
Routine Synthesis of L-[¹⁸F]6-Fluorodopa with Fluorine-18 Acetyl Hypofluorite

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The synthesis of L-[¹⁸F]6-fluorodopa (2.4–10.6 mCi) was done by passing gaseous [¹⁸F]acetyl hypofluorite through a solution of L-methyl-N-acetyl- $[\beta$ -(3-methoxy-4-acetoxyphenyl)]alaninate in acetic acid at room temperature followed by the hydrolysis of the intermediate products with concentrated hydriodic acid. The desired fluorodopa isomer was isolated in 8% EOB radiochemical yield by high performance liquid chromatography in an overall synthesis time of 100 min.

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It has recently been shown that L-[¹⁸F]6-fluorodopa can be used to study cerebral dopamine metabolism in the human brain (1,2). Three synthetic methods for the preparation of this compound have been reported in the literature. The first method is based on the reaction of [¹⁸F]acetyl hypofluorite with a partially blocked dopa derivative in acetic acid (3). The second method involves the reaction of [¹⁸F]F₂ with dopa in liquid HF (4) and the third, most recent method utilizes [¹⁸F]F₂ and a dopa-silane precursor (5). The first two procedures produce a mixture of the three possible aromatic ring fluorinated isomers of dopa out of which can be separated the 6-fluoro isomer by high performance liquid chromatography (HPLC), in low but usable yields (1.3–3% EOS). The third method affords the highest yield (8% EOS) and produces only the 6-fluoro-isomer. We report here an improved procedure based upon the reaction of [¹⁸F]acetyl hypofluorite on a fully derivatized dopa substrate.

This paper describes the reaction of acetyl hypofluorite with L-methyl-N-acetyl- $[\beta$ -(3-methoxy-4-acetoxyphenyl)]alaninate (Fig. 1) (6). To date, this substrate gives the highest yield of L-[¹⁸F]6-fluorodopa and provides the most convenient route to its preparation. The reaction is also a convenient route for the preparation of L-[¹⁸F]2-fluorodopa since it is produced in nearly equivalent amounts with the 6-fluoro-isomer. The re-

sults of the reaction between acetyl hypofluorite and a series of other derivatized dopa precursors will be published elsewhere (7).

MATERIALS AND METHODS

3-O-Methyl dopa was purchased commercially*. Hydriodic acid (55%) was obtained† and was distilled from red phosphorous under H₂ and sealed in ampules in 3-ml portions containing 0.04% hypophosphorous acid as stabilizer.

Thin layer chromatographic analysis (TLC) was performed on aluminum backed TLC plates‡. HPLC purification was carried out on a Waters chromatograph equipped with a NaI-Tl scintillation detector. For the purification of L-[¹⁸F]6-fluorodopa a 50 cm, semi-preparative, reverse phase column§ was used with 0.1% acetic acid in USP water as the eluant at a flow rate of 4 ml/min. The enantiomeric purity of the collected product was analyzed through ligand exchange chromatography as previously reported (8). ¹H and ¹⁹F spectra were recorded at 80 and 254 MHz, respectively, as D₂O solutions containing a trace of HCl. Trifluoroacetic acid was used as an external reference for ¹⁹F nuclear magnetic resonance (NMR) and TMS was used as an external reference for ¹H NMR.

Preparation of L-Methyl-N-Acetyl- $[\beta$ -(3-Methoxy-4-Acetoxyphenyl)] Alaninate 1

To a cold (0°C) stirred suspension of L-3-O-methyl dopa* (5.0 g) in dry methanol was introduced a rapid stream of HCl gas for 1 min. The resultant solution was

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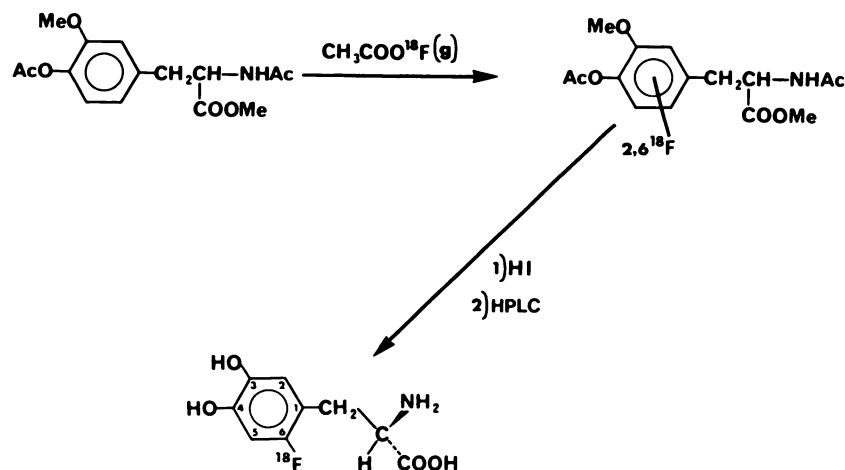


FIGURE 1
Flow scheme for synthesis of L-¹⁸F-6-fluorodopa

stirred at room temperature for 24 hr. The solution was evaporated, dissolved in methanol, and evaporated again. The residue was dried further on the vacuum line for 1 hr to give 5.38 g (94%) of solid material. The crude amino-ester·HCl was suspended in dry pyridine-acetic anhydride (1:1, 25 ml). The mixture was cooled under running water and then stirred at room temperature for 10 min. The mixture was poured into 1M H₂SO₄ (100 ml) and extracted with ethyl acetate 3 × 60 ml. The combined extracts were washed with brine, dried, and concentrated to afford a colorless oil which quickly crystallized when cooled to room temperature. This material was dried under vacuum to give 6.07 g (90% overall yield) of a white solid m.p. 111–113°C. The compound can be recrystallized from ethyl acetate, filtered, and washed with ethyl acetate/hexane (2:1) to give 5.16 g, m.p. 111–113°C. Thin layer chromatography analysis (EtOAc/trace NH₄OH) showed only one spot (UV detection) before and after recrystallization.

¹H NMR (80 MHz-CDCl₃) δ2.0 (s, 3H), 2.3 (s, 3H), 3.1 (d, 2H), 3.7 (s, 3H), 3.8 (s, 3H), 4.9 (m, 1H), 6.7–7.0 (m, 3H). Anal. calcd. for C₁₅H₁₉NO₆: C 58.27, H 6.15, N 4.53. Found: C 58.21, H. 6.20 N 4.52.

Fluorination of 1 with [¹⁸F]Acetyl Hypofluorite

Compound 1 (60 mg, 200 μmol) was dissolved in glacial acetic acid (20 ml) and placed in a glass reaction vessel (2 cm × 10 cm). After target irradiation (9) the ¹⁸F-F₂/Ne (80 μmol F₂) was passed through a solid KOAc/HOAc column to produce gaseous [¹⁸F]acetyl hypofluorite (10) which was bubbled through the above solution via a 1/16-in. O.D. teflon tube at ~50 ml/min at room temperature. After fluorination the acetic acid solution was transferred to a rotary evaporator, allowed to stand for 5 min and evaporated to dryness with a hot air gun as the heat source (Fig. 2). Hydriodic acid (3 ml, see above) was added and the solution heated with an oil bath at 155°C for 15 min. The HI was

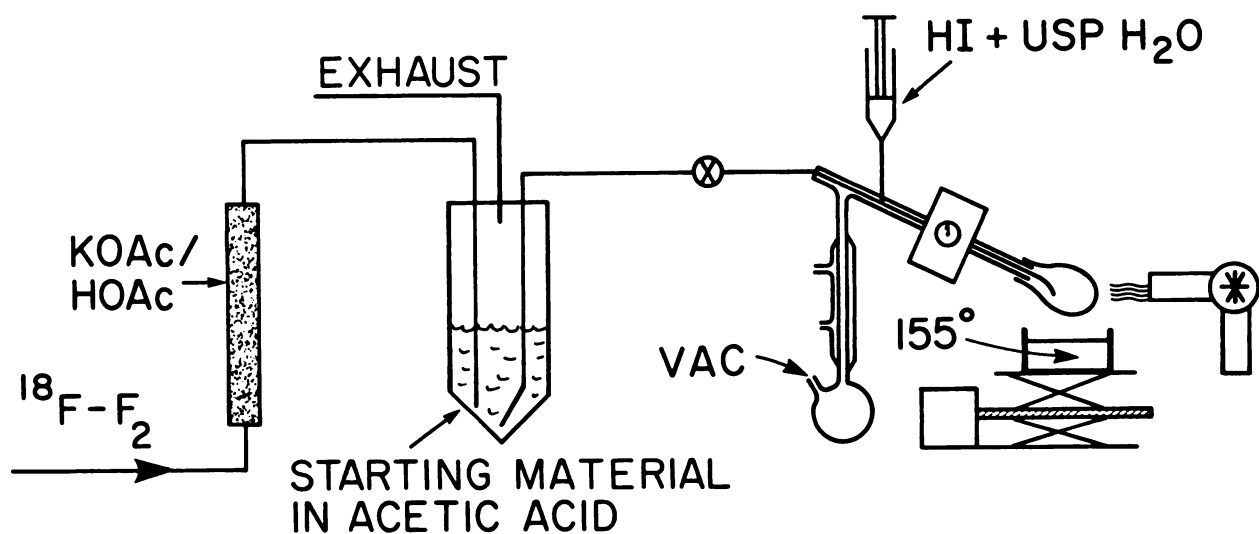


FIGURE 2
Schematic of remotely operated system for preparation of L-[¹⁸F]6-fluorodopa

evaporated and water added (2×5 ml) in two portions and each successively evaporated to remove remaining HI (heat gun used for evaporations). Water (2 ml) was added and the solution filtered through a $0.22 \mu\text{m}$ filter[†] and again evaporated to dryness. The residue was dissolved in concentrated pH 7 phosphate buffer (0.5 ml) and injected into the HPLC. The yield of L-[¹⁸F]6-fluorodopa was on average 8% decay corrected to EOB with a yield range of 5.1–10.7%. The overall synthesis time was 100 min. The yield of L-[¹⁸F]2-fluorodopa was approximately the same as that for the 6-fluoro isomer.

The specific activity of the final product was determined to be about 200 mCi/mmol. Typical preparations for human or animal use yielded 2.3–10.6 mCi (85 MBq–0.4 GBq) at EOS when starting, from 54–258 mCi (2 GBq–9.5 GBq) of ¹⁸F-F₂ (EOB).

6-Fluorodopa ¹⁹F NMR: +50.2 ppm (dd, $J_{\text{F-H}(5)} = 10.9$ Hz, $J_{\text{F-H}(2)} = 7.5$ Hz); ¹H NMR: δ 6.83 ppm (dd, H-5), 6.78 (dd, H-2), 4.2 (dd, $-\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$), 3.2 (m, $-\text{CH}_2-$).

2-Fluorodopa ¹⁹F NMR: +63.5 ppm (broad singlet); ¹H NMR: \approx 6.7 ppm (overlapping doublets, $J = 3.3$ Hz, H-5 and H-6), 4.2 (dd, $J_{\text{H}\alpha\text{-H}\beta} = 7.5$ Hz, $J_{\text{H}\alpha\text{-H}\beta'} = 5.8$ Hz, $-\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$), 3.2 (m, $-\text{CH}_2-$).

HPLC Purification of L-[¹⁸F]6-Fluorodopa

The separation of 2- and 6-fluorodopa was achieved after one pass (15 min) through a semipreparative reverse phase column[§] (Fig. 3) with a mobile phase of 0.1% HOAc/USP H₂O, at 4 ml/min. The fraction containing the 6-fluoro isomer was collected directly from the column and was pushed by the pump through a $0.22 \mu\text{m}$ sterilizing filter^{**} into a multi-injection vial containing saline. The product is collected in ~ 9 ml.

RESULTS AND DISCUSSION

The method described here for the synthesis of L-[¹⁸F]6-fluorodopa is based on the reaction of [¹⁸F]acetyl hypofluorite with a fully derivatized dopa derivative. The radiochemical yield is on average 8% (decay corrected) and requires a synthesis time of 100 min. This yield represents about a three-fold increase over the previously published method using acetyl hypofluorite (3). It is also higher yielding than the latest published method (3% EOS) using ¹⁸F-F₂ on underivatized dopa, in liquid HF and also avoids the significant hazards of using HF.

The yields for the recently described C-Si bond cleavage route to 6-fluorodopa are higher (8% at EOS) than the one described here (8% EOB) but the preparation of the silane precursor is difficult and is afforded in a very low yield. In contrast, the acetylation of the amino and aromatic hydroxyl groups of L-3-O-methyldopa methylester is straightforward and high yielding. In fact, a technician can prepare gram quantities of the product within one working day.

We have found in our studies (7) that the dopa molecule must be fully derivatized to achieve the highest yields with acetyl hypofluorite. If either the amine or aromatic hydroxyl functionalities are left underivatized the yield is dramatically reduced. Presumably this is due to the facile oxidation of these unprotected groups by the highly electrophilic fluorinating agent. Furthermore, the choice of the protecting groups for the catechol system largely influences the radiochemical yield of the final product and the regioselectivity of fluorination. Out of several catechol protecting groups we have tried, the combination of 3-OMe and 4-OAc pro-

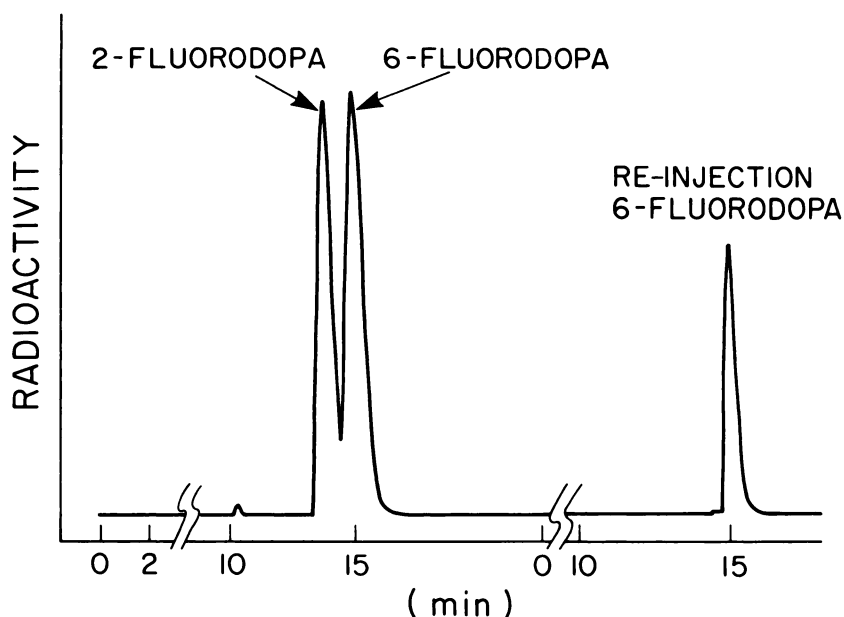


FIGURE 3
Preparative HPLC chromatograms of crude reaction mixture and of re-injection of final product

vides the highest yield of the isomeric fluorodopas. This also affords a ~1:1 mixture of the 2- and 6-fluoro isomers (Fig. 3). Fortunately, these can be easily separated by HPLC. The 5-fluoro isomer is not formed in the reaction. We have used both gaseous or solution phase acetyl hypofluorite and found them to work equally well, but we choose to use the gaseous form routinely because it is more convenient.

It is important to use HI that has been freshly distilled or is purchased as sealed ampules and contains only trace amounts (0.04%) of hypophosphorous acid as stabilizer. The commercially available HI which is purchased in bottles and is stabilized with ~1% H₃PO₂ does not hydrolyze the intermediate products of the fluorination reaction very efficiently.

Figure 3 illustrates a typical radiochromatogram obtained from a production run of 6-fluorodopa. Surprisingly few unwanted fluorinated by-products were obtained and most of the ¹⁸F not incorporated into the substrate during reaction was lost as H¹⁸F or other volatile fluorinated compounds after treatment of the intermediate reaction products with HI. A small amount of activity (2–3%) remains on the front of the HPLC column and this can later be eluted with methanol. It appears that the percentage of the total activity that this portion represents is mainly influenced by the efficiency of the acid hydrolysis. This was inferred by the examination of the ¹H and ¹⁹F NMR spectra of the methanol column wash which revealed the presence of fluorine containing species with couplings (J_{FH}) similar to those of 2- and 6-fluorodopa and a strong proton signal (δ2 ppm region) typical of an acetyl resonance. HPLC (uv detection) analysis of the crude product indicates that dopa and 6-fluorodopa are the major components. This suggests that most of the unreacted starting material is converted into dopa after treatment with HI. HPLC analysis of the final product with UV (280 nm) detection revealed that there was a single chemical component which eluted at the same time as the radioactivity associated with [¹⁸F]6-fluorodopa. The presence of dopa was not detected in the final product.

Due to the difficulty in separating the 2- and 6-fluorodopa isomers the chromatographic purification must be done with care. The separation requires a relatively large (0.94 cm I.D. × 50 cm) reverse phase column (see experimental). Three conditions are critical to the separation: (a) The deprotected material must be dissolved in buffer before injection. This is necessary since even after the water evaporations the solution is still acidic and this leads to poor resolution, (b) it is important to keep the injection volume at approximately 0.7 ml, and (c) keep the amount of starting material at a maximum of 60 mg. If these conditions are met the two isomers can be almost completely separated in one pass through the column (Fig. 3). There is ≤5% of the 2-fluorodopa isomer left after one

pass. Exactly where to cut the fraction will depend on the configuration of the HPLC system and how much dead volume there is between the collection point and the scintillation detector. After time the HPLC column will lose resolving power at which time a single recycle pass through the column successfully separates the two isomers to the previous purity standard. An added feature of this scheme is that equal quantities of L-[¹⁸F]2-fluorodopa may be obtained in this manner. Biological data on this compound have yet to appear in the literature.

The eluant used for the HPLC purification is 0.1% acetic acid/USP water. This eluant is very convenient since it is suitable for human i.v. injection after addition of saline and microfiltration. In our system the product collection tube is connected in series to the pump, a membrane filter, and a multi-injection vial which contains saline (70 mg/0.5 ml). The product is simply pushed directly into the vial from the pump.

The enantiomeric purity was established via HPLC using an in situ prepared chiral column (8). Using ¹⁸F detection it was shown that the product was ≥97% in the L enantiomeric form.

NMR Analysis

Both ¹⁹F and ¹H NMR spectra were obtained for the 2- and 6-fluorodopa isomers collected from typical patient runs. In addition, ¹⁹F NMR spectra were also obtained for an unseparated fraction containing both 2- and 6-fluorodopa (Fig. 4) in order to determine the

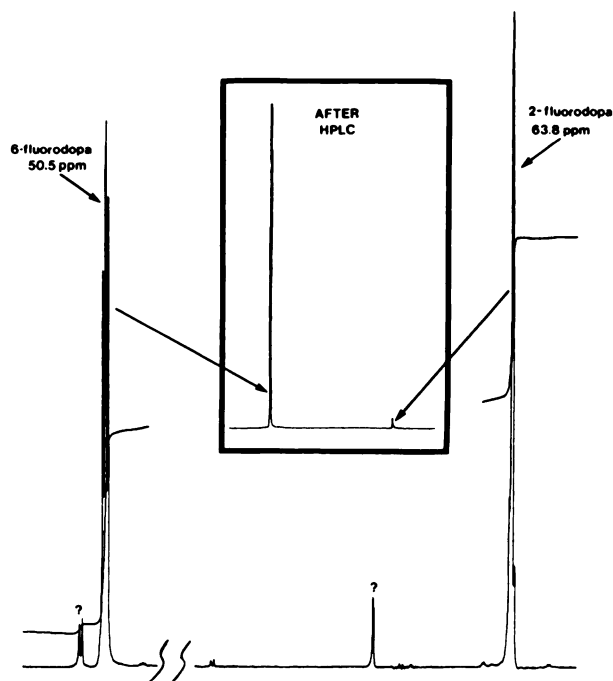


FIGURE 4
¹⁹F NMR spectra of unseparated 2- and 6-fluoro isomers and of final product after HPLC purification (inset)

isomeric ratio resulting from the fluorination reaction. A summary of the characteristic spectroscopic features taken from these spectra appears in the experimental section. The values listed compare favorably with those previously reported (3).

The ^{19}F NMR spectrum of the HPLC collected fraction containing both fluorodopa isomers revealed a 45:55 mixture of the 2- and 6-fluoro isomers respectively.

The ^{19}F NMR analysis of the final product, after HPLC purification, showed the chemical purity of L-[^{18}F]6-fluorodopa was >95% with the only contaminant being a small amount (<5%) of L-[^{18}F]2-fluorodopa (inset, Fig. 4).

FOOTNOTES

* Sigma No. M-1007, Sigma Chemical Corp., St. Louis, MO.

† Aldrich Chemical Co., Milwaukee, WI.

‡ Merck No. 5534, Darmstadt, West Germany.

§ Whatman Magnum 9, ODS-3, 50 cm, Clifton, NJ.

¶ Millipore No. SLGSV255F, Millipore Corp., Bedford, MA.

** Millipore No. SLGSO25OS, Millipore Corp., Bedford, MA.

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