Synthesis of "No-Carrier-Added" α-[^{11}C] Methyl-L-Tryptophan

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Described here is a synthesis of "no-carrier-added" α-[^{11}C]methyl-L-tryptophan based on alkylation with $^{11}CH_3I$ of an anion generated by reacting the Schiff base of L-tryptophan methyl ester with di-isopropylamine. The synthesis requires ~30 min after the end of $^{11}CO_2$ collection and gives α-[^{11}C]methyl-L-tryptophan in a 20–25% radiochemical yield calculated at the end of the synthesis and without correction for radioactive decay. The specific activity of the final radiopharmaceutical, measured at the end of the synthesis, was around 2,000 Ci/mmol. Data confirming the stereospecificity of the synthesis are also presented.

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The concentration of the neurotransmitter serotonin is lower in the brain of patients with certain mental disorders than in the normal brain (*1*). Cerebral metabolism of serotonin is, therefore, obviously a topic of some importance. To date we have not had a means to quantify it in vivo in the human brain.

The metabolism of serotonin has been extensively investigated in animals by tracing labeled tryptophan (Trp) and its metabolic product, serotonin (5-HT), and the serotonin degradation product, 5-hydroxyindole acetic acid (5-HIAA) (*2,3*). It should be noted that this metabolite can be lost from the brain during the course of an experiment. Therefore, although serotonin synthesis can be quantified in animal experiments if HPLC separation of different metabolic products is done, it might be difficult to do so using external monitoring (e.g., PET) and labeled tryptophan as a tracer.

With PET we can measure uptake and, in some instances, follow a part of the metabolic pathway of various labeled substances in human brain (*4,5*). For instance, PET imaging of the neurotransmitter dopamine pool in the living human brain using fluorine-18 labeled 6-fluoro-L-dopa has recently been described (*6*). PET can also be used to examine cerebral metabolism of serotonin by means of labeled tryptophan. To do this, tryptophan should be labeled in a position other than carboxylic.

A synthesis of carbon-11 (^{11}C) tryptophan with the label in the carboxylic position has already been devised (*7,8*). However, any label in that position would be lost during subsequent metabolism, during the conversion of 5-hydroxytryptophan to serotonin, preventing proper quantitation. Yet synthesis of tryptophan with the label in any other position than the carboxylic is presently extremely difficult. Several fluorinated analogs of L-tryptophan have been synthesized (*9,10*), but their usefulness in studying the rate of serotonin synthesis has never been confirmed. For these reasons we decided to investigate analog of labeled tryptophan that, like tryptophan, follow serotonin's metabolic pathway but not metabolized into 5-HIAA.

Sourkes and co-workers (*11,12*) have shown that α-methyl-L-tryptophan follows, at least in part, the metabolic pathway of L-Trp and thus could be a suitable tracer for measurement of the in vivo synthesis of serotonin. They have also shown that the metabolic product of α-methyl-L-Trp is α-methyl serotonin (*11, 12*), which they postulated was the end product of the metabolic degradation of α-methyl-L-Trp. To facilitate measurement of the rate of synthesis of serotonin in vivo we have synthesized α-[^{11}C] methyl-L-tryptophan.

MATERIALS AND METHODS

All chemicals used in this work were of research grade except for high performance liquid chromatography (HPLC) solvents which were of HPLC purity and were obtained from regular suppliers. ^{1}H-NMR spectra were obtained at 200 MHz using a Varian XL-200 spectrometer. Tetramethylsilane was used as an external standard when aqueous solutions were analyzed and as an internal standard when organic solvents

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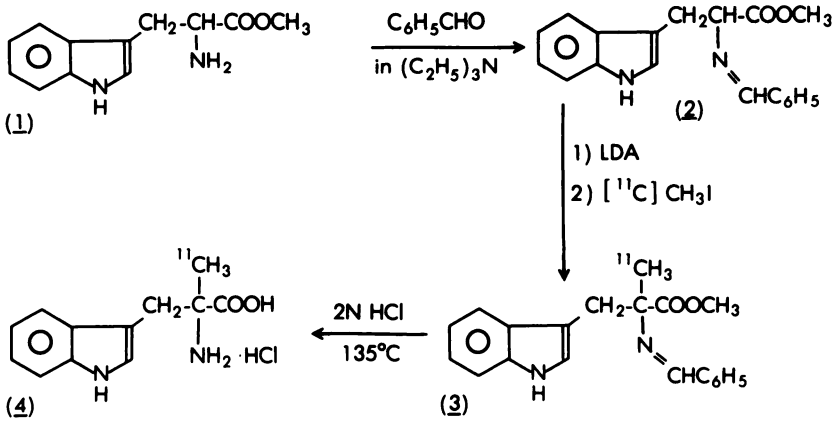

FIGURE 1
Schematic representation of reaction sequences used in the synthesis of α-methyl-L-tryptophan.

were used. Mass spectra were taken with a HP5980A mass spectrometer using chemical ionization (CI) or electron impact (EI) (80 eV) as the ionization mode. High performance liquid chromatography was done on a chiral column (Chiralpak WH, Baker Chemical Co.) using uv (=254 nm) or radioactivity detector. Thin layer radiochromatography (TLRC) was done on a hard layer silica gel plate (Analtech Uniplates, Analtech Inc., AN-47521).Melting points were determined in a block heater and are reported uncorrected. Radioactivity of the positron-emitting radionuclides was measured in an isotope calibrator which was calibrated for various volumes to permit measurement at different stages of the synthesis. These measurements were also used when determining the specific activity.

Preparation of the Starting Material for α-Methylation

The reaction sequences used in the synthesis of benzaldimine of L-tryptophan methyl ester (**2**), the starting material in the synthesis of α-[^{11}C]methyl-L-tryptophan (**4**), are schematically outlined in Figure 1. Synthesis **2** was adapted from the methods described in Ref. *13* and *14*; it should be noted that it was not possible to reproduce the synthesis exactly as described because of instability (at least in our hands) of the L-tryptophan methyl ester in the basic conditions proposed for its preparation in Ref. *13*. The starting material, L-tryptophan methyl ester hydrochloride (1.8 g), was first, therefore, converted into L-tryptophan methyl ester (**1**) by extracting **1** into diethyl ether (80 ml) from 16 ml of cold 1N sodium hydroxide. The ether layer was washed with 30 ml of ice-cold distilled water.

After drying with anhydrous sodium sulfate, the ether was evaporated leaving a viscous liquid (yield 75%). Compound **1** was used without further purification in the synthesis of the Schiff base (**2**). 0.6 g (2.6 mmol) of **1** was mixed with 4 ml of dry triethylamine and the mixture cooled to −10 °C. A mixture of 0.274 g (2.6 mmol) of benzaldehyde in 5 ml of dry triethylamine was then added drop by drop during vigorous stirring. After all benzaldehyde had been added, the reaction mixture was stirred for another 4 hr at a bath temperature between −5 °C and −10 °C. At the end of this period, the reaction mixture was slowly warmed to room temperature as the stirring was continued for an additional hour. Solid KOH (2 g) was added to the reaction mixture, the mixture was left to stand for 30 min, and then filtered. After removal of the solvent, the remaining oil was triturated with diethylether giving white crystals which were recrystallized from benzene-cyclohexane (1:1) (*13*) (yield 50%). Identification data: M_p 124–125 °C, lit. 124–125 °C (*13*); ^{1}H-NMR ($CDCl_3$), δ(ppm) 3.29 (m, 2H,CH_2-); 3.61 (s, 3H, CH_3 (ester)); 4.10 (m, 1H,-CH-), 6.60–7.68 (m, 1H); 7.86 (s, broad, 1H); MS (m/e) 306 (10%, M^+, EI).

Preparation of $^{11}CH_3I$ (No-Carrier-Added)

No-carrier-added $^{11}CH_3I$ was prepared by adapting the method described earlier (*15*) and outlined in the synthesis of

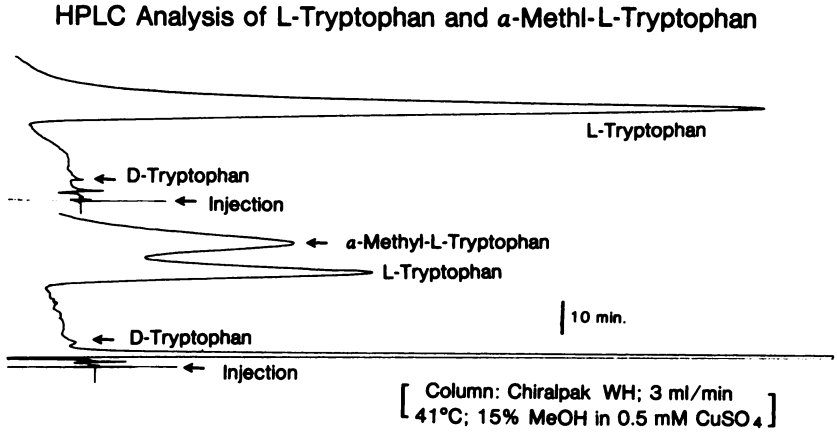

FIGURE 2
HPLC comparison of the enantiomeric purity of α-methyl-L-tryptophan and L-tryptophan used as starting materials. Chromatographic analysis was done on a Chiralpak WH column$^+$ using 15% methanol in 0.5 mM $CuSO_4$ as an elution solvent at 41 °C. The flow was 3 ml/min and a uv detector at 254 nm was used.

^{11}C- choline and choline analogues (*16,17*). Briefly, $^{11}CO_2$ was produced through a ^{14}N (p,α) ^{11}C reaction by irradiating research purity nitrogen (*18*) with 9.8 MeV protons. Carbon-11-labeled CO_2 was reduced to [^{11}C]CH_3OH with $LiAlH_4$. $^{11}CH_3I$ was released with hot HI in the same vessel in which $^{11}CO_2$ had been collected and then reduced to $^{11}CH_3OH$. Carbon-11-labeled methyl iodide dissolved in dry THF was added to a solution of an anion of 2 prepared in dry THF.

Synthesis of α-[^{11}C] Methyl-L-tryptophan (4)

A solution of 0.05 mmol of di-isopropylamine in 1–2 ml of dry THF was cooled to −78°C in a dry ice-acetone bath under nitrogen. A solution of methyl lithium in THF (0.05 mmol in 0.5 ml) was added drop-wise while the solution was stirred in the bath (*in situ* preparation of lithium di-isopropylamine (LDA). After all solution had been added, the mixture was stirred for another 20 min. To this solution 0.05 mmol of 2 in 1 ml of dry THF was added and the reaction mixture was stirred for 20 min. No-carrier-added $^{11}CH_3I$ (2–30 mCi) in THF was injected into the reaction mixture containing an anion of 2. (An alternative method was direct distillation of $^{11}CH_3I$ into the reaction mixture containing 2.) When all of the $^{11}CH_3I$ had been added, the solution was warmed to room temperature (~5 min) and filtered. The solvent was removed under reduced pressure and the residue treated with 1 ml of 2N HCl for 5 min in a bath at 135 °C to obtain α-[^{11}C]methyl-L-tryptophan. However, hydrolysis of the ester might not be necessary; it has been shown that methyl esters of other aromatic amino acids are promptly hydrolyzed in vivo. If removal of benzaldehyde only is desired, this could be done by hydrolyzing the product in 1N HCl. After evaporation of the acid under reduced pressure, 2 ml of sterilized water was added. The final solution was passed through a reverse-phase SEP-PAK column and a 0.2 μm Millipor filter membrane connected in series. The filtration system was washed with an additional 2 ml of sterilized water. The radiochemical yield of the final compound after a 30-min synthesis was around 20–25% calculated at the end of the synthesis (not corrected for radioactive decay). In a clinical run from 100 mCi of CH_3I, ~18 mCi of ^{11}C-labeled α-methyl L-tryptophan was obtained. On TLRC the final product showed one radioactive spot with $R_f = 0.85$ (CH_3OH-NH_4OH; 3.5:0.05), identical to that of an authentic sample prepared by the same procedure and identified by standard physicochemical methods (see below).

The same procedure as described above was used to synthesize nonradioactive α-methyl-L-tryptophan at the 1-mmol level to investigate reaction sequences, timing, and to determine stereoselectivity of the synthesis. The nonradioactive α-methyl-L-tryptophan was identified by standard physicochemical methods (given below) and the final product was purified by triturating the oily residue with ethyl ether. The compound was recrystallized from cyclohexane (*13*).

Identification was done by M_p (methyl ester (1)); 133–136 °C; lit. 135–137 °C (*13*); ^{1}H-NMR (amino acid hydrochloride); $\delta(D_2O)$ ppm; 1.36 (s, 3H, CH_3); 2.95–3.26 (AB-system, 2H, $-CH_2-$); 6.9–7.47 (m, 6H, indol system); MS (methyl ester): m/e (M = 232, 100%, CI).

Chiral Purity

Chiral purity was investigated on both radioactive and nonradioactive compounds using a Chiralpak-WH column at 41 °C and 15% MeOH in 0.5 m*M* $CuSO_4$ in water as the elution solvent (V_{el} = 111 ml, α = 3.1 at 41 °C) (Fig. 2). A chromatogram (Fig. 2) showed the chiral purity of the final product to be the same as that of the starting material, L-tryptophan, with no D-stereoisomer present. The chromatogram also proved that there was no racemization during the synthesis. For comparison, Figure 3 shows a composite chromatogram containing chromatograms of α-methyl-D- and -L-tryptophan and a chromatogram of a mixture of D- and L-stereoisomers.

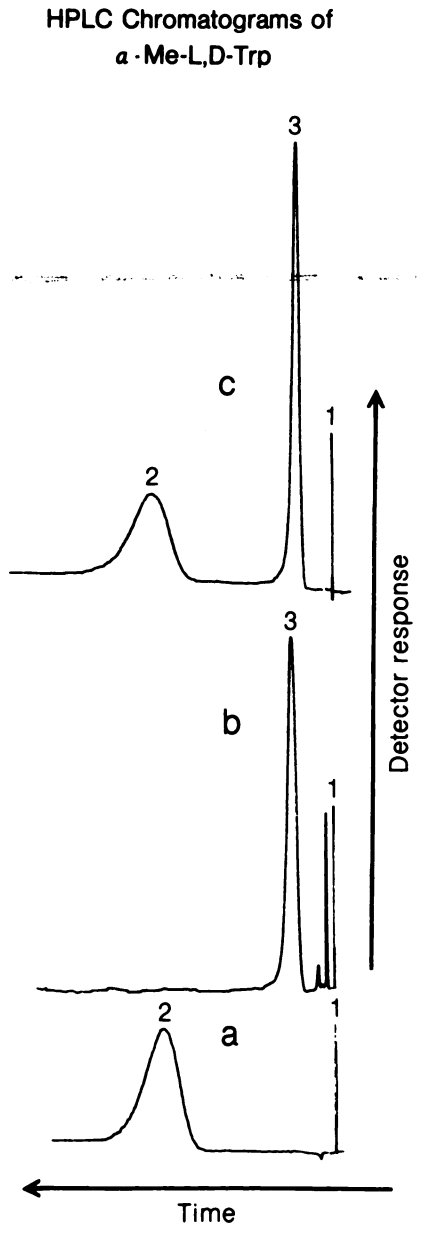

FIGURE 3
HPLC analysis of α-methyl L- and D-tryptophan synthesized separately from L- and D. (A) shows a chromatogram of α-methyl-L-tryptophan, (B) shows a chromatogram of α-methyl-D, and (C) shows a chromatogram obtained after mixing two enantiomers. These chromatograms were done at room temperature. Other conditions were the same as those mentioned in the Figure 2. Numbers 1, 2, and 3 identify the injections, α-methyl-L-tryptophan, and α-methyl-D-tryptophan, respectively.

RESULTS AND DISCUSSION

The synthesis of α-[^{11}C]methyl-L-tryptophan, as described in this manuscript, requires ~30 min to complete from the end of $^{11}CO_2$ collection. It takes ~10 min to convert $^{11}CCO_2$ to $^{11}CH_3I$. This reaction proceeds with a high chemical yield (>90%) relative to the amounts of $^{11}CCO_2$ present in the methyl iodide synthesis vessel (*15–17*).

The synthesis of α-methyl-L-tryptophan described in the literature (*13*) did not work in our laboratory. We had problems obtaining a free base of L-tryptophan methyl ester when following the procedure in the literature (*14*) because the methyl ester is not stable in the basic medium used. To overcome this problem we used ice-cold 1N sodium hydroxide and promptly extracted the L-tryptophan methyl ester into di-ethyl ether. The Schiff base was synthesized by using the procedure from Ref. *13*.

The Schiff base (**2**) was always used in the same week it was synthesized because prolonged storage resulted in partial decomposition and complicated the synthesis. It should be noted that indolic nitrogen in compound **2** was not protected. However, the results suggest the formation of an anion only on the α-carbon of compound **2** as was earlier reported by Brana et al. (*13*). We have not investigated the consequences if any, of the formation of an anion on the indolic nitrogen. This would probably reduce the yield of the final compound because of the side reaction resulting in partial formation of some N-methyl compound. The reaction mixture was analyzed after the acid was added and since no N-methyl compound was observed we concluded that the side reaction does not occur or at least does not have serious consequences on the reaction used in this work.

It is also interesting to note that the reaction is stereospecific. Retention of the configuration of the chiral carbon in similar alkylation reactions was explained as being due to the anchimeric assistance of the imine nitrogen (*19*). An intermediary shown in Figure 4 (**6**) was proposed (*19*) as a compound that allows the stereo-configuration to be "locked" in the position existing in the original amino acid. One should note that because of the lithium-nitrogen bridge, the configuration of the intermediary is not planar.

To prove that the configuration is indeed retained in the reaction used in this work, we have used the same reaction sequences on L,D and separately on the L and D stereoisomers of tryptophan. As shown in Figure 2, which represents chromatograms obtained on the chiral column, the reactions resulted in α-methyl-tryptophan of the same chiral composition as the tryptophan used as a starting material. Figure 3 is a composite containing chromatograms of both stereoisomers separately (Figure 3) and of a chromatogram obtained after mixing two isomers. A slight variation in the retention times was noticed after consecutive injections of compounds but this was not large enough to make identification of the components doubtful. Since there is a large difference in the elution volumes of L- and D-α-methyl tryptophan, $V_L = 123$ ml and $V_D = 24.6$ ml, respectively ($\alpha = 5.66$; room temperature), the identification was relatively straightforward (see Fig. 3). The synthesis proposed here for everyday use (applying SEP-PAK purification) would produce a radiopharmaceutical containing L-tryptophan as a chemical impurity. This is the only chemical impurity seen by the HPLC analysis of the final radiopharmaceutical. We have not found any need for HPLC purification to remove any potential chemical impurity other than tryptophan. If there is any doubt about chemical purity, HPLC purification should be done.

The removal of tryptophan, the starting material, could also be accomplished by HPLC on a chiral column, but the long retention time (V = 85.5 ml) makes this approach impractical (see Fig. 2). However, there are other solvents available to obtain separation of L-tryptophan and L-methyl-tryptophan in a much shorter time period (Sourkes TL, unpublished data). For this reason, the amount of L-tryptophan should be kept to a level that will not change the steady-state plasma concentration of tryptophan after injection of the tracer containing L-Trp. The levels of L-tryptophan used in this work would add < 1% to the total plasma L-tryptophan.

The specific activity obtained in this work was determined by HPLC and uv-detector (SA = 2,000 Ci/mmol at the end of the synthesis). This specific activity is acceptable because an injection of ~20 mCi of the tracer would contain $\sim 1 \cdot 10^{-5}$ mmol of α-methyl-L-tryptophan. Assuming instantaneous dilution in human blood, the concentration of the tracer would be $\sim 2 \times 10^{-9}$ mmol/ml ($2 \cdot 10^{-6}$ m*M*). This concentration is

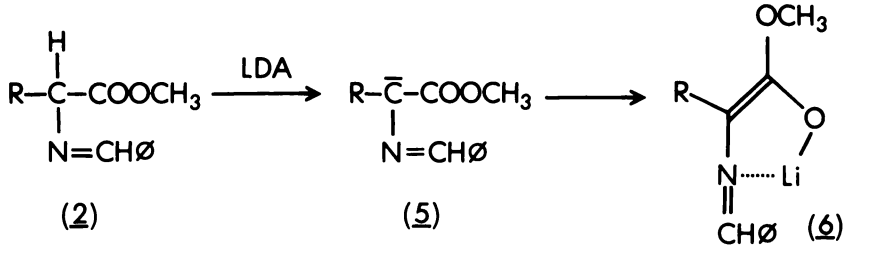

FIGURE 4
Schematic representation of the intermediary from which stereoselectivity is derived. A nitrogen-lithium bridge results in a nonplanar enolate which on alkylation results in retention of the stereoconfiguration of amino acid.

several orders of magnitude below the concentration of L-tryptophan in the blood (around 0.074 m*M*). No special attempts to increase the specific activity were made because the amount of α-methyl-L-tryptophan that would be injected with the final radiopharmaceutical is well below the level that could induce pharmacological effects (*11,12*). The amounts injected will also be well below the K_m value for the blood-brain barrier transport of L-tryptophan (*20*), thereby ensuring the validity of the tracer kinetics.

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