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Synthesis of N-[^{11}C]-Methyl-L-DOPA

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Summary

The radiochemical synthesis of N-[^{11}C-methyl]-L-DOPA was accomplished by N-methylation of the methyl and ethyl esters of L-N-tert-butyl-oxycarbonyl-[ß-(3,4-dimethoxyphenyl)] alaninate with [^{11}C]iodomethane using sodium hydride in tetrahydrofuran and deprotection of the N-methyl intermediate with hydriodic acid. A catalytic influence of Kryptofix on the methylation reaction was observed. Using the ethyl ester precursor, the average specific activity at the end-of-synthesis was 972 mCi/µmole. The synthesis was completed in an average of 45 minutes following the end-of-bombardment.

Key Words: N-[^{11}C-methyl]-L-DOPA, carbon-11, positron emission tomography, dopamine, radiotracer.

Introduction

Over the past decade there has been considerable interest in the study of the dopaminergic neurotransmitter system and its relationship to normal brain function and disease. Tracers have been developed to probe this system through interaction with postsynaptic dopamine receptors and presynaptic dopamine uptake sites (1 - 17). Various elements of the neurotransmitter synthesis and storage pathway have recently been probed using 6-fluoro-DOPA labeled with fluorine-18 (18 - 21). A DOPA derivative that could be synthesized readily from carbon-11 may be useful

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for extending these studies. Known methods for the synthesis of N-methyl-L-DOPA (22, 23) are not applicable to the preparation of the compound labeled with carbon-11 because of the short half-life ($t_{1/2}$ = 20 minutes) of the radioisotope and the methyl group is usually introduced at the beginning of a multistep synthesis. Direct radiomethylation of DOPA or DOPA derivatives is preferable; however, N-methylation of amino acids is not a trivial problem. This report describes the synthesis of N-[^{11}C-methyl]-L-DOPA.

Results and Discussion

The initial attempts to synthesize N-methyl-L-DOPA used the protected amido-ester shown in Figure 1. Only the product of ^{11}C-methylation at the carboxyl moiety and not the expected N-methyl protected product was observed during the labeling of this precursor with [^{11}C]iodomethane in the presence of a strong base.

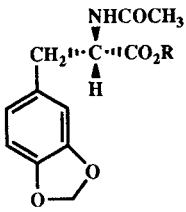

Figure 1. Protected amido-ester of L-DOPA

Over the past several years, new synthetic methods for N-methyl amino acids have been developed. Except for the method of reduction of the imines generated from amino acids esters, formaldehyde and cyclopentandiene (24), the other methods are direct methylation of N-acyl, N-tosyl and N-$POPh_2$ amino acid derivatives using iodomethane in presence of base (NaH, t-BuLi) or Ag_2O (25 - 32). An alternate method for the synthesis of N-[^{11}C-methyl]-L-DOPA was chosen by methylation of the protected precursor - L-methyl-N-tert-butyl-oxycarbonyl-[ß-(3,4-dimethoxyphenyl)] alaninate, **5**, with [^{11}C]iodomethane in presence of the sodium hydride.

The starting material, L-methyl-N-tert-butyloxycarbonyl-[ß-(3,4-dimethoxyphenyl)] alaninate, **5**, was prepared from L-methyl-3-methoxy-L-tyrosinate, **3**, (33) by the route shown in Figure 2 and purified by semipreparative HPLC.

The initial attempts to alkylate **5** with [^{11}C]iodomethane and deprotect the alkylated intermediate, **6**, with hydriodic acid gave the final product, **1**, with total radiochemical yield of only 0.14% (corrected from $^{11}CH_3I$) in 55 minutes from the end-of-bombardment (Figure 3). Methylation of the N-t-BOC amino acid using t-BuLi or t-BuOK, and the use of the high boiling solvent dioxane was also attempted. An unexpected low methylation yield was observed in dioxane, and large quantities of by-products were observed when t-BuLi and t-BuOK were used as the base.

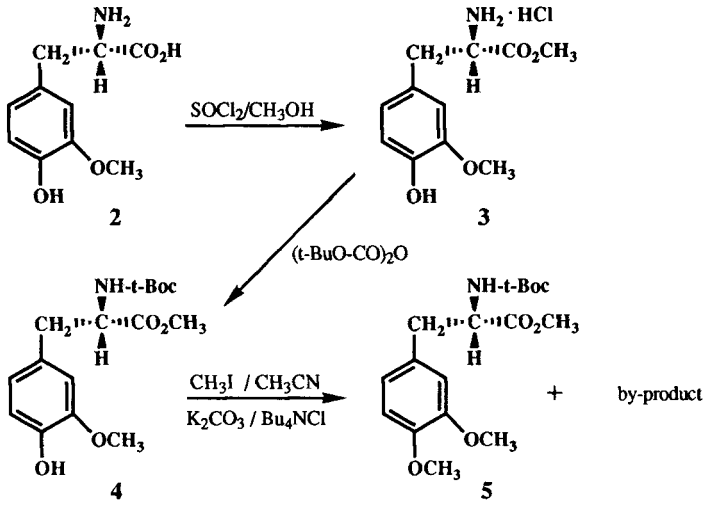

Figure 2: Synthesis of t-Boc protected L-DOPA

Based on analytical HPLC, the specific activity of the final product from this precursor was determined to be 0.15 - 0.3 Ci/μmole at the end of synthesis. This specific activity was considerably lower than that observed for other [^{11}C]-radiotracers prepared in our laboratory; during this same period, specific activities of between 1.5 - 3 Ci/μmole were observed. One possible explanation for the abnormally low specific activity could be an intramolecular methylation of the precursor taking place during the formation of the precursor anion as shown in Figure 4.

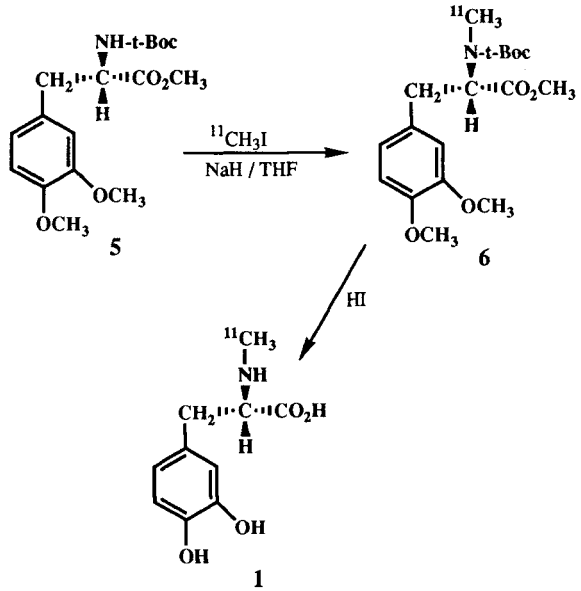

Figure 3: Radiosynthesis of L-N-[^{11}C]-methyl-DOPA.

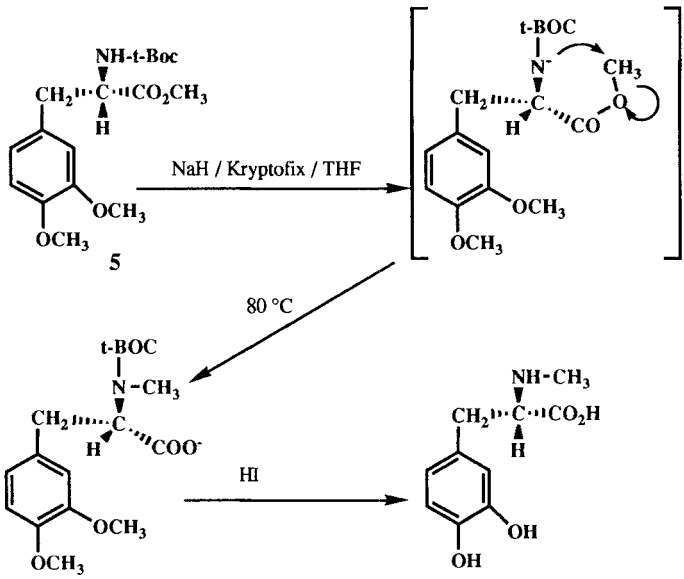

Figure 4: Intramolecular methylation of the methyl ester of protected DOPA.

The formation of carrier N-methyl-DOPA was observed by analytical HPLC without the addition of iodomethane in model reactions (precursor **5**, NaH, Kryptofix, THF). Compared to the final product DOPA, the amount of precursor converted to N-methyl-DOPA was 0.5%, 2%, and 5% after 1, 15, and 35 minutes of heating at 80 °C, respectively.

To improve the specific activity and eliminate the proposed rearrangement, L-ethyl-N-tert-butyloxycarbonyl-[ß-(3,4-dimethoxy-phenyl)] alaninate, **7** (Figure 5) was prepared. Using this precursor, no intramolecular ethylation was observed in identical model reactions. The radiochemical synthesis of N-[^{11}C]-methyl-L-DOPA from **7** provided a final product with a specific activity higher than 1 Ci/µmole.

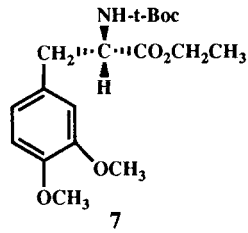

Figure 5: L-ethyl-N-tert-butyloxycarbonyl-[ß-(3,4-dimethoxyphenyl)] alaninate

The synthesis, semipreparative HPLC, and formulation was completed in an average run time of 45 minutes with an average radiochemical yield of 12 % (calculated at end-of-synthesis; corrected for decay). The average specific activity was 972 mCi/µmole at end-of-synthesis. The final formulated solution was

chemically and radiochemically pure as determined by analytical HPLC. Thin layer chromatography was used to determine the presence of Kryptofix in the final product. The Kyptofix concentration was less than the limit of sensitivity of the test; i.e. < 10 μg/ml.

Experimental

All reagents used were ACS or HPLC purity grade. ^{1}H-NMR spectra were recorded on a Bruker AM 300 (300 MHz); chemical shifts (δ) were recorded in parts per million downfield from TMS. Low resolution mass-spectra (MS) were recorded on the Finnigan MAT ITD instrument modified for matrix assisted laser desorption ionization of the analytical sample. High resolution mass-spectra was recorded on an AEI MS-30 (University of Minnesota Mass Spectroscopy Laboratory). Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. High performance liquid chromatographic analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) detector, and a single two inch NaI crystal radioactive detector. HPLC chromatograms were recorded by a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer using Dynamax - version 1.3 software. HPLC semipreparative purifications were performed on an Alltech 10 μ C-18 Econosil column (250 x 10 mm) using a mobile phase of 99.9% water / 0.1% acetic acid at flow rate 7 mL/min. Chemical and radiochemical purity were determined using an Alltech 10 μ C-18 Econosil column (250 x 4.6 mm) with the same mobile phase as in the semipreparative HPLC at a flow rate 2 mL/min for final product, **1**, and using 50% acetonitrile / 50% water (0.1 M ammonium formate) for precursor, **5** and flow rate of 3 mL/min. The Kryptofix concentration in the final product was determined with Macherey-Nagel SIL G UV254 TLC plates (4 x 8 cm) and a mobile phase of CH_3OH - 30% NH_4OH (4:1) (Kryptofix R_f = 0.3, iodine stain). A dose calibrator (Capintec 12R) was used for all radioactivity measurements.

L-methyl-N-tert-butyloxycarbonyl-[ß-(3,4-dimethoxyphenyl)] alaninate, 5.

Thionyl chloride (2.26 g, 19 mmole) was added dropwise over a 5 minute period to a stirred suspension of 3-methoxy-L-tyrosine hydrate (Sigma) (0.5 g, 2.18 mmole) in methanol (10 mL) cooled in a dry ice-ethanol bath. The resulting solution was refluxed for 20 minutes, stirred at room temperature for 1 hour, and evaporated to yield a solid (methyl 3-methoxy-L-tyrosinate hydrochloride, **3**) (33).

The residue was suspended in 5 mL $CHCl_3$, $NaHCO_3$ (0.2 g, 2.38 mmole) and NaCl (0.4 g) in 3.5 mL water. Di-tert-butyl pyrocarbonate (Sigma) (0.517 g, 2.37 mmole) dissolved in two mL of $CHCl_3$ was added. The mixture was refluxed for 90 minutes. After the solution cooled, it was separated and the dark aqueous solution

was extracted with $CHCl_3$. The $CHCl_3$ layer was dried and evaporated to yield an oil. The colorless oil was dissolved in two mL of CH_3CN and evaporated to yield a solid methyl N-tert-butyloxycarbonyl-3-methoxy-L-tyrosinate, **4**. Yield: 0.7 g, 98%. The solid was recrystallized with $CHCl_3$-hexane (1:2) to yield white needles. ^{1}H-NMR, $CDCl_3$ (δ, ppm): 1.40 (s, 9H, t-Bu), 2.99 (m, 2H, CH_2), 3.70 (s, 3H, $COOCH_3$), 3.82 (s 3H, m-OCH_3), 4.76 (dd, 1H, CH), 5.78 (s, 1H, OH), 6.70 (m, 3H, Ar).

A stirred mixture of **4** (0.7 g, 2.15 mmole), potassium carbonate (0.47g, 3.4 mmole), tetrabutylammonium chloride (0.02 g, 72 μmole), iodomethane (0.49 g, 3.5 mmole) and 5 mL dry acetonitrile was refluxed overnight and filtered. The solids were rinsed on the filter with $CHCl_3$ and the filtrate evaporated to dryness under reduced pressure. The oily residue was twice recrystallized with hexane to yield **5** (0.35 g, 47.9%) containing 1% by-product. The analytical HPLC of **5** exhibited one peak (k' = 9; 99%).

The product was purified using semipreparative HPLC column (Alltech 10 μ C-18 Econosil column (250 x 10 mm), eluent 50% acetonitrile / 50% water (0.1 M ammonium formate), flow rate 10 mL/min). The desired product, **5** (k' = 8), was collected and the organic solvent evaporated under reduced pressure. The residue was dissolved in water and extracted with $CHCl_3$. After drying and evaporation of the $CHCl_3$, the solids were recrystallized with hexane to yield white needles of **5** (m.p. 72 °C). Analytical CHN calculated for $C_{17}H_{25}NO_6$: C, 60.18; H, 7.37; N, 4.13. Found: C, 60.08; H, 7.42; N, 4.08. MS of **5**: Low resolution, m/z (rel. intensity), [M$^+$] 339 (100%); High resolution, [M$^+$] 339.1656 (7.82%), calculated for $C_{17}H_{25}NO_6$: M 339.1681. ^{1}H-NMR, $CDCl_3$ (δ, ppm): 1.41 (s, 9H, t-Bu), 3.01 (m, 2H, CH_2), 3.70 (s, 3H, $COOCH_3$), 3.81 (d, 6H, m- and p-OCH_3), 4.75 (dd, 1H, CH), 6.66 (m, 3H, Ar).

L-ethyl-N-tert-butyloxycarbonyl-[ß-(3,4-dimethoxyphenyl)] alaninate, 7.

The synthesis of **7** was similar to the synthesis of methyl ester, **5**, except for the use of ethanol for esterification. After purification by HPLC, the product (k' = 10) was prepared as a clear oil. MS of **7**: High resolution, m/z (rel. intensity), [M$^+$] 353.1870 (2.2%), calculated for $C_{18}H_{27}NO_6$: M 353.1837. ^{1}H-NMR, $CDCl_3$ (δ, ppm):1.21 (t, 3H, $C\underline{H}_3$-CH_2), 1.43 (s, 9H, t-Bu), 3.02 (m, 2H, CH_2-Ar), 3.82 (d, 6H, m- and p-OCH_3), 4.75 (dd, 1H, CH), 6.6-7.2 (m, 3H, Ar).

Synthesis of N-[^{11}C-methyl]-L-3-hydroxytyrosine, N-[^{11}C-methyl]-L-DOPA, 1.

A reaction mixture of dry THF (0.5 mL), **5** (2 mg, 5.9 μmole), sodium hydride (2 mg, 83 μmole), and Kryptofix (2.2 mg, 5.85 μmole) was stirred for 30 minutes at room temperature. [^{11}C]Iodomethane, produced as previously described

(34), was swept by a stream of argon gas into the cooled (-78 °C) reaction vial. The vial was heated at 80 °C for 10 minutes then the solvent was evaporated dryness under reduced pressure. Hydriodic acid, stabilized with hypophosphorous acid, (Aldrich) (1mL) was added. The resulting solution was heated at 140 °C for 15 minutes and evaporated to dryness under reduced pressure. The residue was taken up in an aqueous solution of 0.1 M sodium acetate (1 mL). The solution was applied to the semipreparative HPLC column and the desired product, **1** (retention time = 5.5 minutes; k' = 1.8) was collected. The radiochemical purity and specific activity of final solution were determined by analytical HPLC. The final product eluted with the same retention time (4.3 minutes) as an authentic sample of N-methyl-D,L-DOPA (1).

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