Enantioselective Synthesis of 6-[Fluorine-18]-Fluoro-L-Dopa from No-Carrier-Added Fluorine-18-Fluoride

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Methods: A trimethylammonium veratraldehyde triflate was synthesized and used as a precursor for the asymmetric synthesis of 6-[^{18}F]fluoro-L-dopa. **Results:** Its nucleophilic fluorination with ^{18}F-fluoride produced by the ^{18}O(p,n)^{18}F nuclear reaction on enriched ^{18}O-water led to the corresponding no-carrier-added [^{18}F]fluoroveratraldehyde (45 ± 5% EOB). Diiodosilane was used to prepare the corresponding [^{18}F]fluorobenzyl iodide (36.5 ± 5.3% EOB). Akylation of (S)-1-tert-boc-2-tert-butyl-3-methyl-4-imidazolidinone with this electrophilic agent, hydrolysis and purification by preparative high-pressure liquid chromatography made 6-[^{18}F]fluoro-L-dopa ready for human injection, in a 23% ± 6% decay-corrected radiochemical yield. The enantiomeric purity and the specific activity were above 96% and 1 Ci/μmole respectively. **Conclusion:** Through this procedure, starting from 250 mCi of ^{18}F-fluoride, multimillicurie amounts (32 ± 8.5 mCi) of no-carrier-added 6-[^{18}F]fluoro-L-dopa are now available at the end of synthesis (90 min) with a good radiochemical purity (more than 98%).

Key Words: fluorine-18; fluorodopa; enantioselective synthesis

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The need for reliable production of 6-[^{18}F]fluoro-L-dopa, which is widely used to investigate the regional distribution of dopamine in the human brain by PET (*1*) has led, during the last few years, to reports of various electrophilic and nucleophilic syntheses for this compound (*2*). The electrophilic syntheses of this amino acid often suffer a lack of regioselectivity and require the use of molecular fluorine or acetyl hypofluorite as the fluorinating agent (*3–8*). Although a regioselective fluorination at position 6 has been proposed by Firnau et al. (*9*), the radiochemical yield (1%) of this synthesis was not suitable for routine production. To overcome the problem of radiochemical yields and nonregioselectivity, radiodemetallation reactions were investigated. Radiofluorodesilylation was proposed, but the main limitation of this method was the difficulty in the preparation of the starting precursors (*10*). A synthesis based on fluorodemercuration was also developed (*5,11,12*). To date, this simple and high-yielding route is used in several laboratories for routine production of 6-[^{18}F]fluorodopa. More recently, a fluorodestannylation reaction was proposed as an alternative to the reliable fluorodemercuration method (*13*). This new procedure should allow larger scale production of this radiopharmaceutical for PET studies.

Unfortunately, all these electrophilic methods rely on the use of either neon or ^{18}O-gas targets and hazardous molecular fluorine as the carrier. As a result, the radiopharmaceutical is characterized by a low specific activity (1 mCi/μmole). Although ^{18}F gas can now be produced with a higher specific activity (30 mCi/μmole) in centers that have a small "proton-only" cyclotron, it is nevertheless much more difficult to produce a high level of activity in the electrophilic [^{18}F]F_2 form from the ^{18}O(p,n)^{18}F nuclear reaction on ^{18}O-enriched oxygen gas than no-carrier-added (NCA) nucleophilic ^{18}F-fluoride species (>1 Ci/μmole) from the ^{18}O-enriched water target. Today, the ^{18}O-water method is certainly the method of choice for routine production of the ^{18}F-fluorinating agent (*14*).

Several regioselective nucleophilic syntheses of fluorodopa using this last approach have been described in the last few years (*15–20*). However, all these nucleophilic multistep syntheses proceed with low radiochemical yields (5%–10%) and long synthesis times (more than 110 min).

Because PET applications require high levels of activity, a new approach for the nucleophilic synthesis of this radiopharmaceutical in a NCA form has been developed in this laboratory. The main parameters of this NCA synthesis, presently routinely used in the authors' center for the production of NCA 6-[^{18}F]fluoro-L-dopa for PET studies, are described in this article.

MATERIAL AND METHODS

Starting Materials

Hydriodic acid (57% weight), dichloromethane (HPLC) and the gold-label reagents dimethyl sulfoxide (DMSO) and acetonitrile were purchased from Janssen Chimica (Geel, Belgium) and used without further purification. The aminopolyether Kryptofix 222 [(4,7,13,16,21,24)hexaoxa-1,10 diazabicyclo(8.8.8)hexacosan], po-

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tassium carbonate, (S)-1-(tert-boc)-2-(tert-butyl)-3-methyl-4-imidazolidinone (Boc-BMI, 5) and ethanol were obtained from Merck (Darmstadt, Germany). All the other organic substrates and solvents were of analytic grade from Aldrich Bornem, Belgium. Oxygen-18-enriched water (98.5%) was obtained from Campro Benelux (Veenendaal, Holland).

2-Fluoro-4,5-dimethoxybenzaldehyde (6-fluoroveratraldehyde, 1), (2S,5S)-1-(tert-boc)-2-(tert-butyl)-3-methyl-5-(2'-fluoro-4',5'-dimethoxybenzyl)-4-imidazolidinone (7) and 6-fluoro-L-dopa (8) were synthesized as previously described (*18,21*).

Tetrahydrofuran (THF), the solvent used in the alkylation reaction was dried by successive distillation from Na and K. [^{18}F]fluoride was produced at the CRC Liège cyclotron with a CGR MeV (Versailles, France) cyclotron by the $^{18}O(p,n)^{18}F$ reaction using [^{18}O]H_2O (40%). Radiochemical yields are expressed at the EOB relative to the total amount of the $^{18}F^-$ recovered in ^{16}O-water after the Dowex 1X8 separation.

All melting points (MP) were determined on a Fisher-Johns melting point apparatus and are uncorrected. Tritiated nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Brucker 400 NMR spectrometer. For all compounds, proton chemical shifts were reported in parts per million (delta) down field from the internal standard tetramethylsilane. Mass spectra were recorded using a VG Trio-1 Benchtop GC-MS.

The microwave equipment used was a commercial Bauknecht model MWUT 517 (Kornwestheim, Germany) with nine power settings, starting at 50 W and increasing by 50-W increments to 500 W. A power setting 6 (a nominal 300 W) was used for 1 min for labeling the aldehyde (3).

High-Pressure Liquid Chromatography (HPLC) System

HPLC was carried out with a Waters 501 HPLC pump and a Rheodyne injector. The ultraviolet (UV) absorption and the radioactivity of the effluent of the HPLC column were monitored with a UV Lambda Max detector (model 480, Waters, at 254 and 281 nm) and a well-type NaI scintillation crystal and associated electronics (Eberline, Santa Fe, New Mexico), respectively.

An analytical Lichrosorb Select-B, C-8 Merck Darmstadt column (4 × 250 mm, 10 μ), eluted with a 65:35 solvent mixture of methanol and water at pH 4 (acetic acid 5×10^{-2} *M*) at a flow rate of 0.7 ml/min, was used for the identification of ^{18}F-labeled products (the aldehyde, benzyl iodide derivatives and fluoroalkylated products; Condition A). A semipreparative ODS 3 column (9.4 mm × 500 mm) from Whatman was used for purification of 6-[^{18}F]fluoro-L-dopa. The eluting solvent was 5 m*M* sodium acetate, 1 m*M* ethylenediaminetetraacetic acid (EDTA), 0.1% acetic acid, and 0.01% ascorbic acid, pH 4, at a flow rate of 5 ml/min (Condition B). Analytical conditions for identification of 6-[^{18}F]fluoro-L-dopa were a Lichrosorb Select-B, C-8 Merck Darmstadt column (4 × 250 mm, 10 μ) eluted with 0.1% acetic acid in water at a flow rate of 0.7 ml/min. A ligand-exchange chromatography column (Chiral Prolu Si 100, Serva, Polylab, Antwerp, Belgium) eluted with a solution of $NaH_2PO_4 \cdot H_2O$ (50 m*M*) and 1 m*M* $CuSO_4$ (pH 4) at a flow rate of 1.5 ml/min was used to determine the enantiomeric purity of the final radiopharmaceutical (Condition C). The identity of the different radiolabeled compounds was supported by HPLC coinjection studies.

Determination of the specific activity of 6-[^{18}F]fluoro-L-dopa was achieved on the same previously used analytical Lichrosorb Select-B column, either by indirect quantification of the [^{18}F]labeled aldehyde (Condition A) or directly on the final preparation (Condition B). The UV detector, with a 10-mm analytical cell, was set at 254 nm in the first case and at 281 nm in the second one. The area of the UV absorbance peaks was determined by an automated integrator (Shimadzu C-R5A Chromatopac, Antwerp, Belgium). In both cases, a calibration curve was determined with authentic reference samples.

Thin-Layer Chromatography (TLC) System

TLC analyses were carried out with plastic plates precoated with silica gel (Macherey-Nagel, Düren, Germany). The TLC solvent systems used were either hexane/ether (25:75, Condition D) or dichloromethane (100%, Condition E). Spots were detected by UV illumination where possible or analyzed for radioactivity with a radio TLC-linear analyzer from Berthold (Vilvorde, Belgium).

Silica gel chromatography columns were performed with Kieselgel 60 (70–230 mesh, Merck).

Preparation of 6-Dimethylaminoveratraldehyde (2)

6-Fluoroveratraldehyde (1) (2 g, 10.9 mmole), dimethylamine hydrochloride (1.16 g, 14.2 mmole) and potassium carbonate (1.16 g, 8.4 mmole) were stirred and heated to reflux in a mixture of DMSO (20 ml) and water (8 ml). After 1 hr, small additional portions of K_2CO_3 (5.8 mmole, 800 mg) were added. The progress of the reaction was followed by TLC (hexane/ether 40:60; R_f(2) = 0.37; R_f(1) = 0.61). If needed, dimethylamine hydrochloride and potassium carbonate were added to complete the reaction. Saturated aqueous potassium carbonate (40 ml) was added to the cooled solution, which was twice extracted with ether (2 × 30 ml). The organic layers were washed with water and dried on $MgSO_4$. The yellow oil obtained after filtration and evaporation was purified on a silica gel column (30 × 2.5 cm) eluted with hexane/ether (60:40). The yellow solid was characterized by a MP of 62°C (1.5 g, 66%).

MS(relative intensity): 209 (M^+, 100), 194 (78), 168 (26), 151 (23), 122 (10), 80 (14).

^{1}H-NMR ($CDCl_3$): 10.16 (s, 1H, CHO), 7.24 (s, 1H, Ar-H), 6.53 (s, 1H, Ar-H), 3.89 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.82 (s, 6H, $N(CH_3)_2$).

Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.13; H. 7.24; N, 6.69. Found: C, 63.0; H, 7.29; N, 6.82.

Preparation of 6-Trimethylammoniumveratraldehyde Triflate (3)

To a stirred solution of 6-dimethylaminoveratraldehyde (1.9 g, 9.1 mmole) in methylene chloride (10 ml) kept under N_2 at ambient temperature was added 1.8 ml of methyl trifluoromethanesulfonate (15.9 mmole). The solution was stirred for 3 hr at this temperature. The color of the solution changed gradually from yellow to green and red while a precipitate appeared.

The solid was filtered, washed with cold CH_2Cl_2 (50 ml), cold ether (100 ml) and dried in vacuo. The white crystals (1.5 g, 44.5%) characterized by a MP of 140°C were used without other purification for the labeling reaction (by TLC: CH_3CN/ether 75:25), R_f(3) = 0.55; R_f(2) = 0.84).

MS(relative intensity): 224 (26), 209 (97), 194 (100), 180 (38), 168 (34), 150 (34), 95 (31), 69 (40).

^{1}H-NMR (DMSO): 10.06 (s, 1H, CHO), 7.91 (s, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 4.02 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.74 (s, 9H, $N^+(Me)_3$).

Anal. Calcd for $C_{13}H_{18}NO_6F_3S$: C, 41.82; H, 4.87; N, 3.75. Found: C, 41.81; H, 4.89; N, 3.83.

Preparation of 2-Fluoro-4,5-Dimethoxybenzyl Iodide (4)

To a solution of diiodosilane (DIS), prepared as described subsequently, was added under stirring a solution of 6-fluoroveratraldehyde (15 mg, 81.5 μmole) in 5 ml of CH_2Cl_2. After a 5-min reaction at room temperature, the mixture underwent chromatography on a silica gel column (10 × 1 cm, CH_2Cl_2 100%, 30 ml). Evaporation of the solvent under reduced pressure afforded 22.9 mg of 2-fluoro-4,5-dimethoxybenzyl iodide as a yellow solid (95%, MP 91°C) (Condition E $R_f(4) = 0.54$; $R_f(1) = 0.2$)).

^{1}H-NMR($CDCl_3$): 6.75 (d, 1H, Ar-H, J_{H6}-F=7.2 Hz), 6.54 (d, 1H, Ar-H, J_{H3}-F=10.84 Hz), 4.41 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3).

Anal. Calcd for $C_9H_{10}O_2FI$: C, 36.51; H, 3.41. Found: C, 36.63; H, 3.38.

Preparation of DIS

Iodine (200 mg, 787 μmole) was placed in a dry conical flask equipped with a stirring bar. A mixture of phenylsilane (110 μl, 891 μmole) and ethylacetate (6 μl, 61.4 nmole) was then added. A vigorous exothermic reaction took place immediately. This crude DIS preparation was stirred for 2 min at room temperature and used without purification for the radioactive and nonradioactive reductive-iodination step.

Fluorine-18 Production

NCA aqueous [^{18}F]fluoride was prepared by the $^{18}O(p,n)^{18}F$ reaction on a small volume of enriched water (1.8 ml, 40%) in a silver target. A typical production run (10-μA bombardment for 1 hr) yielded 250 to 280 mCi of [^{18}F]fluoride, which was delivered to the laboratory through a 25-m long Teflon tube.

Fluorination of (3) with NCA Fluorine-18-Fluoride

The [^{18}F]fluoride was trapped from the [^{18}O]water using a Dowex 1X8 anion exchange resin (*22*) and the activity (±250 mCi) eluted from the column with 400 μl of potassium carbonate (7 mg/ml) into a glass vessel (2.5 ml) that contained 22 mg of Kryptofix 222 and 4.2 mg of potassium carbonate. Oxygen-16-labeled water was evaporated under nitrogen flow, and the aminopolyether/^{18}F-fluoride complex was dried by azeotropic evaporation with CH_3CN (3 × 100 μl) at 120°C. A solution of 6-trimethylammoniumveratraldehyde triflate (15 mg, 40.2 μmole) in DMSO (900 μl) was then added to the residue, and the labeling reaction was performed at 140°C for 10 min in an aluminum heating block or with microwaves for 1 min (300 W).

After the reaction, the reaction mixture was diluted with 0.5 N HCl (40 ml) and passed through a C-18 Sep Pak column (1 g, Waters). The cartridge was successively washed with 0.5 N HCl (5 ml) and H_2O (10 ml) and the whole activity finally eluted with CH_2Cl_2 (4 ml). To remove water from the sample, the [^{18}F]fluorobenzaldehyde solution was passed through a $MgSO_4$ column (8 × 1 cm), which was rinsed by CH_2Cl_2 (9 ml). The 6-[^{18}F]fluoroveratraldehyde was identified either using analytical HPLC (retention time 5 min; Condition A) or TLC ($R_f(1) = 0.20$; Condition E).

Conversion of NCA Fluorine-18-Fluoroveratraldehyde into NCA 2-[Fluorine-18]-Fluoro-4,5-Dimethoxybenzyl Iodide (4)

The previous radioactive solution of 6-[^{18}F]fluoroveratraldehyde (**1**) was then added to a preparation of DIS (previously prepared as described earlier). After a 2-min reaction at room temperature, the 2-[^{18}F]fluoro-4,5-dimethoxybenzyl iodide was purified on a silica gel column (10 × 1 cm; eluant CH_2Cl_2 100%, 30 ml). Evaporation of the solvent afforded the purified benzyl iodide derivative (Condition E $R_f(4) = 0.54$).

NCA Alkylation of the (S)-Boc-BMI (5) with 2-[Fluorine-18]-Fluoro-4,5-Dimethoxybenzyl Iodide (4)

In a closed conical vial under stirring, 200 μl of a 0.32 *M* lithium diisopropylamide (LDA) solution was added to the imidazolidinone (20 mg, 78 μmole) in 150 μl of dry THF at −78°C. The mixture was allowed to react for 5 to 15 min at this temperature. The [^{18}F]fluorobenzyl iodide in 200 to 400 μl of THF was then added. The alkylation reaction was performed for 5 min at the same temperature. The alkylated product was characterized by a retention time of 15 min (HPLC, Condition A, flow rate 1 ml/min) (TLC Condition D $R_f(7) = 0.27$; $R_f(4) = 0.60$).

Hydrolysis of the Fluorine-18-Fluoroalkylated Derivative (7) and HPLC Purification

The organic solvents (THF/hexane) were evaporated at 140°C under a flow of nitrogen. To the residue, 57% HI (1 ml previously distilled on red phosphorus) was added and the vial capped. Hydrolysis was performed by heating to 200°C for 20 min. After cooling, partial neutralization of HI was realized with 6 N NaOH (0.75 ml). The resulting solution was injected onto the HPLC column under Condition B. The retention time of 6-[^{18}F]fluorodopa was 12 min.

The enantiomeric purity of the final preparation was determined by ligand-exchange chromatography (Condition C). Under these conditions, the retention times for the D and L isomers were 7.5 min and 16.4 min, respectively.

Formulation

The HPLC fraction eluting from the HPLC column corresponding to 6-[^{18}F]fluoro-L-dopa (±8 ml) was collected from the column directly through a sterilizing 0.22-μ membrane filter (CATHIVEX-GS, Millipore, Brussels, Belgium) into a sterile vial (20 ml) that contained NaCl to ensure the isotonicity of the final solution.

RESULTS AND DISCUSSION

The previously published enantioselective syntheses of 6-[^{18}F]fluoro-L-dopa (*15–20*) involved ^{18}F-fluorination by aromatic nucleophilic substitution of 6-nitroveratraldehyde. Subsequent conversion of the [^{18}F]fluoroveratraldehyde into the corresponding [^{18}F]benzylalcohol and [^{18}F]benzylhalide derivatives was then realized by treatment with $NaBH_4$ followed by $SOBr_2$ (30%–40%) (*18*). Diastereoselective alkylation of the enolate of (S)-(−)-1-tert-boc-2-tert-butyl-3-methyl-4-imidazolidinone (**5**) by the electrophilic aryl bromide was realized at low temperature. After hydrolysis and preparative HPLC purification, a high enantiomeric purity (enantiomeric excess of 96% or more) in 6-[^{18}F]fluoro-L-dopa was obtained without chiral HPLC but with a low radiochemical yield (5%–10% EOB) after a long synthesis time (more than 110 min).

To allow routine production of 6-[^{18}F]fluoro-L-dopa by a nucleophilic approach, each step of this chemical pathway was separately studied and optimized. The improvements mainly consisted of the synthesis of a trimethylammonium-veratraldehyde triflate (**3**) as the starting precursor for the

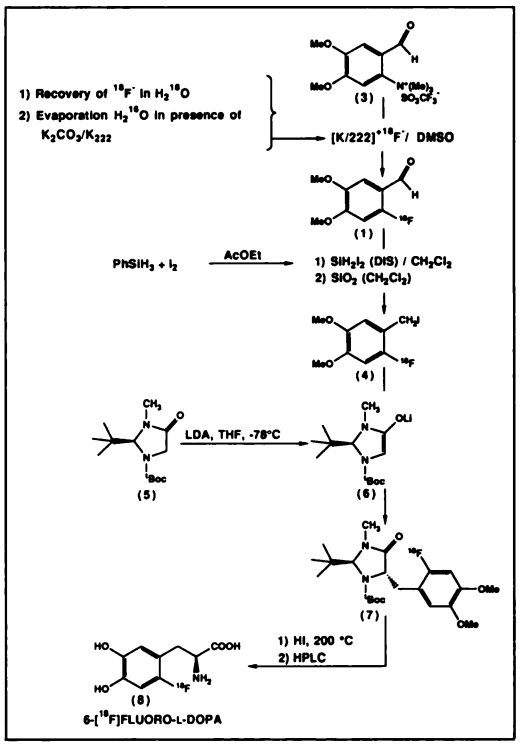

FIGURE 1. Radiolabeling of NCA 6-[^{18}F]fluoro-L-dopa starting from its aryltrimethylammonium precursor.

synthesis and the development of a new method for the preparation of the electrophilic alkylating agent (**4**).

Other parameters, such as labeling temperature, HPLC and TLC conditions, were also optimized. These considerations led to a new chemical pathway, which required four major steps (Fig. 1).

Labeling Reaction

In the previous reports, the initial precursor used for the synthesis of 6-[^{18}F]fluoro-L-dopa was 6-nitroveratraldehyde (*15–20*). With this starting compound, although the nucleophilic substitution by [^{18}F]F^- in the presence of the aminopolyether Kryptofix 222 and potassium carbonate in DMSO proceeded with a radiochemical yield of 50% EOB (*16*), the use of a cationic leaving group, such as a trimethylamonium triflate, instead of the neutral nitro group was considered for two major reasons.

The first one was the similar yield obtained for the incorporation of the [^{18}F]fluoride. For the regioselective nucleophilic aromatic substitution of the 6-trimethylammoniumveratraldehyde triflate by [^{18}F]F^-, a 10-min reaction at 130°C was needed to obtain a [^{18}F]compound (**1**) in 40% to 70% radiochemical yields. These yields were based on the amount of $^{18}F^-$ recovered in ^{16}O-water after the Dowex 1X8 separation. The exceptionally high values (70%) obtained for this labeling step were due to a batch of precursor presenting a high chemical purity difficult to reproduce (discussed later). A mean value of 45% ± 5% was then considered for the radiolabeling step. The use of this quaternary salt as the starting material reduced the overall synthesis time by at least 10 min compared with that of the nitro exchange reaction, which resulted in this case in a slight increase in the end-of-synthesis radiochemical yield. By using a commercial microwave oven, the reaction time for the radiolabeling was reduced to 1 min. Nevertheless, with this approach, lower radiochemical yields in [^{18}F]fluoroveratraldehyde were always obtained (35%–40% EOB). No attempts were made to increase the radiochemical yield through this technique.

The second one was the simplification of the subsequent synthesis and purification steps. Actually, after labeling, the traditional C-18 Sep Pak procedure allowed the isolation of the NCA 6-[^{18}F]fluoroveratraldehyde free from the large excess of unreacted $N^+(Me)_3$ starting substrate. As a result, the final HPLC purification of the amino acid from the starting precursor and byproducts was also greatly simplified.

The starting aryltrimethylammonium precursor (**3**) of this synthesis was prepared according to the general pathway shown in Figure 2. The 6-fluoroveratraldehyde derivative (**1**) was synthesized according to literature method (*21*). Its conversion into the N,N-dimethylamino intermediate and aryltrimethylammonium salt followed the same synthetic approach as previously described for the preparation of other similar compounds (*23,24*). Because methyl trifluoromethanesulfonate is a highly reactive methylating agent, the quaternization reaction proceeded in less than 3 hr at room temperature. If the reaction mixture was not too diluted, the synthesized triflate compound was easily isolated from the reactional mixture by filtration. To obtain a high chemical purity required for a smooth reaction with $^{18}F^-$, washing of the precipitate with a large volume of cold CH_2Cl_2 and ether was of primary importance. Following this procedure, this stable compound, obtained as a white

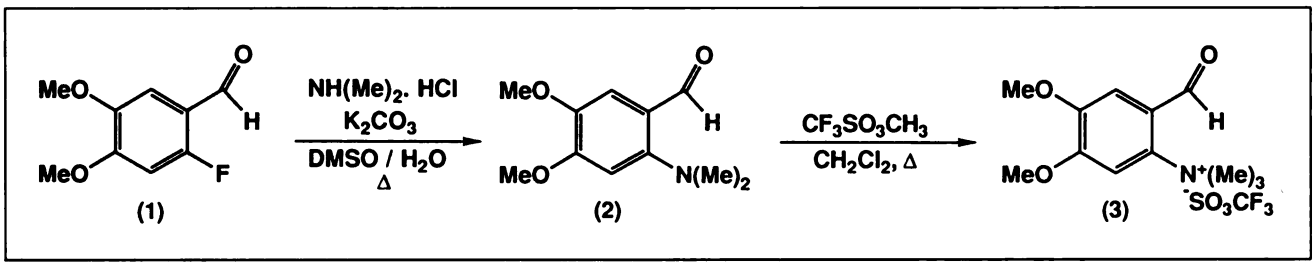

FIGURE 2. Chemical steps for the synthesis of the 6-trimethylammonium veratraldehyde triflate starting from 6-fluoroveratraldehyde.

solid, can be kept and used several months after its preparation without detectable degradation. Nevertheless, its solubilization in DMSO for the labeling reaction produced a clear solution that can darken (slightly yellow to green to red) with time (1 hr) if the chemical precursor's purity is not high enough. A lack of reactivity of the N^+ derivative was also observed after overdrying of the Kryptofix salt. In these conditions, lower radiochemical yields were also achieved (35%–45%). However, high yield (45% ± 5%) in this labeling step can be obtained with the authors' robotics system using an optical probe for the detection of the end of evaporation (*25*). Therefore, this step of the synthesis is highly independent of parameters such as water volume, temperature and nitrogen flow.

Reductive Iodination Reaction

The second step of the synthesis consisted of the preparation of a new electrophilic alkylating agent. In the previous reports on the synthesis of fluorodopa, 2-[^{18}F]fluoro-4,5-dimethoxybenzyl bromide was used as the alkylating agent for the enolate of Boc-BMI. However, high and reproducible yields for the alkylation step were only obtained after a time-consuming purification of the benzyl bromide on a silica gel column (*17,18,26*). The long time required for this purification step (30 min) and the loss of activity as a result of the unexpected secondary reactions on the support were certainly the two main limitations of this method. Owing to these problems, a new preparation method for the [^{18}F]fluorobenzyl halide was then considered which, as illustrated on Figure 1, implies the conversion of the 6-[^{18}F]fluoroveratraldehyde (**1**) into the corresponding [^{18}F]fluorobenzyl iodide derivative (**4**) in a single step. This transformation was realized in presence of DIS, a reagent that allows the reductive iodination of aldehydes (*27*). Although this reaction proceeded with high yield at room temperature with commercially available DIS and a macroscopic quantity of aldehyde (50 mg), the same procedure extended to the preparation of NCA 2-[^{18}F]fluoro-4,5-dimethoxybenzyl iodide resulted in variable yields and poor reliability (5%–90%). To solve this problem, DIS was freshly prepared from iodine and phenylsilane and used, without purification, in the radioactive runs. The progress of the reductive iodination reaction was followed by radio TLC and HPLC. For the HPLC control, elution with CH_3CN and water had to be avoided because degradation of the benzyl iodide occurred on the column. Methanol instead of acetonitrile as the solvent partially solved that problem (Condition A).

The 2-[^{18}F]fluoro-4,5-dimethoxybenzyl iodide (**4**) was quickly and easily purified by passing through a small silica gel cartridge (1 × 10 cm) and eluted with methylene chloride (30 ml). Under these conditions, the electrophilic [^{18}F]-fluorinating agent (**4**) was obtained free from the unreacted aldehyde staying on the column, with a radiochemical yield of 78% to 84% within 10 min from 6-[^{18}F]fluoroveratraldehyde (Table 1).

Alkylation

The third step of this synthesis was the alkylation reaction. After evaporation of CH_2Cl_2, the 2-[^{18}F]fluoro-4,5-dimethoxybenzyl iodide, resolubilized in THF, was added to a previously prepared lithium enolate solution of Boc-BMI (commercially available in both enantiomeric forms) obtained with LDA at dry ice temperature. This asymmetric inductive alkylation step led to the formation of a new carbon-alpha carbon-beta bond with high diastereoselectivity. However, high and reproducible yields in this alkylation step were only obtained (69%–85%, 50 min EOB,

TABLE 1
Main Characteristics of NCA Synthesis of 6-Fluorine-18-Fluoro-L-Dopa

Step Completed	Radiochemical Yield of Each Step (%)	Cumulated Radiochemical Yield (%)	Available Radioactivity (Not Decay Corrected, mCi)	Time from E.O.B. (min)
Recovery of ^{18}F and $H_2{}^{18}O$		100%*	250*	7
Evaporation of $H_2{}^{16}O$, preparation of the [Kryptofix 222]$^+$ $^{18}F^-$ agent,	—	—	—	20
Labeling reaction and	—	—	—	30
Sep pak purification	40–70	45 ± 5	90 ± 10	35
Reductive iodination (DIS), SiO_2; evaporation CH_2Cl_2				37
	78–84	36.5 ± 5.3	68 ± 10	45
Alkylation reaction	69–85	28.1 ± 6.6	49 ± 12	50
Hydrolysis, HPLC and formulation				70
	79–85	23 ± 6	32 ± 8.5	90

*The yield based on ^{18}F recovered in $H_2{}^{16}O$ after Dowex 1X8 are shown as the mean ± s.d. of 40 experiments.

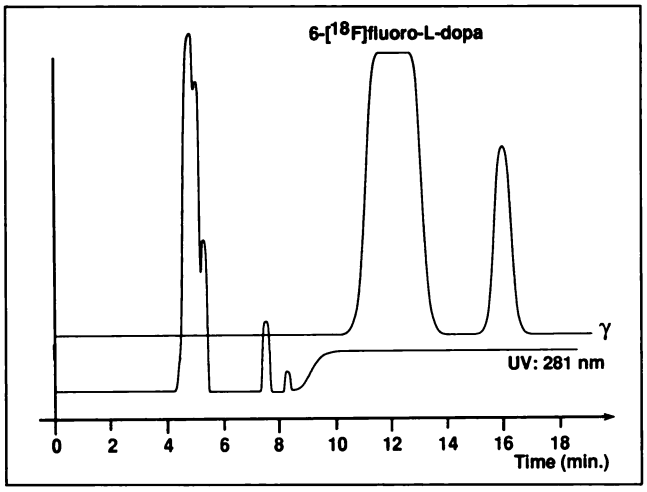

FIGURE 3. HPLC purification profile of 6-[^{18}F]fluoro-L-dopa. Conditions: Whatman ODS 3 (7 μ) column (9 × 500 mm).

Fig. 1 and Table 1) if the [^{18}F]fluorobenzyl iodide was purified as described earlier.

Hydrolysis

The last step of the synthesis implied the removal of the protecting groups. Efficient acid hydrolysis was only observed at a temperature minimum of 200°C (20 min), the overall process occurring in a closed vial. Although the hydrolysis has to be carried out under hard conditions to proceed with high yield, no racemization was observed during the process.

HPLC Purification

After hydrolysis, the acidic solution was cooled at room temperature, partially neutralized with a 6 N sodium hydroxide solution and then injected onto the HPLC column for final purification. For routine HPLC purification of 6-[^{18}F]fluorodopa, two different solvent systems were reported (*2*). Both methods required the elution of a semi-preparative reverse-phase column with either pure 0.1% acetic acid solution or a mixture of sodium acetate, EDTA, ascorbic acid and acetic acid. In the authors' hands, the presence of EDTA and ascorbic acid in the eluent resulted in an almost quantitative recovery for the 6-[^{18}F]fluorodopa injected onto the HPLC column (more than 95%). On the other hand, the use of 0.1% acetic acid as the solvent led to a recovery yield ranging below 50%. Figure 3 illustrates a typical chromatogram obtained from a production run of fluorodopa. The first radioactive peak was 6-[^{18}F]fluorodopa; the second peak was attributed to partially hydrolyzed fluorodopa. Typically, under these conditions, a cumulated radiochemical yield of 79% to 85% was obtained for the hydrolysis and purification steps.

Formulation

The isotonicity of the solution was achieved by adding 800 μl of NaCl (9%) to the 6-[^{18}F]fluorodopa HPLC fraction (±8 ml), which was previously filtered on a 0.22-μ Millipore filter to ensure sterility of the final solution.

Quality Control

Prior to each human injection, the radiochemical purity and enantiomeric excess of the fluorodopa preparation were monitored by HPLC analysis on a Serva Prolu Si 100 chiral column (Condition C). The main radiochemical impurity detected was the D isomer of fluorodopa (less than 2%), which eluted at the same time as the L isomer in all the described methods of preparative purification.

Specific activity, estimated by HPLC analysis after either the first step of synthesis on the [^{18}F]fluoroveratraldehyde or at the end of synthesis on a nondiluted 6-[^{18}F]fluorodopa solution, was in both cases around 835 ± 280 mCi/μmole. In this last case, it was the limit of detectability for the UV detector. These results are consistent with the use of NCA [^{18}F]fluoride and indicates little, if any, dilution of the specific activity by [^{19}F]fluoride of the triflate counterion of the starting precursor.

The formulated solutions of NCA 6-[^{18}F]fluoro-L-dopa were analyzed for stability by HPLC within 2 min, 30 min, 2 hr and 4 hr after preparative separation. No degradation was detected, although sample preparations were left at laboratory temperature without any special care. The radiochemical purity of the 6-[^{18}F]fluoro-L-dopa preparations was around 98.2% (n = 40).

Chemical purity was also assessed by analytical HPLC at two different wavelengths (281 and 220 nm). Under these conditions, no major chemical contaminant was detected. At the pH of the injectable solution (*4*), no darkening of the formulated solution of fluorodopa on prolonged storage time was observed.

Before the first human injection, the radiosynthesis protocol was tested seven times for sterility and apyrogenicity. Thereafter, retrospective testing was done after every 10 productions.

Representative results of the NCA fluorodopa productions are summarized in Table 1. It includes the radiochemical yields of each synthesis step, the elapsed time from EOB and the radioactivity present at various stages in the radiosynthesis (not decay corrected). So, after separation of NCA [^{18}F]fluoride from ^{18}O-enriched water, 250 mCi (100%) of fluoride $^{18}F^-$ in ^{16}O-water is available for this synthesis, which produces, without chiral HPLC purification, multimillicurie amounts (32 ± 8.5 mCi, end of synthesis) of NCA 6-[^{18}F]fluoro-L-dopa with both high radiochemical and enantiomeric purities (L ≥ 98%).

The whole synthesis time was about 90 min. Further improvement should be possible by using shorter reaction times for the alkylation step (1 min) and microwaves for the evaporation, labeling and hydrolysis steps. The decay-corrected radiochemical yield of this synthesis, which mainly depends on the radiochemical yield of the labeling reaction of the N^+ derivative (directly related to its chemical purity) ranged from 17% to 29% (EOB). The synthesized product, after sterile filtration and quality control, is ready for human use.

To avoid excessive radiation exposure to chemists, a robotic system that allows routine production of fluoro-

dopa through this asymmetric synthesis, was developed (*25*).

PET studies were performed on humans with these NCA preparations of fluorodopa (n = 20). The first studies showed that this tracer behaves in the same way as carrier-added preparations of this radiopharmaceutical.

CONCLUSIONS

This highly regioselective (only one of the three positional isomers was synthesized) and stereoselective (e.e. more than 96%) synthesis of NCA 6-[^{18}F]fluoro-L-dopa provides this radiopharmaceutical with both a high specific activity and high levels of activity useful for several PET studies.

In addition, with the same nucleophilic approach, several other aromatic amino acids, in the NCA state, such as 2-[^{18}F]fluoro-L-yrosine are routinely synthesized in our laboratory at a high level of activity (*28*).

Moreover, the synthesis of 6-[^{18}F]fluoro-L-dopa and other [^{18}F]fluoroamino acids from the [^{18}F]F^- nucleophilic precursor greatly simplifies laboratory procedures. Indeed, many other ^{18}F-fluorinated radiopharmaceuticals, such as [^{18}F]fluorodeoxyglucose and ligands for neurotransmission rely on the production of the $^{18}F^-$ by the $^{18}O(p,n)^{18}F$ nuclear reaction on enriched water.

The chemical community will decide over the years to come which one of the electrophilic and nucleophilic approaches is the more useful, but according to the results presented in this article, the nucleophilic method appears to be a promising method for the preparation of 6-[^{18}F]fluoro-L-dopa and a large variety of other [^{18}F]fluoro-amino acids.

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