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NOTE

Further investigation on the radiosynthesis of α-[^{11}C]methyl-tryptophan

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ABSTRACT

Improved procedures for the radiosynthesis of α-[^{11}C]methyl-tryptophan and α-[^{11}C]methyl-tryptophan methyl ester were studied. Following α-deprotonation of tryptophan methyl ester benzaldimine with LDA, 60 - 80% of no carrier added [^{11}C]iodomethane was incorporated in 5 minutes at 27 - 30 °C. After HPLC purification, radiochemically pure α-[^{11}C]methyl-tryptophan or its methyl ester was produced with minimum chemical contamination from tryptophan. The [^{11}C]methyl-tryptophan synthesized, however, was found to be a racemate.

INTRODUCTION

In 1988, Chaly et al. described the radiosynthesis of α-[^{11}C]methyl-L-tryptophan (1) based on the method of Brana et al. (2) for use in the study of *in vivo* serotonin synthesis with positron emission tomography (PET). However, our attempts to repeat the published synthesis of this tracer did not reproduce their results.

This article reports improved synthetic procedures which yield either 100% radiochemically pure α-[^{11}C]methyl-tryptophan or α-[^{11}C]methyl-tryptophan methyl ester with minimum chemical impurities from tryptophan or its methyl ester. The results of the chiral purity of α-[^{11}C]methyl-tryptophan are also reported.

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EXPERIMENTAL

Melting points are uncorrected. NMR spectra were obtained on an IBM NR/80 using $(CH_3)_4Si$ as an internal standard. Infrared (IR) spectra were obtained on a Nicolet 205 FTIR spectrometer. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. HPLC purification and analyses of radioactive mixtures were performed using a previously described system (3). Radioactive and mass peak areas were measured using Hewlett-Packard 3390A recording integrators. Radioactivity measurements were made on a dose calibrator (Capintec CRC-12R). L-Tryptophan methyl ester and α-methyl-D,L-tryptophan were obtained from Sigma Chemical Co.

α-Methyl-tryptophan methyl ester was synthesized according to Brana et al (2). m.p.: 137 - 138 °C (lit. 135 - 137 °C); ir ν cm^{-1}: 1730 (C=O); 1590 (C=C). nmr ($CDCl_3$): δ ppm 1.4 (s, 3, CH_3), 1.95 (s, 1, NH), 3.15 (d, 2, CH_2), 3.50 (s, 3, CH_3), 6.70 - 7.60 (m, 5 ArH).

The benzaldimine of L-tryptophan methyl ester, the precursor for α-methyl-tryptophan, was synthesized according to the same literature. m.p. 126 - 127 °C (lit. 124 - 126 °C); ir ν cm^{-1}: 1740 (C=O); 1640 (N=C). nmr ($CDCl_3$) δ ppm 1.95 (s, 1, NH), 3.3 (m, 2, CH_2), 3.65 (s, 3, CH_3), 4.15 (m, 1, CH), 6.65-7.70 (m, 11,10, ArH), 7.9 (s, 1, CH=N). $[\alpha]_D^{23}$: -145° ($CHCl_3$, c=3).

Lithium diisopropylamide (LDA) was either obtained from a commercial source (1.4 M in THF; Aldrich Chemical Co.) or freshly prepared from n-butyllithium and diisopropylamine.

[^{11}C]Iodomethane was synthesized as previously described (4).

α-[^{11}C]methylation of the benzaldimine of L-tryptophan methyl ester.

LDA (one equivalent based on the amount of the precursor) in 300 μL of dry THF was sealed in a glass vial and placed in a dry ice-ethanol bath (-78 °C). The vial was purged with argon. Five mg of the precursor, the benzaldimine of L-tryptophan methyl ester, was dissolved in 200 μL of dry THF and added to the solution of LDA 20 to 30 minutes prior to the introduction of radioactivity. The solution turned yellow.

No-carrier-added [^{11}C]iodomethane was introduced to the reaction vial with a stream of dry nitrogen. When the radioactivity reached a maximum level, the carrier gas flow was stopped. The reaction vial was removed from the dry ice-ethanol bath and maintained either at room temperature or in a water bath of 25 - 30 °C for 5 minutes. The α-[^{11}C]methylation reaction was monitored by applying an aliquot of the reaction mixture to a HPLC analytical C-18 column (Novapak, 15 cm x 4.5 mm i.d.; Waters Associates) eluted with a mobile phase of acetonitrile-

water (50:50) containing 0.1 M ammonium formate at a flow rate of 2 mL/min.

α-[^{11}C]methyl-tryptophan methyl ester.

After the 5 minute α-[^{11}C]methylation reaction, 0.5 mL of 1 N HCl was added to the reaction mixture. The reaction vial was heated for 1 minute at 110 °C with venting under a stream of argon. After cooling, 0.5 mL of 8.4% sodium bicarbonate was added. The solution was then diluted with HPLC buffer (water-acetonitrile-phosphoric acid-triethylamine: 400/70/0.7/1.4) (5), and applied to a semipreparative C-18 HPLC column (Novapak, 25 cm x 10 mm i.d.; Waters Associates). The column was eluted with the same solvent at a flow rate of 7 mL/min. α-[^{11}C]Methyl-tryptophan methyl ester eluted at 8.1 minutes (k' = 4.8). α-[^{11}C]Methyl-tryptophan, tryptophan and tryptophan methyl ester eluted at 2.0, 1.8 and 4.7 minutes (k' = 0.8, 0.6, and 2.8), respectively.

The HPLC peak containing the radioactivity associated with α-[^{11}C]methyl-tryptophan methyl ester was collected and the solvent was evaporated under vacuum. The residue was dissolved in sterile normal saline (7 mL) and sodium bicarbonate (3 mL, 8.4%).

α-[^{11}C]methyl-tryptophan.

α-[^{11}C]methyl-tryptophan was synthesized from the methyl ester. After the hydrolysis of the [^{11}C]methylated benzaldimine of L-tryptophan methyl ester with 1 N HCl at 110 °C, 0.3 mL of 2 N NaOH was added to the reaction mixture. The solution was then diluted with HPLC buffer (water-acetonitrile-phosphoric acid-triethylamine: 400/25/0.7/1.4), and applied to the HPLC column mentioned above and eluted with the mobile phase at a flow rate of 7 mL/min. α-[^{11}C]Methyl-tryptophan eluted at 5.8 minutes (k' = 3.1), while tryptophan eluted at 4.3 minutes (k' = 2.0). The HPLC peak containing the radioactivity associated with α-[^{11}C]methyl-tryptophan was collected and formulated as above.

Chiral purity determination

The chiral purity of α-[^{11}C]methyl-tryptophan was examined using a chiral HPLC column (Nucleosil chiral 1, 25 cm x 4.5 mm i.d., Alltech Associates) with a mobile phase of water-acetonitrile (90:10) containing 1 mM copper acetate and 1.7 mM of acetic acid (pH 4.7) at a flow rate of 1.5 mL/min. Chiral purity of the unlabeled methyl-tryptophan synthesized and purified according to the literature procedure (2) was also examined using the same HPLC system as α-[^{11}C]methyl-tryptophan.

RESULTS AND DISCUSSION

The syntheses of α-[^{11}C]methyl-tryptophan and its methyl ester were based on the literature procedures (2, 6, 7, 8) with modifications suitable for radiosyntheses with short-lived ^{11}C.

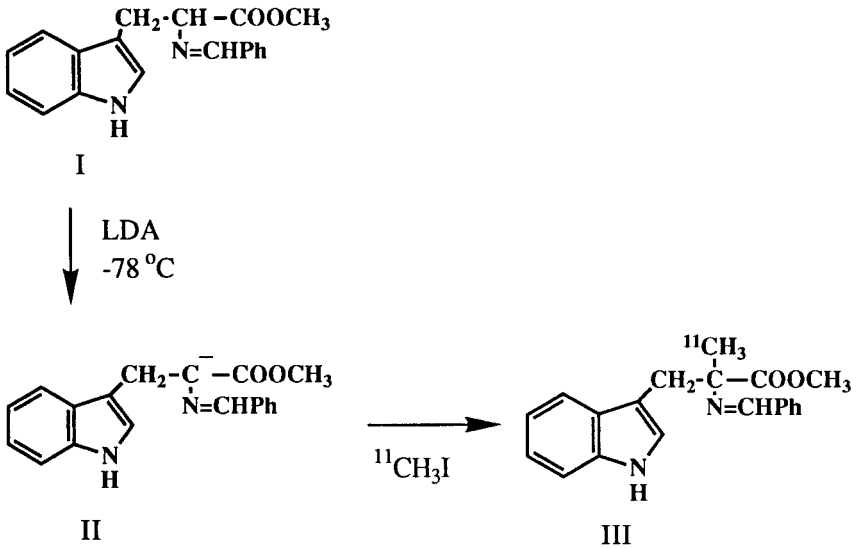

Figure 1. Radiosynthesis of α-[^{11}C]methyl-tryptophan.

The anion (II in Figure 1) formed by α-deprotonation with LDA reacted with [^{11}C]iodomethane rapidly to produce α-[^{11}C]methyl-tryptophan benzaldimine methyl ester (III in Figure 1) in yield of 60 - 80%. The concentration of the precursor which ranged from 10 - 40 mg/mL did not significantly affect the yield. The temperature during the [^{11}C]methylation did affect the yield. The reaction in a water bath of 25 - 30 °C for 5 minutes gave a yield of 60 - 80%, while the reaction at room temperature for 5 minutes gave a yield of only 3 - 5% (Table 1).

Table 1: Yield of α-[^{11}C]methylation of tryptophan methyl ester benzaldimine with LDA.

$^{11}CH_3I$	Temperature	Reaction Time	Yield (%)
n.c.a.	r.t.	5 min.	3.9 ± 0.9 (n=2)
n.c.a.	r.t.	10	54.5 ± 26.0 (n=2)
c.a.	r.t.	10	51.1 ± 7.5 (n=3)
n.c.a.	25-30 °C	5	63.3 ± 12.3 (n=5)

n.c.a.: no carrier added; c.a.: carrier added; r.t.: room temperature

Hydrolysis of the benzaldimine of α-[^{11}C]methyl-tryptophan methyl ester with HCl proceeded rapidly, yielding the α-[^{11}C]methyl-tryptophan methyl ester. After HPLC separation, 100% radiochemically pure α-[^{11}C]methyl-tryptophan methyl ester was obtained in a radiochemical yield of 25 - 30% (decay corrected). The synthesis time including HPLC purification was approximately 25 minutes. The specific activity was approximately 1300 -1500 mCi/μmol (calculated at the end of the synthesis (EOS)). Chemical contamination from unseparated tryptophan methyl ester was approximately 0.1 μmole.

Contrary to the suggestions in the literature (1, 6, 7), hydrolysis of the methyl ester of α-[^{11}C]methyl-tryptophan with HCl was not rapid enough to be compatible with short half life of carbon-11. The hydrolysis reaction was studied using unlabeled α-methyl-tryptophan methyl ester and tryptophan methyl ester (Figure 2). Even with concentrated HCl, only 10 - 15% of the methyl ester was hydrolyzed in 5 minutes.

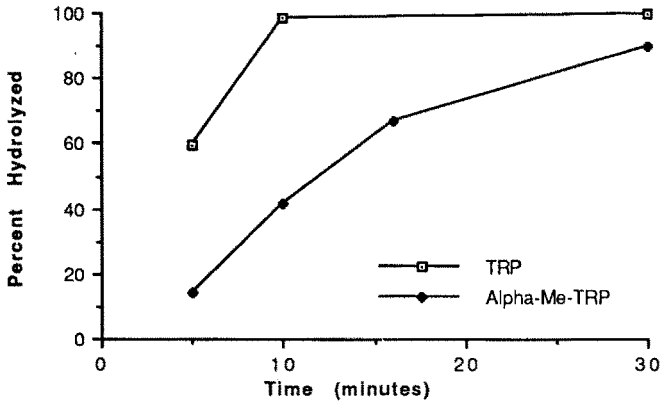

Figure 2. Hydrolysis of α-methyl-tryptophan methyl ester and tryptophan methyl ester with conc. HCl.

In contrast, hydrolysis with 2 N NaOH was faster (9). At pH 8 - 9, 70 - 80% of the α-[^{11}C]methyl-tryptophan methyl ester was hydrolyzed almost instantaneously. However, at high pH, co-precipitation of tryptophan methyl ester and α-[^{11}C]methyl-tryptophan methyl ester occurred, resulting in loss of the [^{11}C]methyl-tryptophan methyl ester. After HPLC purification, 98 - 100% radiochemically pure α-[^{11}C]methyl-tryptophan was obtained in an overall radiochemical yield of 25 - 30% (decay corrected). The synthesis time was approximately 25 minutes. The average specific activity was between 1300 - 1500 mCi/μmole (calculated at EOS). Chemical contamination with tryptophan was approximately 0.2 μmole.

Chiral HPLC of the α-[^{11}C]methyl-tryptophan synthesized as described above revealed that a racemic mixture of L and D isomers was

prepared (Figure 3). The unlabeled α-methyl-tryptophan synthesized according to Brana et al. (2) was also determined to be a racemate. Racemization during deprotonation following enolate formation with lithium has been well studied by Seebach et al. (10, 11). Their work suggested that for enantioselective α-alkylation, the existence of a new chiral center is essential after the original chiral center has been destroyed by deprotonation with LDA.

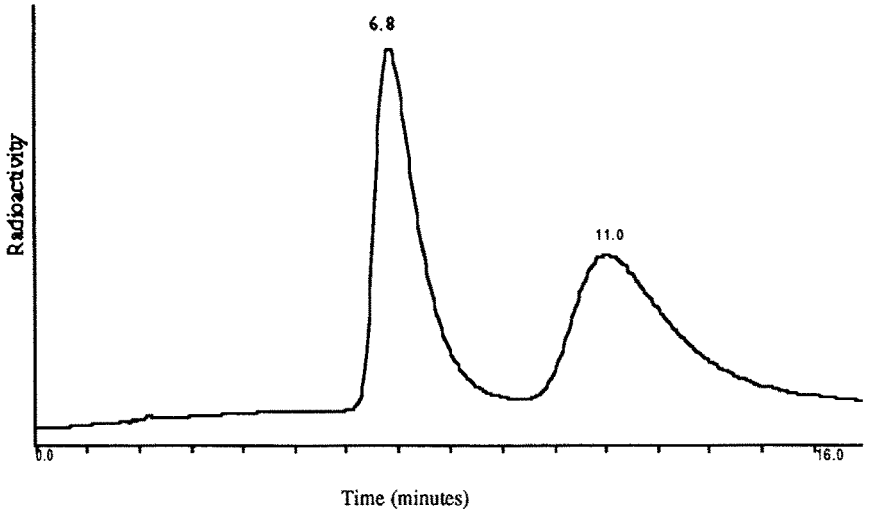

Figure 3. Chiral separation of α-[^{11}C]methyl-D-tryptophan and α-[^{11}C]methyl-L-tryptophan

In view of their concept of "self-reproduction" of chirality, the synthetic procedure using the benzaldimine of tryptophan could easily racemize, since there is no additional chiral center after the achiral enolate has formed. Similarly, α-alkylation with alkyl halides following α-deprotonation of imines of amino acids with LDA have been well described in literature (12 - 15). Some of the syntheses with the Schiff bases prepared from external chiral auxiliary reagents such as (s)proline or (-)menthone are diastereoselective, yielding a 10 - 50% enantiomeric excess (13, 14). In all these cases, a chiral center is still retained after the α-deprotonation destroys the original chiral center. Even with an external chiral auxiliary group retained in molecule, configuration of lithium enolates is changeable (14). No reports have appeared regarding non-racemic products of α-alkylation starting with imines of one of diastereomers of amino acids without an additional chiral center, except for the ones reported by Brana and Chaly (1,2). Following their procedures we obtained a racemate. Therefore, it is very likely that this synthetic procedure gives a racemic mixture of α-[^{11}C]methyl-tryptophan. Since enzyme inhibition by α-amino acids is highly stereoselective, racemization

that resulted from this synthesis is not desirable. Alternative synthesis routes or separation of α-[^{11}C]methyl-L-tryptophan from α-[^{11}C]methyl-D-tryptophan by chiral chromatography will be required.

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