Electrophilic radioiodination of 5-trimethylstannyl-3-((1-*tert*-butoxycarbonyl-2(*S*)-azetidinyl)methoxy)pyridine using chloramine-T and H_2O_2 as oxidizing agents

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ABSTRACT

The radiochemical synthesis of 5-[^{125}I]iodo-3-(2(*S*)-azetidinylmethoxy)pyridine [(5-[^{125}I]-iodo-A-85380) or ([^{125}I]5IA) was accomplished by radioiodination of 5-trimethylstannyl-3-((1-*tert*-butoxycarbonyl-2(*S*)-azetidinyl) methoxy) pyridine using chloramines-T and H_2O_2 as oxidizing agents followed by acidic deprotection. An average radiochemical yields of [^{125}I]5IA was 75 % and 55 % respectively. The reaction proceeds well within 15-30 min and 30-60 min at room temperature (20-25^{o}C) respectively. Different chromatographic analysis techniques (TLC and HPLC) were used to evaluate the radiochemical yields during the process as well as the purity of the final product with a radiochemical purity over 99.9 % and a specific activity of 74 KBq / μg substrate.

Key words : 5-iodo-3-(2(.S')-azelidinylmelhoxy)pyridine / radioiodination / nicotinic acetylcholine receptor / brain / SPECT.

INTRODUCTION

Recently, there are different current investigations have been demonstrated the continuous need and development of the techniques and the methods intended for organic compounds and biomolecules radioiodination with medical useful iodine radionuclides [1,2]. That is due to that iodine atom occupies a similar volume to that of methyl or ethyl group and can substitute for an alkyl group in an organic molecule without unduly perturbing the steric or polar configuration [3,4]. On the other hand, carbon-iodine bonds have similar polarities to carbon-carbon bonds except for active substrates such as compounds have been already containing phenol group or indole group. An oxidizing agent must normally be present such as chloramine-T and iodogen to oxidize I_2 to a better electrophile in acidic pH value [5,6]. Reagents based on boron, silicon, tin, thallium, aluminum or mercury have all been reported. In this type of electrophilic substitution reaction, the hetero group is introduced into a precursor molecule at the position where the radioiodine label is desired. The hetero group is then replaced by electrophilic iodine (iodide and chloramines-T or iodogen) [7,8].

5-Iodo-3-(2(.S')-azetidinyl)methoxy)pyridine (5IA), an A-85380 analog iodinated at the 5-position of the pyridine ring, was evaluated as a radiopharmaceutical for investigating brain nicotinic acetylcholine receptors (nAChRs) by single photon emission computed tomography (SPECT) [9-11].

Changes in the density of nicotinic acetylcholine receptors (nAChRs) have recently been reported in the brains of patients with various disorders, including Alzheimer's disease and Parkinson's disease [12,13]. An increased density of nicotine receptors has also been reported in the brains of smokers [14,15]. In addition, evidence of an important role of nAChRs in cognition and improvement of cognitive function by nicotine have been reported [16,17]. These observations and the superior radiation properties of ^{123}I for single photon emission computed tomography (SPECT) prompted us to develop a radioiodinated ligand that is suitable for in vivo imaging. Therefore, based on structure-activity

relationship studies of nicotine, we developed (-)-5-iodonicotine, a (-)-nicotine analog iodinated at the 5-position of the pyridine ring [18,19]. In vitro receptor binding and in vivo bio-distribution studies of this compound showed that the binding affinity for nAChRs was the same as that of (-)-nicotine, the parent compound, and deiodination in vivo was minimized. These results indicated that position 5 of the pyridine ring of (-)-nicotine was the most practical site for iodination from the viewpoints of minimum disturbance of receptor binding and maximum in vivo stability, but its usefulness was significantly impaired by high nonspecific uptake and rapid clearance from the brain. Impetus for further work on the development of a radioligand for in vivo imaging of nAChRs has been provided by the discovery of epibatidine, an alkaloid from the skill of the poisonous Ecuadorian frog Epipedobatus tricolor with extremely high affinity for nAChRs in the brain [20-24]. More recently, 3-(2(A')-2-azetidiyl)methoxy)pyridine (A-85380) has been developed, which has high affinity comparable to that of epibatidine at nAChRs but with much less toxicity [25]. The favorable properties for in vivo nAChR imaging therefore prompted us to synthesize a radioiodinated A-85380 analog.

The selection of the site introduced radioiodine is important for the development of a radioiodinated nAChR imaging agent, as demonstrated in design of (-)-5-iodonicotine [18,19]. With regard to the nicotinic pharmacophore, the recently developed model suggests that the binding of nicotine to nAChRs is associated with a pyrrolidine nitrogen atom (a cationic center), a pyridine nitrogen atom (an electronegative atom), planarity of the pyridine ring (a dummy point or an atom to define a line along which the hydrogen bond may form), and the distance between the two nitrogen atoms [25,26]. Therefore, when a cationic center, a pyridine nitrogen atom and planarity of the pyridine ring were selected as essential pharmacophoric elements and A-85380 was superimposed on (-)-5-iodonicotine, the position of iodine in (S)-5-iodonicoline was superimposed on position 5 of the 3-pyridyl fragment in A-85380. Therefore, since (-)-5-iodonicotine showed the same affinity as (S)-nicotine without disturbance of receptor binding, it has been suggested that the 5 position of the 3-pyridyl fragment is the best site for iodination in A-85380.

In fact, Keren et al. recently synthesized all of the A-85380 derivatives iodinated at possible positions of the 3-pyridyl fragment, that is 2-, 4-, 5- and 6-iodinated derivatives of A-85380 and found that an A-85380 derivative iodinated at the 5 position of the 3-pyridyl fragment, 5-iodo-3-(2(,S')-azetidinyl)methoxy)pyridine (5IA), had the highest affinity for nAChRs among all synthesized derivatives [27]. Furthermore, they performed bio-distribution studies in mice and SPECT image studies in baboons, and suggested that [^{123}I]51A is a promising SPECT imaging agent of nAChRs [28,29].

In this work, we have investigated a simple method for radioiodination of BOC-5-IA. It is used in nuclear medicine as SPECT agent for studies of central nAChRs in human subjects. The radioiodine atom was incorporated in the 5-position of the pyridine ring which facilitate the electrophilic radioiodination [16, 30,31] as shown in Fig. (1).

The different reaction parameters affecting the rate of the reaction were the concentration of the substrate, hydrogen ion concentration, type of the oxidizing agent and time. The method afforded a high radiochemical yield of pure 5-[^{125}I]IA and high specific activity up to 74 KBq / μg Boc-5IA.

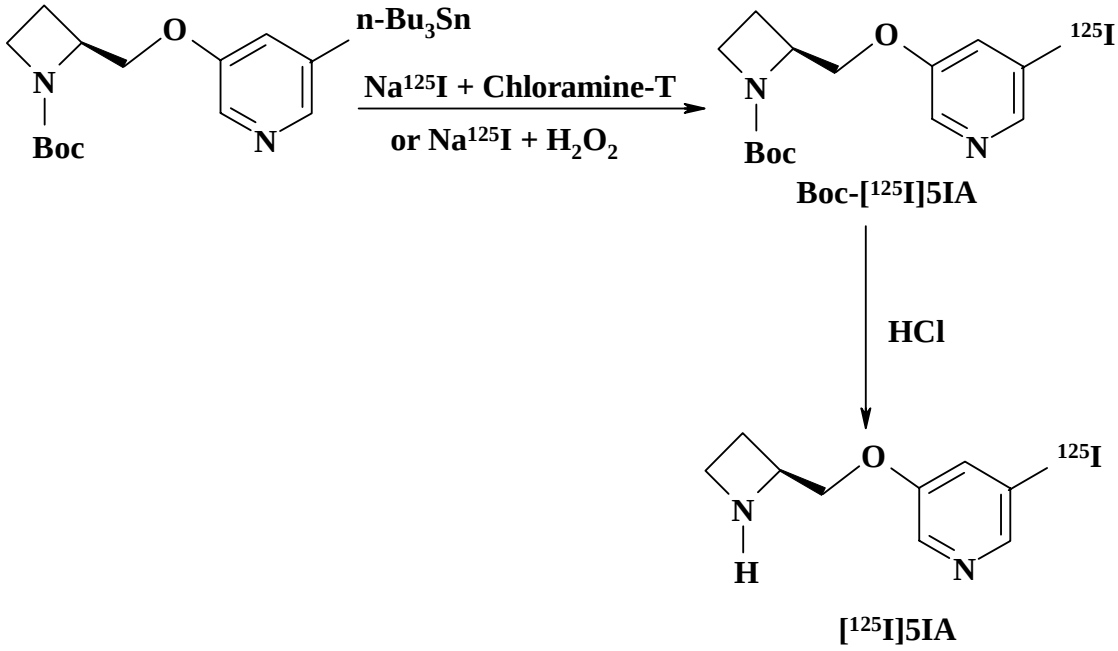

Fig. (1) : Radiosynthesis of Boc-[^{125}I]5IA and [^{125}I]5IA

EXPERIMENTAL

Materials

All reagents used were of analytical grade. 5-iodo-A-85380 and the precursor (5- trimethylstannyl-3-(1-tert-butoxycarbonyl-2(S)-azetidinylmethoxy)- pyridine) [BOC-5IA] were taken as a gift from Hideo Saji (graduate school of Pharmaceutical Sciences and graduate school of medicine, Kyoto university, Japan). Na^{125}I was purchased from Nordion Co. Belgium, as a no carrier added in NaOH of specific activity 15.5 MBq/μg.

Chromatographic techniques :

Thin Layer Chromatographic Analysis (TLC)

Thin layer chromatography (TLC) is used for the determination of the radiochemical yield and the radiochemical purity. TLC plates : Silica Gel G-60, Solvent: 10% ammonium acetate : methanol (1:1) R_f of 5IA = 0.55-60, I^- = 1.0

High Performance Liquid Chromatography (HPLC)

Two aliquots (10-20 μl) were withdrawn from the reaction solution after varying investigated time intervals. The first aliquot was withdrawn into a test tube to determine the total activity. The second aliquot was injected onto a HPLC-column [(Shimadzu LC-9A pump) on reversed phase RP-18 column (250 x 4 mm) Lichrosorb, Merck] to separate the product peaks. Each component of the reaction mixture was eluted after a definite retention time by a mixture of CH_3CN:H_2O:CF_3COOH (9:91:0.2) as eluant and at a flow rate of 1.2 ml/min [(9)]. Retention time for iodide was 2 min (identified by UV absorption of cold KI solution), BOC-5IA was 8 min. and [^{125}I]5IA was found to display at 9 min. The aliquots were determined by a gamma counter. The radiochemical yield was determined by

collecting the radioactive peaks and comparing their activities with those of a non-chromatographed sample of identical volume. The radiochemical yield % was calculated as follows :

$$\text{Radiochemical yield (\%)} = \frac{\text{Activity of the product}}{\text{Total activity}} \text{ X } 100$$

Method

A solution of 5-trimethylstannyl-3-(1-*tert*-butoxycarbonyl- 2(*S*)-azetidinylmethoxy)pyridine (2mg in 1 ml methanol) was added into the shipping vial with $Na^{125}I$ followed by solution of chloramines-T in a methanol-acetic acid mixture (1:1) (1mg in 1ml mixture) at room temperature for 30 min, and the reaction was terminated by addition of an aqueous solution of $Na_2S_2O_3$ (1 mg in 50 ml). Volatile radioactivity was removed from the vial by purging it with nitrogen gas. The reaction mixture was injected onto an RPC18 column and eluted with a mixture of CH_3CN:H_2O:CF_3COOH 9:91:0.2 at a flow rate of 1.2 ml/min. The radioactive peak, with a retention time of 9 min corresponding to [^{125}I]5IA was collected, and the solvent was removed under a stream of nitrogen. The product was redissolved in water (1 ml). The radiochemical purity exceeded 99%. Figures 2,3 show the retention time of each component.

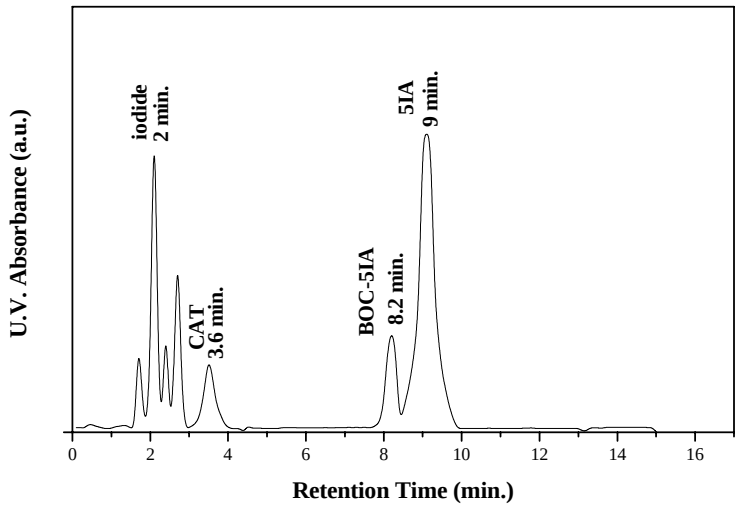

Fig. (2) : U.V. absorption chromatogram of the reaction mixture of Boc-5IA, CAT and KI

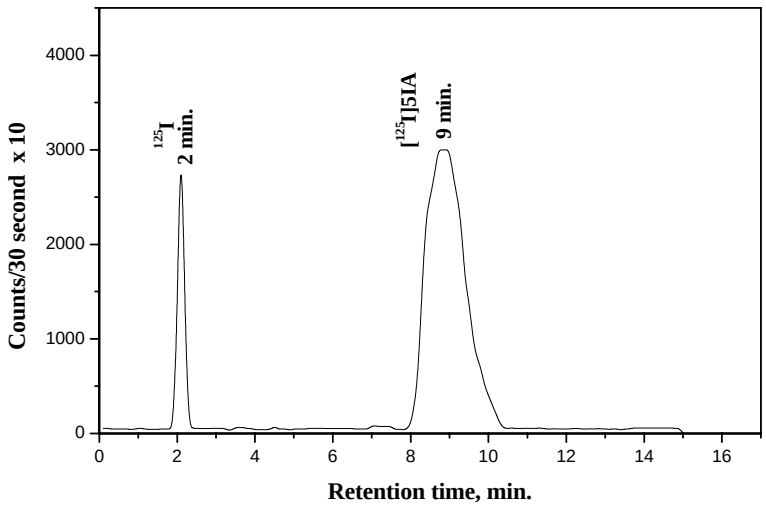

Fig. (3) : Radiochromatogram of the reaction mixture of Boc-5IA, CAT and Na*I

RESULTS AND DISCUSSION

The work presents herewith indicates that the reaction of iodonium ion ($^{125}I^{+}$) with the C-5 position of the reactive pyridine ring of the Boc-5IA molecule which may be categorized as electrophilic substitution reaction proceeds well at room temperature (20-25^{o}C). The electrophilic reaction was instantaneously well proceeded with time as shown in Fig. (4).

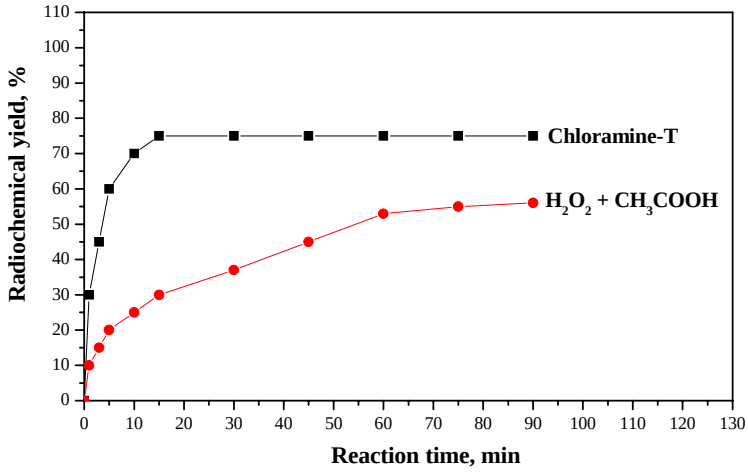

Fig. (4) : Variation of the radiochemical yield % of [^{125}I]5IA with reaction time using Chloramine-T and H_2O_2 as oxidizing agents.

The radiochemical yield increases with increase in the substrate content as shown in Fig. (5).

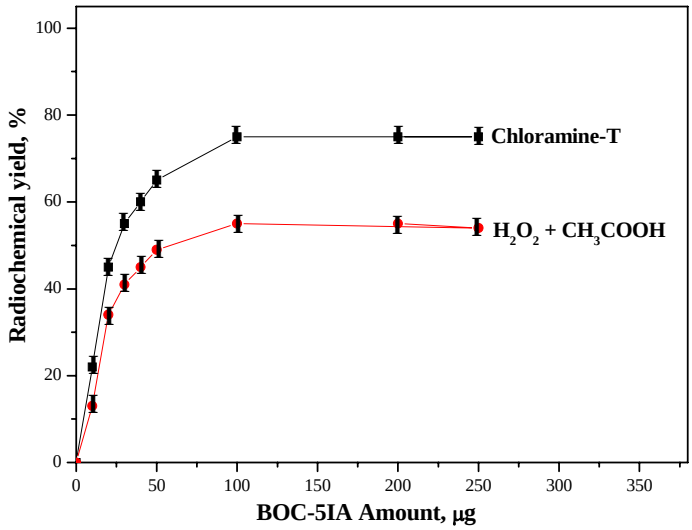

Fig. (5) : Variation of the radiochemical yield % of [^{125}I]5IA with substrate amount (µg).

The reaction has been occurred by using iodinating agents, Na^{131}I and chloramine-T or Na^{125}I and chloramine-T. The different parameters affecting the electrophilic substitution were investigated to achieve the desired radiochemical yield % of [^{125}I]5IA up to 70 % in time 15-30 min at 20-25^{o}C. Additionally, the results obtained revealed that electrophilic radioiodine generated by chloramine-T (N-chloro-p-toluene sulfonamide sodium salt) appears to be the most effective oxidizing agent than peracetic acid (hydrogen peroxide and acetic acid) as shown in Fig. 6.

Chlorinated oxidizing agents such as chloramine-T and iodogen produce chlorinated side products especially in case of chloramine-T. These chlorinated side products are responsible for decreasing the radiochemical yield.

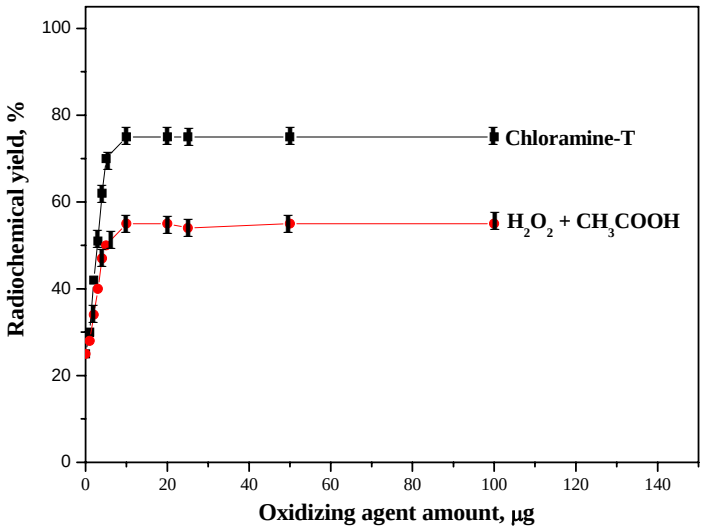

Fig. (6) : Variation of the radiochemical yield % of [^{125}I]5IA as a function of oxidizing agents (Chloramine-T and H_2O_2) amount (µg).

Similarly, in a comparative experimental study our results confirmed that a decrease in the radiochemical yield % up to 10 % was obtained on using peracetic acid as oxidizing agent. The reaction was completely different from the other previously reported due to its no-carrier-added and temperature independent. A very important and vital aspect must be considered in this reaction is the molarity of sodium hydroxide solution in which most of radioactive iodine are dispensed. The presence of excess base has a negative pronounced influence on the electrophilic substitution process. Basically, $Na^{125}I$ in 0.1 M NaOH pH 8 – 9 which was delivered from Nordion, reductant free and carrier free must be diluted to attain a molarity of 0.03 M NaOH (pH $\simeq$ 7) using dilute hydrochloric acid to adjust the pH up to 7.

The method allows a best utilization of other cyclotron produced medical radionuclide (i.e. ^{123}I). That is by using the decay energy (148 KeV) produced from the decay of ^{123}Xe to ^{123}I for the excitation of the reaction rate without intermediate isolation of the radioiodine gas to $Na^{123}I$ solution as the following equations :

$$^{124}Te\ (p, 2n) \longrightarrow {}^{123}Xe \xrightarrow[148\ KeV,\ 2.1\ h]{\beta+,\ E.C.} {}^{123}I\ \ gas \qquad or$$

$$^{122}Te\ (d, n) \longrightarrow {}^{123}Xe \xrightarrow[148\ KeV,\ 2.1\ h]{\beta+,\ E.C.} {}^{123}I\ \ gas$$

CONCLUSION

The preliminary results permit the following conclusions :

1- The technique have proven to be useful to incorporate radioiodine radionuclides onto the pyridine ring of the Boc-5IA (as a reactive group) at room temperature in reasonable time up to 60 min, where chloramine-T appears to be the most effective oxidizing agent than H_2O_2.

2- An advantage of reactions of this type is the accessibility of classes of compounds that are thermolabile and other bio-molecules containing reactive groups such as pyridine, phenol, imidozoles and indoles without perturbation of the initial structure of the substrate molecules.

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الإحلال الإلكتروفيلى لليود المشع فى جزئ 5-(ثلاثى ميثيل زئبق)-3(1-رباعى بيوتوكسى كربونيل-2-أزيتيدينيل ميثوكسى) بيريدين باستخدام كلورامين-ت وفوق أكسيد الهيدروجين كعوامل مؤكسدة

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القاهرة – مصر

ملخص البحث

يشتمل هذا البحث على تحضير المركب المرقم 5-(يـود-125)-3(2-أزيتيـدينيل ميثوكـسى) بيريدين وذلك عن طريق الإحلال الإلكتروفيلى لمركب 5-(ثلاثـى ميثيـل الفـصديم)-3(1-ريـاعى بيوتوكسى كربونيل-2-أزيتيدينيل ميثوكسى) بيريـدين باسـتخدام الكلـورامين-ت وفـوق أكـسيد الهيدروجين كعوامل مؤكسدة ثم التحال الحامضى. وقد تم الحصول على متوسط عائد ترقيمى 75 %، 55 % بالترتيب. وقد تم التفاعل فى فترة زمنية مناسبة 15-30 دقيقة، 30-60 دقيقة عند درجـة حرارة مـن 20_ 25°م بالترتيـب. وتـم إسـتخدام طـرق التحليـل الكرومـاتوجرافى المختلفـة (TLC and HPLC) لتعيين عائد الترقيم وأيضا النقاوة الإشعاعية للمركب الناتج وهى حوالى 99,9 % ونشاط إشعاعى نوعى 74 كيلوبيكريل/ ميكروجرام من المادة المراد ترقيمها.