposide, etoposide sulfate, cytarbine, taxotere side chain, neverapine, mefloquin, olanzapine, atorvastatin,



Scheme 30.3 Chiral pool approach for the synthesis of naturally occurring slagenin B and slagenin C.



Scheme 30.4 Enantioselective synthesis of stemoamide.

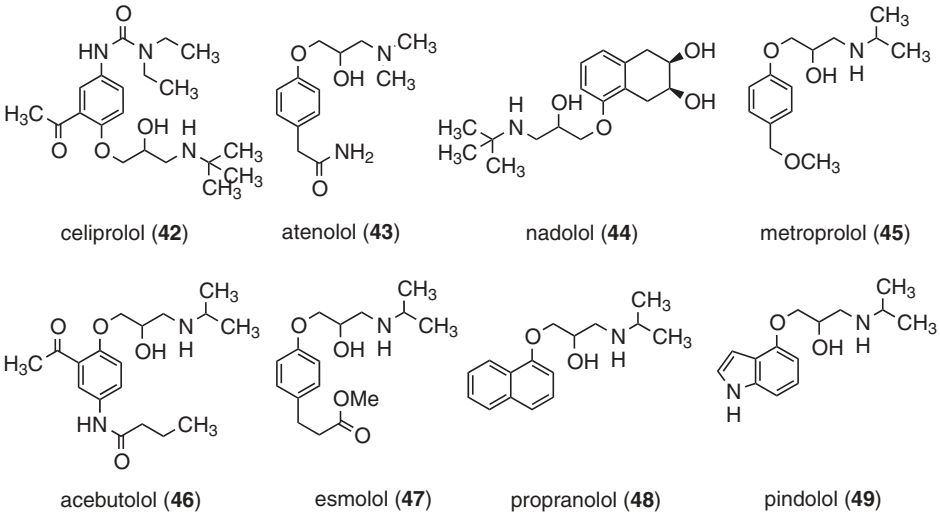


Scheme 30.5 Process for ziprasidinone·HCl.

donapezil, venlafloxacine, cisapride, and azithromycin. The NCL's process for ziprasidone[6] (Scheme 30.5) was a fascinating study wherein comprehensive investigation of nucleophilic substitution on trichloronitrobenzenes resulted in uncovering of a novel didecarboxylation of C2-arylmalonates, thereby generating a cost-effective process for a key oxindole derivative.

30.2.1 Synthesis of β-Blockers

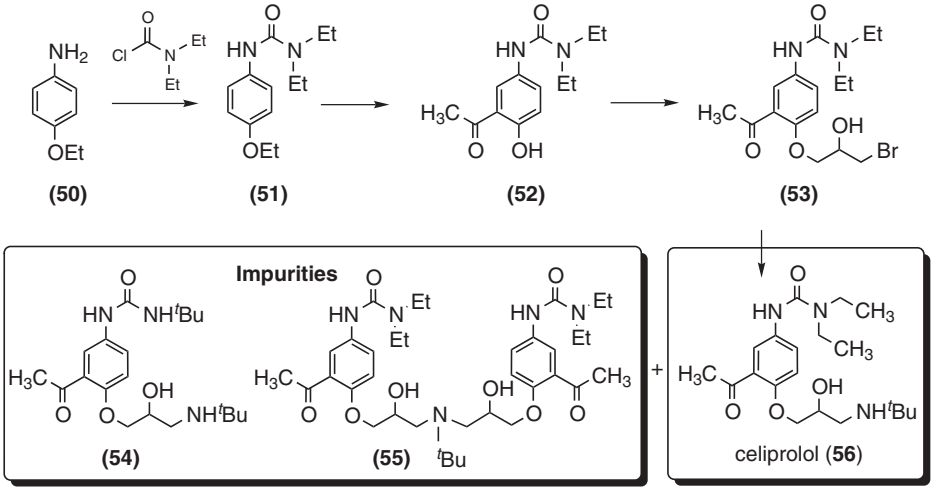
Drugs belonging to the class of aryloxypropanol amines are useful β-blockers. The β-blockers (adrenergic-receptor blocking drugs) comprise a group of drugs prescribed for treating cardiovascular disorders, such as cardiac arrhythmia or ischemic heat disease and hypertension. A large number of β-blockers, such as propranolol, atenolol, metoprolol, nadolol, cervidolol, and celiprolol, are currently marketed (Scheme 30.6). Contributions from our group include asymmetric processes for preparation of several aryloxypropanol amines. Many of these processes, founded on our modification of Jacobsen's hydrolytic kinetic resolution, have been successful in resolving aryl glycidyl ethers. The HKR methodology, initially developed by Eric Jacobsen,[7] has many advantages: (1) water used as medium and reagent, (2) the accessibility of racemic terminal epoxides and racemic aryl glycidyl ethers, and (3) low loadings and recyclability of the commercially available catalyst and the ease of product separation from unreacted epoxide due to large boiling point and polarity differences.



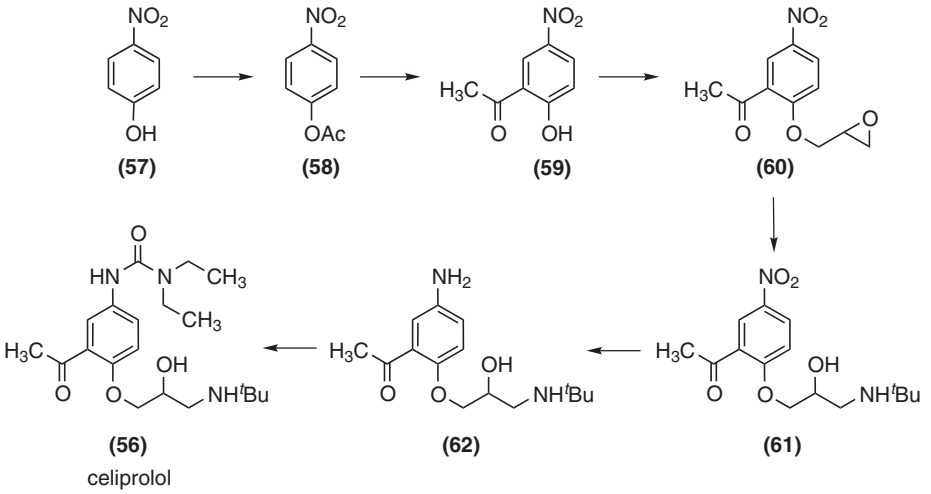
Scheme 30.6 Commercial propanolamine-based β-blockers.

Turnkey Process for Celiprolol Celiprolol has a unique *N*,*N*-diethylurea in its structural framework; we wished to integrate HKR into a process. The existing route starts with 4-ethoxyaniline which was treated with diethylcarbamoyl chloride in the presence of potassium bicarbonate to give *N*-*p*-ethoxyphenylacetamide (Scheme 30.7). Friedel–Crafts acylation with acetyl chloride and anhydrous aluminum chloride followed by acid hydrolysis furnished the acetophenone derivative. Reaction of the urea derivative with epichlorohydrin followed by treatment with hydrobromic acid yielded a bromohydrin. Celiprolol was obtained as a free base by reaction of this bromohydrin with *tert*-butylamine in the presence of triethylamine, and was later converted to its hydrochloride salt.

The introduction of *N*,*N*-diethylurea is carried out at a fairly early stage of the process with an expensive reagent, diethylcarbamoyl chloride (DECC). Being labile, this unit



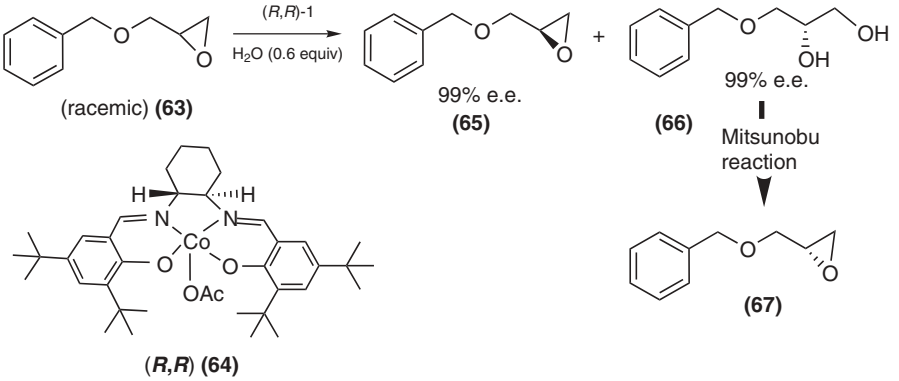
Scheme 30.7 Commercial route for celiprolol hydrochloride.



Scheme 30.8 NCL's route for celiprolol hydrochloride.

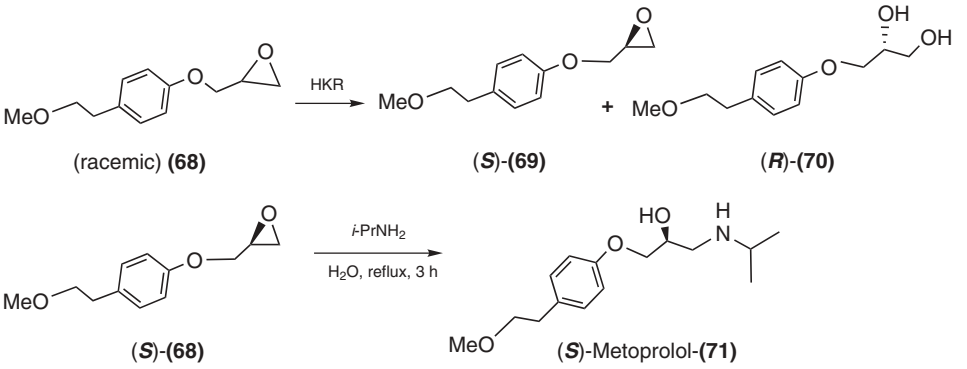
undergoes side reactions, resulting in two by-products that require extensive purification. We wished to circumvent the above-mentioned difficulties; introduction of the *N*,*N*-diethylurea segment as late as possible would be critical to our success. We therefore devised a new approach starting with easily available 4-nitrophenol (Scheme 30.8). An added advantage of the nitro group in the aromatic ring was expected to be the provision of crystalline intermediates, making their purification simple.

Acetylation followed by Fries migration of 4-nitrophenol gave 2-hydroxy-5-nitroacetophenone. This was treated with an excess of epichlorohydrin in the presence of potassium carbonate and a catalytic amount of triethylbenzylammonium chloride to secure the crystalline aryl glycidol derivative (Scheme 30.9). Ring opening of the resulting epoxide A with *tert*-butylamine in water yielded the solid amino alcohol, the nitro group of which was reduced over catalytic Pd/C in methanol. Although two amino groups are present in substrate B, introduction of *N*,*N*-diethylurea moiety using DECC in the presence of Et_3N resulted in exclusive formation of celiprolol base, presumably due to steric hindrance from the *tert*-butyl group. This process led to diminution of costs and did not involve tedious

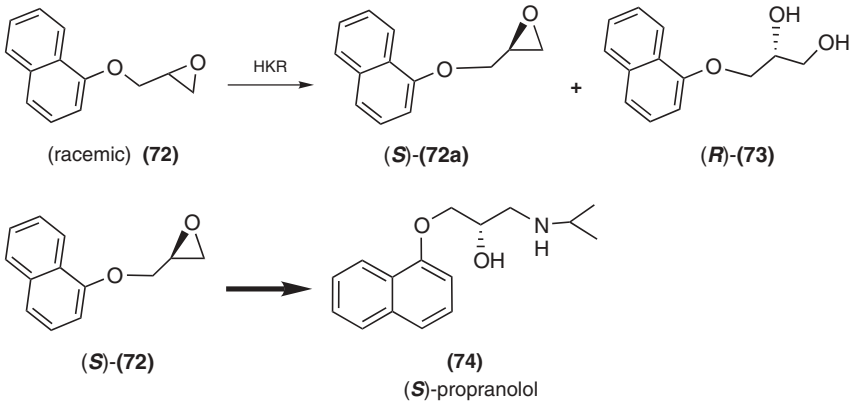


Scheme 30.9 Hydrolytic kinetic resolution of aryl glycidyl ether.

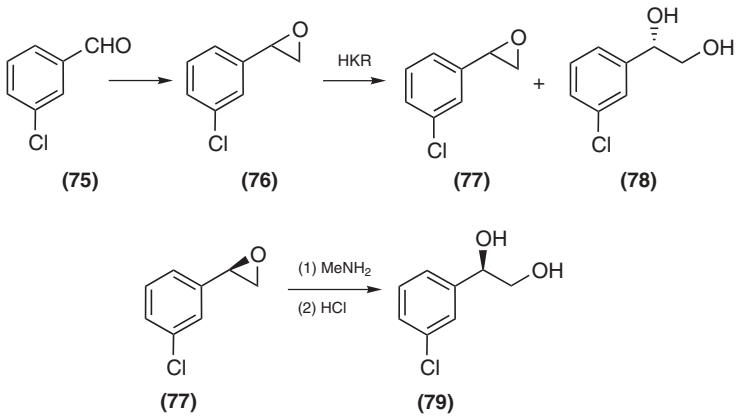
separations. Several intermediates were crystalline and offered multiple purification points for control. We have demonstrated the use of HKR protocol in the synthesis of β-blockers (*S*)-metoprolol[8] (**71**) (Scheme 30.10), (*S*)-propranolol (**74**) (Scheme 30.11), and (*R*)-phenylephrine[9] (**79**) (Scheme 30.12) as representative examples.



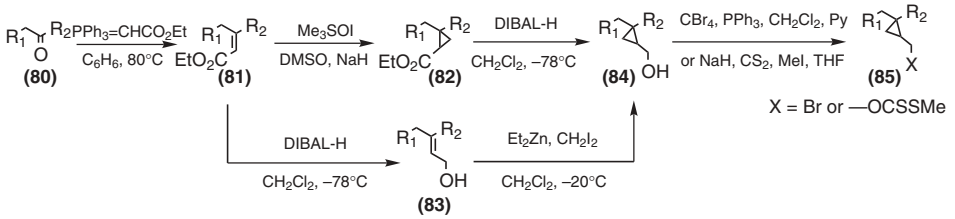
Scheme 30.10 Synthesis of chiral (*S*)-metoprolol.



Scheme 30.11 Synthesis of chiral (*S*)-propranol.



Scheme 30.12 Synthesis of chiral (*R*)-phenylepherine.



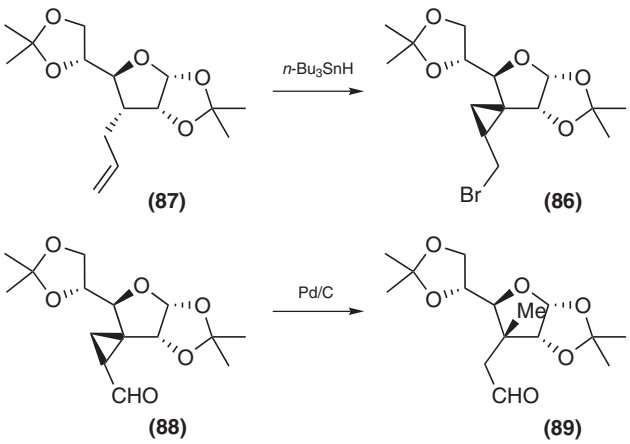
Scheme 30.13

30.2.2 Ring-Closing Metatheses as a Pathway to Chiral Compounds[10]

Interest in developing new protocols that can produce spiro derivatives grew considerably, due primarily to the inherent rigidity displayed by the spiro functionality (Scheme 30.13). Molecules containing a spiro group find innumerable applications, particularly in peptides, nucleosides, and carbohydrates. The synthesis of spiro derivatives was a difficult endeavor until the advent of novel catalysts by Schrock and Grubbs[11] used in ring-closing metathesis (RCM). The RCM-based approaches have made the introduction of a spiro group in a structural framework of an organic molecule an easy proposition.

The synthesis of *gem*-diallyl derivatives can be achieved by double alkylation of active methylene groups. We realized that installation of *gem*-diallyl functionality on a carbon atom, not activated by any electron-withdrawing group, is a difficult proposition. The problem becomes insurmountable on carbohydrate precursors because base-catalyzed reactions lead to tandem elimination of water molecules, resulting in the formation of complex mixtures. We observed interesting reactions with carbohydrate cyclopropyl precursors. For example, the radical-mediated cyclopropyl scission of the spirocyclopropyl bromide (**86**) with *n*-Bu_3SnH gave the C-allyl derivative (**87**) in a stereo-controlled fashion. On the other hand, hydrogenation of the cyclopropylaldehyde derivative (**88**) over Pd/C provided **89** with a quaternary chiral center (Scheme 30.14).

We were delighted by the radical ring-opening reaction described above and envisaged that quenching of in situ–generated homoallyl radical (Fig. 30.1) with allyl tri-*n*-butyltin should lead to the formation of *gem*-diallyl derivative.[10]



Scheme 30.14

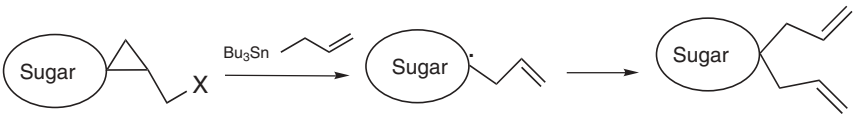
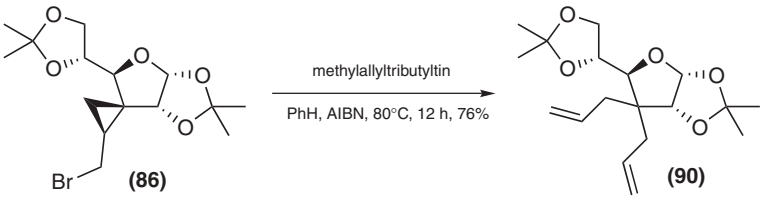


Figure 30.1 Regiospecific diallylation of carbohydrate scaffolds.



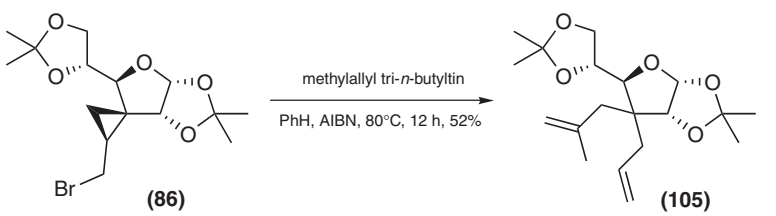
Scheme 30.15

The requisite precursor (**1**) was treated with allyl tri-*n*-butyltin and catalytic 2,2′-azokisisobutyronitrile (AIBN) to give the *gem*-diallyl derivative **90** in 76% yield (Scheme 30.15).

To establish the versatility of this reaction, a number of spirocyclopropylmethyl bromides were prepared[6] (Table 30.1), according to the general strategy depicted in Fig. 30.1. The carbonyl derivative was subjected to the Wittig reaction with $Ph_3P{=}CHCO_2Et$ in refluxing benzene. For entries 1 to 3, the resulting unsaturated ester was cyclopropanated with $Me_3S(O)I$–NaH in DMSO and then reduced with DIBAL-H in CH_2Cl_2 at −78°C to afford the cyclopropylmethanol derivatives. For entry 4, the unsaturated ester was first reduced with DIBAL-H in CH_2Cl_2 at −78°C to provide an allylic alcohol which was subsequently cyclopropanated (entry 5 as well) with a modified Simmons–Smith reaction using Et_2Zn–CH_2I_2 in CH_2Cl_2 at −20°C. Conversion of the cyclopropylmethanol to the corresponding cyclopropylmethyl bromide was accomplished with CBr_4–Ph_3P–pyridine in CH_2Cl_2.

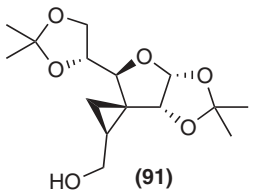
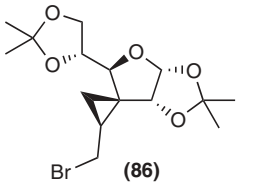
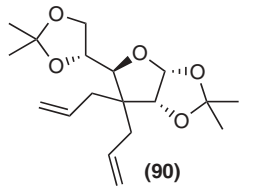
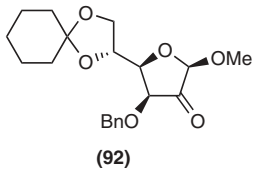
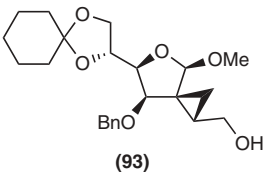
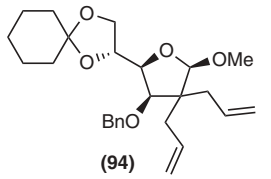
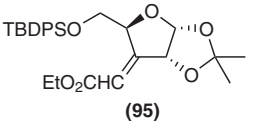
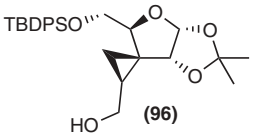
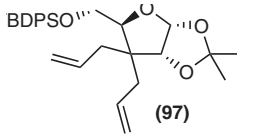
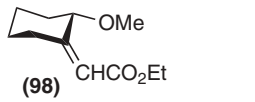
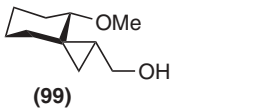
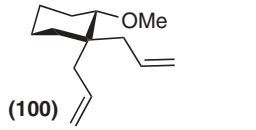
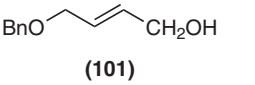
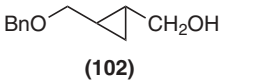
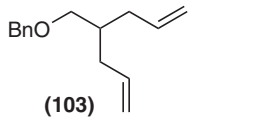
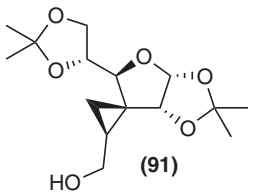
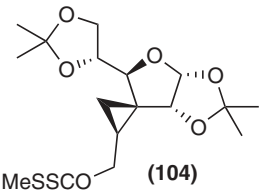
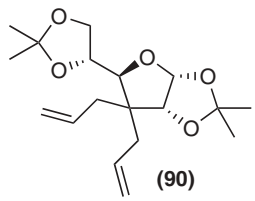
The preparation of the xanthate derivative (entry 6) from cyclopropylmethanol was accomplished with a well-defined protocol using CS_2–NaH–MeI in THF. The bromo/xanthate derivatives (entries 1 to 6) were treated with allyl tri-*n*-butyltin–AIBN to provide the *gem*-diallyl product in good yields. Application of this concept to install two different allylic functionalities on the same carbon was also explored when methylallyl tri-*n*-butyltin was treated with **86** to give rise to 3-deoxy-3-β-allyl-3-β-methallyl derivative (**105**) (Scheme 30.16). The absolute stereochemistry of **105** was established by NOE studies.

Our next task was to perform ring-closing metathesis reactions to secure novel spirocyclopentyl derivatives. Compound **90** was treated with Grubbs' catalyst[11] in CH_2Cl_2 at



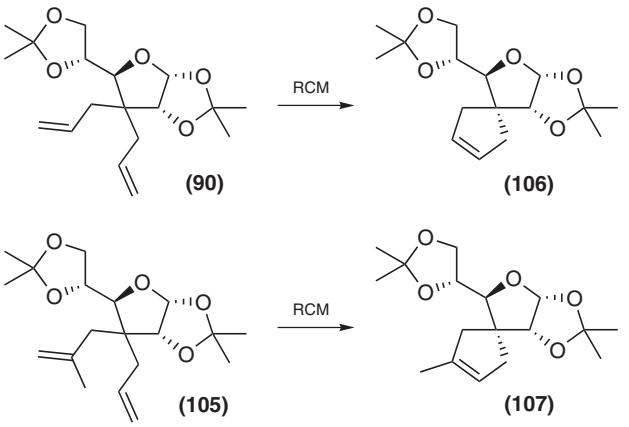
Scheme 30.16

TABLE 30.1 Spirocyclopropylmethyl Bromides

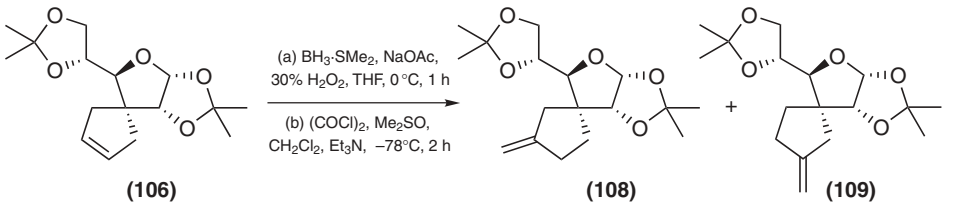
Entry	Substrate	Product	Yield (%)
1	 **(91)**	 **(86)**	 **(90)**	76
2	 **(92)**	 **(93)**	 **(94)**	44
3	 **(95)**	 **(96)**	 **(97)**	81
4	 **(98)**	 **(99)**	 **(100)**	60
5	 **(101)**	 **(102)**	 **(103)**	53
6	 **(91)**	 **(104)**	 **(90)**	62

room temperature to provide the spirocyclopentenyl derivative **106**. Interestingly the RCM reaction of **105** gave **107** in 75% yield (Scheme 30.17).

Adoption of an iterative approach of these two strategies helped us prepare some novel bis-spirocyclopentyl derivatives of carbohydrates. For this endeavor, compound **106** was subjected to a hydroboration–oxidation reaction, which led to the formation of a complex mixture of diastereomers (Scheme 30.18). The mixture was oxidized under Swern oxidation conditions to give two products (**108** and **109**), separated by silica gel chromatography. The correct assignment of structures **108** and **109** was achieved by NOE experiments.



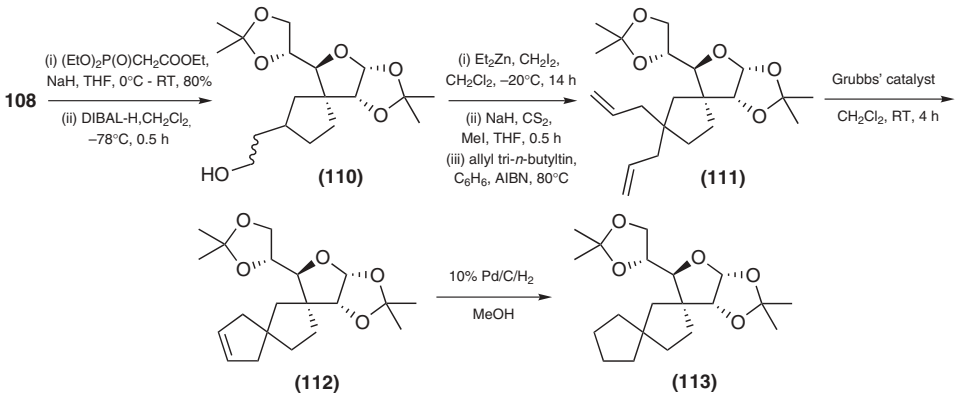
Scheme 30.17



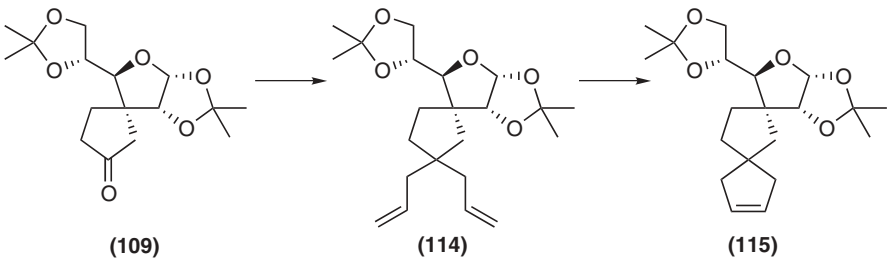
Scheme 30.18

Compound **108** was subjected to the following sequence of reactions: Wittig olefination with $(EtO)_2P(O)CH_2CO_2Et$, reduction of an ester group with DIBAL-H to obtain **111**, Simmons–Smith cyclopropanation, and the corresponding xanthate preparation with NaH–CS_2–MeI in THF (Scheme 30.19).

Radical-induced diallylation of xanthate gave **111**; RCM reaction and catalytic hydrogenation produced the bis-spiro derivative (**113**). Similarly the other isomer



Scheme 30.19



(109) (114) (115)

Scheme 30.20

(**24**) was also converted to the diallyl derivative (**114**) and the bis-spiro derivative **115** (Scheme 30.20).

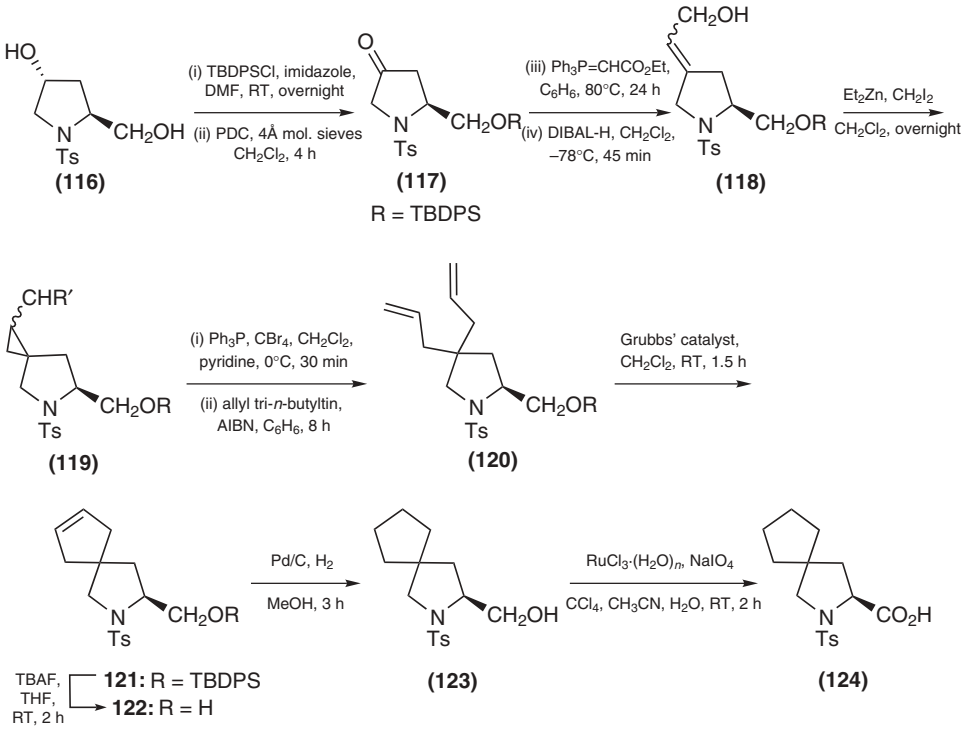
Spiroproline derivatives are of interest in biological studies as an inhibitor and mechanistic probe of prolyl-4-hydroxylase. To develop potent inhibitors of angiotensin-converting enzyme (ACE), lipophilic and sterically hindered spiroproline derivatives have been substituted in the structural framework of peptides. In addition, spiroprolines could offer interesting scaffold precursors, particularly in synthesis of combinatorial libraries of peptides. Earlier synthetic efforts aimed at spiroproline analogs involved building the proline nucleus on the structural backbone of the alicyclic system followed by resolution. We decided to try to expand the potential of our synthesis of spirocyclic compounds to proline derivatives, with the hitherto unknown 2-aza-spiro[4.4]-nonane carboxylic acid (**124**) derivative as the target.

The known *trans*-*N*-(*p*-toluenesulfonyl)-4-hydroxy-L-prolinol (**116**) was converted into 4,4′-cyclopentyl-L-proline derivative by essentially following the route already discussed and shown in Scheme 30.21. The purity of the final product was determined by chiral HPLC (95%).

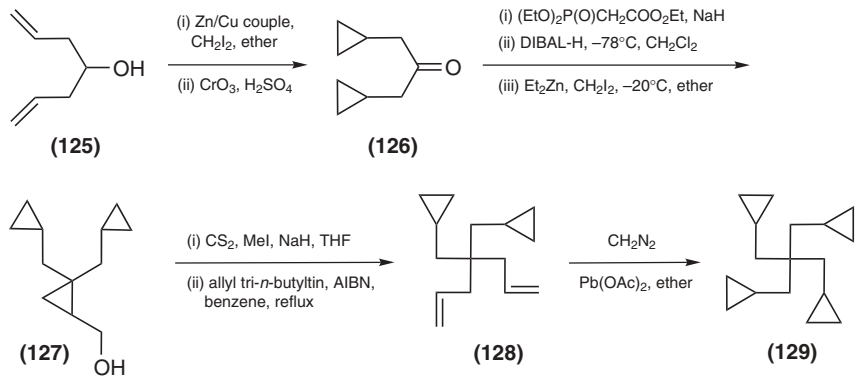
The art and science of organic synthesis encompasses an enormous range of activities in which synthesis of the designed symmetric molecules is an aesthetically pleasing endeavor. We have synthesized (Scheme 30.22), a structurally unique and symmetric, nonnatural product, tetrakis(cyclopropylmethyl)methane (**129**) and studied its conformation by single-crystal x-ray as well as by low-temperature NMR spectroscopy.

30.3 SYNTHESIS OF PHARMACEUTICALLY RELEVANT CHIRAL TETRAHYDROFURANS

Asthma is a chronic inflammatory disease complicated by periodic acute inflammatory changes. Arachidonic acid and certain closely related polyunsaturated fatty acids are well-known precursors for a multitude of biologically active substances, such as prostaglandins, thromboxanes, and leukotrienes, that are all formed by oxygenation and further transformation of these fatty acids. Prostaglandins and thromboxanes have long been associated with inflammatory conditions; leukotrienes have evoked special interest because their biological potency often greatly exceeds that of prostaglandins and thromboxanes. The role of leukotrienes, the metabolites of arachidonic acid that are produced by action of the 5-lipoxygenase (5-LO) enzyme, in inflammatory and allergic responses, including arthritis, asthma, psoriasis, and thrombotic diseases, has been well recognized.[12–14]

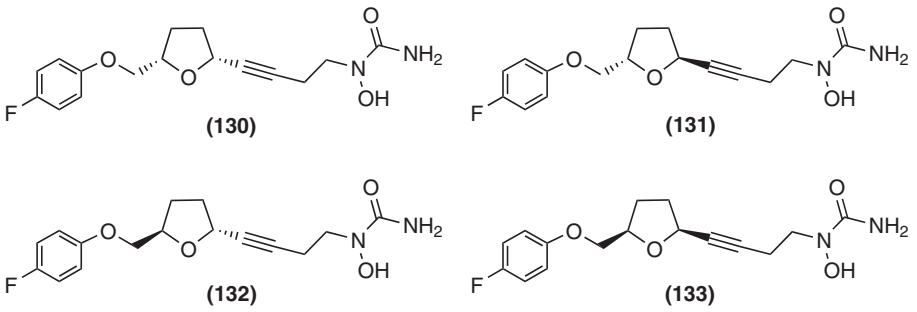


Scheme 30.21



Scheme 30.22 Synthesis of tetrakis(cyclopropylmethyl)methane.

The 5-LO enzyme acts on arachidonic acid, leading to the formation of leukotriene B4 (LTB4) and the cysteinyl leukotrienes (LTC4, LTD4, and LTE4). The physiological effects of leukotrienes mimic the recognized pathophysiological features of asthma, including bronchoconstriction, increased airway responsiveness, increased microvascular permeability, and hypersecretion of mucus. SRS-A (slow-reacting substance of anaphylaxis) has long been implicated as a mediator of airway anaphylaxis mainly because it is a



Scheme 30.23

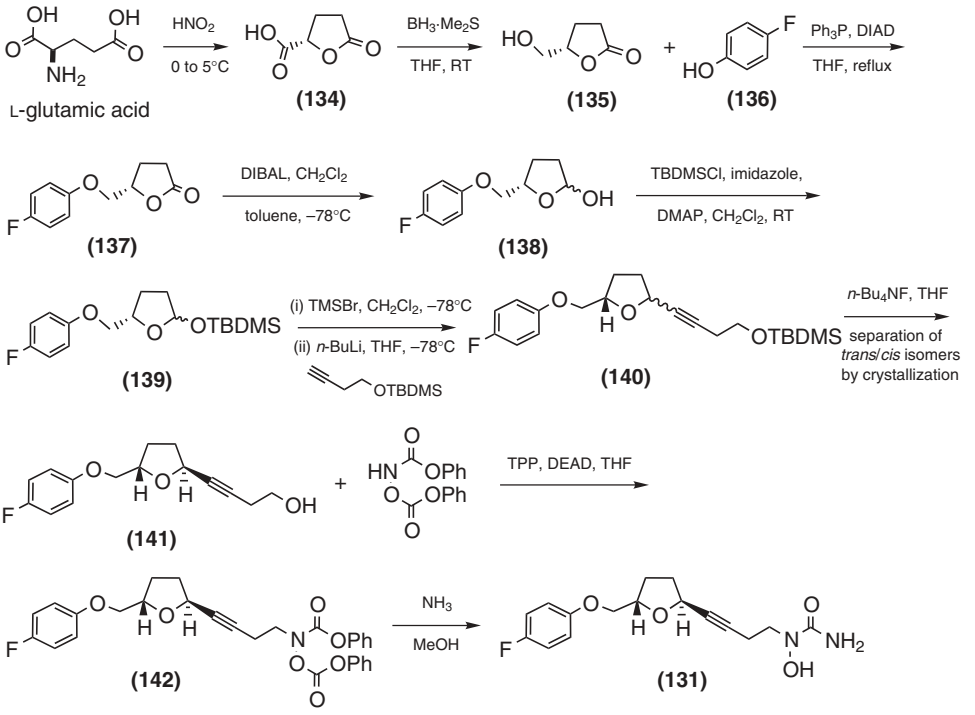
bronchoconstrictor and is released in conjunction with antigen–antibody reactions in the lung. The discovery that SRS-A is a mixture of three cysteinyl-containing leukotrienes C4, D4, and E4 has generated immense interest and stimulated efforts to clarify the possible role of these substances in pulmonary hypersensitivity reactions. The enzyme 5-lipoxygenase is the first specific enzyme in the arachidonic acid cascade leading to the biosynthesis of leukotrienes. A selective inhibitor of the 5-lipoxygenase pathway would block both the release of leukotrienes and the bronchial contractions that ensue from allergen challenge of lungs in asthmatic patients.[12–14]

Chiral tetrahydrofurans are integral structural features of a variety of natural products.[14] The chiral tetrahydrofuran (**131**) was explored as a potent 5-lipoxygenase (5-LO) inhibitor that blocks production of leukotrienes and with several other compounds has been investigated for the prophylactic treatment of chronic asthma.[15,16] It showed a high degree of potency and revealed an exceptionally favorable safety profile in extensive animal studies. It broadly blocked the production of leukotrienes while significantly alleviating both bronchoconstriction and bronchial inflammation. The compound exhibited high oral bioavailability as well as a pharmacodynamic profile suitable for an attractive therapeutic agent for chronic asthma.

Inhibition of LTB_4 production in ionophore-stimulated human whole blood was employed in the evaluation of the isomers of **131**. All these belong to the lignan family of 2,5-disubstituted tetrahydrofurans, featured with diverse substitution and *trans*-juxtaposition of ring substituents. Of the four possible isomers **130** to **133**, the (*S*,*S*)-isomer (**131**) was found to have the best potency and was selected for further development. Compound **131** is a single enantiomer with an all-*trans* configuration. Compounds **130** and **131** and their stereoisomers (**132** and **133**, Scheme 30.23) have also been investigated for their inhibitory action against 5-lipoxygenase.[15,16] Compound **131** was found to be a potent and orally active leukotriene modulator that works by inhibiting the action of 5-lipoxygenase (5-LO) to block the generation of cysteinyl leukotrienes and LTB_4. Further studies on **132** showed a high degree of potency, excellent oral bioavailability, and exceptionally favorable safety profile compared to **131**.[15,16]

30.3.1 Discovery Route

The discovery route selected for compound **131** is outlined in Scheme 30.24.[17] Commercially available (*S*)-(+)-hydroxymethyl-γ-butyrolactone (**135**) was chosen as the chiral synthon. Diisopropylazodicarboxylate/triphenyphosphine-mediated Mitsunobu coupling



Scheme 30.24 Discovery route for 131.

with 4-fluorophenol (**136**) yielded chiral lactone (**137**). This material revealed a propensity to unravel to the open-chain derivative; dissolution of the material in toluene followed by treatment with pyridinium *p*-toluenesulfonate and chromatography furnished the requisite lactone (**7**) as a white crystalline solid.

Reduction of the lactone **137** to the corresponding lactol **138** necessitated use of DIBAL; quenching the reaction with methanol followed by workup with aqueous potassium sodium tartarate furnished the product as a clear viscous oil that solidified on standing to a white solid. The anomeric hydroxyl group was conveniently protected by the formation of *tert*-butyldimethylsilyl ether by treatment with *tert*-butyldimethylsilyl chloride, imidazole, and *N*,*N*-dimethylaminopyridine. Further activation of the anomeric position in compound **139** with trimethylsilyl bromide followed by treatment with 1-*t*-butyldimethysiloxy-3-butyne and *n*-butyllithium yielded a *trans*/*cis* mixture (1 : 1) (**140**) that was used without further purification. Reaction of compound **140** with tetrabutylammonium fluoride led to deprotection of the hydroxy functionality. The resulting *trans*/*cis* mixture of the alkynols was subjected to extensive chromatography and repeated crystallization to obtain a *trans*-alcohol (**141**) as a white crystalline solid. Further elaboration to **131** was carried out by appropriate modifications of a literature procedure.[17]

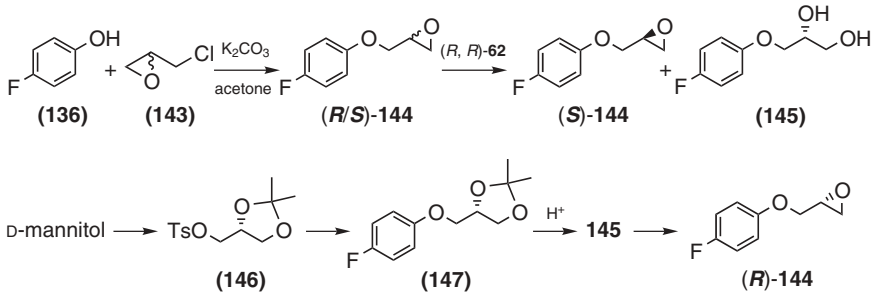
The discovery route was fraught with several tandem difficulties, such as expensive and difficult-to-source reagents, elaborate protection–deprotection sequences, many cryogenic and capricious reactions, poor diastereoselectivity, and unfavorable waste

streams.[17] Many reactions were conducted at −78°C. Silyl-protecting groups were used in numerous instances; the reagents were expensive and unstable for extended storage/shipping and handling. Deprotection of the silyl-protecting groups involved the use of expensive TBAF. The atom economy in the protection–deprotection sequence was not in the direction desired and led to a large waste stream that was difficult to handle. The chiral lactone starting material was single sourced; an old literature procedure used to prepare it was inefficient. Coupling of 4-fluorophenol to the lactone proceeded via a Mitsunobu reaction[18] that used toxic reagents such as DIAD. Separation of the triphenylphophineoxide side products involved laborious chromatography. Subsequently, during C—C bond formation using *n*-BuLi, a 1 : 1 mixture of *trans*/*cis* isomers was obtained whose separation to furnish *trans* material involved tedious chromatography and presented difficulty. These problems would mitigate against efficient scale-up and cost-effective production of the target molecule and had to be eliminated or ameliorated to develop a cost-effective process for the target molecule.

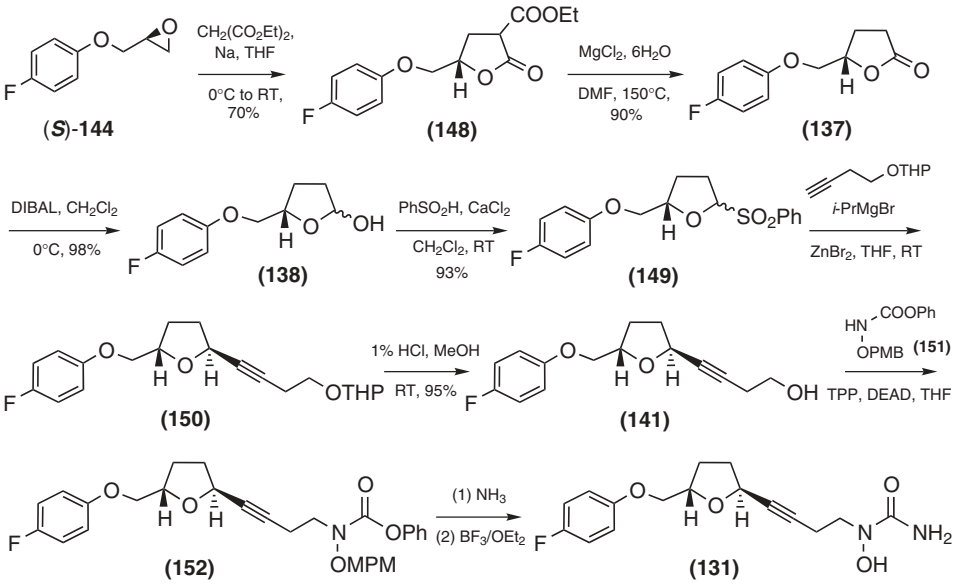
The interesting characteristic stereochemical features of the molecule coupled with the inefficiency of the existing synthetic protocols prompted us to undertake route selection. An ideal synthesis would encompass the facility and felicity of reaction conditions and ready accessibility of raw materials; allied with high and reproducible yields in all steps of the synthetic sequences.

We therefore devised three diverse routes to the target molecule that obviate the problems cited above.[19] The first route afforded the following advantages: (1) *p*-fluorophenol and glycidyl tosylate are inexpensive, commercially available starting materials the sequence leading to the arylated lactone was smooth and proceeded in high yield; (2) reaction with phenylsulfinic acid followed by a Grignard reaction yielded a *trans*/*cis* alcohol ratio of 70 : 30; (3) all cryogenic reactions were eradicated and all the silyl-protecting groups were replaced; and (4) facile introduction of sulfur and nitrogen in place of the oxygen of the five-membered ring led to new analogs for pharmacological and preclinical evaluation.

The relevant starting material, the (*R*)- and (*S*)-4-fluorophenylglycidyl ethers (***R***)-**144** and (***S***)-**144**, were prepared by a four-step procedure (Scheme 30.25). O-Alkylation of *p*-fluorophenol with (±)-epichlorohydrin (**143**) in anhydrous acetone in the presence of K_2CO_3 as base provided the ether (**144**) in nearly quantitative yield as a colorless liquid. Hydrolytic kinetic resolution, according to our modification of Jacobsen's protocol[7] with (*R*,*R*)Salen and Co(III)OAc catalyst and 0.5 mol of water, afforded the (***S***)-**144** (45%,



Scheme 30.25 HKR approach to key glycidyl ethers (***R***)-**144** and (***S***)-**144**.



Scheme 30.26 Route I for synthesis of **131**.

92% e.e.) and the diol (**145**) (46%, 97% e.e.) after column chromatography. The diol was converted to the corresponding (***R***)-**144** following a one-step procedure developed by us.

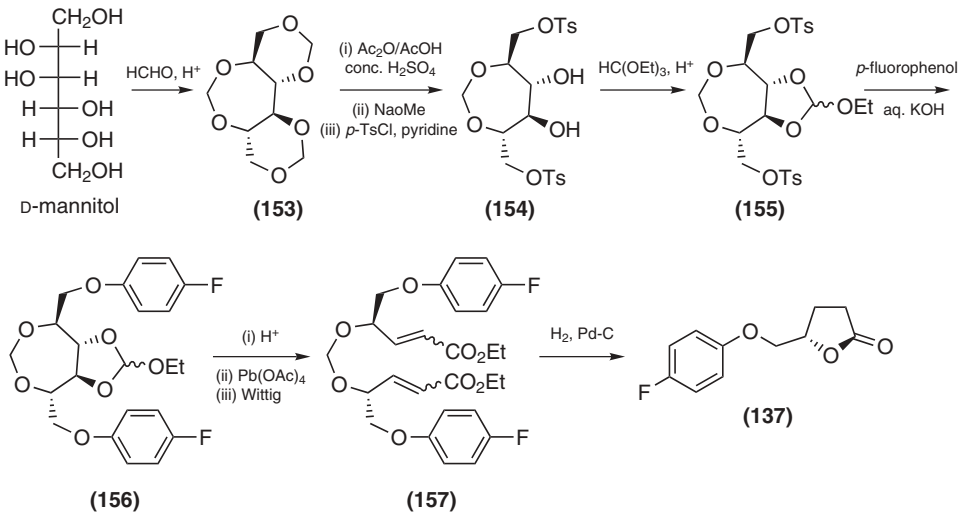
Route I The pivotal intermediate was defined to be the lactone (**7**) on which the key C—C bond-forming reactions could be explored as outlined in Scheme 30.26. (***S***)-Glycidyl ether [(***S***)-**144**] was chosen as the chiral precursor because of inherent stereochemical coherence within the C2 chiral center of **131**. The sodium salt of diethylmalonate on treatment with (***S***)-**144** furnished the corresponding 2-carboethoxy-γ-butyrolactone (**148**) in 70% yield. This α-esterlactone was conveniently decarboxylated under modified Krapcho conditions to afford lactone (**137**) in excellent yield. On reduction with DIBAL-H, lactone **137** provided lactol (**138**) in almost quantitative yield. Following a methodology pioneered by Ley,[20] treatment of lactol (**138**) with benzenesulfinic acid and calcium chloride in dichloromethane provided the sulfone (**149**) in 93% yield. Treatment of the sulfone (**139**) with Grignard reagent in the presence of zinc bromide in anhydrous THF provided **150** as a diastereomeric mixture which on THP deprotection gave a mixture of alkynols with a *trans*/*cis* ratio of 70 : 30 (HPLC analysis) (compared to the 50 : 50 mixtures obtained earlier). Crystallization (twice) of the crude product from ether–light petroleum yielded the pure *trans*-alcohol (**141**) in 35% yield with HPLC purity above 95%. The melting point, optical rotation, and spectral data were in excellent accord with an authentic sample.

We next focused our efforts on introduction of the hydroxyureidyl moiety by a patent noninfringing process. We tried two different protecting groups of N and O in hydroxylamine in order to have a convenient appendage for selective manipulation at N and O, to generate substituted hydroxyureidyl substitutes for future studies. *O*-4-Methoxybenzylhydroxylamine hydrochloride, prepared from *N*-hydroxyphthalimide in

a two-step sequence, was treated with phenylchloroformate–triethylamine in dichloromethane to provide diprotected hydroxylamine **151** in 50% yield. The Mitsunobu reaction of protected hydroxylamine (**151**) with the *trans*-alcohol (**141**) in the presence of triphenylphosphine–diethylazodicarboxylate in anhydrous THF provided the alkynyl urethane (**152**) in 98% yield. Treatment of **152** with methanolic ammonia followed by treatment of the crude urea with freshly distilled $BF_3{\cdot}Et_2O$ provided **131**. Charcoal treatment and recrystallization from ethyl acetate–light petroleum furnished pure **131** in 62% yield.

Route II Carbohydrates are endowed with a number of chiral centers and are available in various chain lengths. D-Mannitol is an inexpensive commodity chemical endowed with a C2 axis of symmetry. A synthesis of the key intermediate γ-butyrolactone (**7**) from D-mannitol was envisaged in which chiral centers at C2 and C5 are correlated with C2 of the lactone intermediate (Scheme 30.27). This would, in effect, provide two molecules of the lactone from one molecule of D-mannitol.

The first step involved the known conversion of D-mannitol into 1,3; 2,5; and 4,6-tri-*O*-methylene-D-mannitol (**153**) using formalin and concentrated HCl in 92% yield. The selective hydrolysis of the 1,3 and 4,6 acetal functionalities was achieved by treating trimethylene derivative (**153**) with Ac_2O/AcOH/concentrated. H_2SO_4. Under the preferred protocol, acetolysis occurred to furnish the tetraacetate, which under Zemplen deacetylation conditions (NaOMe/MeOH) rendered crystalline 2,5-*O*-methylene-D-mannitol in an overall yield of 51%. At this stage, arylation that would differentiate primary C1 and C6 hydroxyl groups was planned. Toward this end, the tetraol was converted into its 1,6-ditosylate (**154**) using 2.2 equiv of tosyl chloride in pyridine at ambient temperature. Treatment of **154** with triethylorthoformate in the presence of catalytic PTSA gave an orthoester (**155**) in quantitative yield. S_N2 displacement of tosyl groups with *p*-fluorophenol/aqueous KOH in refluxing acetonitrile afforded di-4-fluorophenoxy compound (**156**). Deprotection of the orthoester using 1% aqueous HCl in THF provided the diol that was subjected to oxidative



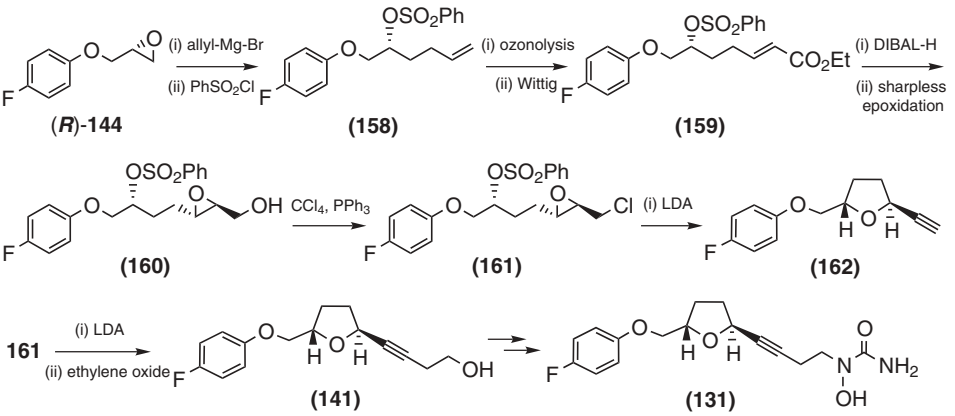
Scheme 30.27 Route II, a chiral-pool approach for synthesis of lactone (**137**).

cleavage with lead tetraacetate in dichloromethane to give a dialdehyde which was subjected immediately to Wittig olefination using carboethoxymethylene–triphenylphosphorane in dichloromethane to provide the (*Z*,*E*) unsaturated ester (**157**) in 74% yield after chromatography. Unsaturated ester (**157**) hydrogenated in the presence of Pd-C at 40 to 50 psi followed by treatment with 10% H_2SO_4 in refluxing ethanol cleaved the acetal; concomitant intramolecular cyclization furnished (4*S*)-(4-fluorophenoxymethyl)-γ-butyrolactone (**137**) in 87% yield after chromatography. The melting point, optical rotation, ^{1}H NMR, and mass spectral data were in accordance with the authentic sample and the sample obtained via route I.

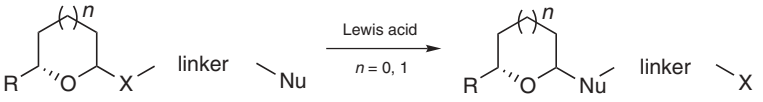
Route III To circumvent the lack of diastereoselectivity associated with routes I and II, we formulated an alternative strategy wherein the chiral centers at C2 and C5 would be generated simultaneously followed by in situ cyclization leading to the tetrahydrofuran ring (Scheme 30.28). This would expand the range of analogs and also facilitate preparation of radiolabeled materials.

Cu(I)-catalyzed regioselective opening of epoxide (***R***)-**144** with allyl magnesium bromide furnished the alcohol with a terminal double bond: this alcohol was then protected as its benzene sulfonate ester (**158**). Reductive ozonolysis of the terminal double bond in **158** furnished aldehyde, which was then elaborated by reaction with stable Wittig ylide to provide the corresponding α,β-unsaturated ester (**159**). The predominant (*E*)-olefinic ester, obtained after chromatographic removal of minor (*Z*)-isomer on reduction with DIBAL-H followed by Sharpless asymmetric epoxidation with (+)-diisopropyltartarate/titanium tetraisopropoxide/cumene–hydroperoxide yielded an epoxy alcohol (**160**) in 98% yield. Alcohol (**160**) was converted into the corresponding epoxy chloride (**161**) with triphenylphosphine/carbon tetrachloride/catalyzed $NaHCO_3$; additional formation of ring-opened products in substantial ratio was discernible. The formation of side products was subdued considerably by addition of $CHCl_3$ to enable and maintain the solubility of starting material in reaction medium: the product was now obtained in gratifying 64% yield.

The key transformation[21] was next effected by exposure of **161** to 3 equiv of LDA,[19] which resulted in the generation of propargyl alkoxide through double elimination



Scheme 30.28 Route III, a double-elimination and intramolecular S_N2 approach for synthesis of lactone (**137**).



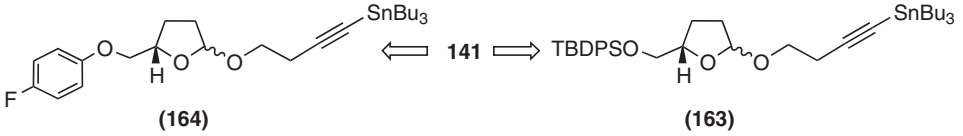
Scheme 30.29 Requirements for the anomeric O—C rearrangement.

followed by concurrent intramolecular S_N2 cyclization to provide the THF–acetylene derivative (**162**). This central transformation expediently formed the main skeleton in one pot with appropriate substitution and crucial stereochemistry in accordance with the envisaged strategy. Further homologation to the homopropargyl alcohol (**141**) was achieved by in situ generation of lithium acetylide with *n*-BuLi in THF and treatment with ethylene oxide under Lewis acid (BF_3:OEt_2) catalysis, in 90% yield as a solid. The poor NOE enhancement observed during the double irradiation of protons at C2 and C5 suggested that these protons are not in close proximity (i.e., the relative stereochemistry of junction is *trans*).

The salient features of this route are double elimination of epoxy chloride[21] with LDA with concomitant cyclization to a tetrahydrofuran ring. These stepwise operations occurring sequentially in one pot were indeed the most pleasing aspect of this work for us. The versatility of route III has been demonstrated by its successful extension to six-membered and higher homologs by essentially manipulating the Grignard reagent attacking the epoxide (***R***)-**144**. We have successfully prepared the six- and seven-membered ring analogs. It is worthy to mention that this route also led to other isomers of **141** by appropriate juggling with the catalyst during Jacobsen's kinetic resolution and later during Sharpless asymmetric epoxidation.

In collaboration with Steven Ley,[22] we developed an alternative synthesis at Cambridge, UK, that capitalized on an oxygen-to-carbon rearrangement of an anomerically linked alkynyl stannane tetrahydrofuranyl ether derivative as the key step.[20] The Ley group had recently introduced a new general method[20] for the efficient synthesis of tetrahydropyran (THP) and tetrahydrofuran (THF) rings containing substituents adjacent to the oxygen atom (Scheme 30.29). A range of anomerically linked carbon-centered nucleophiles, including electron-rich alkenes, silyl enol ethers, and alkynylstannanes have been shown to rearrange in the presence of Lewis acid to afford carbon-linked products in good yields. This methodology is particularly suited to target-oriented synthesis, as it necessarily combines anomeric activation with side-chain heteroatom protection.

Two different approaches to **141** were conceived, which differed in the timing of the introduction of the *p*-fluorophenol substituent (Scheme 30.30). Thus in route (i) it was envisaged that the bulky *tert*-butyldiphenylsilyl (TBDPS)-protecting group in precursor **163** would bias the diastereoselectivity of the rearrangement reaction to favor formation of a *trans*-disubstituted THF ring, as had been observed previously in the synthesis of



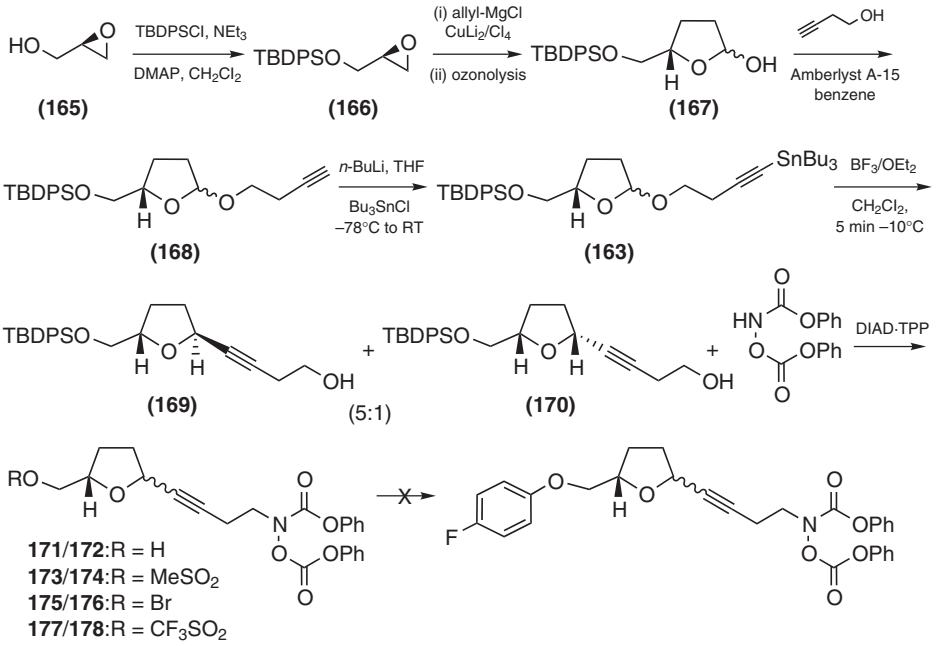
Scheme 30.30 Proposed synthetic routes to **141**.

muricatetrocin C.[20] Conversely, route (ii) incorporated the moiety (precursor **164**) prior to the rearrangement step. This allowed a shorter route to **141**; however; the diastereoselection of the key step was less predictable.

Execution of Routes I and II

Route I Lactol **167** was readily available in 70% yield over three steps starting from (*R*)-glycidol (**165**) (Scheme 30.31). Heating **167** in the presence of homopropargylic alcohol and catalytic Amberlyst A15 in benzene at reflux afforded the tetrahydrofuranyl ether **168** in 95% yield as a 3 : 2 mixture of anomers. Deprotonation of **168** with butyllithium (1.2 equiv) at −78°C followed by treatment with tributyltin chloride (1.15 equiv) afforded, after workup, the tributylstannylated material. This material was not purified but was dissolved in dichloromethane, cooled to −10°C and treated with boron trifluoride etherate (3.0 equiv) for 5 minutes before the reaction mixture was quenched by the addition of sodium hydroxide. Aqueous workup and inspection of the crude ^{1}H NMR indicated that the reaction had generated the carbon-linked products (**169** and **170**) in a 5 : 1 ratio favoring the *trans*-product. Unfortunately, at this stage the diastereoisomers were not separable by chromatography.

Introduction of the hydroxyurea portion proceeded by displacement of the hydroxyl group released during the rearrangement step following the established Mitsunobu protocol. Initial attempts at removing the TBDPS group using TBAF in THF failed, producing an intractable mixture of compounds. However, reaction in the presence of hydrofluoric

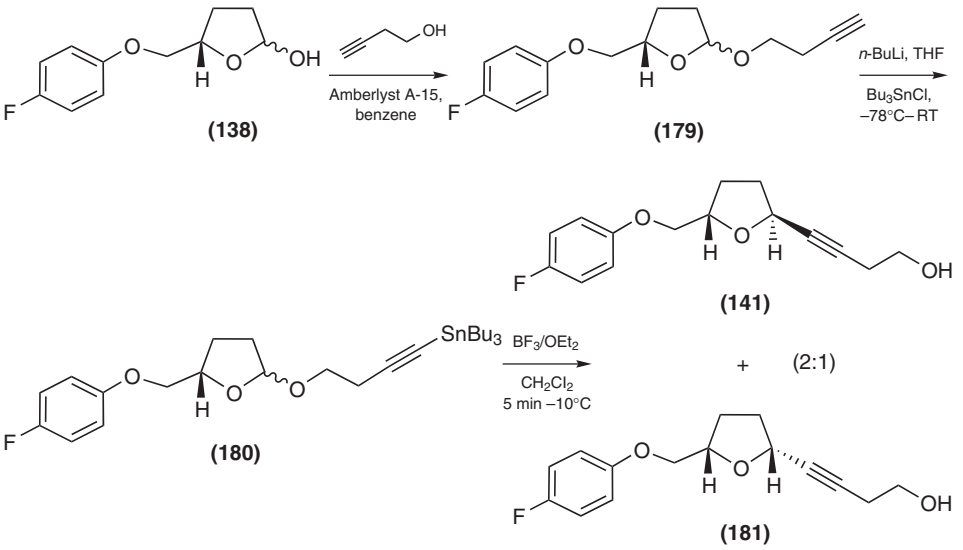


Scheme 30.31 Route I executed based on Ley's protocol.

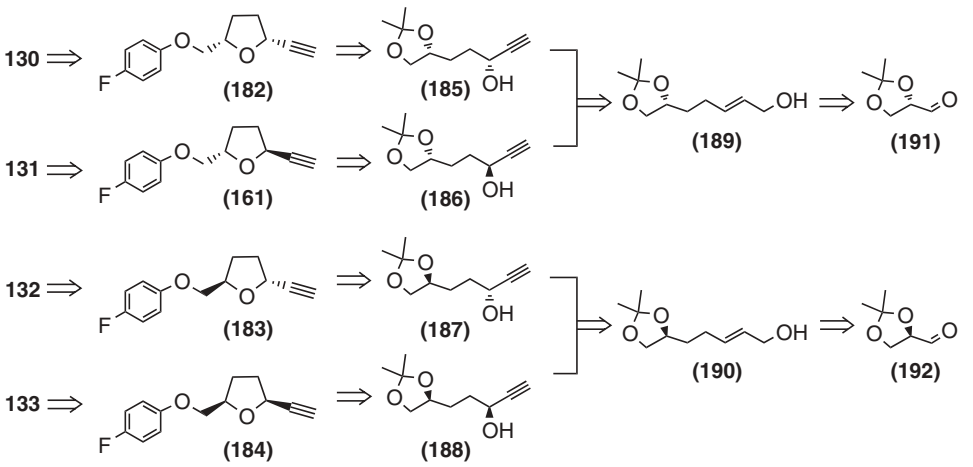
acid–pyridine complex in THF slowly (60% conversion after 2 days) cleaved the silyl ether to afford **171**–**172** cleanly. Unfortunately, neither transformation permitted chromatographic separation of the epimeric mixture. At this stage, introduction of the aryl unit followed by aminolysis was required to complete the synthesis. Thus, **171**–**172** mixture was subjected to the Mitsunobu conditions in the presence of *p*-fluorophenol. Disappointingly, this failed to realize the desired aryl ether, resulting instead in complete decomposition of the starting material. Other attempts at this transformation focused on the prepreparation of an activated leaving group, which could subsequently be displaced by the phenol moiety. Functional groups incorporated included a mesylate (**173**–**174**), a bromide (**175**–**176**) and a triflate (**177**–**178**), however none of these compounds underwent displacement to afford any appreciable amounts of the aryl ether. It was also disappointing to find that in the preparation of these compounds no separation of the epimeric mixture was observed.

Route II It appeared that the phenoxycarbonate protecting group of the *N*-hydroxyurea was extremely base sensitive, and that as soon as this *N*-hydroxyl was free it became a competitive nucleophile, resulting in failure to realize any conversion to the desired aryl ether. Although the potential existed for a change in the protecting group strategy, the lack of any observed separation of the epimeric mixture was also a concern. Thus, with these results in mind, it was decided to abandon this route and pursue the potentially more rewarding route shown in Scheme 30.32.

As predicted, the *trans*-selectivity of the reaction decreased due to the reduced steric bulk of the aryl ether side chain. However, pleasingly in this case, the rearrangement products were found to be completely separable by MPLC to afford diastereomcrically pure **141** in 57% isolated yield. Furthermore, single-crystal x-ray diffraction of **141** was obtained, providing unambiguous proof of the assigned *trans*-stereochemistry.



Scheme 30.32 Route II executed based on Ley's protocol.



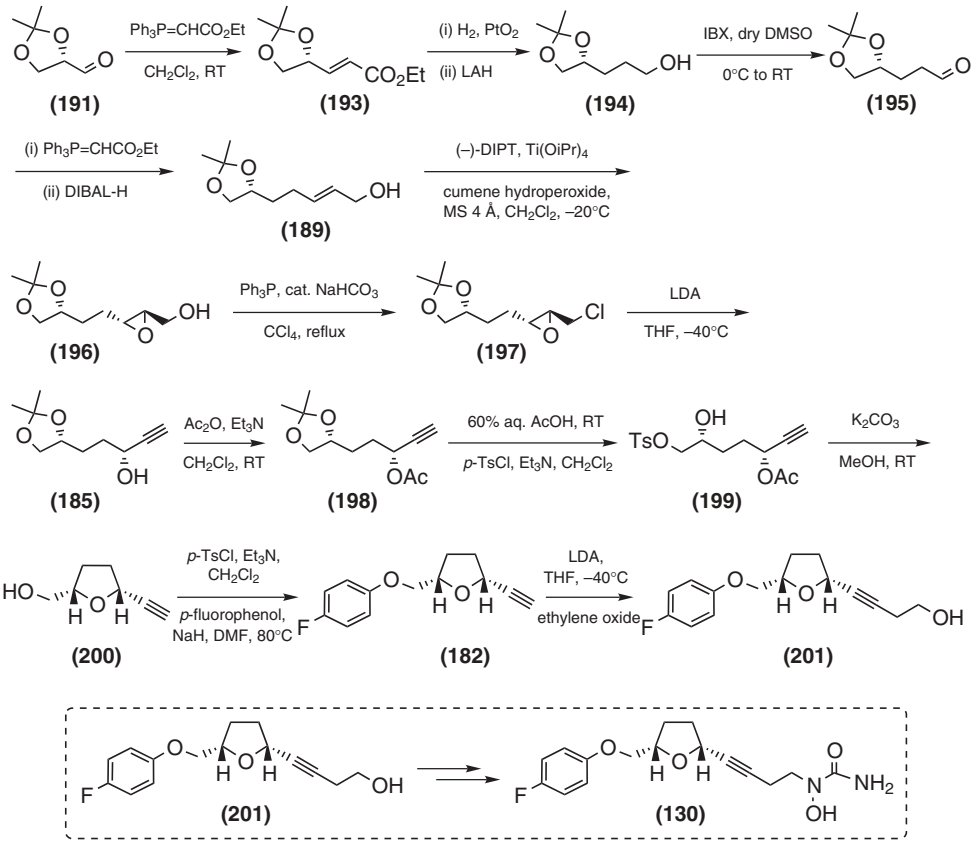
Scheme 30.33 General sequence established for the synthesis of **130** to **133.**

30.3.2 Alternative Strategies for Synthesis of Compounds 130 to 133 Based on C-Alkynyl Furan Derivatives

Our continued investigations resulted in several new routes being defined. During these investigations we developed a unified strategy that addresses the synthesis of **130** to **133** in an enantioselective fashion.[23,25] As show in the general retrosynthetic analysis (Scheme 30.33), **130** to **133** were prepared from the respective alkynes **161** and **182** to **184**, which in turn can be derived from alkynols **185** to **188** by an intramolecular nucleophilic substitution. The synthesis of diastereomers **185/186** could be envisaged from (*S*)-glyceraldehyde (**191**) following a sequence of Wittig and reductions, and of **187/188** from the (*R*)-glyceraldehyde derivative (**192**). Thus, of the two requisite chiral centers, one was obtained from either (*S*)- or (*R*)-glyceraldehyde derivative, while the other was introduced on the allylic alcohol by the SAE[24] method using (+) and (−)-DIPT.

Synthesis of Compound 130 Accordingly, Wittig olefination of **191** (prepared from L-ascorbic acid) with (carbethoxymethylene)triphenyl phosphorane in CH_2Cl_2 gave the ester **193** (Scheme 30.34), which on catalytic hydrogenation with PtO_2 at room temperature afforded **194** in quantitative yield. The ester (**194**) was reduced with LAH in THF to furnish the known alcohol **195** (97%), which on oxidation with IBX in DMSO afforded aldehyde (**196**) (84%). Subjecting **196** to Wittig olefination with (carbethoxymethylene)triphenyl phosphorane, in benzene at reflux, resulted (65%), followed by selective reduction with DIBAL-H in CH_2Cl_2 at −20°C furnished allylic alcohol (**189**) (98%).

Sharpless asymmetric epoxidation[24] of **189** using (−)-DIPT, Ti(O·*i*-Pr)$_4$ and cumene hydroperoxide at −20°C furnished (2*R*,3*R*)-epoxide (**196**) (76%), which on subsequent reaction with Ph_3P in CCl_4 in the presence of $NaHCO_3$ (cat.) at reflux gave **197** (68%). The fragmentation of chiral epoxy chloride (**197**) on treatment with LDA at −40°C afforded **185** (95%); further acetylation (Ac_2O, Et_3N) furnished the corresponding acetate (**198**) (97%). Hydrolysis of **198** with 60% aqueous AcOH at room temperature (73% yield) followed by tosylation (*p*-TsCl, Et_3N) in CH_2Cl_2 afforded monotosylate **199** in 71% yield. Cyclization of tosylate (**199**) with K_2CO_3 in methanol at room temperature afforded the 2,5-disubstituted tetrahydrofuran (**200**) (99%), which on tosylation and further

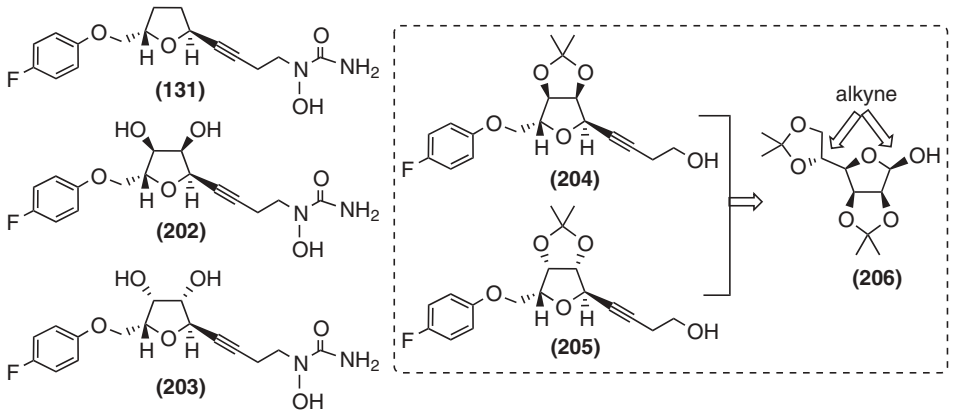


Scheme 30.34 Representative synthesis of isomeric tetrahydrofuran **130**.

treatment with 4-fluorophenol in the presence of NaH in DMF at 80°C afforded **182** in 86% yield. Treatment of lithiated alkyne (**182**) with ethylene oxide provided the key building block (**202**), which was further elaborated to the requisite **130** by a known two-step sequence.

Thus, the four stereoisomeric tetrahydrofuran derivatives (**130** to **133**) were prepared by following this flexible approach employing (*S*)- and (*R*)-glyceraldehyde derivatives and Sharpless asymmetric epoxidation and double elimination of substituted epichlorohydrin as the key steps.

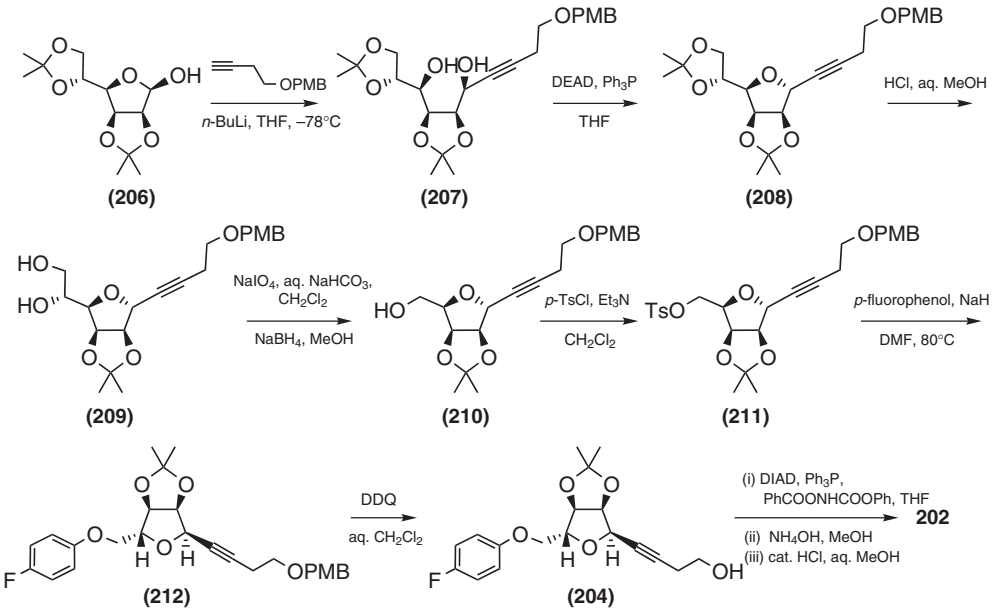
Synthesis of Polyhydroxyfuran Derivatives Related to Compound 131 After evaluating various approaches founded on catalytic asymmetric transformations or chiral pool sources and a combination of both, we next turned our attention to synthesizing derivatives of compound **131**, where the tetrahydrofuran is densely functionalized. Allured by the rich stereochemical diversity, abundant availability, and their biological significance, we have opted to integrate carbohydrates templates in their furanose form and append both functional groups at two ends (Scheme 30.35). The D-*ribo*- and D-*lyxo*-configured derivatives (**202** and **203**) were synthesized in this context.[24] The beauty of our approach is that the synthesis of the requisite alkyne derivatives (**204** and **205**) is achieved from a



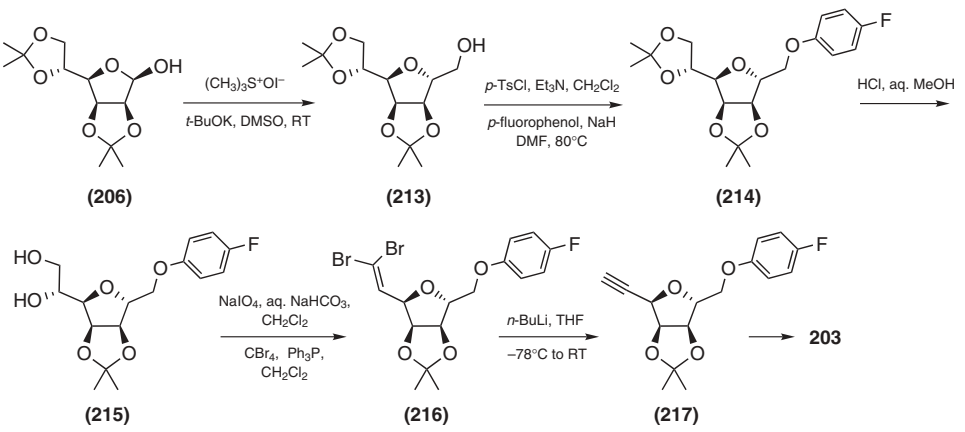
Scheme 30.35 Hydroxyderivatives of **131** and their retrosynthesis.

common chiron (**206**) (i.e., mannosediacetonide). Choosing an appropriate terminus for the introduction of alkyne (i.e., either C1 or C5) lends elegance to our approach.

Synthesis of Alkynyl Furan (202) The synthesis began with the addition of lithiated 1-(4-methoxybenzyloxy)-3-butyne (Scheme 30.36) in THF with **206** followed by cyclization of the major diol (**207**) (43%, in a 19 : 1 ratio) under Mitsunobu conditions to afford **208** (93%) exclusively. Selective deprotection of terminal acetonide (70%), oxidative cleavage of the resulting diol with $NaIO_4$ and aq. $NaHCO_3$ in CH_2Cl_2 (96%), and subsequent reduction with $NaBH_4$ in MeOH afforded **210** in 60% yield. Tosylation of **210** followed by treatment with 4-fluorophenol and NaH in DMF furnished **211** (75%). Oxidative deprotection of the PMB



Scheme 30.36 Synthesis of **204**.

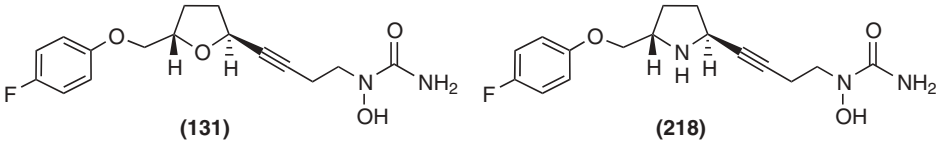


Scheme 30.37 Synthesis of **203**.

group in **211** using DDQ in aqueous CH_2Cl_2 (1 : 19) provided the advanced intermediate (**204**) (69%), which was converted into a urea derivative (**202**) following an established two-step sequence.

Synthesis of Alkynylfuran (203) The key issue in the synthesis of **203** is stereoselective introduction of a hydroxymethylene group at C1 and an alkyne at C4 (Scheme 30.37). The synthesis started with the treatment of **206** with trimethyl sulfoxonium iodide in the presence of *t*-BuOK in DMSO to afford **213** selectively.[25] Reaction of **135** with *p*-TsCl and Et_3N in CH_2Cl_2 and subsequent treatment with sodium 4-fluorophenolate furnished **214** in 53% yield. Selective terminal acetonide deprotection of **214** with catalytic concentrated HCl in aqueous MeOH at room temperature afforded a diol (**215**). Oxidative cleavage of **214** with $NaIO_4$ in the presence of aqueous $NaHCO_3$ in CH_2Cl_2 and treatment of intermediate aldehyde with CBr_4 and Ph_3P in CH_2Cl_2 at room temperature afforded a dibromo derivative (**216**). The key double elimination of dibromoalkene (**216**) by using *n*-BuLi afforded **217** in 77% yield. Following the established sequence (see Scheme 30.34) compound **217** was converted to **203** in four steps.

Synthesis of Aza Analog of Compound 131 Considering the potential of **131** to inhibit LTB_4 production in ionophore-stimulated human whole blood, we were interested to study the effect of replacing the ring oxygen with nitrogen, and this led to the synthesis of the aza analog (**218,** Scheme 30.38). The rationale was that nitrogen is an indispensable part of many natural compounds, including alkaloids, amino acids, azacarbohydrates, and macromolecules (including proteins, DNA, RNA, etc.). These compounds play a

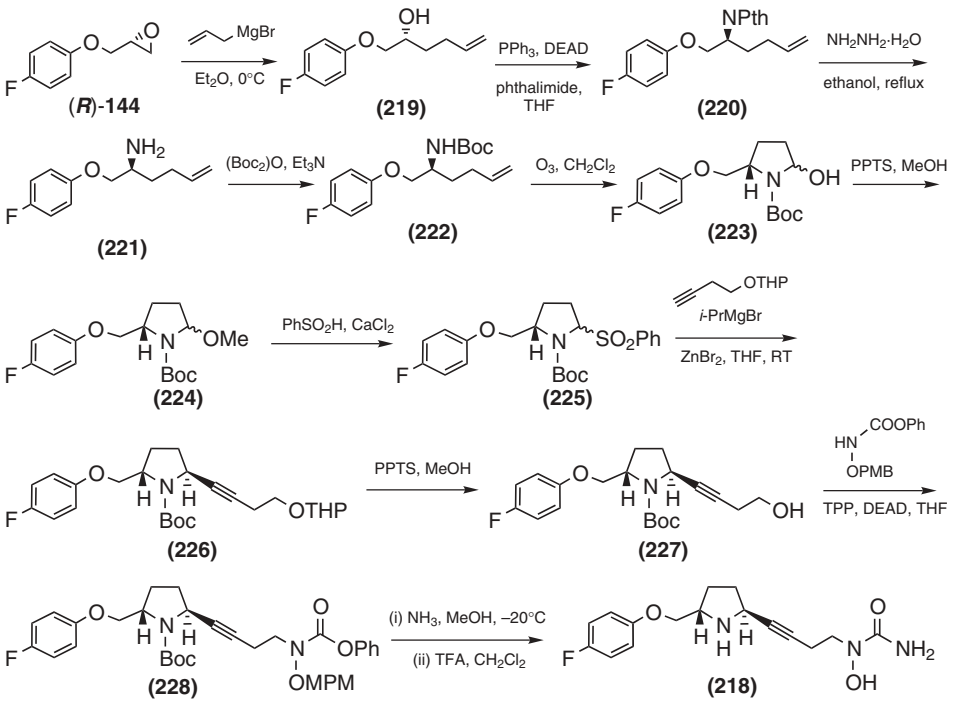


Scheme 30.38 Designed aza analog (**218**) of **131**.

major role in the life-making process, chemical communication in mammals to aging and photosynthesis. We have employed the glycidyl ether, (*R*)-**144**, as the starting chiral precursor and the aza variation of Ley's protocol to construct a 2,5-disubstituted pyrrolidine derivative. Though hemiaminal compounds have been reported to undergo nucleophilic substitution in the presence of various Lewis acids, especially BF_3:OEt_2, $TiCl_4$, $SnCl_4$, and so on, only a few nucleophiles, such as allyl trimethylsilane, TMSCN, $TMSN_3$, and so on, work well with variable degrees of stereoselectivity. A modification of the methodology pioneered by Steven Ley has been very successful in our hands. The attractive features of this methodology include the stability and crystalline nature of most sulfone derivatives, facilitating easy purification by recrystallization, applicable to a broad range of nucleophiles, efficient stereoselectivity, and no requirement for external Lewis acid.

Our synthetic exploration for aza analog **218** started with the opening of the epoxide (***R***)-**144** (Scheme 30.39) with allylmagnesium bromide–CuCN to provide **219**. Treatment of **219** with phthalimide under Mitsunobu conditions using diethyl azodicarboxylate and triphenyl phosphine in THF gave **220**. Hydrolysis of phthalimide derivative was done with hydrazine hydrate in refluxing ethanol to get free amine (**221**), which was protected with Boc anhydride/triethyl amine to afford **222**. Ozonolysis of **222** in CH_2Cl_2 for 2 h gave the cyclized 2-hydroxypyrrolidine derivative (**223**) as a diastereomeric mixture.

Our next concern was to affix the side chain at C2 position via a Lewis acid–mediated nucleophilic addition to *N*-acyliminium ion diastereoselectively using Ley's phenyl sulfone protocol. However, conversion of the hemiaminal (**223**) using $PhSO_2H$



Scheme 30.39 Synthesis of the aza analogue (**218**).

in CH_2Cl_2 was found to be a difficult proposition. To circumvent the problem, we converted **223** to the corresponding 2-methoxypyrrolidine derivatives (**224**) and were gratified to note that the reaction of **224** with $PhSO_2H$ in CH_2Cl_2 in the presence of $CaCl_2$ was facile and afforded the desired 2-benzenesulphonylpyrrolidine derivative (**225**).

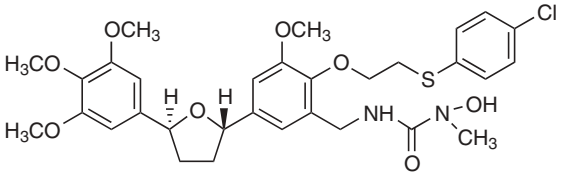
The sulfone derivative (**225**), when treated with dialkyl zinc reagent prepared in situ from $ZnBr_2$ and 4-tetrahydropyranyloxy-1-butyl magnesium bromide in THF for 10 h, reacted smoothly with the substrate to provide homopropargyl pyrrolidine derivative (**226**). The THP group was subsequently removed by exposure to catalytic amount of PPTS in MeOH to retrieve the homopropargyl alcohol derivative (**227**). The diastereomeric excess could probably not be quantified from the ^1H NMR spectrum, because of the rotameric distortion of signals. The diastereomers were not separable by flash chromatography either. However, the mixture was found to be in a 87 : 13 ratio through analytical HPLC. The Mitsunobu reaction of propargyl alcohol derivative (**227**) with *N*,*O*-bis(phenoxycarbonyl) hydroxylamine in the presence of PPh_3 and DEAD provided a fully protected urethane derivative (**228**). Compound **228** on exposure to the methanolic solution of NH_3 simultaneously cleaved benzoate ester and converted urethane into urea, thus affording *N*-hydroxyurea derivative (**160**) in 80% yield. Finally, deprotection of the Boc group with trifluoroacetic acid in CH_2Cl_2 at room temperature culminated in total synthesis of the target compound (**218**).

It was interesting to note that the HPLC analysis of the final compound showed only one peak with both reverse phase and chiral column, indicating that the minor *cis* isomer might have been removed from the mixture during purification. There was no NOE enhancement observed during the irradiation of protons at C2 and C5, which suggested that these protons are not in close proximity, thus establishing the *trans* stereochemistry.

30.4 DRUGS FOR THE TREATMENT OF SKIN DISORDERS

Atopic (hereditary) dermatitis is a chronic disease[13] that affects the human skin and is characterized by red papulovesicular oozing lesions that, with persistence, transform into raised scaling plaques. The disease affects about 20 million people throughout the world. Psoriasis is a chronic skin disease characterized by scaling and inflammation affecting about 6 million people in the world. It is a common, papulosquamous disease of the skin manifested clinically by scaly, erythematous, thickened plaques of various sizes favoring the extensor surfaces of the body. Elevated levels of PAF and LTs were detected in the blister fluids from patients with atopic dermatitis and psoriasis. The only treatment available to date for these diseases was the use of antibiotics, corticosteroidal applications, phototherapy, and other topical treatment such as vitamin D_3 ointment, vitamin A (retinoids), coal tar, and anthralin. There was no specific drug available that could act as a dual inhibitor.

Very recently, (±)-*trans*-2-[3-methoxy-4-(4-chlorophenylthioethoxy)-5-(*N*-methyl-*N*–hydroxyureidyl)methylphenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (**229**), a tetrahydrofuran derivative has been identified as a dual inhibitor for its activity as a PAF receptor antagonist and as a 5-LO enzyme inhibitor (Scheme 30.40). Armed with the advantage of concomitant inhibition, compound **229** offered many therapeutic advantages in terms of efficacy, pharmacodynamics, and cost for the treatment of such skin disorders.



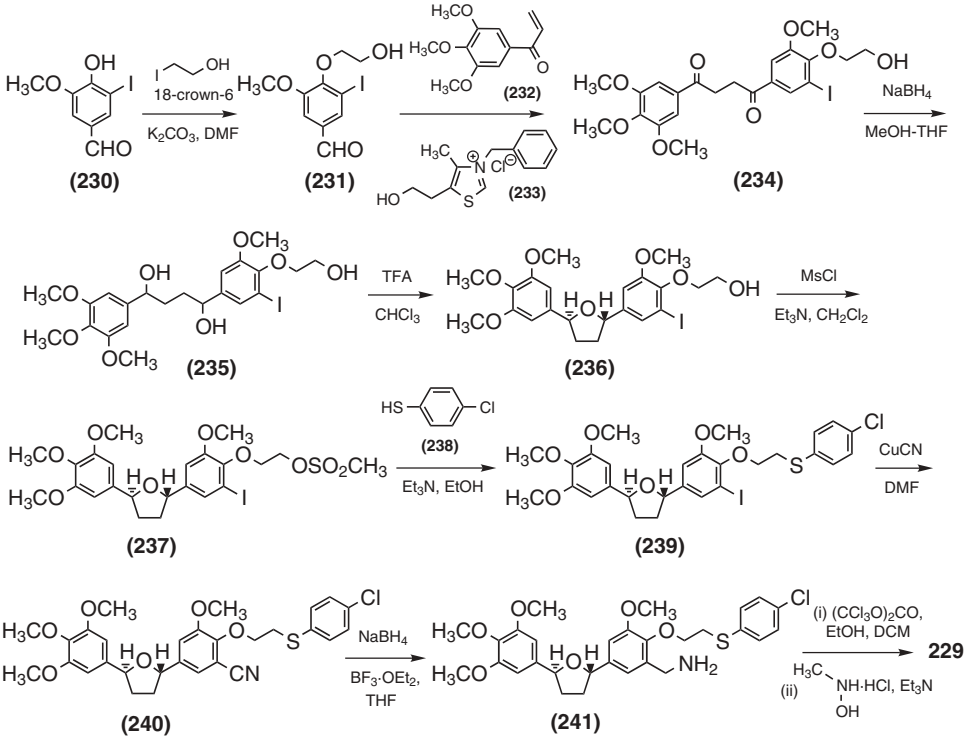
(229)

(+)-*trans*-2-[3-methoxy-4-(4-chlorophenylthioethoxy)-5-(*N*-methyl-*N*-hydroxy ureidyl)methylphenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran

Scheme 30.40 Dual 5-LO inhibitor and PAF receptor antagonist.

30.4.1 Discovery Synthesis of Compound 229

The only synthesis published to date by the medicinal chemists Cai et al. was not amenable to scale-up. The discovery route started with the O-alkylation of 5-iodovanillin (**230**) with 2-iodoethanol in the presence of K_2CO_3 and 18-crown-6 ether in DMF to give compound **231** (Scheme 30.41). The Stetter reaction of 3,4,5-trimethoxyphenyl vinyl ketone (**232**) with aldehyde **231** in the presence of 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride catalyst (**233**) and triethylamine in DMF gave the 1,4-dione (**234**). Reduction of **234** with sodium borohydride in a MeOH–THF mixture gave the corresponding 1,4-diol (**235**). Diol **235** was cyclized with 5% trifluoroacetic acid in $CHCl_3$ to furnish a mixture of *cis* and *trans* isomers of 2,5-diaryl tetrahydrofurans.



Scheme 30.41 Discovery route for **229**.

The pure *trans*-isomer (**236**) which was isolated in 20% yield was converted to its mesylate (**237**). Reaction of **237** with *p*-chlorothiophenol (**238**) in the presence of triethylamine and EtOH under reflux gave an *S*-alkylated compound (**239**), which on heating with cuprous cyanide in DMF furnished a cyano compound (**240**). Reduction of **240** with sodium borohydride–borontrifluoride etherate yielded an aminomethyl compound (**241**) in 20% yield. Finally, treatment of **241** with triphosgene followed by *N*-methylhydroxylamine hydrochloride in the presence of triethylamine afforded the target compound (**229**), (Scheme 30.41).

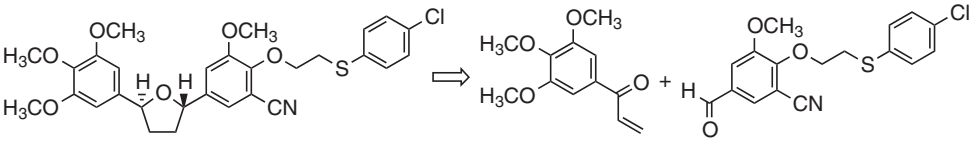
This synthetic route had several demerits regarding commercial feasibility. Additionally, the product obtained was a waxy low-melting solid that proved to be difficult to work with because of its instability to light, heat, and moisture. More significantly, it was difficult to formulate and speculation centered on its intrinsic instability, especially when hydroxylic solvents were used in formulations. It was therefore imperative to develop alternative procedures for synthesis and formulations.

30.4.2 Process Innovation for Compound 229: Systematic Investigation[27]

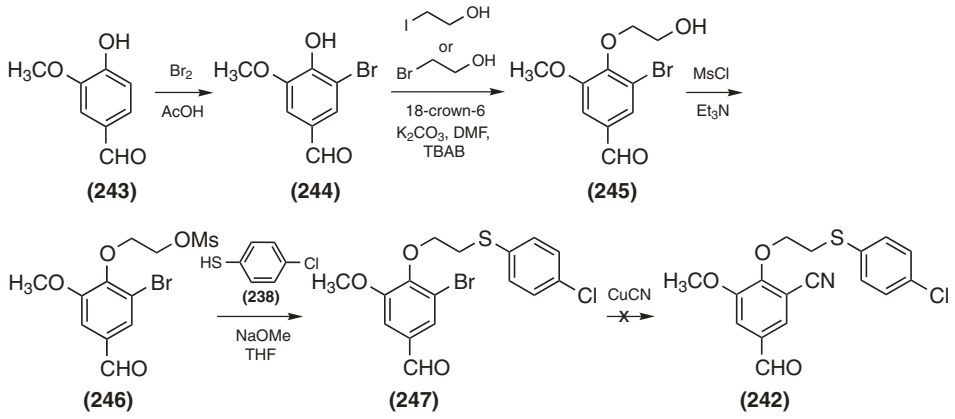
To provide an operationally and commercially feasible alternative process for **229**, every operation of the discovery route was analyzed systematically and the key issues to be addressed and the problems found were attempted one by one. One of the first problems with this discovery synthesis was that it was linear and all the building blocks were added in a stepwise fashion. To provide a convergent synthesis, we planned the synthesis of the key intermediate (**240**) using the Stetter reaction of an enone (**232**) with an advanced cyanoaldehyde (**242**) (Scheme 30.42).

Synthesis of Cyanoaldehyde (242) We opted to synthesize the cyanoaldehyde (**242**) from iodovanilin (**230**), which was made from readily available vanillin (**243**) on iodination with iodine monochloride in acetic acid. Handling iodine monochloride on a multikilogram scale was a very dangerous proposition, as it was known to be highly corrosive and dangerous to handle: the effects are usually irreversible. As an alternative, we opted for using the corresponding bromo derivative (**244**), which in turn could be readily prepared by bromination of vanillin (**243**) with a mixture of bromine and AcOH. Bromo compound (**244**) on reaction with 2-iodoethanol in the presence of K_2CO_3 and tetrabutylammonium bromide, a phase-transfer catalyst, in DMF gave an O-alkylated product (**245**, Scheme 30.43).

Replacing 2-iodoethanol with 2-bromoethanol further modified the preparation of **245**, as the latter was nearly one-sixth as expensive and the O-alkylated product (**244**) was obtained in higher yields. Compound **245** on reaction with methanesulfonyl chloride and triethylamine in methylene chloride gave a mesylate (**246**), which was further reacted with



Scheme 30.42 Alternative convergent approach opted for the advanced intermediate (**240**).

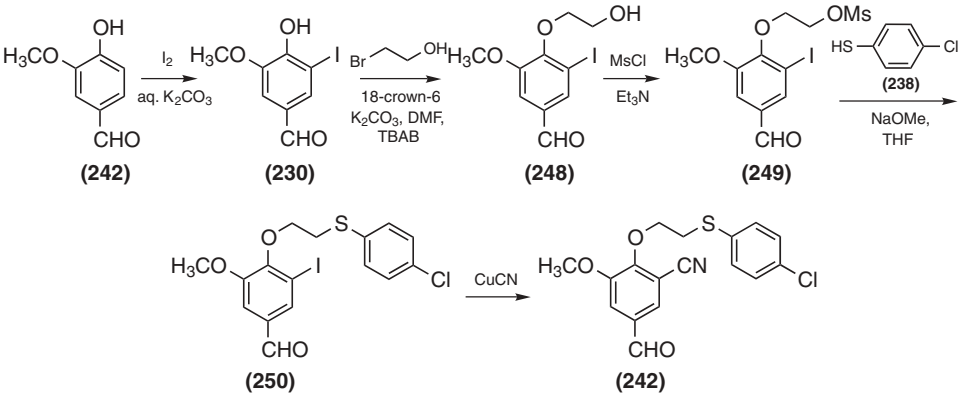


Scheme 30.43 Attempted synthesis of advanced cyanoaldehyde (**242**).

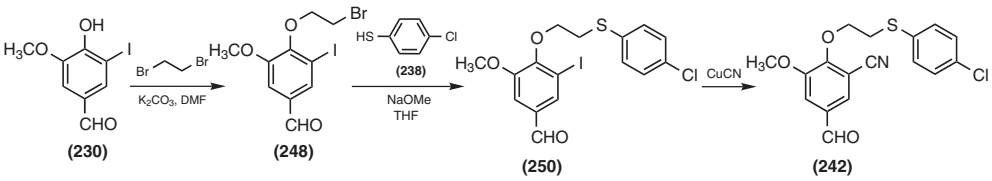
p-chlorothiophenol (**238**) to furnish an S-alkylated product (**247**), albeit, in low yields. Discouragingly, the reaction of a bromo derivative (**247**) with cuprous cyanide in the presence of tetrabutylammonium bromide in DMF at reflux temperature did not yield **242** as expected. The nucleophilic substitution reaction failed even at temperatures above 200°C in solvents such as *N*,*N*-dimethylacetamide and *N*-methylpyrrolidinone.

As the replacement of iodo with bromo was found to an ineffective and difficult proposition we next attempted at least to replace the hazardous iodine monochloride for iodination of vanillin on a multi-kilogram scale. After careful experimentation we concluded that iodine in aqueous sodium bicarbonate solution is as effective as ICl. Iodination under these revised conditions afforded 5-iodovanillin (**2**) in 82% yield (Scheme 30.44). When subjected to the same sequence of reactions as in Scheme 30.43, 5-iodovanillin (**230**) gave the cyano aldehyde (**242**) in moderate yields (Scheme 30.44).

To reduce the number of steps involved in the synthesis of **242**, an alternative route where a mesyl equivalent leaving group is placed in the beginning of the synthesis (i.e., by replacing bromoethanol with dibromoethane) was explored. As shown in Scheme 30.45, 5-iodovanillin (**230**) was heated with 1,2-dibromoethane and K_2CO_3 in DMF to afford the



Scheme 30.44 Synthesis of advanced cyanoaldehyde (**242**).

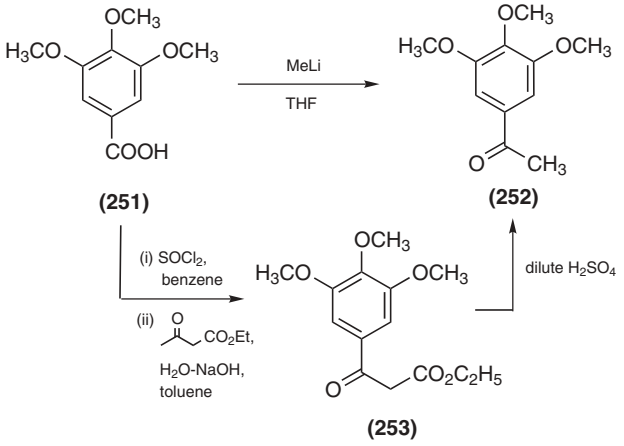


Scheme 30.45 Synthesis of advanced cyanoaldehyde (**242**).

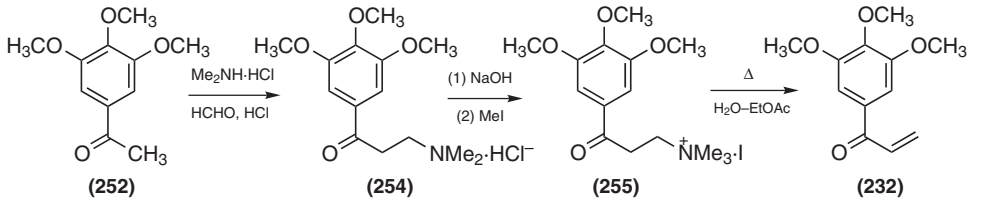
O-alkylated product **250** in 76% yield. Reaction of compound **250** with *p*-chlorothiophenol (**238**) and sodium methoxide powder in THF gave the *S*-alkylated derivative (**250**) in 72% yield. On heating with cuprous cyanide in DMF, compound **250** afforded a cyano compound (**19**) in 72% yield.

Synthesis of Vinyl Ketone 232 3,4,5-Trimethoxy phenyl vinyl ketone (**232**) was conveniently prepared on a large scale from 3,4,5-trimethoxyacetophenone (**252**). The initial synthesis of **251** was achieved in moderate yields by reacting commercially available 3,4,5-trimethoxybenzoic acid (**251**) with methyllithium (Scheme 30.46). However, the classical approach of converting the acid to the β-keto ester and then decarboxylating was found to be better suited for large-scale preparation of **252** and was hence deployed. The acid (**251**) was heated with $SOCl_2$ in benzene and catalytic amounts of DMF to give 3,4,5-trimethoxybenzoyl chloride (95% yield), which was condensed immediately with ethyl acetoacetate in a H_2O–NaOH toluene mixture to secure the β-keto ester (**253**), in 50% yield. Compound **253,** on refluxing in dilute H_2SO_4, furnished **251** in 90% yield.

Acetophenone (**252**) was converted into the vinyl ketone (**232**) by the procedure of Girotra et al. Mannich reaction of compound **252** with *N*,*N*-dimethylamine hydrochloride and *p*-formaldehyde in refluxing isopropyl alcohol in the presence of a catalytic amount of HCl resulted in formation of the Mannich base (**254**) in 77% yield. Subsequently, treatment of **254** with aqueous NaOH and methyl iodide in ethyl acetate gave a quaternary salt (**255**) in 83% yield. On heating in a H_2O–EtOAc biphasic system, compound **255** underwent a



Scheme 30.46 Synthesis of acetophenone (**252**).

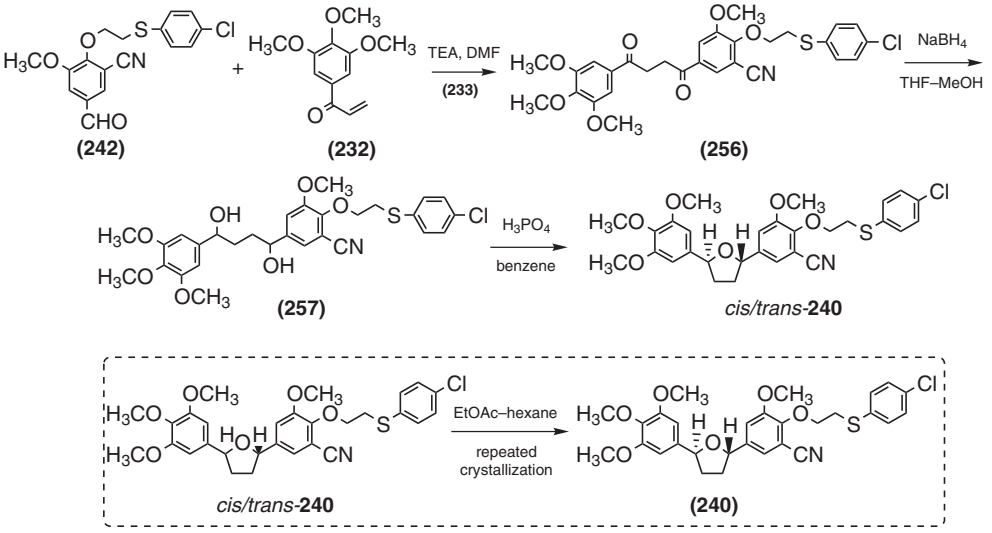


Scheme 30.47 Synthesis of vinyl ketone (**232**).

thermal elimination reaction to afford a vinyl ketone (**232**) in 50% overall yield starting from **252** (Scheme 30.47).

After having both coupling partners (**232** and **242**) in hand, we next focused our attention on their coupling, employing a Stetter reaction to obtain the 1,4-diketone moiety (**256**). Accordingly, the aldehyde (**242**) was reacted with a vinyl ketone (**232**) in the presence of TEA and thiazolium catalyst (**233**) in DMF to give a 1,4-diketone (**256**) in 70% yield (Scheme 30.48). On reduction with $NaBH_4$ in a THF–MeOH mixture, compound **256** gave a 1,4-diol (**257**) in nearly quantitative yield. It is known that 1,4-diols are easily converted to tetrahydrofurans by dehydration. Accordingly, on refluxing with *o*-phosphoric acid, **257** afforded a racemic *cis–trans* tetrahydrofuran mixture (**258**) in 99% yield. HPLC analysis revealed the presence of a 60 : 40 *cis*/*trans* mixture.

Preliminary biological data indicated that *trans*-2,5-diaryl tetrahydrofurans were more potent in anatagonizing PAF than their corresponding *cis*-isomers. It was observed that on repeated crystallization from an EtOAc–hexane mixture, a crude *cis*-/*trans*-tetrahydrofuran mixture (**240**) afforded *trans*-tetrahydrofuran (**240**) in 30% yield. HPLC analysis revealed that the *trans*-isomer (**240**) had purity greater than 97% and contained less than 1% of the contaminating *cis*-isomer. The residue obtained from the mother liquors of the initial crystallization was enriched with the *cis*-isomer. When dissolved and stirred in a mixture of trifluoroacetic acid and chloroform at room temperature, the *cis*-isomer isomerized to



Scheme 30.48 Synthesis of the advanced intermediate (**240**).

the *trans*-isomer in a 1 : 1 ratio. The product was purified by crystallization from an ethyl acetate–hexane mixture. The *trans*-isomer thus obtained was subjected to a combination of repeated crystallizations. Two such isomerizations gave the required *trans*-isomer (**240**) in 70 to 75% yields.

Reduction of Cyano Group in Compound 240 Reduction of **240** with $NaBH_4$–BF_3–Et_2O to the aminomethyl compound (**241**) was not feasible on a large scale due to risk of a thermal runaway reaction. Alternatively, reduction of **240** with lithium aluminum hydride ($LiAlH_4$) gave **241** in about 50% yield. To improve this step we opted for using aluminum hydride, known as alane for this transformation.

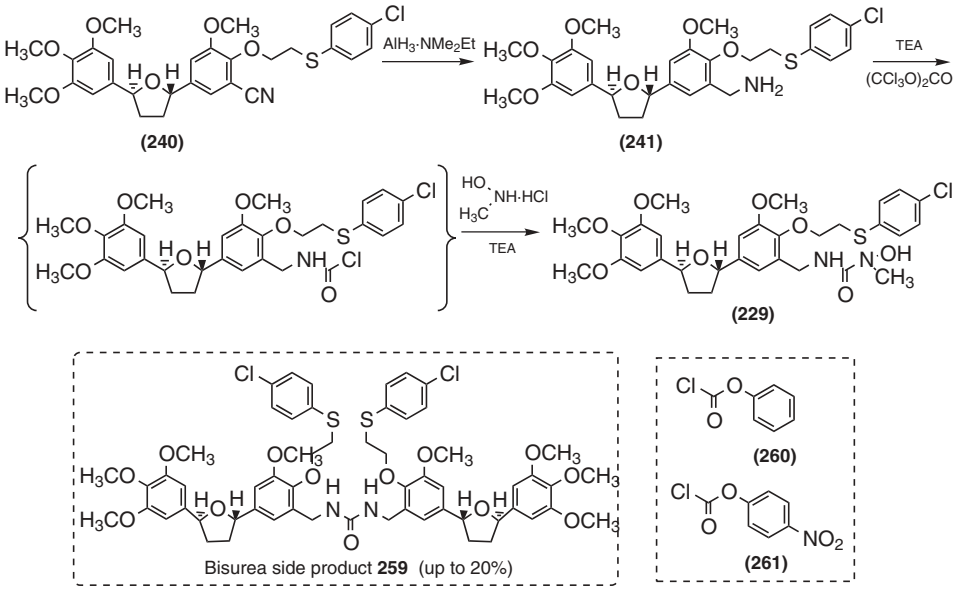
Alane (AlH_3) is a useful reducing agent[26] prepared by reacting $LiAlH_4$ with concentrated H_2SO_4 in THF. The cumbersome procedures of preparing standardized THF solutions of alane on a large scale made this an expensive proposition. Moreover, the alane in THF afforded a slow THF cleavage reaction. Hence an alane–amine complex was considered to be a plausible alternative to this reagent. Everett and Park reported the preparation and use of a number of amine–alane complexes, among which the dimethylethyl amine (DMEA)–alane complex was seen as a very useful reducing agent in terms of selectivity, efficiency, and effectiveness. The DMEA–alane complex (**258**) was prepared by reacting DMEA with $LiAlH_4$ at 0°C in toluene. The insoluble trilithium aluminum hexahydride was filtered under N_2 and the toluene solution containing the complex was used directly for reduction:

$$3\ LiAlH_4 + Me_2EtN \xrightarrow{\text{toluene}} Li_3AlH_6 + 2AlH_3{\cdot}NMe_2\ Et$$

(258)

Alane solutions obtained in this manner had a concentration of 0.5 to 0.55 M. Accordingly, reduction of **240** with DMEA–alane complex (**258**) gave an aminomethyl compound (**241**) in 95% yield (Scheme 30.49) and was taken to the next step without further purification. The final reaction in the preparation of the target compound (**229**) involved the introduction of the N-substituted urea functionality. It was known that N-substituted ureas were usually prepared from amines through the intermediate formation of the generally unstable carbamoyl chlorides that convert to the isocyanates. Carbamoyl chlorides were prepared by reacting amines with reagents such as phosgene gas or triphosgene [bis (trichloromethyl)carbonate(BTC)], a stable phosgene substitute. Triphosgene was preferred over phosgene gas as the latter had a lower vapor pressure and was considered to be highly potent and toxic. Despite its exorbitant cost, triphosgene could still be used, as only 0.33 mol of this reagent was required in most nucleophilic reactions. The aminomethyl compound (**241**) was reacted with triphosgene and subsequently with *N*-methylhydroxylamine hydrochloride in presence of triethyl amine to yield the final product (**229**) as a viscous mass (Scheme 30.49).

The crude product obtained was purified by column chromatography over silica gel with 1 : 1 ethyl acetate–hexane as the eluant to secure the final compound (**229**) in 55% yield. Careful isolation and characterization showed that one of the major by-products of this reaction to be the bisurea derivative (**259**) in yields of up to 20%. We explored phenylchloroformate as a substitute to triphosgene in the preparation of unsymmetrical urea derivatives. Phenylchloroformate (**260**) in the presence of an appropriate base on reaction with an amine (**241**) gave the corresponding carbamate, which on further reaction with *N*-methyl hydroxylamine gave a urea derivative (**229**). However, the formation of bisurea



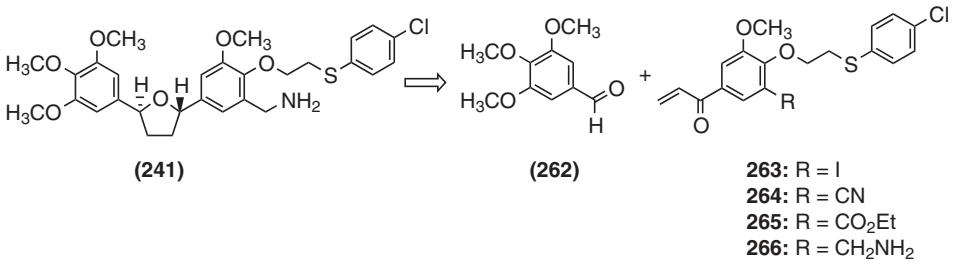
Scheme 30.49 Successful synthesis of **229**.

could not be entirely avoided in this reaction. Phenylchloroformate was therefore replaced by commercially available *p*-nitrophenylchloroformate (**261**), wherein the reaction was facilitated by the ability of *p*-nitrophenol to act as a better leaving group. Accordingly, aminomethyl compound (**241**) on reaction with *p*-nitrophenylchloroformate and *N*-methyl hydroxyl amine hydrochloride in the presence of triethylamine, afforded the final compound (**229**) in 98% yield with less than 2% of bisurea as contaminant.

***Purification and Crystallization of Compound* 229**[28] The product (**229**) obtained hitherto was not pure enough (95 to 96%) for clinical research. Moreover, the compound was not able to retain its crystallinity for long and decomposed when exposed to light, air, or moisture into a sticky mass. After crystallization from various solvents, it was observed that when crystallized from isopropanol, **229** retained its crystalline state for longer periods when stored at reduced temperatures. Compound **229** was dissolved in isopropanol and then refrigerated at −20°C; the white solid that crystallized out was highly pure (greater than 98% by HPLC analysis) and was stable at room temperature. X-ray diffraction studies indicated that IPA formed a complex with compound **1**; the IPA content in the molecule, which was about 5 to 6% (GC analysis), was essential for compound **1** to retain its crystalline state and its stability. Furthermore, it was observed that when the compound was dried under vacuum at 80°C and the IPA content slowly reduced, reaching a limit of about 3%, the molecule collapsed again into a jellylike glossy mass.

30.4.3 Alternative Routes for Compound 229

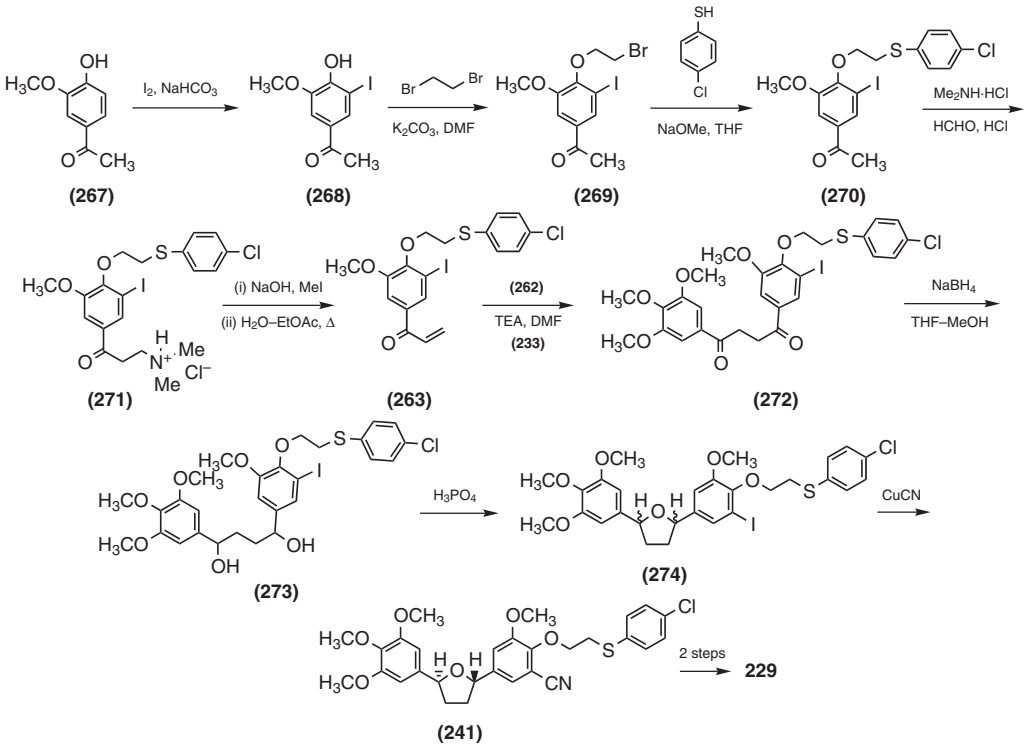
After establishing the convergent synthesis of **229** and the feasibility of the Stetter reaction between modified aldehyde and enone, in the interest of greater efficiency we modified[27] the coupling partners further (Scheme 30.50) by selecting an amino compound (**241**) as the target. Considering the easy availability of 3,4,5-trimethoxybenzaldehyde (**262**), which



Scheme 30.50 Alternative convergent approach opted for the advanced intermediate (**241**).

potentially should make the process much simpler, we have interchanged the functional groups of coupling partners and executed their feasibility in the synthesis of **241** in general and in the Stetter reaction in particular.

Route 1: Synthesis of Compound 241 Employing Vinyl Ketone 263 Acetovanillone (**267**) on iodination with I_2 in aqueous $NaHCO_3$ gave 5-iodoacetovanillone (**268**) in 85% yield (Scheme 30.51). Further reaction with 1,2-dibromoethane and K_2CO_3 in DMF afforded an O-alkylated compound (**269**) in 75% yield. The *S*-alkylated compound (**270**) was obtained by following the established method and was subjected to the Mannich reaction, wherein the active methyl group was condensed with *p*-formaldehyde and *N*,*N*-dimethylamine hydrochloride in the presence of a catalytic amount of HCl in IPA to give



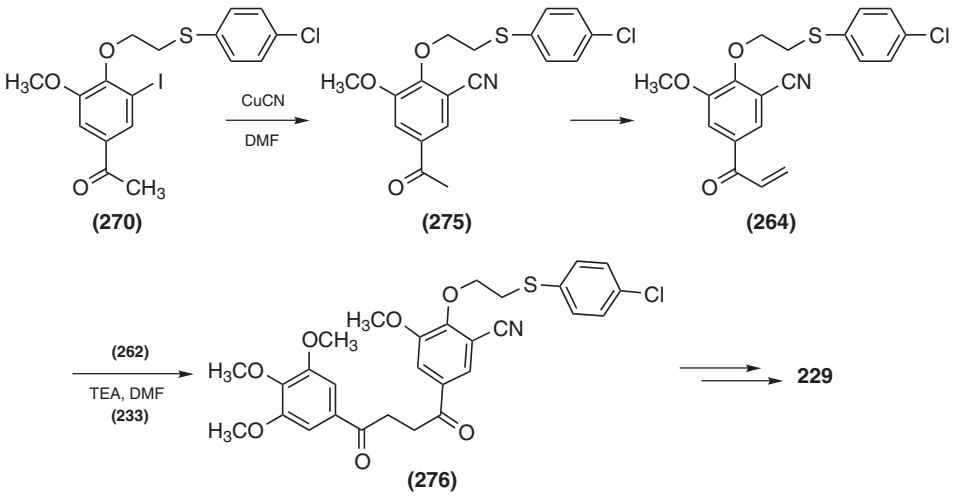
Scheme 30.51 Synthesis of **229** using vinyl ketone **263**.

the Mannich salt (**271**) in 54% yield. Compound **271** on treatment with aqueous NaOH and MeI in EtOAc followed by the pyrolysis of the resulting quaternary salt gave a vinyl ketone (**263**). After having the vinyl ketone fragment (**263**) in hand, the next task was to couple this with an aldehyde (**262**) to obtain the corresponding 1,4-diketone compound (**272**). This was achieved by the Stetter reaction, wherein **263** was reacted with **262** using a thiazolium catalyst (**233**) and triethylamine in DMF to give1,4-dione (**272**) in 80% yield (Scheme 30.51). Dione **272** on reduction with $NaBH_4$ in THF–MeOH afforded the 1,4-diol (**273**) in quantitative yield. Diol **273** on cyclization with H_3PO_4 in benzene[10] gave the iodotetrahydrofuran (**274**) in 94% yield, which without further purification was reacted with CuCN in DMF under reflux conditions to give tetrahydrofuran (**241**) in 74% yield (Scheme 30.51).

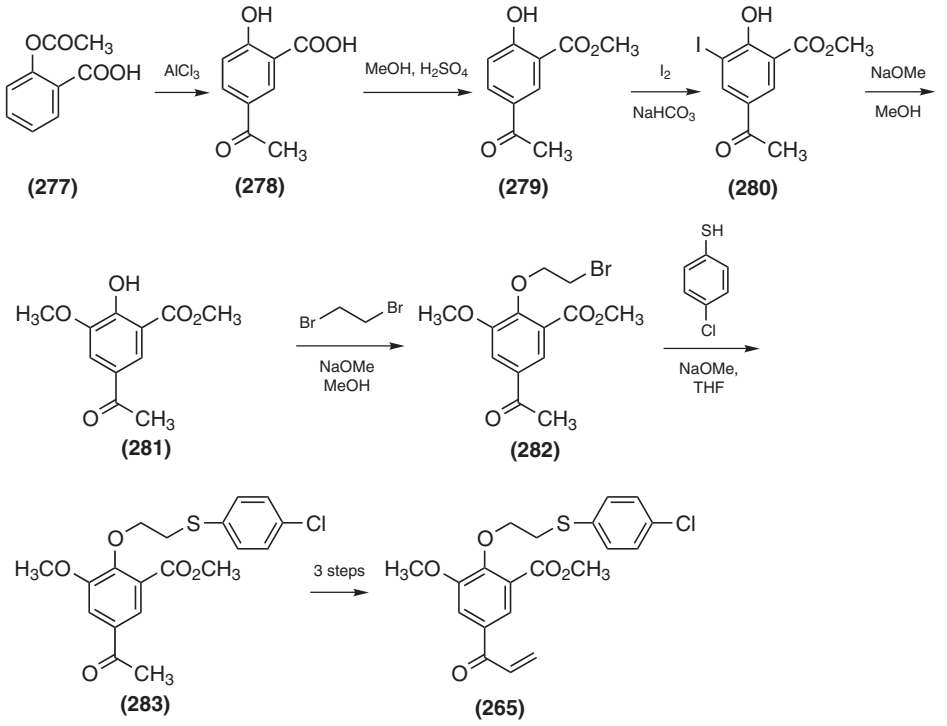
Following the established purifications and isomerization in trifluoroacetic acid and chloroform at room temperature provided the pure *trans*-tetrahydrofuran (**241**) in 54% overall yield starting from **274**. The *trans*-isomer (**241**) on reduction with alane–dimethylethylamine complex afforded the amino methyl compound in 95% yield, which was converted to the requisite urea (**229**) by using *p*-nitrophenylchloroformate.

Route 2: Synthesis of Compound 241 Employing Vinyl Ketone 264 Compound **270** of Scheme 30.51 was treated with cuprous cyanide in DMF at reflux to furnish a cyano compound (**275**) in 65% yield (Scheme 30.52). Following established methods, **275** was converted to the corresponding vinyl ketone (**264**), which was condensed with aldehyde (**262**) under Stetter conditions to provide a diketone (**276**). A regular sequence has been employed to convert **276** to the final urea (**229**).

Route 3: Synthesis of Compound 241 Employing Vinyl Ketone 265[27,28] As shown in Scheme 30.53, aspirin (**277**) was converted to a synthon (**265**), which in turn was reacted with 3,4,5-trimethoxy benzaldehyde (**262**) to give the corresponding 1,4-dione (**284**, Scheme 47). Dione **284** could then be logically taken to the final compound (**229**) following the sequence of reactions depicted in Scheme 30.54. During the conversion of aspirin (**277**)

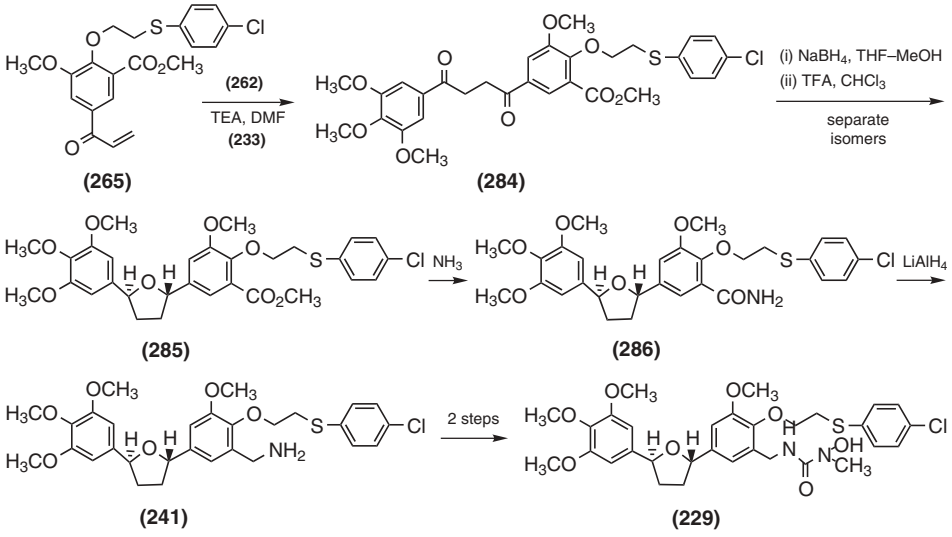


Scheme 30.52 Synthesis of **229** using vinyl ketone **264**.



Scheme 30.53 Conversion of Aspirin to vinyl ketone **264**.

to 3-carboxy-4-hydroxy acetophenone (**278**) by Fries rearrangement, it was observed that the optimal reaction conditions involved mixing the two components with an excess of $AlCl_3$ at 130°C using ethylene dichloride as the preferred solvent.



Scheme 30.54 Synthesis of **229** from a vinyl ketone (**264**).

30.5 CONCLUSIONS

India is often described as a rich country where poor people live. Its wealth is due to a rich biodiversity and traditional knowledge base, including traditional medicinal systems. To exploit this, a program has been launched to discover and develop bioactives. Twenty laboratories have been networked together in this. Several companies are scouting in India for new ideas and technologies. The ability to assemble and manage an effective global knowledge network rather than developing in-house capability is becoming the key determinant of competitiveness. India is becoming an exporter of knowledge.

As of January 1, 2005, the Patents Act of India has been amended, via an ordinance that will enforce all international product patent laws there according to the GATT agreement, including those relating to pharmaceuticals. This should open the floodgates to foreign collaboration, as witnessed by the extensive outsourcing currently under way. Researchers and strategic partners must be geared to providing tomorrow's solutions today. According to Dr. Mashelkar:

> Rapid paradigm shifts taking place in the world as it moves from super power bipolarity to multipolarity, as industrial capitalism gives way to green capitalism and digital capitalism, as information technology creates netizens out of citizens, as the nations move from "independence" to "interdependence", as national boundaries become notional, and as the concept of global citizenship gets evolved, we will see a world full of new paradigms and new paradoxes. There is no doubt that the rapid advance of science and technology will directly fuel many of these.

The Indian pharmaceutical, and in particular the contract R&D organizations have seen a dramatic change in their capabilities and sophistication. International pharmaceutical companies should now be ideally poised to seek collaborations to bring innovative drugs to consumers at an affordable price.

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31

PRINCIPLES AND PRACTICE OF CLINICAL DRUG DEVELOPMENT

COLIN SCOTT

Novacal Pharmaceuticals
Emeryville, California

31.1 INTRODUCTION

After the discovery of a drug candidate, there remain a number of hurdles to overcome before it can be marketed. The ultimate goal is to prove that the drug is safe and efficacious. The watershed between research and development has been reached. Here the overall process of evaluation of the drug candidate changes. The chemistry group and other scientists provide the development group with an opportunity to take to the clinic a chemical with therapeutic utility. The mandate of the development team is to take the chemical entity and change it into a drug that regulatory authorities are prepared to license.

In any pharmaceutical company, big or small, transitioning from research to development is probably the largest paradigm shift. The laws of science are supplemented with three additional primarily nonscientific disciplines, adherence to the ethical code of medical research, proof of efficacy and safety, and compliance with regulatory requirements. Scientific discipline and development discipline are horses of a different color. In research it is a requirement to get the "best possible" entity; in development it is the requirement to choose the potential drug that will be proved to have therapeutic utility in a reasonable development time frame and will be approved by regulatory authorities to be marketed. Research is always looking for the "best"; development is always looking for the "best registrable." The rules governing the two disciplines are separated by a bureaucratic chasm that requires the research group to be acceptant of a significant up-regulation of bureaucratic process. Development requires much more red tape.

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31.2 HISTORY OF ETHICAL MEDICAL RESEARCH

Drug development is wrapped in two interlocking but independent disciplines. On the one hand, there are the regulatory and quasiscientific disciplines, which are dealt with later in this chapter, and on the other hand, there is the moral responsibility for the subject, the ethics of clinical research. Clinical research was being conducted long before there were moves to require proof of safety and efficacy before a drug could be put on the market. In the early days of medical research, ethics were considered to be covered by the medical treatment ethical codes and were not considered as a separate entity. Possibly the best known early medical ethics code was from ancient Greece, the Hippocratic Oath, a modernized version of which is still in use. The American Medical Association's current *Code of Medical Ethics* states that the Hippocratic Oath "is an expression of ideal conduct for the physician." The oath in its original version referred exclusively to the role of the physician in patient management and therefore is not directly relevant to research. However, the oath contained the statement, "I shall keep them from harm and injustice," a sentiment that encapsulates the essence of the more modern codes of ethics.

There is some controversy over who was the original pioneer of clinical research ethics. One school cites Thomas Percival, an Edinburgh University, Scotland–trained English physician (1740–1804), as the originator of clinical research ethics. He published an ethical code of conduct in 1794, *Medical Ethics or a Code of Institutes and Precepts Adapted to the Professional Conduct of Physicians and Surgeons.* This code was adapted by the American Medical Association for use by American physicians in 1847. Percival's detractors point out that the Percivilian code focused on the responsibility of physicians to care for the sick. Although it contained guidance on the use of experimental techniques on patients ("[these] should be scrupulously and conscientiously governed by sound reason"), it did not mention one of the essential modern-day concepts, the consent of the subject. Another school cites William Beaumont, a Connecticut-born physician (1785–1853) and sometime army surgeon who became known as the "father of gastric physiology." It is said that in 1833 he espoused an ethical research code which included the requirement for voluntary consent by any research subject, cessation of the research if it caused distress to the subject, and the provision that the subject was free to withdraw from the research whenever he or she wanted. Beaumont's detractors question whether this code ever existed. Whoever has the greater claim to be the father of clinical research ethics, it is apparent that the basic precepts of clinical research ethics were laid down over 100 years before the barbaric deviation from ethical conduct which led to the creation of today's clinical research ethics.

The modern era in medical research ethics began with the Nuremberg Code (1947). It is no small irony that the clearest pronouncements on the pivotal role of consent were promulgated in Germany at the beginning of the twentieth century. Indeed, in 1931 the German Reichs Minister of the Interior forbad medical experimentation unless the "subject or his legal representative has unambiguously consented to the procedure in the light of relevant information provided in advance." Just 15 years later, over 20 physicians who worked for the German armed forces stood trial for atrocities committed during World War II. Some were sentenced to death, most because they had conducted human medical experimentation to which no sane person would have consented. Germany did not hold the monopoly of unethical research. The Office of Scientific Research and Development in the United States was accused of using subjects for experimentation without them giving informed consent.

The Nuremberg Code of 1947 defined the voluntary consent of the subject in the following terms;

> This means that the person involved should have legal capacity to give consent; should be so situated as to be able to exercise free power of choice … and should have sufficient knowledge and comprehension of the elements of the subject matter involved as to enable him to make an understanding and enlightened decision. This latter element requires that before the acceptance of an affirmative decision by the experimental subject there should be known to him the nature, duration and purpose of the experiment; the methods and means by which it is to be conducted; all inconveniences and hazards reasonably to be expected; and the effects upon his health or person which may possibly come from his participation in the experiment.

This clear definition of informed consent was supplemented by the following principles:

- Investigators must be scientifically qualified.
- Research must be purposeful and necessary for the benefit of society.
- Appropriate measures should be taken to avoid or protect subjects from injury or unnecessary physical or mental suffering.
- The risks to the subjects shall not be greater than the humanitarian importance of the problem.
- Subjects may terminate the experiment at any time.
- Research must be based on animal studies or other rational justification.

The Nuremberg Code encompasses all that has to be adhered to for appropriate clinical research to be conducted without harming the subject who volunteers.

The field of clinical research ethics is no more immune from the reinvention of the wheel than any other quasiscientific discipline. The desire for improvement is both laudable and counterproductive. It is fueled by the reality of policy versus practice.

Porton Down in the UK was a biochemical research facility. In the mid-1950s, part of its research activities were concerned with the effects of nerve gases. One young serviceman was asked to participate in an experiment to find a cure for the common cold. It is claimed that he was actually exposed to a nerve toxin, sarin, and subsequently died. As recently as 2002 the British High Court gave the go-ahead for a new inquest into the death. Ethical issues in medical research have a habit of not going away until there is complete resolution.

Some experiments that were on shaky or clearly nonethical grounds at their inception lived on even after the adoption of the Nuremberg Code. Probably the best known is the Tuskegee Syphilis Study. In 1932, three hundred and ninety-nine African Americans who were diagnosed as suffering from syphilis were entered into the study, as were 200 healthy African Americans who acted as controls. There was no informed consent. The purpose of the study was to prove that the antisyphilitic medications available at the time were not just ineffective but were harmful. The requirement to generate data to prove this hypothesis might be considered laudable, but the methods used to populate the study were not ethical. After the study started, the way that the participants were managed raised major ethical concerns. By 1947, penicillin was considered a safe and effective treatment for syphilis. The Tuskegee syphilis patients were not evaluated as to whether they might benefit from this new medication. One justification was that their disease was too advanced for them to be candidates for benefit. The study continued and

would no doubt have ended only when all 599 participants had died had its existence not been leaked to the press in 1970. In 1972, an advisory panel determined that the study was medically unjustified. The study was closed and a class action law suit led to a multimillion dollar restitution to the survivors and the surviving family members of this misguided and unethical study. In 1997, President Clinton formally apologized to the Tuskegee study participants.

With human rights abuses such as these, there is more than enough justification to continue to legislate against abuse or at least to try to define what is unethical. The Nuremberg Code formed the basis of the Declaration of Geneva Physician's Oath (1948). This was adopted by General Assembly of the newly formed World Medical Association (WMA). It was looked on as a modernization of the Hippocratic Oath and was an attempt to focus the individual physician's attention on medical ethics.

Physician's oath At the time of being admitted as a member of the medical profession:

- I solemnly pledge myself to consecrate my life to the service of humanity.
- I will give to my teachers the respect and gratitude which is their due.
- I will practice my profession with conscience and dignity; the health of my patient will be my first consideration.
- I will maintain by all the means in my power, the honor and noble traditions of the medical profession; my colleagues will be my brothers.
- I will not permit considerations of religion, nationality, race, party politics or social standing to intervene between my duty and my patient.
- I will maintain the utmost respect for human life from the time of conception, even under threat, I will not use my medical knowledge contrary to the laws of humanity.
- I make these promises solemnly, freely and upon my honor.

The WMA went on to adopt an International Code of Medical Ethics (1949). This was an attempt to develop international standards of medical ethics and sought to summarize the most important principles. It did not specifically address clinical research ethics. That topic was brought to the attention of the WMA Medical Ethics Committee in 1953. After several years of discussion and research a draft declaration was finally tabled in 1961. It was adopted at the 18th WMA General Assembly, held in Helsinki in 1964. The main points in the declaration are:

- Clinical research should be based on adequately performed laboratory and animal experimentation.
- It must be conducted by scientifically qualified persons.
- There must be a protocol which will be reviewed by an ethical committee.
- The risk-benefit ratio must be favorable.
- Informed consent must be obtained.

The Declaration of Helsinki was the first time that medical ethics impinged on the regulation of new drugs. The first item could be said to be the start of the requirement for documentation to establish that sufficient preclinical research has been conducted to allow a drug to be given to a human being. In the United States, this documentation is called the investigational new drug (IND) application. It could be argued that its existence is due

largely to the ethical considerations that were behind the Declaration of Helsinki. From this requirement there grew a massive industry dedicated to preclinical research in animals. This has led to additional ethical considerations regarding the use of animals in the development of a drug. At one extreme it is argued that the use of animals in biomedical research is unnecessary because equivalent information can be obtained by alternative methods. The fact is that it may be possible sometime in the future, but is not at present. Some animal studies that were at one time considered essential for an IND, such as the LD_{50} (the lethal dose of drug required to kill 50% of the animals to which it is given), have been reevaluated and dropped from the preclinical requirements. Nonanimal evaluation can supplement animal testing and can reduce the use of animals in research (e.g., in the UK the number of laboratory animals used annually has almost halved in the past 20 years), but the technology does not exist to supplant animal studies completely. Relevant research requires intact physiological systems, and they cannot be mimicked accurately at present. It is not acceptable ethically to risk the health of a human being on the basis of nonphysiological data alone.

The Declaration of Helsinki remained unchanged until 1975 when an extensive revision, conducted on behalf of the WMA, was adopted at the 29th General Assembly in Tokyo. This amendment included an expansion of the basic principles and categories developed to address clinical research combined with therapeutic care and clinical research for purely scientific purposes.

Further revisions occurred in 1983 (Venice amendment), 1989 (Hong Kong amendment), and 1996 (Somerset West, Republic of South Africa, amendment). The last of these revisions caused something of an uproar in the medical research community. What made the balloon go up was the language of paragraph 29: "The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic and therapeutic methods. *This does not exclude placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists*" [author's italics]. To put it another way, a placebo-controlled study design could not be used ethically in drug studies unless no other treatment was available. This interpretation effectively excluded the placebo-controlled study from clinical research. Most regulatory authorities considered that the placebo-controlled clinical trial design was vital to the evaluation of drug efficacy and safety in the majority of medical conditions. Here again, as with the advent of the Declaration of Helsinki, medical ethics impinged on clinical research and drug regulatory process.

It took four years before that controversy was partially resolved. In 2000 at the General Assembly in Edinburgh, Scotland a note of clarification was attached to the Declaration of Helsinki; it read:

> [A] placebo controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:
>
> - Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety….
> - Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk of serious or irreversible harm.

This masterpiece of bureaucratic language was meant to blunt the debate, but the controversy still has not gone away. The Declaration of Helsinki continues to evolve and its presence will ensure that medical ethics and clinical research will forever be intertwined.

In the United States it was as a direct result of the revelation of the Tuskegee Syphilis Study that the next U.S. medical ethics initiative emerged. The National Research Act of 1974 was passed (Public Law 93348), which required regulatory protection for human subjects and created the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. In 1979 this commission produced the Belmont Report, named after the Smithsonian Institution's Conference Center, where the discussions were first held in 1976. The report established three ethical principles to allow problems to be solved in the area of ethics in clinical research: (1) respect for persons, (2) beneficence, and (3) justice. In general terms, these categories were equivalent to informed consent, risk–benefit assessment, and an appropriate choice of subjects for the research.

U.S. federal regulations were developed from the Belmont Report. They were adopted, in 1991 by 17 federal departments and agencies: hence the term "the Common Rule." This governs research conducted or supported by these departments and agencies. The regulations are called Title 45, Code of Federal Regulations 46 (45 CFR 46): Federal Policy for the Protection of Human Subjects. There are four parts:

1. *Subpart A*: Department of Health and Human Services (DHHS) policy for the protection of human research subjects
2. *Subpart B*: DHHS protections pertaining to research, development, and related activities involving fetuses, pregnant women, and human in vitro fertilization
3. *Subpart C*: DHHS protections pertaining to biomedical and behavioral research involving prisoners as subjects
4. *Subpart D*: DHHS protections for children involved as subjects in research

The Food and Drug Administration (FDA) has a different set of regulations governing human research, including:

- 21 CFR 50: Informed Consent
- 21 CFR 56: Institutional Review Boards
- 21 CFR 312: Investigational New Drug Application

All clinical research is subject to these and other FDA regulations. Medical ethical issues are now enshrined in U.S. law. Let the final word on ethics come from the National Bioethics Advisory Commission: "It is essential that the research community come to value the ethics of research as central to the scientific process."

31.3 HISTORY OF THE REGULATION OF MEDICAL RESEARCH

Today, there is general acceptance that clinical testing of proposed therapeutic entities is mandatory. No drug will be approved without compelling clinical evidence of safety and efficacy. Clinical studies cannot be initiated without a clearly defined preclinical development program which forms an integral part of the IND.

At the beginning of the twentieth century, preclinical testing of drugs was not obligatory, and the requirement of clinical trials to demonstrate that a drug was safe, let alone efficacious, had never been seriously considered. In the final years of the nineteenth century, Dr. Harvey W. Wiley was appointed chief chemist at the Department of Agriculture.

He embarked upon a crusade to protect the public from adulterated food and established in 1903 a volunteer "poison squad," who agreed to eat food that was treated with chemical preservatives to establish whether they were injurious to health. Among the chemicals fed to the poison squad were salicylic acid, formaldehyde, benzoic acid, and borax. After five years it was concluded that chemical preservatives should be used in foods only when necessary, a sentiment with which few would argue. Wiley expanded his interest to drugs, and by persistent lobbying and campaigning was a major force behind the inclusion of provisions to protect the public against "misbranded" or adulterated drugs in the Pure Food and Drug Act signed by President Theodore Roosevelt in 1906. This act was concerned only with violations of the food and drug regulations after they occurred. There were no provisions for testing new drugs. Three decades later the act was still in force. There were no requirements to determine the clinical safety of drugs. It took a major disaster in health care to set in motion the regulatory processes that we consider indispensable today to ensure patients' safety.

In 1932, Gerhard Domagk demonstrated that a chemical called prontesil protected mice against some bacterial infections. Subsequent evaluation showed that prontesil was metabolized to *p*-aminobenzenesulfonamide, which was known as sulfanilamide. As prontesil had been discovered in 1908, there were no intellectual property issues. Many pharmaceutical companies, including Merck, Parke-Davis, and Eli Lilly, had obtained the backing of the American Medical Association Council on Pharmacy and Chemistry (AMACPC) to market sulfanilamide in capsules and tablets for streptococcal infections. It should be noted that this AMACPC review was not a legal requirement before a drug could be marketed. In 1937 sulfanilamide was being used extensively in the treatment of a variety of infectious diseases. A small pharmaceutical company, S. E. Massengill of Bristol, Tennessee, became aware that there was an unmet need for a liquid preparation. The company's head chemist was instructed to develop such a product. The reason that a liquid formulation was not available was that a suitable solvent had not been identified. The formulation that the Massengill Company produced comprised diethylene glycol (better known now for its use as an industrial solvent), water, and flavorings, including raspberry extract. The liquid formulation was called an *elixir*. This term was reserved exclusively for formulations that contained ethanol. Elixir Sulfanilamide did not, a fact that played an important role in minimizing an iatrogenic catastrophe. The liquid formulation of sulfanilamide was "tested", but the tests that the company conducted were based on its marketability and included appearance and flavor acceptability. No toxicity testing was conducted; none was required by the Food and Drugs Act of 1906.

In the fall of 1937, Massengill's Elixir Sulfanilamide was distributed and was used by approximately 350 patients. Nearly one-third of those patients died, due primarily to renal failure. The Massengill Company's response to this disaster was as forceful as it could be; they sent out over 1000 telegrams requesting the return of Elixir Sulfanilamide from the distributors. As the extent of the tragedy became apparent, a government department, the FDA, moved with commendable alacrity to seize the remainder of the manufactured batch. Without a legal nicety in the act of 1906, the FDA would have been powerless to prevent the distribution of the remainder of the first manufactured batch. However, as Elixir Sulfanilamide did not contain ethanol, it was "misbranded." A misbranded product could be seized. Such was the fine line drawn by bureaucratic language, which prevented a tragedy from becoming a medical catastrophe. It was the medical enforcement equivalent of Al Capone being sent to prison for tax evasion rather than bootlegging, racketeering, and murder. Had the entire batch of Elixir Sulfanilamide been distributed and consumed, the death

toll would have reached several thousand. In Massengill's defense, however, it should be stated that they had contravened no laws other than the issue of misbranding.

This medical tragedy produced one positive outcome. The U.S. Congress was galvanized into passing the Federal Food, Drug and Cosmetic Act, which was introduced by Senator Royal S. Copeland and signed by President Franklin Roosevelt in 1938. It replaced the original drug legislation from 1906. This act began regulation of the pharmaceutical industry. Drug manufacturers were henceforth required to provide scientific proof of safety of new drug products before they were allowed to market them. In addition, proof of fraud was no longer necessary before action could be taken to prevent false claims being made for drugs. Hitherto, wild exaggerations were common. Labeling claims for drugs emblazoned with such names as "Warner's Safe Cure for Diabetes" could be stopped only if it could be proved that the manufacturer of the medication did not believe the claim was justified. The reversal of this absurd interpretation of freedom of speech was a major step forward in protecting consumers from unsubstantiated claims but fell well short of protecting the public from being exposed to nonefficacious "snake oil" products. Proof of efficacy was the next watershed in the protection of the public from those pharmaceutical manufacturers who were prepared to take the money but not deliver the therapeutic goods.

The legislation that was to be enacted to require proof of efficacy was forced on Congress by a most unusual and tragic set of circumstances. It was recognized by the legislators that additional controls on pharmaceutical products were required, although their concept of what type of controls did not assign efficacy a major role. In 1960, Senator Estes Kefauver initiated hearings to control unfair marketing practices. The main thrust of what became Kefauver's bill dealt with pricing and intellectual property. It paid lip service to the proof of efficacy. The bill was not popularly received and would probably never have gained sufficient support to be enacted had it not been for another horrendous medical tragedy.

Chemie Grunenthal was a German company which manufactured a wide variety of over-the-counter and prescription drugs that were sold by many different companies. One of the drugs that it manufactured was called thalidomide. It was a tranquilizer that was recommended for, among other indications, the treatment of morning sickness in pregnant women. First marketed in the mid-1950s, by 1962 it was on the market in 46 countries.

Thalidomide was marketed in West Germany in 1957, and reports started to be released concerning potentially drug-related neural toxicity. In addition, there were reports of congenital malformations in babies born to women who had taken thalidomide. The predominant malformations were limb deformities, including shortening or missing arms, with hands extending from the shoulders, and similar problems with legs. This malformation was not unknown; it had been reported as early as the eighteenth century and was called *phocomelia* after the Greek word for "seal limbs." The drug continued to be marketed despite increasing evidence that it was toxic, because preclinical testing of the drug in pregnant rats, mice, hamsters, dogs, and primates had not shown this teratogenic potential. However, in 1962 the drug was withdrawn voluntarily because of increasingly negative public opinion.

In the United States in 1960, Richardson-Merrell sought marketing approval for thalidomide under the brand name Kevadon. It never reached the market because of the resistance of an FDA medical reviewer, Dr. Francis Kelsey. It is said that she was influenced by her previous experience with the antimalarial drug quinidine, which had teratogenic activity. Her misgivings were based on concerns that peripheral neuritis had been observed in adults. This mixture of concern about safety and previous experience combined to overrule the considerable body of preclinical evidence that the drug was safe. Kelsey

exercised the bureaucrats' power to delay the approval process and thereby prevented a major medical disaster in the United States. It is believed that mine babies were born with thalidomide-induced phocomelia in the United States, whereas in the rest of the world the total is conservatively estimated at 10,000. As a result of her actions, Kelsey was given the President's Distinguished Federal Civilian Service Award by President John F. Kennedy, the highest civilian honor that can be conferred on a government employee.

The major change in drug legislation caused by the thalidomide disaster was induced by an increased public awareness and demand for drug safety. This public need was the motivation for the Kefauver bill to be redrafted. The revised Kefauver–Harris Amendment was signed into law by President Kennedy on October 10, 1962. One of the most significant effects of this legislation was the requirement that drugs were to be proven effective before they could be marketed in the United States. A safety issue was transformed into a requirement for proof of efficacy. It was the second major change in drug legislation and it was enacted almost as an afterthought.

Proof of efficacy was defined as the requirement that two adequate and well-controlled studies confirm appropriate activity. This language, crafted in the early 1960s, was to haunt the drug approval process for the next 35 years. The dye was cast for drug development. The Elixir Sulfanilamide disaster led to the requirement for proof of safety, and the thalidomide disaster was the vehicle which ensured that a drug has to be proved effective before it can be marketed. Everything relating to the drug approval process as we know it today relates back to the requirement to prove safety and efficacy. The FDA had been transformed from an agency that responded to negative drug issues to an agency that proactively scrutinized new drug development.

Since the adoption of the Kefauver–Harris Amendment, there have been innumerable modifications, additions, and changes made to the remit and responsibilities of the FDA. For example, clinical research has had to respond to the challenges of the global market. Different countries in the developed world required different data to obtain marketing approvals. This led to slower, more expensive development programs. It was proposed that within reasonable limits, a safe and effective drug should not require vastly different clinical development programs to gain approvals to be marketed in different parts of the world. Europe had pioneered harmonization in the European Community in the 1980s and initiated discussions with Japan and the United States on the harmonization of drug development requirements. These discussions culminated in the birth of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). This occurred at a meeting of the European Federation of Pharmaceutical Industries and Associations in April 1990 in Brussels. The ICH comprises representatives of the regulatory bodies and research-based industry from Europe, Japan, and the United States.

The terms of reference for the ICH were agreed on and the topics selected for harmonization were safety, quality, and efficacy. The focus was on harmonizing the technical content of blocks of data where it was apparent that there were significant regional variations in requirements. This resulted in over 60 guidelines and revisions being published in the first 10 years that the ICH existed. This phase of development was deemed complete at the 5th International Conference on Harmonization (ICH 5) held in San Diego in November 2000. The main focus of the ICH switched to harmonization of the format and content of registration applications. Ultimately, this will result in a common technical document. Other areas targeted for harmonization include new technological advances, new innovative medicines, and postmarketing issues.

The U.S. drug regulatory process is now more closely linked to Europe and Japan than ever before. The ICH guidelines are implemented after publication in the *Federal Register*. Fundamentally, however, with regards to drugs, the societal mandate in the United States has remained the same: The drug must be proven safe and effective. This led to the development of arguably the most important piece of documentation in drug development in the United States, the *new drug application* (NDA). If that document passes the FDA approval process, the drug is deemed safe and effective within the scope of the clinical program and may be given to patients. The content and format of the NDA will continue to evolve, but the basic tenets have endured: Ensure full comprehension of the participant as to the risks of the study and its methods of eliciting usable results, establish safety and efficacy, and going full circle historically, return to the Hippocratic Oath, keep [subjects] from harm and injustice. Ethics and development have come together through tortuous paths.

31.4 PRECLINICAL DEVELOPMENT

The aim of a clinical development program is to generate sufficient data to satisfy the regulatory requirements for allowing the drug to be marketed. The endpoint of this process is to prove that the drug is safe and effective in humans. The doorway to testing the drug in humans in the United States is to generate an *investigational new drug* (IND) application.

The initial animal studies to determine pharmacological effects are usually conducted using laboratory-scale drug synthesis. After the initial in vitro and in vivo tests have shown preclinical "proof of principle", the scale-up process is begun. The initial scale-up is usually between a few hundred grams to a kilogram, depending on the complexity of the synthesis and whether the synthetic route is scalable (i.e., chromatography steps can be accommodated in the scale-up or there are no potentially explosive steps that would preclude scale-up). For drugs that have little or no toxicity, the scale-up will have to be on the order of tens of kilograms, as the IND enabling toxicology evaluations may have to go to 100-fold the expected human dose. The next major event is the manufacture of good manufacturing practice (GMP) material.

Good manufacturing practice is a system for ensuring that products are produced consistently and controlled according to quality standards. This system forms an integral part of the manufacturing process. It is considered necessary for the following reasons:

- To assure consistency between and within batches of the investigational product and thus assure the reliability of clinical trials
- To assure consistency between the investigational product and the future commercial product and therefore the relevance of the clinical trial to the efficacy and safety of the marketed product
- To protect subjects of clinical trials from poor-quality products resulting from manufacturing errors (omission of critical steps such as sterilization, contamination and cross-contamination, mix-ups, wrong labeling, etc.) or from starting materials and components of inadequate quality
- To document all changes in the manufacturing process

In the early clinical trials the dosage form may be different from the commercial product (e.g., capsule instead of tablet), but for the pivotal studies on which registration is based

it should be the same as for the commercialized product. It is accepted that validated analytical procedures may not always be available for the early clinical trials, so provisional production parameters and in-process controls should be deduced from experience with analogous products. In short, the GMP system evolves during the clinical development but should be the same as is required by marketed product for the drug that is used in the pivotal trials.

It is not necessary to manufacture material for any of the preclinical studies to GMP. Some companies like to use GMP material for the IND-enabling toxicology studies, but a reasonable compromise is to use material that has a certificate of analysis. The progression in GMP manufacture is usually facilitated if the last non-GMP batch is larger than the GMP batch. The latter faces only the stringency of the in-process controls and intermediate and API release specifications, not the potential problems of those controls applied to a scale that has not previously been manufactured. The aim in the succeeding scale-up is not to produce a drug that is significantly different (API or impurities) from the drug that was used in the IND-enabling in vitro and in vivo studies.

There are two main reasons for conducting preclinical in vitro and in vivo studies:

- To characterize the drug and investigate its utility in models of possible therapeutic targets
- To satisfy the regulatory requirements to allow clinical development of the drug to advance

The characterization and assessment of possible utility are not subject to any stringent controls other than ethical committee approval when animals are used. As an example of such a program, here is a potential feasibility assessment of a topical application of a drug to treat atopic dermatitis. The initial study could be to determine activity: for example, applying the drug topically to prevent an injection of zymosan-inducing paw edema in a mouse model, or topical application to prevent a delayed-type hypersensitivity response to 2,4-dinitroflurobenzene in a mouse model. The next stage in characterization could be assessing penetration of the drug to the dermis, which is the target site. The mini-pig has skin which is structurally similar to human skin, so that an analysis of a cutaneous punch biopsy after topical application of the drug would determine penetration. The third model in the proof of principle program would be topical application of the drug to a mini-pig model of atopic dermatitis to assess efficacy. In three simple studies, answers are found as to whether the drug delivered topically is active, penetrates to the required site of action, and is effective. It must be accepted that animal models are not necessarily predictive of human efficacy or safety (e.g., the failure of animal models to predict the mutagenic activity of thalidomide), but a positive set of efficacy results in animal studies is usually grounds for advancing the development program. Should the results of the animal studies be negative, there is scant reason to continue the program. These studies are not usually subjected to the preclinical documentation requirement of *good laboratory practice* (GLP), the equivalent for preclinical testing to GMP for manufacturing. However, these studies, if conducted pre-IND, will be used in the pharmacology/toxicology section of the IND to establish a rationale for exposing the drug to humans and must therefore be adequately documented.

In the same way as the bureaucratic noose tightens the GMP requirement as the development program approaches the pivotal clinical studies, so the bureaucratic requirements for preclinical testing strengthen as the studies become the determinant of whether a drug can be given to humans. Throughout the clinical development, additional nonclinical studies

are required, but initially the requirement is for IND enabling studies so that the drug can be given to humans. The standard requirements are:

1. Single- and repeat-dose toxicity studies
2. Pharmacokinetic, toxicokinetic, and absorption, distribution, metabolism, and excretion (ADME) studies
3. Genotoxicity studies
4. Safety pharmacology studies

It should be noted that all of the studies listed above relate to drug safety, and none to preclinical proof of concept. This is the dividing line between studies that require the bureaucratic vigor of GLP and those that do not. The IND-enabling studies have to be conducted to GLP standards. GLP was developed to promote the quality and validity of the test data used for determining the safety of chemicals and chemical products. The FDA published GLP regulations for nonclinical studies in 1976, and they provided the basis of the Organisation for Economic Co-operation and Development guidelines that made GLP international in 1978. Like the Declaration of Helsinki and the ICH guidelines, the OECD GLP guidelines remain under continuous review and are updated periodically.

Good laboratory practice is a quality system concerned with the organizational process and the conditions under which nonclinical health and environmental safety studies are planned, performed, monitored, archived, and reported. The guidelines examine the requirements for test facility organization and personnel, such as qualifications, training (including training logs of updates), and standard operating procedures. They define the requirements of the quality assurance program, including documentation, inspections, and sign-off of final reports. There are guidelines on facility utilization, apparatus, materials and reagents, test systems, test articles, study performance, report quality, and the storage and retention of records and materials.

1. *Toxicity studies*. One program in the safety evaluation straddles the requirement for GLP. The toxicology program frequently starts with dose-finding studies to determine the high, middle, and low doses that will be used in the repeat-dose toxicity studies to support phase I clinical studies. These studies can include single-dose escalation and short-duration multiple-dose studies (approximately 5-day dosing). Usually, these studies record gross pathology without histology. They are not subject to GLP but will, of course, be reported in the IND. The IND-enabling repeat-dose toxicity studies should be conducted under GLP. These studies are usually conducted in two mammalian species (one nonrodent) and should be equal or exceed the duration of the human clinical trials proposed. If, in the later stages of development, the duration of the dosing period increases in the human clinical trials, additional toxicity studies must be conducted of sufficient duration to support those trials. For a chronic treatment it is necessary to conduct a six-month study in rodents and at least a nine-month study in nonrodents.

2. *Pharmacokinetic, toxicokinetic, and ADME studies.* As a part of the toxicity studies, or in other studies, toxicokinetics should be performed. *Toxicokinetics* is defined as the generation of pharmacokinetic data in order to assess systemic exposure. These data can be used in the interpretation of toxicology findings and their relevance to clinical safety. For meaningful results to be generated, analytical methods must have been developed with the analytes (API, metabolites, etc.) and matrices (plasma, whole blood, tissue,

etc.). These methods are under continuous review as additional information is gathered on metabolism and species differences. The drug and metabolite distribution in tissues should be determined. The ICH members are in agreement that single-dose distribution studies should form part of the preclinical evaluation. There are circumstances when it will be necessary to conduct repeat-dose tissue distribution studies. They would be appropriate for compounds that have a long half-life, incomplete elimination, or unanticipated organ toxicity.

3. *Genotoxicity*. Tests are designed to show whether a drug can induce genetic damage. The ICH guidance on the standard battery for genotoxicity testing of drugs advocates the following as an initial assessment:

- A test for gene mutation in bacteria
- An in vitro test of chromosomal damage
- An in vivo test of chromosomal damage using rodent hematopoietic cells

If these tests are negative, it is usually considered that no additional testing of genotoxic activity is required. Positive tests will require additional evaluations.

4. *Safety pharmacology studies*. Safety pharmacology studies are those studies that determine undesirable pharmacodynamic effects of a drug on physiological functions. The most important of these are effects of physiological functions that are critical for life, cardiovascular, respiratory, and central nervous systems. However, if a drug is targeted to affect a disease process in another system with a specific effect such inflammatory cell migration in the gastrointestinal tract, closer scrutiny of that system may be necessary.

Hot topic issues will always ensure that this preclinical category remains under consideration. Recent acknowledgment of enhanced proarrhythmic risk is an example. Much attention is being paid to nonclinical evaluation of the potential for delayed repolarization (QT interval prolongation) by pharmaceuticals.

Like the process chemistry modifications and formulation development, the nonclinical evaluation process continues well past the IND enabling phase. Additional preclinical evaluation will be necessary. For example, if human beings of reproductive age will be treated by the drug, reproductive toxicology will be necessary before an NDA can be filed, and if the drug is to be dosed chronically, carcinogenicity studies will be required. The nonclinical evaluation process continues throughout the clinical development.

Once sufficient preclinical data have been gathered, there is an opportunity to meet with the FDA for a prefiling IND assessment. This pre-IND meeting allows the sponsor to outline the basic elements that will be in the IND and to seek the FDA's view as to whether the filing appears to meet the requirements that will allow the IND to be approved and thus allow clinical trials to begin. The structure of the IND is detailed below.

1. *Introductory statement*. This includes the name of the drug and all active ingredients, the pharmacological class, the structural formula, the method of formulation, the route of administration, and a summary of previous human experience.

2. *General investigational plan*. This should cover the investigations that will be conducted during the next year and a rationale for this approach.

3. *Investigator's brochure*. The most important feature of this document, which is the primer for the investigating physician, is the summary of safety and efficacy and the pharmacokinetics and biological distribution of the drug in animals.

4. *Protocols*. These give the details of the types of studies that will be conducted, in which subject population, for how long, with what variables. The protocols also list the qualifications of the investigators and subinvestigators and the name and address of the investigational review board.

5. *Chemistry, manufacturing, and control* (CMC) *information*

a. Drug substance [active pharmaceutical ingredient (API)], including the general methods of preparation, the analytical methods used to assure identity, strength, quality, and purity, and data supporting the stability of the drug substance for the duration of the toxicology studies

b. Drug product, including all components, analytical methods for release, a brief description of the manufacturing and packaging procedures, and sufficient data to assure the product's stability during the planned clinical studies

6. *Pharmacology and toxicology (pharm/tox) information.* This includes data from animal and in vitro studies:

a. Pharmacological effects and mechanisms of action and drug disposition, including effects and mechanisms of action and information on absorption, distribution, metabolism, and excretion. Safety pharmacology data must be available on the effects in animals on vital functions such as cardiovascular, central nervous, and respiratory systems.

b. Integrated summary of toxicological effects of the drug in animals and in vitro. The clinical studies proposed determine the duration of toxicology testing required, whether reproductive toxicology is required, and whether special toxicity tests due to the drug's route of administration are required. Prior to human exposure, in vitro tests for the evaluation of mutations and chromosomal damage are generally required.

There are a number of other sections that may be required, depending on the drug being evaluated. These include sections on previous human experience and dependence and abuse potential.

The data-driven elements in the IND are the CMC and pharm/tox Sections. The mechanism of drug synthesis has to have been defined and analytical methods developed to ensure reproducible quality that is sustainable over time. It is recognized that as manufacturing scale-up occurs, changes will take place in the synthetic pathway, but care has to be taken to ensure that the drug substance and impurity profile remain the same as for the batches of drug substance used in the IND enabling toxicology or safety pharmacology studies. If there is a significant deviation from the analytical release specifications, it could invalidate the results of those studies, which would have to be repeated; bridging studies conducted to show that the current drug is essentially similar in its effects to the original drug; or in the worse-case scenario, a new IND would have to be filed.

The filing of an IND takes the development process to the next level. If the IND is accepted by the FDA [i.e., if it conforms to the content and format laid down in the Code of Federal Regulations (21 CFR 312.23)], the FDA has 30 days to comment. At the end of this time, the clinical study may be started whether or not comments have been received. If the FDA determines that there is inadequate information to justify administering the

drug to humans, the program is put on clinical hold until such times as adequate data are provided to support giving the drug to humans, once this is achieved, the clinical phase of the development program has begun.

31.5 CLINICAL DEVELOPMENT

The IND is a living document that remains open for as long as the drug remains under investigation. As each new study is designed, the sponsor must submit to the FDA a protocol amendment containing the protocol for the next study. Safety reports must be made to the FDA within 15 calendar days of the sponsor being made aware of either a serious and unexpected adverse experience or any finding from nonclinical studies which suggests a significant risk to human subjects, such as reports of mutagenicity, teratogenicity, or carcinogenicity. The sponsor must submit annual reports that contain individual study information and summary information which comprises all information obtained during the previous year's clinical and nonclinical investigations, including a summary of all safety reports generated during the year. Information amendments are made to the IND when essential information is not contained in any protocol amendment, safety report, or annual report. An example of the content of an information amendment would be new chemistry or other technical information.

The clinical phase of the development program is the most complex and costly. The overriding concern is for the safety of the clinical trial subjects. Like the manufacturing code of practice (GMP), and the preclinical evaluation code of practice (GLP), there is a code of practice for the human investigation phase of the development program, *good clinical practice* (GCP). Unlike GMP and GLP, where there are development stages that do not need to adhere to these regulations, all clinical trials must conform to GCP. The ICH GCP guidance is broadly in line with the Declaration of Helsinki. The main sections deal with:

- The Institutional Review Board
- The responsibilities of the sponsor of the clinical trial and the investigator who will conduct the trial
- The clinical trial protocol
- The investigator's brochure
- The essential documents for the conduct of a clinical trial

This aim of GCP guidance is to minimize the potential for harm to befall a clinical trial subject. This governs the entire structure of the clinical development program. For example, the numbers of patients entered into studies would form an inverted pyramid. The earliest evaluations will be conducted in less than 100 subjects, while the last studies in the clinical development program may include hundreds or even thousands of subjects. This is an attempt to minimize exposure to the drug at a stage when the least is known about its possible toxicity and maximize it to determine safety and efficacy in advance of applying for approval to market the drug.

The goal of the clinical development program is to generate sufficient clinical data of acceptable quality to file a new drug application (NDA) with the FDA so that they may determine whether the drug can be approved for marketing. Probably the single most important document in an NDA is the *label text*, also called the *package insert*, a summary

of the drug that is made available to prescribers and patients. The information that is contained in the label text summarizes what the FDA has approved to be marketed. Any other use is called *off-label* and is not sanctioned by the FDA. If something goes wrong, the physician has no defense to the accusation that he misused the drug. The marketing company is also bound completely by the label text. There can be no advertising using information outside the label text. The power of the document dictates that it should be constructed before clinical development is started so that the clinical research efforts are focused on that endpoint. When marketing, medical, and regulatory departments first develop the label text there is often a tendency to craft language that looks like: "tastes like chocolate, cures cancer, and costs a buck." As long as there is some justification based on the nonclinical data for aiming at these best possible goals, they must be considered feasible endpoints. Of course, it must be accepted that this exercise will require continual modification as hard data become available, but ultimately, by focusing the clinical development program on the maximum potential of the drug, the end result should be the best that the drug can deliver for the patients who take it.

If the preclinically devised label text is the esoteric goal of the clinical development program, the operational aspect of the program is dictated by need for clinical trial subject safety. The traditional terminology divides the program into four temporally related phases. There are well-established definitions of these phases:

Phase I This phase starts with the first administration of the drug to human subjects. The initial study in phase I (called phase Ia) is directed at an evaluation of safety. As it often consists of single ascending doses, there may be no opportunity to evaluate therapeutic efficacy endpoints. As the subjects that are most often used in these studies are healthy volunteers, no disease-related efficacy endpoint is possible. The subjects are usually male, due to the requirement for reproduction toxicology data before women of childbearing potential can be entered into clinical trials and there being no necessity to submit such studies in the IND. The subjects in the majority of phase I studies are admitted to hospital or a specialized phase I unit and monitored continuously. The initial trial will use an open design; in other words, both the investigator and the subject know that all doses will be test articles and the doses of the test articles will be administered using an ascending-dose regimen. A safety evaluation will be made after each dose is administered before the next dose is given. The assessment of pharmacokinetics and initial data on the characterization of the drug's absorption, distribution, metabolism, and excretion will be sought. There are too many exceptions to this standard phase I evaluation of drugs to allow a comprehensive account of all types of phase I programs. The following are common variants:

- The subjects are patients because the inherent toxicity of the drug under evaluation precludes it from being given to volunteers; such a situation may arise in the early evaluation of an anticancer drug.
- The subjects are patients because the disease process makes it irrelevant what happens in volunteers; topically applied dermatological drugs for the management of skin conditions are usually evaluated for safety in patients that have a disrupted skin surface, as the healthy skin is a natural barrier.
- A surrogate endpoint exists (e.g., a marker that shows activity but is not predictive of therapeutic activity) so that pharmacodynamic data can be generated. For example, a drug for asthma that exerted its effect by reducing inflammatory mediator release

from white cells had a pharmacodynamic evaluation conducted in the initial phase I single-ascending-dose study. Peripheral white cells were harvested and the release of the inflammatory mediator leukotreine B_4 (LTB_4) was monitored after inflammatory mediator release was stimulated by calcium ionophore. The less LTB_4 released after the stimulation, the better the dose of the drug was working.

After the initial single-ascending-dose safety study is completed, the next step is to conduct a multiple-dose safety study (phase Ib). The aim in these studies is to get to steady-state plasma concentrations (i.e., the highest concentrations that will ever be achieved form a particular dosage regimen). The same potential variations in populations and endpoint exist in this late phase I design as did in the first administration to human subjects in the single-ascending-dose study. The multiple-dose study is often conducted using an ascending-dose protocol where the lowest dose is given first and a safety evaluation is conducted before administering the next-highest dose. In cases where the nonclinical and phase Ia safety profiles are benign, it may be possible to run the different doses in parallel, thereby saving a considerable amount of development time.

The total population required for the phase I studies is on the order of 20 to 100. The industry norm for the duration of phase I is less than one year. Of the drugs that enter phase I studies, approximately 70% are progressed to the next phase.

Phase II Once the drug has been shown to be safe, it will be tested for efficacy. The population to be tested will be patients. Care is still taken to minimize the risk to the study population. The inclusion and exclusion criteria which define patients that are eligible for the study usually exclude significant concomitant disease other than the disease or condition that the drug is intended to prevent, diagnose, or treat. There are also usually restrictions on the use of concomitant therapy. Considerable emphasis is still laid of safety evaluation, but the patients may be treated as outpatients unless the disease requires hospitalization. One of the most important endpoints of the phase II is the definition of the dose or dose range to be taken into Phase III. The objectives will include an assessment of the minimum dose that is maximally or sufficiently effective and free of significant toxicity. An evaluation of the primary endpoint to be used to determine efficacy in phase III is usually a major objective. For example, the results in nonclinical studies may suggest that an investigational drug will accelerate the healing of a chronically painful skin lesion and will be assessed in phase II with that endpoint. The proposed label text will have language related to speed of healing. If, during phase II it becomes apparent that healing is not accelerated but chronic pain is controlled, the option will exist to advance to phase III, with the primary variable being speed of healing in the hope that the phase II results were anomalous, or switch to pain relief as the primary variable understanding that the proposed label text will have to be changed to reflect the change in study endpoint.

The FDA describes phase II trials as follows: “Phase II includes controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common or short term side effects and risks associated with the drug. Phase II studies are typically well-controlled, closely monitored, and conducted in a relatively small number of patients.” Whereas the phase I studies were usually conducted with an open design, the phase II studies are randomized. One or more groups of patients, depending on the number of doses being studied, will receive the study drug while another group, the control group, will receive a placebo with or without standard therapy. These studies

are usually double-blind; neither the patient nor the investigator knows who is getting test drug, what dose of test drug is being administered and who is getting placebo. This increases the complexity of the operational aspects of drug packaging and allocation of treatments. The investigational drug must be packaged in an identical manner to the placebo and which must be given to the patients in accordance with a pre-determined randomization code.

The overall aim of the phase II studies is to define the type of studies that will be conducted in phase III, including dose, duration of dosing, frequency of dosing, patient population, and primary variable. When this evaluation is poorly conducted, the phase III outcome becomes much more of a guessing game than is necessary. Additional time spent in phase II to establish the best design for phase III is likely to be returned by a shorter successful phase III. Companies that truncate phase II so that they can start pivotal phase III studies as early as possible do so at their own peril. The end result is often a case of more haste, less speed.

Once phase II is completed, the option exists to meet with the FDA before starting phase III. The FDA regulations state that the purpose of the end-of-phase II meeting is:

- To determine the safety of proceeding to phase III
- To evaluate the phase III plan and protocols
- To identify any additional information necessary to support a marketing application for the uses under investigation

As the third bullet point suggests, this meeting is not confined to evaluating only the phase III program. The FDA definition of the end-of-phase II meeting states that the focus should be "directed primarily at establishing agreements between FDA and the sponsor of the overall plan for phase III and the objectives and design of particular studies. The adequacy of technical information to support phase III studies and/or a marketing application may also be discussed." Phase II is usually conducted in a few hundred patients. Of the seven out of 10 drugs that complete phase I successfully, only four will complete phase II and advance to phase III.

Phase III The studies conducted in phase III are the NDA-enabling studies. In the Federal Food, Drug and Cosmetic Act of 1938 (FFDCA), Drug Amendments of 1962, language regarding clinical study approval requirements read: "… adequate and well controlled investigations …," which was interpreted by the FDA as a minimum of two such phase III studies. In the mid-1990s Carl Peck, M.D., the ex-director of the FDA Center for Drug Evaluation Research, who had left the FDA to start the Center for Drug Development Science, at Georgetown University in Washington, DC, questioned the validity and utility of this interpretation. Over the next few years this evolved into the concept of single clinical trial submissions, where only one adequate and well-controlled phase III study may be required in certain circumstances.

In 1997, President William Jefferson Clinton signed the Food and Drug Administration Modernization Act. The language in Section 115a related to the evidence of effectiveness required to approve a new drug. It was substantially different from the FFDCA with Drug Amendment of 1962: "…data from one adequate and well-controlled investigation and confirmatory evidence." The implication is clear; it is no longer mandatory that two adequate and well-controlled studies (i.e., two phase III studies) are necessary for proof of effectiveness required to approve a new drug. A debate still rages over what constitutes "confirmatory evidence."

One option for the basis of regulatory approval is a single phase III clinical study plus causal confirmation. *Causal confirmation* has been described by Peck et al. as proof that "the drug, through its pharmacological action, favorably alters the clinical condition of those who are treated with it." In other words, proof of pathophysiologic and pharmacologic mechanisms is sufficient to be considered confirmatory.

Simplistically, approval to market a drug could be based on one positive, adequate, and well-controlled phase III study supported by a phase II study where the endpoint was a surrogate endpoint marker. For example, if it is accepted that chest x-ray findings may be indicative of pulmonary function deterioration and the claim for the drug will be preservation of pulmonary function, one adequate and well-controlled phase III study with a pulmonary function endpoint supported by one phase II chest x-ray positive finding adequately fulfills the requirements of the act.

There are a number of additional questions that should be addressed in the phase III program program. The primary requirement is to confirm the findings of safety and efficacy in the phase II program. Inclusion and exclusion criteria to define the study patient population tend to be less rigorous in the phase III program, so the patients more closely resemble the population who will be administered the drug when it is marketed. For chronic treatments there is a requirement to dose for longer periods than are normally considered in phase II. The ICH in its efficacy guideline E1 has determined that the exposure to assess clinical safety for drug intended for chronic administration in non-life-threatening conditions should be at lease 500 patients for six months and 100 patients for one year.

Different drugs require different programs to develop sufficient evidence to gain marketing approval. Special population clinical study requirements are important (e.g., the elderly or children). Ultimately, the aim of this gargantuan clinical development program is to establish sufficient cause, safety, and efficacy to allow a new drug to be added to the physician's therapeutic armamentarium for the benefit of patients.

The total patient population required to complete phase III is highly variable. The ICH states that a minimum of 1500 patients should be exposed to the drug before a marketing application should be assessed, but that may be wholly inadequate in terms of the number of patients who might be required in clinical trials to satisfy the statistical considerations for proof of efficacy. Alternatively, in rare conditions, dealt with in 21 CFR 360.20, Orphan Drug Regulations, significantly fewer patients may be required to satisfy the phase III commitment. (Orphan drug status in the United States can be sought for a drug intended for diseases or conditions affecting less than 200,000 patients per year. In Europe the patient population should not exceed 185,000 patients per year, and in Japan, 50,000 patients per year.) Approximately 70 to 90% of drugs that enter phase III eventually make it to the market.

Phase IV There is one additional phase of drug development which is unrelated to providing documentation for the initial NDA. This is called phase IV. These studies are run to generate additional data on the drug that are not necessary for the approval process but might be used to modify the label text. In phase IV the following information may be sought:

- Relative efficacy compared to different drugs used to treat the same disease
- Cost-effectiveness of the drug
- Assessment of the improvement in the patient's quality of life
- Safety assessment in an unselected patient population
- Opportunities for additional indications

The clinical development of drugs is usually defined as sequential testing through phases I to III for approval and phase IV for additional definition. The process, however, should not be thought of as strictly sequential development. In ICH guideline E8, General Considerations for Clinical Trials, an alternative nomenclature is proposed which more appropriately reflects the development process. Whereas it is usually correct to say that phase II does not start until an adequate phase I is conducted and phase III will not begin until an appropriate phase II has been completed, just because the drug is being evaluated in phase III does not mean that assessments usually associated with phases I and II are not continuing to be developed.

E8 defines the development process as follows:

- *Human pharmacology* (roughly equivalent to phase I)
  - Assess tolerance.
  - Define pharmacokinetics and pharmacodynamics.
  - Estimate activity.
- *Therapeutic exploratory* (roughly equivalent to phase II)
  - Explore use for targeted indication.
  - Define dose range for subsequent studies.
  - Generate data to determine study designs.
- *Therapeutic confirmatory* (roughly equivalent to phase III)
  - Confirm efficacy.
  - Demonstrate safety as well as can be defined within the limited exposure of a clinical program.
  - Establish a dose–response relationship.
- *Therapeutic use* (roughly equivalent to phase IV)
  - Refine risk–benefit relationship.
  - Identify less common adverse reactions.
  - Refine dosing recommendations.

The concept of a logical serial development of a drug has much to commend it, but frequently there are misunderstanding as to the actual stage of development by misinterpreting the underlying basis of the four temporal phases. Frequently, in the more sophisticated development programs there may be as much or more human pharmacology assessment during phase III as there was during phase I.

31.6 CONCLUSIONS

When a hypothesis has been developed, when a chemical synthesis program has been developed, when an exploratory preclinical program has been completed, and when the clinical study evaluation all contribute to the data that generates an approved NDA, all the disciplines of research and development come together to provide a tool to the physician to improve the life of his or her patients.

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