High Yield Synthesis of 6-[^{18}F]Fluoro-L-Dopa

Raman Chirakal, Gunter Firnau, and E. S. Garnett

Division of Radiology and Nuclear Medicine, McMaster University Medical Centre, Hamilton, Ontario, Canada

The radiofluorination of L-dopa with [^{18}F]F_2 was investigated with the purpose of improving the yield of 6-[^{18}F]fluoro-L-dopa. When boron trifluoride was added to the reaction mixture in hydrogen fluoride (HF), the yield was increased threefold. Nine millicuries of 6-[^{18}F]fluoro-L-dopa were produced from 100 mCi [^{18}F]F_2 routinely and reliably after 2 hr of preparation. If acetonitrile or water were substituted for HF, little or no 6-fluoro-L-dopa was made.

J Nucl Med 27:417–421, 1986

The cerebral distribution of the neurotransmitter dopamine in humans can be visualized with positron emission tomography and 6-[^{18}F]fluoro-L-dopa as tracer (*1*). Using this technique, we have demonstrated the biochemical lesion in patients with unilateral Parkinson's disease (*2,3*). Soon it will be possible to measure the kinetics of regional cerebral dopamine in disorders of locomotion and mood (*4*).

Several syntheses have been described for the tracer 6-[^{18}F]fluoro-L-dopa (*5–7*). Thus far, the direct fluorination of L-dopa with [^{18}F]F_2 in liquid hydrogen fluoride (HF) has proved to be the most reproducible (*7*). However, moderate radiochemical yield and the need for special equipment to handle liquid HF may be an obstacle to the widespread application of this method.

The chemical study we report here is aimed at improving the radiochemical yield of 6-[^{18}F]fluoro-L-dopa through a better understanding of the fluorination reaction. We approached this goal in two ways. First, we investigated the effects of the addition of the fluoride acceptors, borontrifluoride (BF_3), arsenic pentafluoride (AsF_5), and silicon tetrafluoride (SiF_4), on the formation of 6-[^{18}F]fluoro-L-dopa. Second, we tried *O*-methylated derivatives of L-dopa as substrates. In addition, we studied the direct fluorination reaction in acetonitrile (CH_3CN) and in water in an attempt to avoid the use of liquid HF as solvent.

Received May 15, 1985; revision accepted Oct. 28, 1985.

For reprints contact: Gunter Firnau, PhD, Div. of Radiology and Nuclear Medicine, Chedoke-McMaster Hospitals, 1200 Main St. W., Hamilton, Ontario, Canada, L8N 3Z5.

MATERIALS AND METHODS

Fluorine-18- (^{18}F) labeled fluorine gas was produced by the nuclear reaction ^{20}Ne (d,α) ^{18}F using the 15 meV deuteron beam of the McMaster Tandem Van de Graaff Accelerator (*8*). The radiofluorination reaction was carried out in an apparatus similar to that described previously (*7*). The PTFE reaction vessel contained a solution of L-dopa or one of its *O*-methylated derivatives dissolved in 5.0 ml of either HF, CH_3CN, or H_2O, the latter acidified with 1 mmol HCl (Table 1). BF_3, AsF_5, or SiF_4 was then condensed on top of the substrate solution at liquid nitrogen temperature. The amounts of gaseous BF_3, AsF_5, or SiF_4 that were added were determined by measuring the pressure of each gas at a known volume. [^{18}F]Fluorine gas (190 μmol) diluted with neon (0.5% F_2, 99.5% Ne, Scott Specialty Gases) was then passed for 30 min through each solution of the substrate (470 μmol) either at −70° for HF, −20°C for CH_3CN, or 0°C for H_2O. The amount of ^{18}F retained in the reaction vessel was measured in a calibrated ionization chamber when the bubbling had finished. The amount of ^{18}F retained in the reaction vessel was expressed as a fraction of ^{18}F that had been produced in the target. The amount of ^{18}F in the target was calculated from the energy, intensity of the deuteron beam, time of irradiation, and the known thick target yield, 91 mCi/μA-h (*9*).

The solvent—HF, CH_3CN, or H_2O—was then evaporated in vacuum and the remainder was dissolved in 10 ml of 0.1 *M* HCl. The HCl was then evaporated and the residue was taken up in 1.5 ml water.

The aqueous reaction mixture was chromatographed with high pressure liquid chromatography (HPLC, stationary phase: two C18 semipreparative columns, 0.78 cm × 30 cm, in series; mobile phase: 0.1% acetic acid in water at 2 ml/min; detector: uv detector at 280 nm and NaI (Tl) scintillation detector to monitor continuously 120 μl of the elute). The

TABLE 1
Effect of Substrate, Solvent, and Lewis Acid on Yield of 6-Fluoro-L-Dopa During Radiofluorination in HF

Reaction	Substrate	Solvent	Lewis acid	Fraction of ^{18}F trapped in reaction vessel	Fraction of ^{18}F incorporated in all [^{18}F]fluoro-L-dopas	Isomer distribution (%) 2-, 5-, 6-isomer			Fraction of ^{18}F in 6-fluoro-dopa
						Fluoro-L-dopas			
1	L-dopa	HF	—	0.65	0.24	35	5	60	0.14
2	L-dopa	HF	BF_3	0.40	0.34	35	5	60	0.20
3	L-dopa	HF	AsF_5	0.10	0.04	29	16	55	0.02
4	L-dopa	HF	SiF_4	0.33	0.09	38	5	57	0.05
5	L-dopa	CH_3CN	BF_3	0.64	0.10	47	39	14	0.01
6	L-dopa	CH_3CN	SiF_4	Not determined	Not determined	50	50	0	0
7	L-dopa	H_2O	—	0.50	0.01	56	44	0	0
8	L-dopa	H_2O	BF_3	0.60	Not determined	56	44	0	0
9	3,4-di-*O*-methyl-L-dopa	HF	BF_3	0.43	0.19*	52	9	39	0.08*
						Fluoro-*O*-methyl dopas			Fraction of ^{18}F in 6-fluoro-isomer
10	3-*O*-methyl-L-dopa	HF	—	0.60	0.34	3	54	43	0.14
11	3-*O*-methyl-L-dopa	HF	BF_3	0.49	0.37	3	54	43	0.16
12	4-*O*-methyl-DL-dopa	HF	BF_3	0.32	0.20	20	26	54	0.11

4.2 mmol of either BF_3, SiF_4, AsF_5 were added.

* Fluorinated 3,4-*O*-methyl-L-dopa was hydrolyzed by treatment with HBr to obtain [^{18}F]fluoro-L-dopas.

elution fraction that contained either the fluoro-L-dopas or the fluoro-*O*-methyl-dopas was collected and its ^{18}F content measured. The amount of ^{18}F that was associated with the fluoro-dopas was expressed as the fraction of ^{18}F that was produced in the target and corrected for radioactive decay.

The isomeric distribution of the fluoro-dopas was determined by ^{19}F- nuclear magnetic resonance (^{19}F-NMR) spectroscopy. A sample of the mixture of the fluoro-L-dopas obtained by HPLC was dissolved in D_2O and its ^{19}F-NMR spectrum was recorded at 235 MHz at a field strength of 5.87 Tesla on a spectrometer† using a 5-mm probe at 21°C. The sample concentration was ~0.1 *M*. The chemical shifts measured downfield from the signal of the external standard $CFCl_3$ were −139.7 ppm, −135.6 ppm, and −126.4 ppm for 2-, 5-, and 6-fluoro-L-dopa, respectively (*6*). The distribution of the isomers of fluoro-L-dopas was determined from the integrated intensity of the ^{19}F-NMR signals. The proportion of each isomer was then expressed as a percentage of the combined intensities of the signals from 2-, 5-, and 6-fluoro-L-dopa.

In experiment 2 (Table 1), 6-[^{18}F]fluoro-L-dopa was actually isolated by the chromatographic procedure described earlier (*7*). The product was identified by gas chromatography-mass spectroscopy and by ^{19}F-NMR (*7*). The chemical and radiochemical purities as well as the specific activity were determined by analyzing a sample of the final product solution with HPLC. The HPLC system described above for the isolation of 6-[^{18}F]fluoro-L-dopa was also used for the quality assurance. The elution times were 28, 29, and 31 min for 2-, 5-, and 6-fluoro-dopa, respectively. The response of the uv detector was calibrated with known concentrations of 6-fluoro-L-dopa. In this way, the amount of 6-fluoro-L-dopa in the sample of the final product was quantitated. Chemical and radiochemical purity were >97%. The specific activity was 220 mCi/mmol.

RESULTS

Table 1 summarizes the results of all the experiments performed. It is shown that the highest yield of fluoro-L-dopas was obtained when L-dopa was the substrate, HF was the solvent and BF_3 was the Lewis acid. Table 2 shows that the yield of fluoro-L-dopas is enhanced by increasing the amount of BF_3 added, up to 4.20 mmol.

Figure 1 illustrates the variety and relative amounts of ^{18}F containing reaction products that were formed under the various reaction conditions. The peak at 16 min comprised ^{18}F containing by-products. The [^{18}F]fluoro-L-dopas eluted at 30 min. More by-products than [^{18}F]fluoro-L-dopas were made when CH_3CN with 4.2 mmol BF_3 was the solvent (Fig. 1A). The use of HF with 2.1 mmol BF_3 reduced the by-products considerably (Fig. 1B). When HF with 4.2 mmol BF_3 was used, the number and amount of the by-products were reduced even further. Partial separation between the combined 2- and 5-fluoro-L-dopas and 6-fluoro-L-dopa can be seen (Figs. 1B and C).

TABLE 2
Effect of BF_3 on Yield of Fluoro-L-Dopas During Radiofluorination of L-Dopa in HF

Amount of BF_3 added to reaction mixture (mmol)	Yield of [^{18}F]fluoro-L-dopas (%)
0	37
2.08	52
4.16	84
4.86	74
5.98	81

Improved Synthesis Procedure for 6-[^{18}F]Fluoro-L-Dopa

As the result of the experiment described, we have altered our original method for the preparation of 6-[^{18}F]fluoro-L-dopa (*7*) by addition of BF_3 to the reaction mixture. Thus, L-dopa (94 mg, 470 μmol) is dissolved in 5 ml liquid HF in a reaction vessel of the apparatus previously described (*7*). Boron trifluoride (4.2 mmol) is then condensed into the reaction vessel at −196°C. After this solution has been warmed up to −65°C dilute radioactive fluorine gas (0.5% F_2 in Ne, total amount of F_2 190 μmol) is passed through it at 80 ml/min. The solvent HF is then evaporated in vacuo at room temperature. From this point, the workup and chromatographic procedure for the isolation of 6-[^{18}F]fluoro-L-dopa, is identical to that previously described (*7*).

DISCUSSION

Dilute fluorine has been described as a "highly reactive and correspondingly unselective (fluorinating) reagent" (*10*). Indeed, when we used it to fluorinate L-dopa we found a variety of fluorination products (Figs. 1A and B). This is not surprising considering the structural complexity of L-dopa and its derivatives. Both are particularly vulnerable to reaction with fluorine because the amino acid side chain can react easily with fluorine radicals. In addition, the substituents of the aromatic ring (—OH, —CH_2R) are electron-donating groups known to promote electrophilic reaction with fluorine. Furthermore, catechols are oxidized readily by F_2. During our investigation, we searched for reaction conditions that have a taming influence on either fluorine or L-dopa. The type of substrate, the reaction solvent, and the Lewis acid all affect isomer distribution and yield.

Effect of Substrate

When *O*-methylated L-dopas were used as substrates the yield of the 6-fluoro isomer decreased with the extent of the methylation of the hydroxyl groups. Under comparable reaction conditions the fraction of ^{18}F in 6-fluoro-dopa was highest when unprotected L-dopa was the substrate (Reactions 2, 11, 12, and 9, Table 1). The use of methylated substrates requires the removal of the methyl group (usually by acid hydrolysis for 30 min). This additional time for hydrolysis would lower the practical yield even further. Thus, the protection of the catechol in L-dopa by a methyl group is not a suitable strategy towards a high yield synthesis for 6-fluoro-L-dopa.

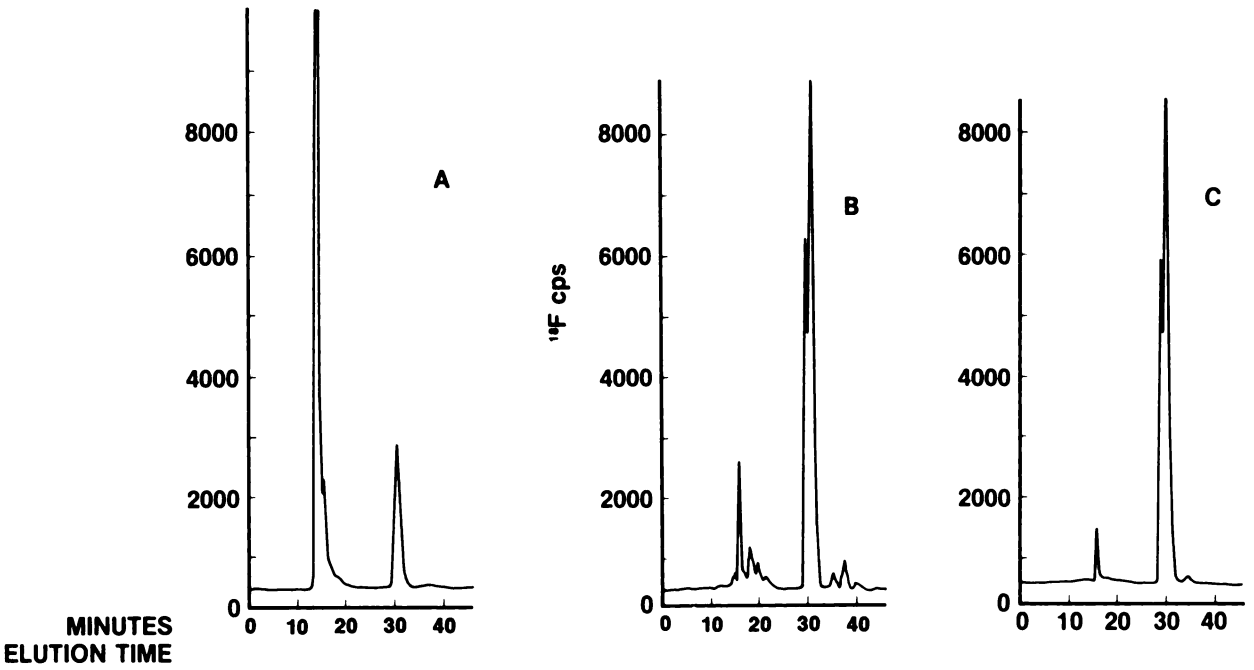

FIGURE 1
Reverse-phase HPLC. Radiochromatograms of reaction mixtures after radiofluorination of A: L-Dopa in CH_3CN with 4.2 mmol BF_3. B: L-Dopa in HF with 2.1 mmol BF_3. C: L-Dopa in HF with 4.2 mmol BF_3

Effect of Solvent

When CH_3CN-BF_3 was used as the solvent instead of HF-BF_3 the fraction of ^{18}F in 6-fluoro-L-dopa dropped from 0.20 to 0.01 (Reactions 5 and 2, Table 1). When water was used, no 6-fluoro-L-dopa was formed. The acidity of HF-BF_3 (*11*) is apparently optimal for both a high incorporation of ^{18}F and for the formation of the desired 6-fluoro isomer.

Effect of Fluoride Acceptors

When the Lewis acid, BF_3, or AsF_5 is added to HF the acidity of the solvent is increased; AsF_5 has the greater effect (*11*). Concommitantly, the two catechol oxygens in dopa can be expected to be protonated (*12*). Protonated oxygens no longer behave as electron-donating but as electron-withdrawing groups. The latter are known to retard electrophilic aromatic substitution (*13*). Indeed, we have observed a decreased rate of reaction between F_2 and L-dopa or its derivatives as reflected in a lower fraction of ^{18}F that is trapped (Reactions 1, 2, 3, 10, and 11, Table 1). This reduced reaction velocity in HF-BF_3 was accompanied by an increase in the yield of the 6-fluoro isomer. The addition of ~4.2 mmol BF_3 produced the maximum yield of fluoro-dopas (Table 2).

Unlike BF_3 and AsF_5, SiF_4 does not increase the acidity of HF. Consequently, SiF_4 did not increase the yield of the fluoro-L-dopas (Reaction 1 and 4, Table 1). The polar solvents CH_3CN-BF_3, CH_3CN-SiF_4, H_2O-HCl, and H_2O-BF_3 not only decreased the yield of the fluoro-L-dopas, they also drastically changed the substitution pattern; fluorine preferentially entered the 2- and 5-position at the expense of the 6-fluoro isomer (Reactions 5 to 8, Table 1).

Lewis acids have been used by many to catalyze electrophilic fluorination reactions. In these reactions they polarize fluorinating agents, like XeF_2 (*14*) and ClF_3 (*15*), to facilitate the electrophilic fluorination. In the present work we believe BF_3, AsF_5, and SiF_4 affect the substrate and not the fluorinating agent F_2. This notion is supported by two observations. First, if BF_3 AsF_5, or SiF_4 did interact with F_2, the specific activity of the product would be drastically lowered because of isotopic exchange between $[^{18}F]F_2$ and the stable fluorine in a common intermediate. Second, the small size of the fluorine molecule makes it unlikely that the F—F bond in F_2 is polarized by fluoride acceptors, such as BF_3 or AsF_5 (*16*). The mechanism by which F_2 reacts with L-dopa remains to be elucidated.

In their pioneering work, Cacace et al. (*10*) have warned against interpreting fluorination reactions according to a common reaction mechanism because "the attack of elemental fluorine on aromatic substrates could conceivably involve multiple, and possibly overlapping pathways." We speculate that the fluoro-L-dopas may be formed by electrophilic action of F_2 on L-dopa. Other reactions also occur because we observe a variety of ^{18}F containing reaction products.

We conclude that (a) our original synthesis of 6-[^{18}F]-fluoro-L-dopa (*7*) can be improved by addition of BF_3 to the reaction medium, (b) the solvent HF cannot be replaced without loss in yield, and (c) use of *O*-methylated L-dopa derivatives as substrates does not increase the yield of 6-fluoro-L-dopa.

We now add 4.2 mmol of BF_3 to the solvent HF for the routine production of the radiopharmaceutical 6[^{18}F]fluoro-L-dopa. Typically, we produce 9 mCi of 6-[^{18}F]flouro-L-dopa with a specific activity of 220 mCi/mmol from 100 mCi $[^{18}F]F_2$. The procedure takes 2 hr.

FOOTNOTES

* Waters Chromatography Div., Millipor, Milford, MA (μBondapak).

† Bruker Instruments Inc., Billerica, MA (WM-250).

ACKNOWLEDGMENT

The authors thank Dr. M. Albring, Degussa-Pharma Gruppe Frankfurt, Germany for 3,4-O-methyl-L-dopa and Dr. C. R. Creveling, National Institute of Health, Bethesda, Maryland, who provided 3-(4-hydroxy-3-methoxy phenyl)-DL-alanine.

This work was supported by Medical Research Council of Canada and the Parkinson Foundation of Canada.

Note

Work with HF is potentially harmful. Skin contact with even small amounts of HF results in painful chemical burns. Before beginning work with HF, the first-aid treatment procedure (*17*) should be available and known to all laboratory personnel.

REFERENCES

1. Garnett ES, Firnau G, Nahmias C: Dopamine visualised in the basal ganglia of living man. *Nature* 305:137-138, 1983
2. Garnett ES, Nahmias E, Firnau G: Central dopaminergic pathways in hemiparkinsonism examined by positron emission tomography. *Can J Neurol Sci* 11:174-179, 1984
3. Nahmias C, Garnett, ES, Firnau G, et al: Striatal dopamine distribution in Parkinsonian patients during life. *J Neurol Sci* 69:223-230, 1985
4. Garnett ES, Firnau G, Nahmias C, et al: Blood brain barrier transport and cerebral utilization of dopa in living monkeys. *Am J Physiol* 238:R318-R327, 1980
5. Firnau G, Chirakal R, Sood S, et al: Aromatic fluorination with xenon difluoride: L-3,4-dihydroxy-6-fluorophenylalanine. *Can J Chem* 58:1449-1450, 1980
6. Chirakal R, Firnau G, Course J et al: Radiofluorination with ^{18}F-labelled acetyl hypofluorite: [^{18}F]L-6-fluorodopa. *Int J Appl Rad Isot* 35:651-653, 1984

7. Firnau G, Chirakal R, Garnett ES: Aromatic radiofluorination with [^{18}F]fluorine gas: 6-[^{18}]fluoro-L-dopa. *J Nucl Med* 25:1228-1233, 1984
8. Chirakal R, Firnau G, Schrobilgen G, et al: The synthesis of [^{18}F]xenon difluoride from [^{18}F]fluorine gas. *Int J Appl Rad Isot* 35:401-404, 1984
9. Carsella V, Ido T, Wolf AP, et al: Anhydrous F-18 labelled elemental fluorine for radiopharmaceutical preparation. *J Nucl Med* 21:750-757, 1980
10. Cacace F, Giacomello P, Wolf AP: Substrate selectivity and orientation in aromatic substitution by molecular fluorine. *J Am Chem Soc* 102:3511-3515, 1980
11. O'Donnell TA: On the acidity of hydrogen fluoride. *J Fluor Chem* 25:75-82, 1984
12. Cotton FA, Wilkinson G: *Advanced Inorganic Chemistry, 2nd Edition*, New York, John Wiley & Sons, pp 384, 1966.
13. Baker JW, Moffitt WG: The nature of the alternating effect in carbon chains. Part XXXIII. The nitration of some aromatic sulphonium and selenomium salts. *J Chem Soc* 1722:1930
14. Korytnyk W, Valentekovic-Horvat S: Reactions of glycals with xenon fluoride. *Tetrahedron Lett* 21:1493-1496, 1980
15. Boudakian MM, Hyde GA: Substitutive aromatic fluorination with chlorine pentafluoride. *J Fluor Chem* 25:435-446, 1984
16. Christe, O: On the reality of positive fluorine. *J Fluor Chem* 22:519-520, 1983
17. Reinhardt CF, Hume WG, Linch AL, et al: Hydrofluoric acid burn treatment. *J Chem Ed* 46:A171-A179, 1969