Aromatic Radiofluorination with [^{18}F]Fluorine Gas: 6-[^{18}F]Fluoro-L-Dopa

Gunter Firnau, Raman Chirakal, and E. Stephen Garnett

McMaster University Medical Centre, Hamilton, Ontario, Canada

A new synthesis is described for the routine production of 3,4-dihydroxy-6-[^{18}F]fluoro-phenyl-L-alanine (6-[^{18}F]fluoro-L-dopa). The reaction between [^{18}F]fluorine gas and 3,4-dihydroxyphenyl-L-alanine (L-dopa) in liquid hydrogen fluoride gave 2-, 5-, and 6-[^{18}F]fluoro-L-dopa. 6-[^{18}F]Fluoro-L-dopa was isolated by reverse-phase high-pressure liquid chromatography. From 100 mCi [^{18}F]F_2, the method produces 3 mCi of 6-[^{18}F]fluoro-L-dopa at the end of synthesis.

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Several times during the past decade it has been suggested that the amino acid 3,4-dihydroxyphenyl-L-alanine (L-dopa), labeled with a positron-emitting radionuclide, could be used as a tracer with which to visualize the dopamine-containing structures in the human brain by positron-emission tomography (*1–3*). Recently the basal ganglia, rich in dopamine, have been imaged with the L-dopa analog, 6-fluoro-L-dopa labeled with fluorine-18 (*4,5*). Measurements of the regional cerebral metabolism of dopamine will now be possible in health and in disease, so that the precise relation between the neurotransmitter dopamine and disorders of mood and locomotion can be elucidated (*6*). These exciting clinical possibilities create a demand for [^{18}F]fluoro-L-dopa in millicurie quantities for each patient. The synthetic methods published previously did not yield these quantities. Earlier approaches to the radiofluorination of dopa used nucleophilic reactions. Neither the Schiemann nor the Wallach reaction produces millicurie quantities of [^{18}F]fluoro-dopa—their radiochemical yields were never better than 0.1% (*1*, Firnau G, Tewson TL, Welch M, unpublished data). Electrophilic fluorinating agents, such as xenon difluoride or acetyl hypofluorite, have been used with more success to introduce fluorine into the aromatic ring of dopa (*7,8*). In our experience xenon difluoride has been the mildest of the fluorinating agents, so that a catalyst is required to make it react with the dopa derivative 3-methoxy-4-hydroxyphenylalanine ethylester to yield 6-fluoro-L-dopa. The most effective catalyst was hydrogen fluoride when it was used in an amount equal to that of xenon difluoride. The F-18 in the labeled xenon difluoride exchanged with the fluorine in hydrogen fluoride, and thus lowered the radiochemical yield of 6-[^{18}F]fluoro-L-dopa drastically to <1% after 4 hr of synthesis time. Acetyl hypofluorite in glacial acetic acid was more reactive towards the dopa derivative than xenon difluoride. It produced all three possible structural isomers: 2-, 5-, and 6-fluoro-L-dopa. The radiochemical yield of 6-fluoro-L-dopa was only 1.3% after 3 hr of synthesis time.

Fluorine gas is an even more aggressive fluorinating agent. At first glance molecular fluorine seems to be too strong a reagent for the easily oxidizable L-dopa. However, if the oxidative properties of fluorine and its tendency to form fluorine radicals could be suppressed (they fluorinate organic substrates indiscriminately), it may react electrophilically with L-dopa. We therefore decided to react L-dopa with dilute fluorine gas (0.5% F_2 in neon) at −65°C in liquid hydrogen fluoride. The choice of hydrogen fluoride is important, because it is both a very polar solvent and a strong acid, and it has been found to promote electrophilic fluorination of phenols (*9,10*). Furthermore, liquid hydrogen fluoride may protect L-dopa against oxidation by fluorine gas. The oxidation of L-dopa involves the deprotonation of the hydroxyl groups. Liquid hydrogen fluoride, a strong acid, keeps

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For reprints contact: Gunter Firnau, PhD, Dept. of Nuclear Medicine, McMaster University Medical Centre, 1200 Main St. W., Hamilton, Ontario, Canada, L8N 3Z5.

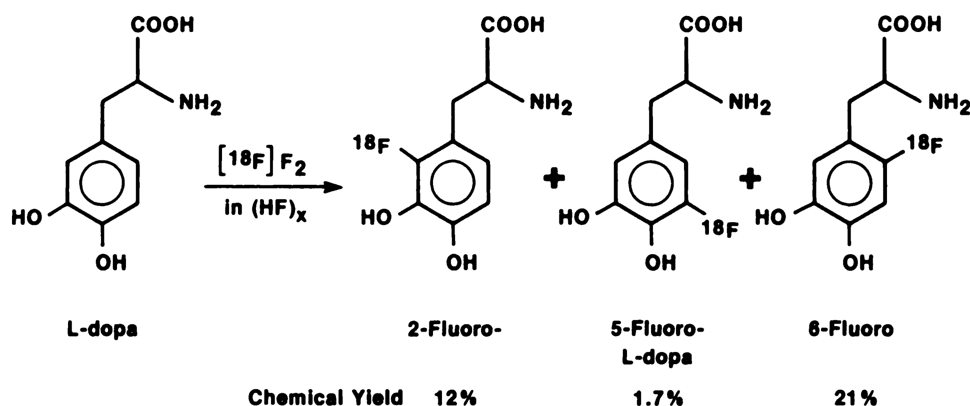


FIG. 1. Reaction scheme.

the two hydroxyl groups well protonated. Fluorine-18 in fluorine gas does not exchange with the fluorine in hydrogen fluoride in the gas phase at room temperature (*11*). The risk of isotopic exchange in the liquid phase at −65°C was therefore considered minimal.

We report here the direct action of $[^{18}F]F_2$ on L-dopa, which leads to the simultaneous formation of F-18-labeled 2-, 5-, and 6-fluoro-L-dopa (Fig. 1). 6-$[^{18}F]$Fluoro-L-dopa in millicurie quantities is then isolated chromatographically.

EXPERIMENTAL METHODS AND RESULTS

The reaction between $[^{18}F]F_2$ and L-dopa. The apparatus for the semiremote handling of fluorine-18 and hydrogen fluoride is shown in Fig. 2. Dilute fluorine gas labeled with fluorine-18 (0.5% F_2 in Ne, total amount of fluorine 180 μmol, 80–110 mCi) was produced by a tandem Van de Graaff accelerator as described previously (*12*). The radioactive gas was passed first through a cooling coil at −70°C and then through a reaction tube that contained a solution of L-dopa (86 mg, 430 μmol) in 5 to 10 ml of anhydrous hydrogen fluoride at −65°C. Gas flow was maintained at 60 ml/min. After the reaction the hydrogen fluoride was evaporated in vacuo at room temperature. The residue in the reaction tube was dissolved in 0.1 *N* hydrochloric acid (3 ml) and transferred into a rotary evaporator. The reaction tube was rinsed once with water (3 ml), which was also transferred into the rotary evaporator. The hydrochloric acid and water were evaporated, the residue was again dissolved in water, and the water evaporated. The final residue was again dissolved in water (1.5 ml) to give a yellow solution, which, after filtration through a 0.45 μm filter, was ready for chromatography.

Chromatographic separation of 6-$[^{18}F]$fluoro-L-dopa. The high-pressure liquid-chromatographic separation was done in two stages because of the large amount of

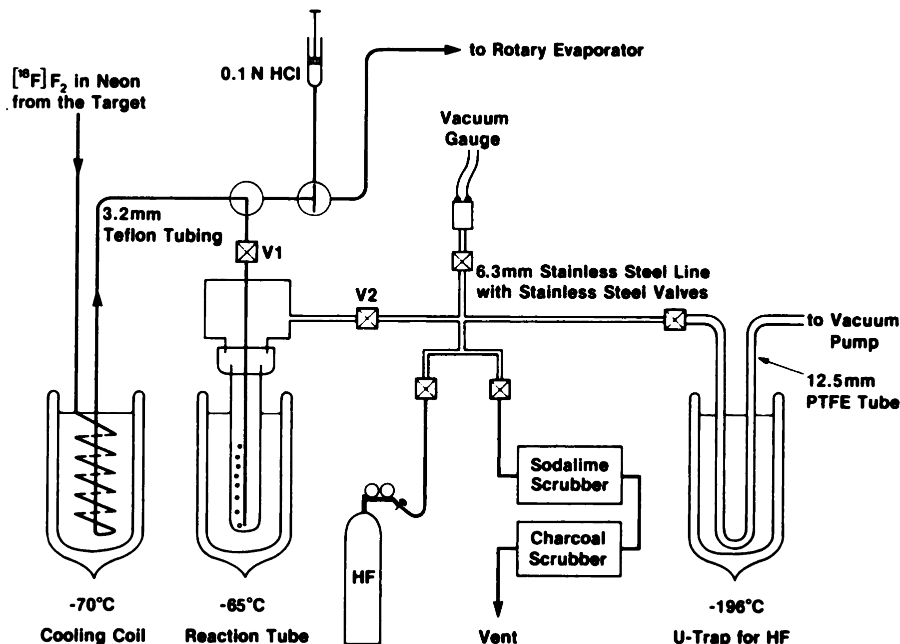


FIG. 2. Apparatus for fluorination of L-dopa in liquid hydrogen fluoride. 125-mm PTFE reaction tube is detachable from its head by CAJON-Ultra-Torr fitting.

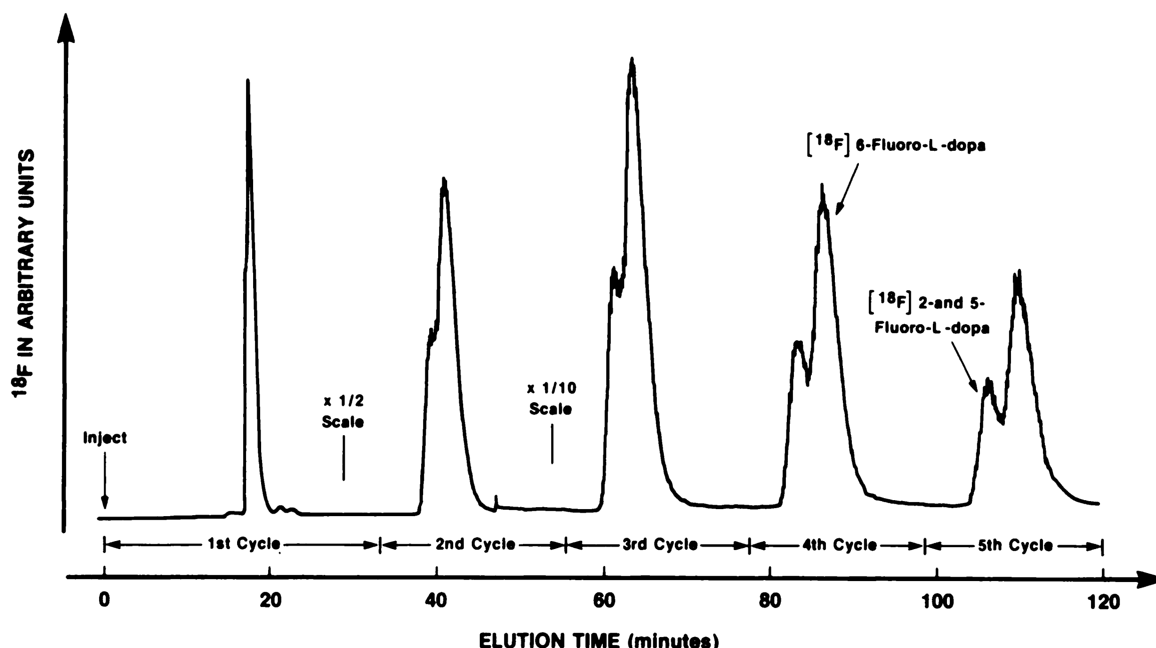


FIG. 3. Isolation of 6-[^{18}F]fluoro-L-dopa by recycling high-pressure liquid chromatography.

starting materials (86 mg). First, the whole reaction mixture (1.5 ml) was applied to a semipreparative reverse-phase column*, eluted with 0.1% acetic acid in water with 5% methanol, at 4 ml/min. The eluate from the column was monitored for both uv absorption at 280 nm and for gamma radioactivity. The chromatogram showed three major uv absorption peaks. The first, at 8 min, contained little fluorine-18; the second, at 10 min, contained the unreacted L-dopa. The third contained the bulk of the fluorine-18 and was only poorly separated from the L-dopa peak. Its elution position was identical to that of authentic 6-fluoro-L-dopa (7). This radioactive fraction was collected and assayed for fluorine-18. It contained 35% of the original F-18. Fluorine-19 NMR spectroscopy (Section 3) comprised all three structural isomers of fluoro-L-dopa.

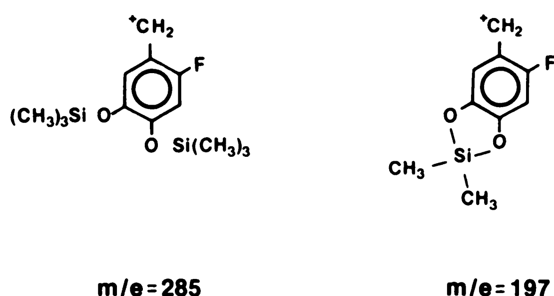
For the second stage of the chromatographic separation recycle, chromatography was used to isolate pure 6-[^{18}F]fluoro-L-dopa. The fraction from the first chromatographic separation, which contained the isomers of fluoro-L-dopa, was evaporated to dryness and redissolved in less than 1 ml in water. It was filtered and applied to two semipreparative reverse-phase columns in series.† These were eluted with 0.1% acetic acid in water at 2 ml/min. The eluant was monitored by the flow through the radioactivity detector. The outlet of that detector was connected to the recycling valve on the solvent delivery system.‡ With this facility the eluate could either be discarded or returned onto the columns for additional separation cycles. Figure 3 shows the effect of recycling on the separation of the isomers. Separation improved with each cycle, although baseline separation was not achieved after five cycles. Under production conditions, the isomers, 2- and 5-fluoro-L-dopa, which eluted early, were shaved from the main peak during the first and second cycles. This maneuver made each subsequent separation cycle more efficient. In this way chromatographically pure 6-fluoro-L-dopa was collected after only three cycles. The solvent was evaporated and the residue redissolved in 5 ml of sterile saline containing 1 mg ascorbic acid to prevent oxidation of fluoro-L-dopa during handling. For clinical use the solution of 6-[^{18}F]fluoro-L-dopa in saline was passed through a 0.22-μm filter. The clear colorless solution contained 5.4 mg (=25 μmol) and 3 mCi of 6-[F-18]fluoro-L-dopa at the end of synthesis (specific activity 120 mCi/mmol).

The chemical content of 6-fluoro-L-dopa in a known aliquot of this solution was determined by analytical high-pressure liquid chromatography. The eluate was monitored by a uv detector at 280 nm and a radioactivity detector. The uv absorption of the detector was calibrated with authentic 6-fluoro-L-dopa (7). The molar absorptivity is 2543 at λmax = 286 nm. The radiochemical purity was 96%.

Characterization of 6-fluoro-L-dopa. *Gas chromatography—mass spectroscopy.* A freeze-dried sample (0.5 mg) of the fluoro-L-dopa that was obtained was converted into its trimethylsilyl derivative. Fifty microliters of a mixture of nine parts *N,O*-(bistrimethylsilyl) trifluoroacetamide, one part trimethylcholorosilane, and 50 μl of tetrahydrofuran were added to the dry sample. After 1 day, the mixture was diluted with toluene, and aliquots were taken for mass spectroscopy (*13*). A sample of authentic L-dopa was treated in the same way. The instrument for GC-MS was a quadrupole

mass spectrometer with the INCOS data system. It was run under the following conditions: column 30 m capillary DB1-30W; helium gas flow 30 cm/sec; temperature programmed from 90°C to 280°C at 5°C/min with a 1-min delay; injector, transferline, and ion source at 280°C; ionization voltage 70 eV. Both spectra were rather featureless except for the two dominant mass fragments at m/e = 267 and 179 for L-dopa, and m/e = 285 and 197 for fluoro-L-dopa. The two sets differ by 18, due to the mass of fluorine (+F − H = 19 − 1 = 18).

We assigned:



Scheme I

Scheme I. Mass spectrometric fragments of 6-fluoro-L-dopa.

This suggests that the fluorinated product was fluoro-L-dopa.

Fluorine-19 NMR spectroscopy. The samples were dissolved in D_2O. The F-19 NMR spectrum was recorded at 235 MHz at field strength of 5.8719 tesla on a spectrometer using a 5 mm probe at 21°C. One thousand scans gave adequate signal-to-noise ratio with a typical sample concentration of 25 m*M*. Fluorotrichloromethane was used as external chemical shift reference. The spectrum of the material from the first stage of chromatography showed: three distinct well-separated signals downfield of $CFCl_3$, a multiplet at −126.4 ppm, a doublet at −135.6 ppm, and a broad singlet at −139.7 ppm. We assigned the observed chemical shifts to the three possible isomers of ring-fluorinated dopa: 2-, 5-, and 6-fluoro-dopa, respectively. The assignment was based upon the splitting pattern of the observed signals, the comparison of the chemical shifts with authentic 5- and 6-fluoro-dopa (*1,7*), and the literature values for 2-, 5-, and 6-fluoro-dopamines (*14*). The relative intensities of the integrated F-19 signals were 59, 35, and 5% for the 6-, 2-, and 5-isomer, respectively. This means that the radiochemical yield was 21% for 6-fluoro-L-dopa, 12% for 2-fluoro-L-dopa, and 2% for 5-fluoro-L-dopa.

A sample of the chromatographically pure 6-fluoro-L-dopa fraction was analyzed for isomeric purity by F-19 NMR spectroscopy as described earlier. Although the major signal was that of 6-fluoro-L-dopa, some 2-fluoro-L-dopa was also detected. Based on the relative intensities of the integrated signals, the fraction contained 96% 6-fluoro-L-dopa.

Proof of chirality. The chirality of the 6-fluoro-L-dopa was established by the chiral reverse-phase high-pressure liquid chromatography method described by Gil-Av et al. (*15*), modified by the addition of a F-18 detector. A single peak was observed at the elution volume of 6-fluoro-L-dopa.

The optimum molar ratio of the reactants. An excess of L-dopa substrate was considered desirable so that $[^{18}F]F_2$ would be used efficiently. On the other hand, it is difficult to process large amounts of L-dopa chromatographically and other products during the workup after the reaction. To find the optimum excess of L-dopa, we varied the molar ratio of L-dopa to F_2 between 0.5 and 3.8, and recorded the radiochemical yields at EOB of all fluoro-dopa isomers combined after the first stage of the chromatographic processing. The optimum was found at L-dopa/F_2 = 2.4 (Fig. 4). Thus, 180 μmol F_2 and 180 × 2.4 = 430 μmol L-dopa were used for routine production runs.

DISCUSSION

The present method has three practical features. First, it uses L-dopa as substrate, and therefore does not require a specially synthesized dopa derivative, unlike the fluorinations with xenon difluoride and acetyl hypofluorite. Second, L-dopa does not racemize during the reaction. Third, the method is rapid and reproducible. A preparation takes just under 2 hr from the end of bombardment. All manipulations can be automated easily. This method produces 6-[^{18}F]fluoro-L-dopa in the highest radiochemical yield (3% at end of synthesis) and in the shortest time compared with all the previously published methods. The disadvantage of the method is that the fluorination reaction is not regiospecific.

Fluorinations of aromatic compounds with fluorine gas do no seem to follow the pattern that one would expect of a classic electrophilic substitution reaction. The bromination of L-dopa, an example of a classic electrophilic reaction, leads to 6-bromo-L-dopa only (*16*),

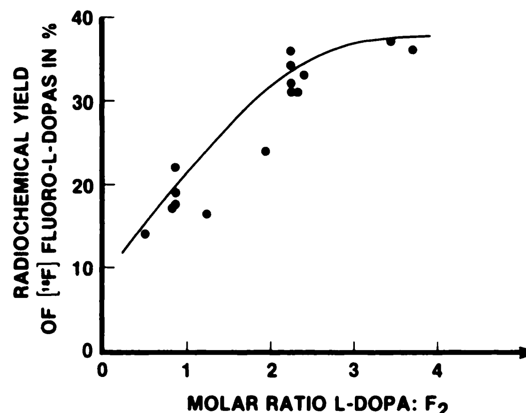


FIG. 4. Effect of relative amounts of reactants on radiochemical yield, corrected to EOB.

whereas our fluorination produced not only 6-fluoro-L-dopa but also the 2- and 5-fluoro isomers. Cacace et al. have characterized fluorine gas as an unselective reagent for aromatic substitution (*17*). This behavior of fluorine has also been described by Misaki, who found that when either phenol or cresol were fluorinated in hydrogen fluoride, as much fluorine entered the ortho as the para position relative to the hydroxyl group (*9*). The isomeric distribution of our reaction products therefore appears plausible.

The elucidation of the reaction mechanism and attempts to make the direct fluorination of L-dopa more regiospecific are in progress in our laboratory.

The isolation of pure 6-fluoro-L-dopa is necessary for use in vivo. We have shown earlier that 5-fluoro-L-dopa is *O*-methylated by catechol-*O*-methyl transferase (E.C. 2.1.1.6) four times as fast as dopa. The reason for this accelerated *O*-methylation was attributed to the presence of fluorine adjacent to the hydroxyl group (*18*). By the same argument, we would expect that 2-fluoro-dopa will also be *O*-methylated faster than dopa. The bulk of the *O*-methylation occurs in the liver, so that minutes after the injection of 5-fluoro-dopa, appreciable amounts of *O*-methylated 5-fluoro-dopa are found in the blood (*19*). As a result, the brain is supplied simultaneously with both fluoro-dopa and *O*-methyl-fluoro-dopa. *O*-Methyl-fluoro-dopa, like 3-*O*-methyl-dopa (*20*), is taken up by the brain, nonselectivity (Firnau G, et al., unpublished data). Use of our mathematical model (*19*) to quantify the metabolism of fluoro-L-dopa requires that only fluoro-dopa should cross the blood-brain barrier. Thus, the presence of 2- and 5-fluoro-L-dopa tend to vitiate the model. 6-Fluoro-L-dopa, on the other hand, is expected to resemble native L-dopa more closely than the other isomers with regard to the action of catechol-*O*-methyltransferase. Based on the preliminary observations of Chiueh et al. (*21,22*), who has found little *O*-methylation of 6-fluoro-dopamine in vivo, one can speculate that 6-fluoro-L-dopa may be *O*-methylated to an even lesser extent than dopa itself. If this is confirmed, the 6-fluoro isomer would be an almost perfect tracer for L-dopa.

The isomeric purity of 96% is acceptable. Clearly the purity depends upon the way in which the second chromatography is run. If greater losses of 6-[^{18}F]fluoro-L-dopa are tolerable, the fractions can be cut in such a way that the final fraction contains 100% isomerically pure material.

The specific activity of 6-[^{18}F]fluoro-L-dopa (120 mCi/mmol at the end of synthesis) is adequate. When an individual is given a typical dose of 6-[^{18}F]fluoro-L-dopa, 3-mCi, he receives 25 μmol. This is diluted in the blood to give an instantaneous plasma concentration of 8 μM. Fluoro-dopa, and dopa, share a transport mechanism common to all large neutral amino acids. The K_m value for both dopa and 5-fluoro-dopa is 360 μM (*18*). If one assumes that the K_m for 6-fluoro-dopa does not differ significantly from that value, the plasma concentration is well below the K_m value. Thus, 6-fluoro-L-dopa at the present specific activity will behave as a true tracer at the blood-brain barrier.

FOOTNOTES

* Whatman M9, Partisil 10/50, ODS-2, 0.9 × 50 cm.

† Waters μBondapak C-18, 0.78 × 30 cm.

‡ Waters Model 6000A.

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