PREPARATION OF 15-HYDROXY-$\Delta^{13,14}$-MILBEMYCIN A_4 AND ITS TRANSFORMATION TO 13-ALKYLMILBEMYCINS

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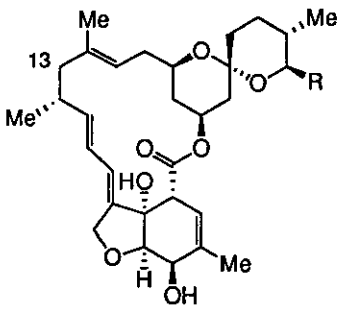
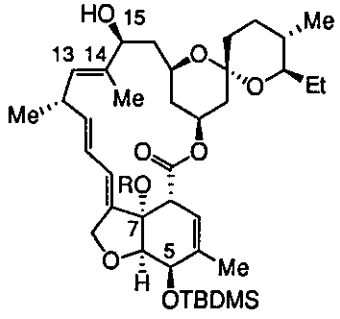
Abstract—13-Alkylmilbemycins were synthesized regiospecifically by Claisen rearrangement of a 15-hydroxy-$\Delta^{13,14}$-milbemycin derivative, which was prepared selectively from a 14,15-epoxymilbemycin derivative by isomerization of the 14,15-epoxide moiety induced by ethylaluminum bis(2,6-diphenylphenoxide).

Milbemycins (**1**), isolated from *Streptomyces hygroscopicus*, are a family of 16-membered ring macrolides and exhibit acaricidal, insecticidal and anthelmintic activities.[1] During the course of our investigations on chemical modification of milbemycins with the aim of improving their original anthelmintic activities, introduction of substituents at C_{13} of milbemycins intrigued us because such modification would change their pharmacokinetics. In our preceding paper,[2] we disclosed the synthesis of 13-alkyloxymilbemycins and reported that such modification was found to be successful in improving the anthelmintic activities of the parent milbemycins. These findings prompted us to synthesize 13-alkylmilbemycins for further investigation. In this report, an efficient procedure for preparing a 15-hydroxymilbemycin derivative and transformation of the 15-hydroxymilbemycin to 13-alkylmilbemycins are described.

Scheme 1.



1a: milbemycin A_4 (R=Et)
1b: milbemycin A_3 (R=Me)
1c: milbemycin D (R=*i*-Pr)

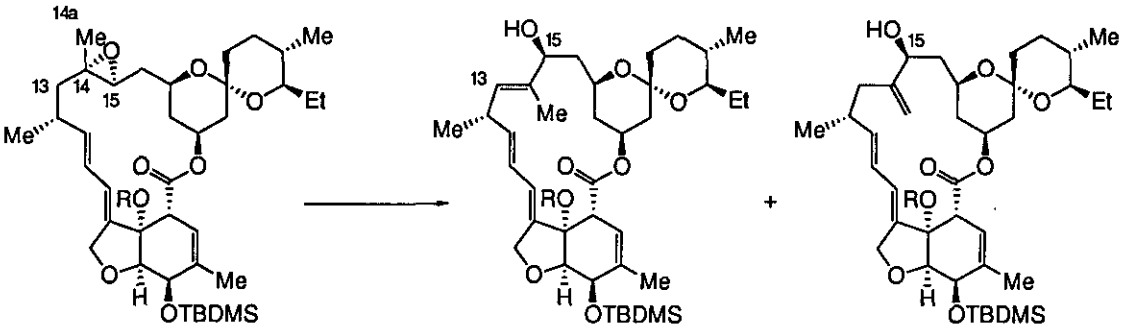


2 R=Me_3Si-
TBDMS=*tert*-$BuMe_2Si$-

Several methods[3] are known to introduce substituents at C_{13} of milbemycins by forming carbon-carbon bonds; albeit, these methods suffer from production of regio- and stereoisomers. For the selective introduction of alkyl substituents at C_{13} of milbemycin A_4 (**1a**), 15-hydroxy-$\Delta^{13,14}$-milbemycin A_4 (**2**) has emerged as the key intermediate. It was assumed that Claisen rearrangement at the C_{13}-C_{15} allylic alcohol moiety of the compound (**2**) would give 13β-alkylmilbemycins selectively.

Firstly, preparation of **2**[4] was investigated. Isomerization[5] of the 14,15-epoxide moiety of **3**[6] by reactions with 9-borabicyclo[3.3.1]nonyl triflate[7] (9-BBNOTf) and trimethylsilyl triflate[8] (TMSOTf) gave **2** in good yields; however, in both reactions exo-methylene derivative (**2exo**) was also isolated as a by-product (Table 1, entries 1, 2). Then, organoaluminum reagents[9] bearing hindered phenoxy substituents on aluminum were examined for the isomerization reaction with the hope that the amphoteric property of aluminum would work well for activation of the carbon-oxygen bond of the epoxide moiety and removal of the hydrogen at C_{13} or C_{14a}. After surveying a variety of phenoxy groups on aluminum and the reaction conditions, ethylaluminum bis(2,6-diphenylphenoxide),[10] $EtAl(DPP)_2$, was found to be the reagent of choice for converting the epoxide (**3**) to the allylic alcohol (**2**). In the reaction of **3** with $EtAl(DPP)_2$, the isomer (**2exo**) was not detected by hplc analysis (Table 1, entry 4).

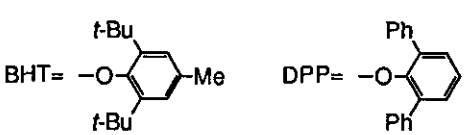
Table 1. Isomerization of epoxide (**3**) to allylic alcohol (**2**).



3 (R=trimethylsilyl) **2** (R=trimethylsilyl) **2exo** (R=trimethylsilyl)

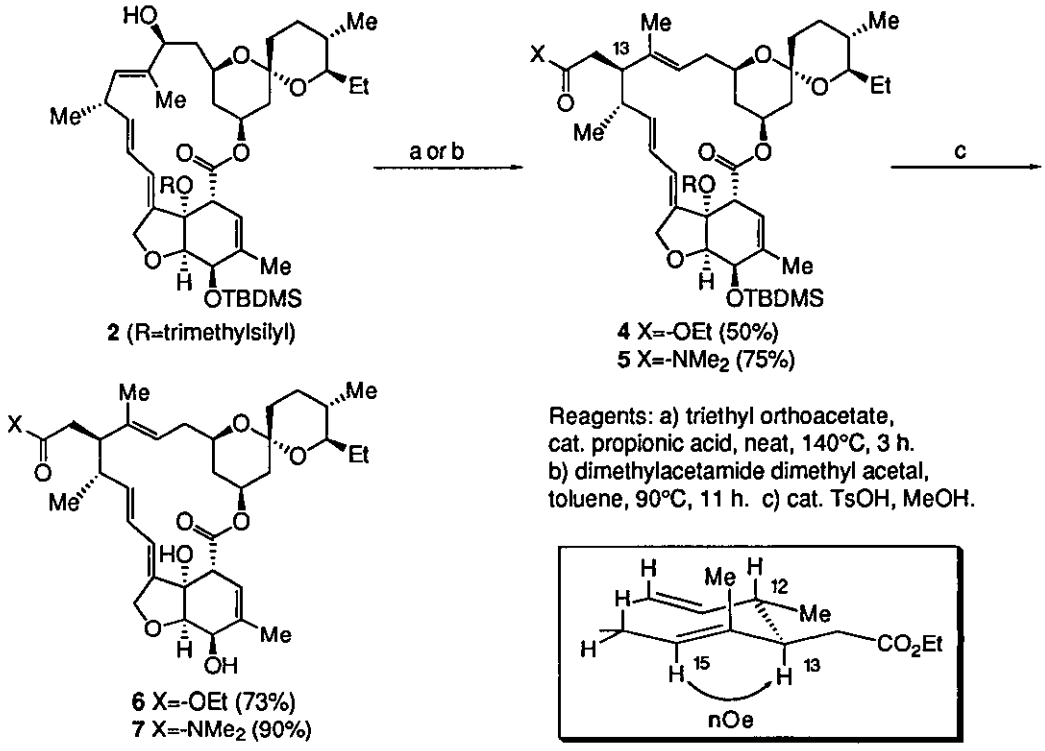
entry	reagents	solvent	temperature[b]	time (h)	yield (%)	
					2	**2exo**
1	9-BBNOTf/2,6-lutidine	hexane	0°C~rt	2	84	10
2	TMSOTf/2,6-lutidine[a]	hexane	0°C	19	88	6
3	$EtAl(BHT)_2$ (3.0 equiv)	toluene	-78°C~rt	1	72	9
4	$EtAl(DPP)_2$ (3.0 equiv)	toluene	0°C~rt	5	91	-

[a] Deprotection of the trimethylsilyl group at C_{15} was carried out after the isomerization reaction.
[b] rt: room temperature.



Having secured an efficient procedure to obtain the 15-hydroxide (**2**), Claisen rearrangement of (**2**) was next carried out. Meerwein-Eschenmoser-Claisen[11] and Johnson-Claisen[12] rearrangements were applied to the compound (**2**), and the corresponding rearranged products (**4**) and (**5**) were isolated in 50 and 75 % yields, respectively. Subsequent deprotection of the silyl groups of the compounds (**4**) and (**5**) in methanol containing a catalytic amount of *p*-toluenesulfonic acid afforded 13-alkylmilbemycins (**6**) and (**7**) (Scheme 2.).

Scheme 2.



The stereochemistry at C_{13} of the synthesized 13-alkylmilbemycins was confirmed to be the β-configuration on the basis of nOe difference experiments, taking account of the local structure of the milbemycin skeleton.[1a,b,e] In the case of the compound (**6**), for example, irradiation of the C_{15} hydrogen at δ 5.11 gave an nOe enhancement of the C_{13} hydrogen at δ 2.36.

In summary, a 14,15-epoxymilbemycin derivative was selectively converted to a 15-hydroxymilbemycin derivative in excellent yield by employing ethylaluminum bis(2,6-diphenylphenoxide) for isomerizing the 14,15-epoxide moiety, and 13β-alkylmilbemycins were regiospecifically synthesized from the 15-hydroxymilbemycin derivative by Claisen rearrangement.

EXPERIMENTAL

Milbemycin A_4 isolated from *Streptomyces hygroscopicus* was used as the starting material, which was

purified by column chromatography prior to experimentation and showed >96% purity by hplc analysis. All compounds were characterized by nmr spectrometry on a JEOL GSX 400 or a JEOL GX 270 spectrometer in $CDCl_3$ solution with tetramethylsilane as internal reference; by mass spectrometry on a JEOL JMS-AX 505H model; and by ir spectrometry on a JASCO FT/IR-830, and were in full agreement with the assigned structures. Unless otherwise indicated all common reagents and solvents were used as obtained from commercial suppliers without further purification.

14,15-Epoxy-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilylmilbemycin A_4 (3).

3 was prepared according to the literature procedure.[6]

13-Dehydro-15-hydro-15-hydroxy-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilyl-milbemycin A_4 (2).

To a solution of **3** (75 mg, 0.1 mmol) in toluene (0.3 ml) at 0 °C was added 1M-toluene solution of ethylaluminum bis(2,6-diphenylphenoxide), which was prepared by reacting Et_3Al (1M in toluene) and 2 equiv of 2,6-diphenylphenol.[10] The reaction mixture was stirred at room temperature for an additional 5 h, and poured into 0.1 N-HCl solution, extracted with CH_2Cl_2, and the extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel chromatography to give 70 mg of **2** (91% yield).

2: Ir(KBr)cm^{-1} 1745, 1250, 1167, 989, 840; ms m/z 744(M^+), 484, 167, 73; ^{1}H-nmr(400 MHz, $CDCl_3$) δ 5.80(1H, dd, *J*=11.4 and 14.7 Hz, H-10), 5.65(1H, br d, *J*=11.4 Hz, H-9), 5.49(1H, br s, H-3), 5.23(1H, dd, *J*=10.2 and 14.7 Hz, H-11), 5.16(1H, d, *J*=8.9 Hz, H-13), 4.64-5.19(2H, m, H-19 and H-8a), 4.54(1H, dd, *J*=2.2 and 14.2 Hz, H-8a'), 4.37(1H, m, H-5), 4.08(1H, m, H-15), 3.86(1H, d, *J*=5.6 Hz, H-6), 3.42(1H, m, H-17), 3.30(1H, m, H-2), 3.06-3.15(1H, m, H-12), 3.00(1H, dt, *J*=2.4 and 9.5 Hz, H-25), 1.78(3H, br s, H-4a), 1.62(3H, s, H-14a), 1.12(3H, d, *J*=6.5 Hz, H-12a), 0.98(3H, t, *J*=7.3 Hz, H-25b), 0.94(9H, s, *t*-butyl-Si), 0.82(3H, d, *J*=6.5 Hz, H-24a), 0.15(3H, s, MeSi), 0.14(3H, s, MeSi), 0.12(9H, s, Me_3Si); *Anal.* Calcd for $C_{41}H_{68}O_8Si_2$: C,66.09; H,9.20. Found: C,65.64; H,9.21.

13-Ethoxycarbonylmethyl-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilylmilbemycin A_4 (4).

A mixture of **2** (75 mg, 0.1 mmol), triethyl orthoacetate (0.2 ml, 1.1 mmol) and propionic acid (2 mg, 0.03 mmol) was heated at 130 °C for 2 h. The resulting mixture was concentrated *in vacuo* and the residue was purified by preparative thin layer chromatography to give 41 mg of **4** (50% yield).

4: Ir(KBr)cm^{-1} 1741, 1251, 1169, 989, 840; ms m/z 814(M^+), 572, 223, 167, 73; ^{1}H-nmr(400 MHz, $CDCl_3$) δ 5.77(1H, dd, *J*=11.5 and 14.7 Hz, H-10), 5.65(1H, br d, *J*=11.5 Hz, H-9), 5.46(1H, m, H-3), 5.42(1H, dd, *J*=9.9 and 14.7 Hz, H-11), 5.18(1H, m, H-15), 4.78-4.86(1H, m, H-19), 4.65(1H, dd, *J*=2.1 and 14.2 Hz, H-8a), 4.56(1H, dd, *J*=2.1 and 14.2 Hz, H-8a'), 4.39(1H, m, H-5), 3.99-4.16(2H, m, -CO_2CH_2-), 3.79(1H, d, *J*=5.2 Hz, H-6), 3.54-3.60(1H, m, H-17), 3.22(1H, m, H-2), 3.02(1H, dt, *J*=2.3 and 9.5 Hz, H-25), 1.79(3H, s, H-4a), 1.50(3H, s, H-14a), 1.20(3H, t, *J*=7.2 Hz, -$CO_2CH_2CH_3$), 1.05(3H, d, *J*=6.6 Hz, H-12a), 0.95(3H, t, *J*=7.1 Hz, H-25b), 0.93(9H, s, *t*-butyl-Si),

0.81(3H, d, *J*=6.5 Hz, H-24a), 0.14(3H, s, MeSi), 0.13(3H, s, MeSi), 0.12(9H, s, Me_3Si); *Anal.* Calcd for $C_{45}H_{74}O_9Si_2$: C,66.30; H,9.15. Found: C,65.50; H,8.90.

13-Dimethylaminocarbonylmethyl-5-*O*-*tert*-butyldimethylsilyl-7-*O*-trimethylsilyl-milbemycin A_4 (5).

A solution of **2** (75 mg, 0.1 mmol) and *N*, *N*-dimethylacetamide dimethyl acetal (133 mg, 1.0 mmol) in toluene (1 ml) was heated (80~90 °C) for 11 h, and poured into water, extracted with ethyl acetate, and the extract was dried over Na_2SO_4 and evaporated under reduced pressure. Purification by silica gel chromatography gave 61 mg of **5** (75% yield).

5: Ir(KBr)cm^{-1} 1741, 1653, 1251, 1168, 1129, 1094, 989, 840; ms m/z 813(M^+), 571, 262, 223, 195, 167, 73; ^{1}H-nmr(270 MHz, $CDCl_3$) δ 5.77(1H, dd, *J*=11.2 and 14.2 Hz, H-10), 5.66(1H, br d, *J*=11.2 Hz, H-9), 5.40-5.49(2H, m, H-3 and H-11), 5.22(1H, m, H-15), 4.78-4.86(1H, m, H-19), 4.66(1H, dd, *J*=2.0 and 14.2 Hz, H-8a), 4.55(1H, dd, *J*=2.0 and 14.2 Hz, H-8a'), 4.38-4.40(1H, m, H-5), 3.79(1H, d, *J*=5.4 Hz, H-6), 3.50-3.63(1H, m, H-17), 3.21-3.24(1H, m, H-2), 2.80-3.08(7H, m, H-25 and Me_2N-), 1.79(3H, s, H-4a), 1.51(3H, s, H-14a), 1.06(3H, d, *J*=6.4 Hz, H-12a), 0.95(3H, t, *J*=7.3 Hz, H-25b), 0.93(9H, s, *t*-butyl-Si), 0.80(3H, d, *J*=6.3Hz, H-24a), 0.13(6H, s, Me_2Si), 0.11(9H, s, Me_3Si).

13-Ethoxycarbonylmethylmilbemycin A_4 (6).

4 (41 mg, 0.05 mmol) was treated with *p*-toluenesulfonic acid (3 mg, 0.015 mmol) in methanol (1 ml) for 2 h. The reaction mixture was poured into saturated $NaHCO_3$ solution, extracted with CH_2Cl_2, and the extract was dried over Na_2SO_4. The residue obtained on concentration was chromatographed on silica gel to give 23 mg of **6** (73% yield).

6: Ir(KBr)cm^{-1} 3460, 1736, 1717, 1181, 1169, 991; ms m/z 628(M^+), 500, 195, 167, 151, 95, 55; HRms(EI) calcd for $C_{36}H_{52}O_9$(M^+) 628.3613, found 628.3635; ^{1}H-nmr(400 MHz, $CDCl_3$) δ 5.80(1H, br d, *J*=11.3 Hz, H-9), 5.73(1H, dd, *J*=11.3 and 14.0 Hz, H-10), 5.45(1H, dd, *J*=9.9 and 14.0 Hz, H-11), 5.41(1H, br s, H-3), 5.32-5.38(1H, m, H-19), 5.09-5.13(1H, m, H-15), 4.64-4.73(2H, m, H-8a and H-8a'), 4.28-4.30(1H, m, H-5), 3.99-4.15(3H, m, 7-OH and -CH_2CO_2-), 3.96(1H, d, *J*=6.0 Hz, H-6), 3.51-3.58(1H, m, H-17), 3.26-3.28(1H, m, H-2), 3.05(1H, dt, *J*=2.4 and 9.3 Hz, H-25), 2.36(1H, m, H-13), 1.88(3H, br s, H-4a), 1.47(3H, s, H-14a), 1.20(3H, t, *J*=7.0 Hz, -$CO_2CH_2CH_3$), 1.02(3H, d, *J*=6.6 Hz, H-12a), 0.99(3H, t, *J*=7.4 Hz, H-25b), 0.82(3H, d, *J*=6.4 Hz, H-24a).

13-Dimethylaminocarbonylmethylmilbemycin A_4 (7).

5 (42 mg, 0.05 mmol) was treated as descrbed for **6** to provide **7** (29 mg, 90% yield).

7: Ir(KBr) cm^{-1} 3450, 1736, 1711, 1645, 1180, 1168, 990; ms m/z 627(M^+) 195, 167, 87, 72, 55; HRms(EI) calcd for $C_{36}H_{53}NO_8$(M^+) 627.3773, found 627.3768; ^{1}H-nmr(270 MHz, $CDCl_3$) δ 5.68-5.82(2H, m, H-9 and H-10), 5.33-5.51(3H, m, H-3, H-11 and H-19), 5.16(1H, m, H-15), 4.69(2H, m, H-8a and H-8a'), 4.30(1H, m, H-5), 3.96(1H, d, *J*=5.9 Hz, H-6), 3.49-3.58(1H, m, H-17), 3.27(1H, m, H-2), 2.85-3.09(7H, m, H-25 and -$CONMe_2$), 2.49(1H, m, H-13), 1.88(3H, br s, H-4a), 1.49(3H, s, H-14a), 1.03(3H, d, *J*=6.4 Hz, H-12a), 0.98(3H, t, *J*=7.3 Hz, H-25b), 0.82(3H, d, *J*=6.3 Hz, H-24a).

REFERENCES

1. a) H. Mishima, M. Kurabayashi, C. Tamura, S. Sato, H. Kuwano, A. Saito, and A. Aoki, Abstract Papers 18th Symp. Chem. Natural Products, pp. 309-316, Kyoto, 1974. b) H. Mishima, M. Kurabayashi, C. Tamura, S. Sato, H. Kuwano, A. Saito, and A. Aoki, *Tetrahedron Lett.*, 1975, 711. c) Y. Takiguchi, H. Mishima, M. Okuda, M. Terao, A. Aoki, and R. Fukuda, *J. Antibiotics,* 1980, **33**, 1120. d) M. Ono, H. Mishima, J. Ide, and S. Muramatsu, *J. Antibiotics,* 1983, **36**, 980. e) H. G. Davies and R. H. Green, *Natural Product Reports*, 1986, **3**, 87. f) T. Blizzard, M. H. Fisher, H. Mrozik, and T. L. Shih, 'Recent Progress in the Chemical Synthesis of Antibiotics,' ed. by G. Lukacs and M. Ohno, Springer-Verlag, Berlin, 1990, pp. 65-102. g) H. G. Davies and R. H. Green, *Chem. Soc. Rev.*, 1991, **20**, 211. h) H. G. Davies and R. H. Green, *Chem. Soc. Rev.*, 1991, **20**, 271. i) K. Tanaka, *Nippon Nogeikagaku Kaishi* , 1987, **61**, 1601.
2. A. Saito, S. Naito, M. Kobayashi, M. Tsuchiya, T. Toyama, S. Kaneko, T. Nanba, and Y. Morisawa, *J. Antibiotics,* 1993, **46**, 1252.
3. a) A. C. O'sullivan and F. Bruno, *ACS Symp. Ser., Synth. Chem. Agrochem. III,* 1992, **504**, 239. b) P. Maienfisch, Eur. Pat. 282 456, Sep. 14, 1988 (*Chem. Abstr.*, 1989, **110**, 75167).
4. B. Frei, P. Huxley, P. Maienfisch, H. B. Mereyala, R. Gunther, and A. C. O'Sullivan, *Helv. Chim. Acta,* 1990, **73**, 1905.
5. J. G. Smith, *Synthesis,* 1984, 629.
6. S. Naito, A. Saito, Y. Furukawa, T. Hata, Y. Nakada, S. Muramatsu, and J. Ide, *J. Antibiotics,* 1994, **47**, 812.
7. T. Inoue, T. Uchimaru, and T. Mukaiyama, *Chemistry Lett.*, 1977, 1215.
8. S. Murata, M. Suzuki, and R. Noyori, *J. Am. Chem. Soc.*, 1979, **101**, 2738.
9. H. Yamamoto and K. Maruoka, *Pure Appl. Chem.*, 1988, **60**, 21.
10. K. Maruoka, A. B. Concepcion, N. Murase, M. Oishi, N. Hirayama, and H. Yamamoto, *J. Am. Chem. Soc.*, 1993, **115**, 3943.
11. a) A. E. Wick, D. Felix, K. Steen, and A. Eschenmoser, *Helv. Chim. Acta,* 1964, **47**, 2425. b) D. Felix, K. Gschwent-Steen, A. E. Wick, and A. Eschenmoser, *Helv. Chim. Acta,* 1969, **52**, 1030. c) H. Meerwein, W. Florian, N. Schön, and G. Stopp, *Liebigs Ann. Chem.*, 1961, **641**, 1.
12. W. S. Johnson, L. Werthemann, W. R. Bartlett, T. J. Brockson, T-T. Li, D. J. Faulkner, and M. R. Peterson, *J. Am. Chem. Soc.*, 1970, **92**, 741.

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