Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXI, No. 1

SYNTHESIS AND SEPARATION OF 3-O-METHYL-2- AND 6-[^{18}F]-FLUORODOPA

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Abstract

3-O-Methyl-2- and 6-[^{18}F]-fluorodopa were synthesized in 8 % radiochemical yield by the direct fluorination of a protected L-dopa derivative with [^{18}F]-acetyl hypofluorite. The 2- and 6-fluoro isomers were separated and purified by reverse phase HPLC.

Key Words: fluorodopa, dopamine metabolism

Introduction

One of the major metabolites of 6-[^{18}F]fluorodopa (6FD), in the blood and brain, is 3-O-Methyl-6-[^{18}F]fluorodopa (Me6FD) (1). In order to derive an accurate model for 6FD one must also understand the kinetics of Me6FD. The kinetics of 6FD measured in the striatum with PET reflect transport and decarboxylation in nigro-striatal neurons and may reflect storage in the dopamine vesicles of the same neurons. However, 6FD is converted to Me6FD which is freely transported between brain and plasma. Since Me6FD is cleared from the plasma slowly, its accumulation in brain is proportional to the time integral of the plasma 6FD concentration, and is thus nearly identical to that of trapped 6FD. Isolation of 6FD uptake kinetics in the striatum thus require time course data measured in other brain regions and assumptions about the relationship between Me6FD kinetics in these regions. Also, if 6FD has been synthesized by the direct fluorination method (2) some 2FD may be present which will later be metabolized to Me2FD.

In order to test these assumptions and to understand the effect of possible Me2FD contamination, we have synthesized MeFD, as described below, for administration in primates.

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0362–4803/92/010039–05$05.00

Received 5 August, 1991
Revised 3 September, 1991

Experimental

General

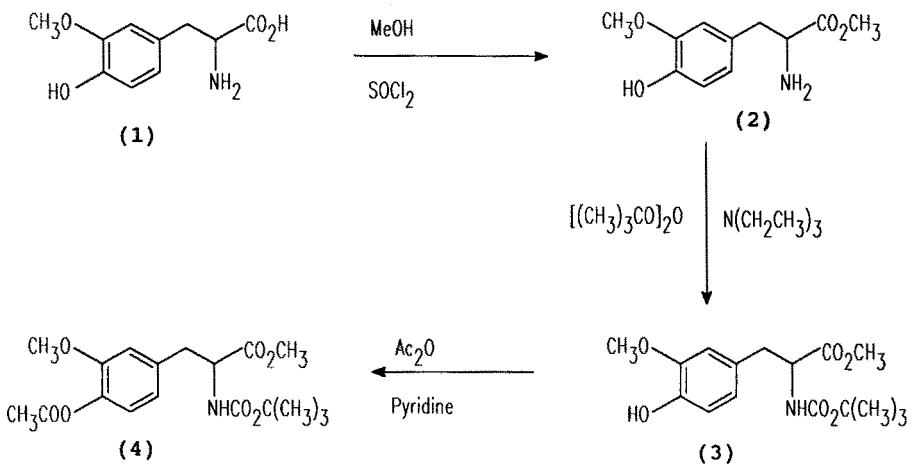
3-Methoxy-L-tyrosine monohydrate was purchased from Sigma Ltd. Thionyl chloride and Di-tert-butyl dicarbonate were purchased from Aldrich. Acetic anhydride, acetonitrile, ACS methanol, hydrochloric acid (37 %), dichloromethane, ethyl acetate, glacial acetic acid, hydrobromic acid (48 %), magnesium sulfate, petroleum ether, potassium phosphate, pyridine, sodium chloride, sulfuric acid (95 %), and triethylamine were obtained commercially. Thin layer chromatographic analysis (TLC) was performed on silica gel TLC plates (Merck No. 5534). The TLC plates were visualized by ultra violet light. ^{1}H and ^{19}F spectra were recorded at 200 MHz.

Microanalyses were performed by Canadian Microanalytical Service, Ltd., Delta, B.C. All melting points were determined on a capillary oil bath instrument and are uncorrected. High Performance Liquid Chromatography (HPLC) was carried out on a Waters system equipped with a Nucleosil C-18 preparative column (250 mm × 22.5 mm)(Phenomenex, Inc.) using a 5% acetonitile/0.07 M KH_2PO_4 eluant at a flow rate of 6 mL/min.

Synthesis of L-methyl-(β-(3-methoxy-4-hydroxyphenyl)] alaninate hydrochloride (2)

To 3-methoxy-L-tyrosine monohydrate **1** (4.88 g, 21.3 mmol) was added methanol (30 ml), and the suspension was cooled to 0 °C. Whilst stirring and over nitrogen, thionyl chloride (1.91 mL, 26.2 mmol) was added dropwise. The resultant faint yellowish solution was refluxed for four hours, and subsequently the solvent was evaporated to yield 5.7 g of white powder m.p. 176-178 °C (lit. 173-174 °C).

A stirred suspension of the methyl ester hydrochloride **2** (5.7 g, 21.8 mmol) in dichloromethane (20 ml) was cooled to 0 °C. Whilst under nitrogen, one equivalent of triethlyamine (2.2 g, 3 mL), was added dropwise, until the pH of the solution was neutral. Di-tert-butyl-dicarbonate (4.8 g, 5 mL) was added dropwise followed by another equivalent of triethylamine (3 mL). The mixture was left stirring overnight. The volatile solvent was evaporated and the resulting white precipitate was triturated with ethyl acetate (30 ml), using an ultrasonic water bath. The precipitate was filtered and washed with



Synthesis of L-methyl-N-carbo-tert-butoxy-[β-(3-methoxy- 4-acetoxyphenyl)] alaninate (4)

additional ethyl acetate (30 ml). The combined organic filtrate was washed with water (2 X 10 ml), then dried with magnesium sulfate, filtered, and evaporated to yield a crude yellow oil of **3**.

The crude carbamate **3** was dissolved in dichloromethane (30 ml). To this stirred solution was added a solution of pyridine and acetic anhydride (1:1, 12 mL) The solution was cooled to 0 °C and was stirred for one hour at room temperature. After about one hour the solution was poured into 1M H_2SO_4 (20 ml) and extracted with ethyl acetate (4 X 25 ml). The combined extracts were washed

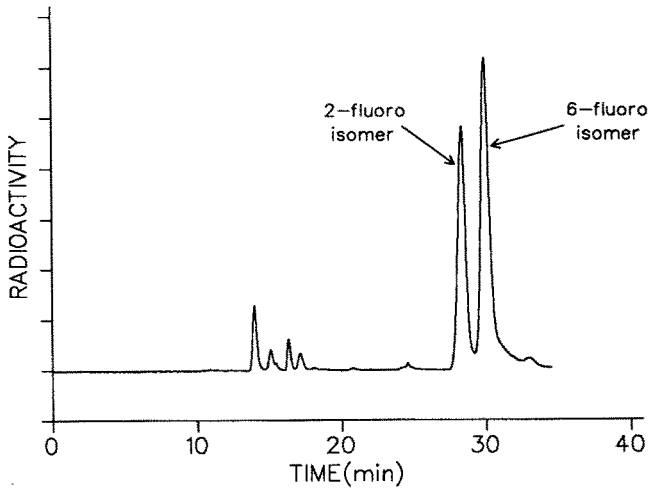
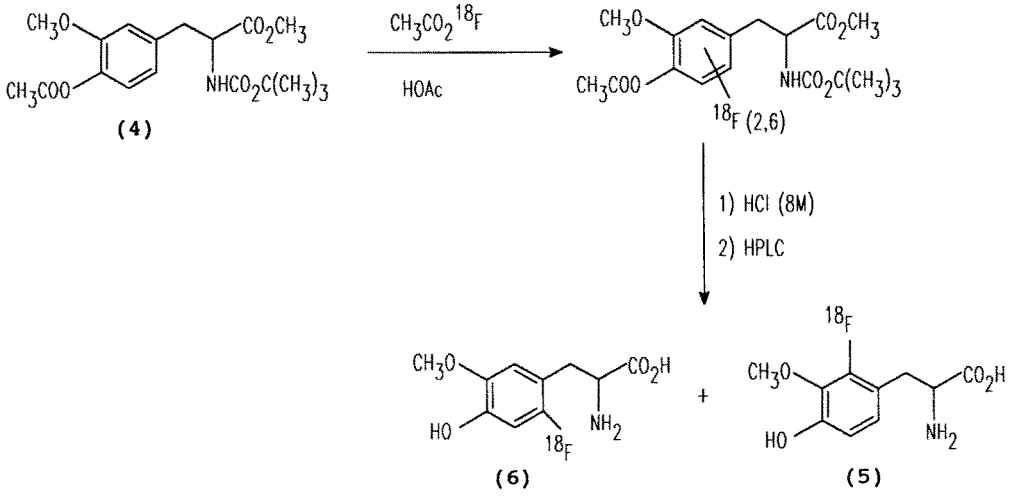


Figure 1: HPLC radiochromatogram of 2- and 6-MeFD

with saturated NaCl solution (2 X 10 ml). The organic layer was dried with $MgSO_4$ and the solvent evaporated. The resulting pale yellow oil was chromatographed by Flash Chromatography using 30% EtOAc/Hexane followed by crystallization from ethanol to give 1.52 g (20% overall yield from **1**) of **4**, m.p. 72-74°. ^{1}H-nmr ($CDCl_3$): δ 1.44 [s, 9H, $NC(CH_3)_3$], δ 2.28 [s, 3H, CH_3CO_2Ar], δ 3.08 [m, 2H, CH_2], δ 3.74 [s, 3H, CO_2CH_3], δ 3.81 [s, 3H, CH_3O], δ 4.58 [m, 1H, CH], δ 4.99 [d, 1H, NH], δ 6.73 [m, 2H, ArH], δ 7.15 [d, 1H, ArH].

Synthesis of 3-O-Methyl-2- and 6-[^{18}F]-Fluorodopa (5 and 6)

The fully protected carbamate **4**(55 mg), was placed in a glass reaction vessel (2 x 10 cm) and dissolved in glacial acetic acid (12 ml). ^{18}F-Acetyl hypofluorite (2) was bubbled into the mixture at a flow rate of 120 mL/min. The product was transferred to a rotary evaporator, and the glacial acetic acid was removed. To the residue was added HCl (8 M, 3 ml) and the mixture boiled for 30 min. The mixture was evaporated to dryness and water added (2 x 5 ml) in two portions and each successively evaporated



to remove any remaining acid. The crude product was dissolved in potassium dihydrogen phosphate buffer (0.06 M, 1.5 ml) and purified by HPLC using a Nucleosil C-18 column (5 μ, 250 mm x 22.5 mm) with an eluant of 5% acetonitrile/0.07 M KH_2PO_4 at a flow rate of 6 mL/min. The product was detected with a UV detector at 254 nm and a radioactivity detector. The two fractions eluting at 27 and 29 min were collected, and the ^{19}F-nmr were recorded. The collected fractions were determined

to be the 2-fluoro and 6-fluoro isomers respectively (Fig 1). ^{19}F-nmr (D_2O, referenced to CF_3COOH):. 6-fluoro isomer, δ -48.9 (dd, $J_{H5,F}$ = 10.4 Hz, $J_{H6,F}$ = 7.3 Hz.); 2-fluoro isomer, δ -58.1 (d, $J_{H6,F}$ = 7.8 Hz).

Results and Discussion

The synthesis of Me2FD and Me6FD was achieved in a similar synthetic route to our earlier synthesis of 6-fluorodopa (2) except that in the synthesis of MeFD, HCl was used instead of HI to deprotect and a t-Boc group was used to protect the amine instead of an acetamide. Using this precursor the radiochemical yield of both isomers was 8% (based on target $^{18}F_2$, decay corrected to EOB) and the radiochemical purity was >95%. The overall synthesis time from EOB was 115 minutes and the specific activity was 200 - 500 mCi/mmol.

Although the synthesis is not stereoselective it does offer the advantage of providing both fluorinated ring isomers for use in animal studies. The stereoselective synthesis of Me6FD via a mercury-dopa derivative has been reported but few details of the synthesis were given (3). In our own studies (4) we have found that the 2-fluoro isomer is transported through the blood brain barrier at a different rate than is the 6-fluoro isomer and that it is metabolized differently. This may be important information for those synthesizing 6-fluorodopa by the direct fluorination method (2) which potentially may yield a product contaminated with the 2-fluorodopa.

Acknowledgements

We wish to thank the Medical Research Council of Canada for generous financial support. We also would like to thank Lawrence Lee for assisting with the synthesis of these compound.

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