No-Carrier-Added Regioselective Preparation of 6-[^{18}F]Fluoro-L-Dopa

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This paper describes the preparation of 6-[^{18}F]fluoro-L-dopa by a no-carrier-added method based on the nucleophilic displacement of nitro groups of two commercially available substrates, 3,4-dimethoxy-2-nitrobenzaldehyde (nitroveratraldehyde) and 6-nitropiperonal. Fluorination was conducted in DMSO with fluorine-18 (^{18}F) in the presence of the aminopolyether Kryptofix 222 and potassium carbonate. The condensation of the fluorinated aldehydes with phenyloxazolone and the subsequent hydrolysis with HI/P yield, after purification by HPLC, only the 6-(D, L) isomers. The racemic mixture (50/50) was resolved on an analytical scale chiral column. The method, which requires 100 min (EOB) to complete, produces 6-[^{18}F]fluoro-L-dopa with a decay-corrected radiochemical yield of 10%, an enantiomeric purity >99%, and a specific activity of 1.2 Ci/μmole.

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Current interest in the 6-[^{18}F]fluoro-L-dopa as the main presynaptic radiotracer for the study of the dopamine metabolism in the normal and pathological brain (*1*) has created a continual increasing demand for this radiopharmaceutical for positron emission tomographic investigations. Use of such an important compound in PET centers is mainly limited by the complex aspects of its synthesis.

The methods reported to date for the preparation of the 6-[^{18}F]fluoro-L-dopa are based on the use of electrophilic labeling with fluorinating agents, such as ^{18}F-F_2 (*2,3,4,5*), $^{18}F(XeF_2)$ (*6,7*) or ^{18}F (CH_3COOF) (*5,8,9–13*), leading at most to specific activities lower than 2 Ci/mmol at the end of the synthesis.

Ideally a preparation of 6-[^{18}F]fluoro-L-dopa should fulfill the following requirements:

1. An overall radiochemical yield (decay not corrected) of production >10%, with an enantiomeric purity better than 95%.
2. A total chemical processing time not exceeding 60 min.
3. A quantitative removal of protecting groups under non-drastic conditions.
4. An easy availability of the starting material showing high chemical stability and reproducible purity.
5. A simplicity in the sequence of the radiochemicals steps, allowing to set up a possible remote controlled or automated synthesis unit for repeated routine productions.

A no-carrier-added (n.c.a.) radiofluorination method of preparation, via the ^{18}F nucleophilic substitution, should meet the above goals in addition to these three significant advantages:

1. The accessibility to this compound by means of ^{18}F produced either with neutrons from a nuclear reactor or with protons from a single particle cyclotron. These nuclear reactions yield very high levels of [^{18}F]fluoride radioactivities (>1 Ci/batch) which are, in theory, quantitatively exchangeable with an adequate leaving group of a specific precursor.
2. The availability for the first time of injectable activities of 6-[^{18}F]fluoro-L-dopa in the n.c.a. state, answering therefore the pending question of the need for the n.c.a. form of this tracer.
3. The preparation of higher radioactivities of the final n.c.a. L compound in a one run synthesis with a radiochemical yield similar to those obtained with the most efficient synthesis using F_2 and CH_3COOF.

This paper reports a regioselective preparation of 6-[^{18}F]fluoro-L-dopa using the nucleophilic displacement by n.c.a. ^{18}F- of the activated nitro groups of two commercially available derivatives of o-nitrobenzaldehyde, 3,4-dimethoxy-2-nitrobenzaldehyde (nitroveratraldehyde) and 6-nitropiperonal. As shown in the scheme outlined in Figure 1, this synthesis follows a similar production pathway as that for the preparation of [^{18}F]L-p-fluorophenylalanine previously reported (*14*).

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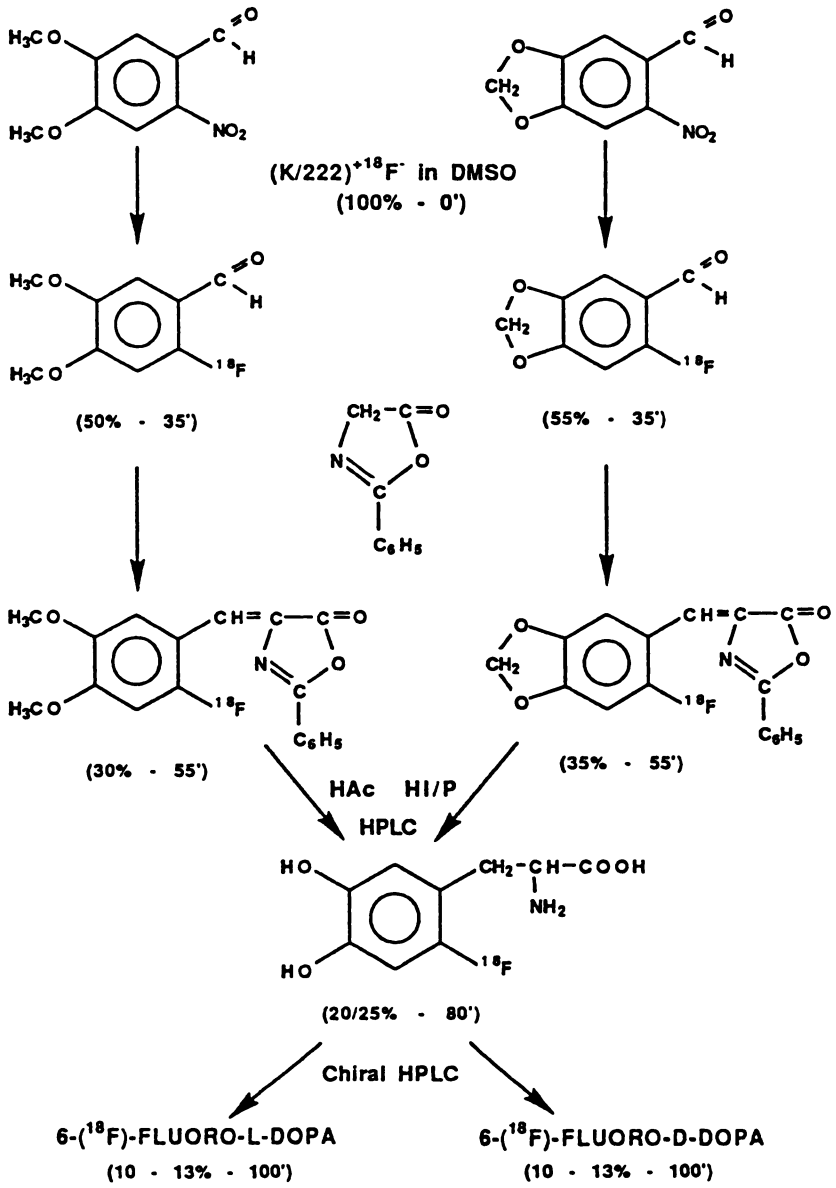

FIGURE 1
Comparison in terms of radiochemical yields and processing times of two n.c.a. synthesis routes to 6-(^{18}F)-fluoro-D,L-dopa.

MATERIALS AND METHODS

Starting Materials

The nonradioactive precursors, nitroveratraldehyde and nitropiperonal were purchased from Janssen Pharmaceutica (Beerse, Belgium) as were the gold label reagents dimethylsulfoxide (DMSO) and acetonitrile, which were used without further purification.

The aminopolyether Kryptofix 222, (4,7,13,16,21,24) hexaoxa-1,10 diazabicyclo [8.8.8] hexacosane, potassium carbonate, and ethanol were obtained from Merck, Darmstadt, FRG. The 2-phenyl-5-oxazolone, not commercially available, was synthesized according to a literature procedure (*15*) and characterized by standard methods.

HPLC System

High-performance liquid chromatography (HPLC) was conducted by means of a Waters 6000 A pump and a U6K injector with a Waters Lambda Model 481 LC ultraviolet spectrophotometer (254 nm) (Waters-Millpore, Brussels, Belgium) and a NaI (Tl) scintillation detector in series for absorbance and radioactivity measurements.

Fluorine-18 Production

No-carrier-added aqueous [^{18}F]fluoride was prepared by the ^{18}O(p,n)^{18}F reaction (*16*) on a small volume of enriched water (1.8 ml, 15% ^{18}O) in a nickel target. The target contents were delivered through 25 m of polyethylene tubing into a conical reaction vessel in the chemistry laboratory.

[K/222]+^{18}F-

No-carrier-added [K/222]+^{18}F- was prepared according to the method reported by Coenen et al. (*17*) in 1986. The aqueous [^{18}F]fluoride solution was added to a 2.5-ml sealed vial containing 7 mg K_2CO_3 and 22 mg Kryptofix 222. Under nitrogen bubbling, this solution was held for a few minutes on an aluminum heating block at 110°C. Five hundred microliters of CH_3CN were added and then evaporated to dryness. The evaporation step was repeated with CH_3CN and finally a dry residue of [K/222]+18F- was obtained.

No-carrier-added ^{18}F-aldehydic Compounds

A solution of 15 mg of nitroaldehyde in 1 ml of DMSO was added to the previous residue. The vial equipped with a screw cap and a silicon septum was tightly closed and heated to 130°C for 20 min.

The DMSO mixture was then diluted with 15 ml of water and the whole solution was passed through two C-18 SEP-PAK cartridges in series. After successively washing with 5 ml of 0.1N HCl, 10 ml H_2O and 500 μl of tetrahydrofuran (THF) as predrying agent, the cartridges were swept with a N_2 flow for 10 to 20 sec. The whole activity was finally eluted into a conical vial through a small Na_2CO_3 column (L: 5 cm, ø: 0.5 cm) with 3 × 1.5 ml ethanol.

HPLC analysis was carried out using the Lichrosorb select-B, C-8 reverse phase column (250 × 4 mm, 10 μm) eluted with methanol-pH 4 water ($5\ 10^{-2}$ *M* HOAc) (65/35) at a constant flow rate of 0.7 ml/min^{-1}. The radioactivity peak showed a retention time of 8 min in accordance with a previously run mass peak of the fluoroaldehydic compounds.

No-carrier-added ^{18}F-azlactone Compounds

A solution of 2-phenyl-5-oxazolone (50 mg, 0.31 mmol) and 1,4-diazabicyclo[2,2,2]octane (DABCO) (100 mg, 0.81 mmol) in 1 ml ethanol was added to the solution containing the [^{18}F]fluoroaldehyde. The mixture was refluxed at 140°C for 10 min. After the condensation, the solvent was removed under a slight nitrogen flow and the residue directly hydrolyzed.

Analytical HPLC was carried out with the same C-8 reverse phase column and conditions similar to those followed for the ^{18}F-aldehyde compounds.

Hydrolysis Procedure

Reduction and hydrolysis of the azlactone derivatives were carried out using freshly distilled hydriodic acid (1.5 ml, stabilized with 50 μl hypophosphorous acid) and red phosphorus (100 mg). The mixture was refluxed under nitrogen at 220°C for 15 min for complete hydrolysis. After partial neutralization with 1 ml of 6N NaOH, red phosphorus was removed by filtration of the mixture on a cartridge of glass wool. The C-18 Sep-Pak procedure, used to remove apolar compounds, produced 4 ml of a prepurified radioactive solution.

HPLC preparative purification was conducted on a Whatman Partisil10 ODS-3 reverse phase column (500 × 9.4 mm, 10 μm) using 0.1% acetic acid in water as eluent. The racemic mixture of 6-[^{18}F]fluoro-L-dopa was eluted at a flow rate of 4 ml/min with a retention time of 17 min. The fluorodopa collected fraction amounted a total volume of 12 ml.

Analytical HPLC of the product was carried out on the select-B C-8 column under the same conditions as reported above for the preparative separation of this compound. The radioactive peak corresponding to 6-[^{18}F]fluoro-L-dopa had the same retention time as an authentic inactive sample prepared according to a preparative scale synthesis (*9*).

Chiral Separation

After reduction of the 12 ml aqueous solvent under vacuum, to a final volume of ~1 ml, a chiral HPLC separation was conducted on a chiral column, according to the Grierson method (*18*), purchased from Polylab-Serva (Antwerp, Belgium) as chiral ProCu = Si 100 column (250 × 4.6 mm, 5 μm). Elution was performed with an aqueous solution of NaH_2PO_4 ($5\ 10^{-2}$ *M*) and $CuSO_4$ (10^{-3} *M*) at pH 4 at a flow rate of 1.5 ml/min^{-1}. Under these conditions, the retention times for D and L forms were 4.5 and 9 min, respectively. The enantiomeric purity of the final preparation was determined with the same column.

RESULTS AND DISCUSSION

The radiosynthesis of 6-[^{18}F]fluoro-L-dopa by a nucleophilic pathway required different parallel investigations for the optimization of the total radiochemical yield. One of them was focused on the effect of the chemical form and concentration of the starting substrate on the nucleophilic fluorination yield.

Two commercially suitable precursors with the same activated nitro group have been compared as starting material; 3,4-dimethoxy-2-nitrobenzaldehyde (nitroveratraldehyde) and 6-nitropiperonal. Fluorination of these compounds was conducted in DMSO with ^{18}F in the presence of the aminopolyether Kryptofix 222 and potassium carbonate. As shown in Figure 2, the yield for both starting compounds increased as a function of the substrate concentration up to a concentration of 15 mg/ml. At higher concentrations, a plateau of 50%–55% is reached. This high fluorination yield has to be regarded as a significant advantage of the nucleophilic pathway. The complete synthesis route leading to the fluorodopa from both compounds was investigated and evaluated in terms of radiochemical and deprotection yields.

The condensation of the fluorinated aldehydes with phenyloxazolone was conducted in presence of a base (DABCO). A freshly prepared 2-phenyl-5-oxazolone which had to be stored under inert atmosphere to prevent its degradation to hippuric acid was required to achieve a maximum radiochemical yield of 70%–75%.

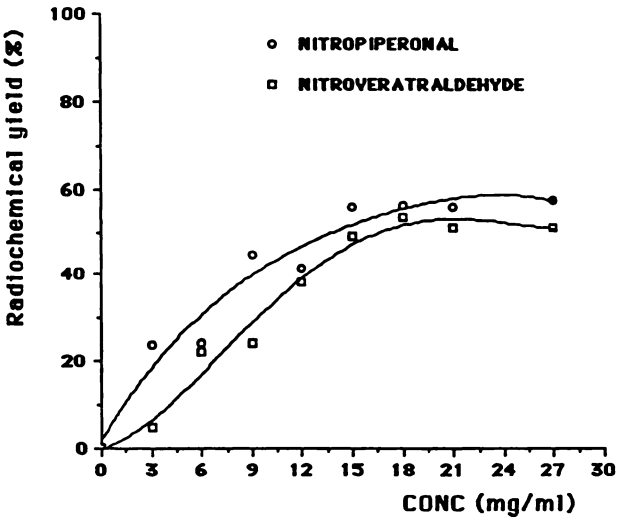

FIGURE 2
Radiochemical yield of n.c.a. ^{18}F-fluorination of 6-nitropiperonal and 6-nitroveratraldehyde as depending on the concentration (for conditions see experimental).

The following hydrolysis with HI/P yielded only the 6 structural isomer of 6-[^{18}F]fluoro-L-dopa. After the previous condensation and the following step of ring opening hydrogenation and hydrolysis of the protecting groups, the resulting decay-corrected yield was 25%.

Initial purification of the n.c.a. racemic amino acid, with an overall yield of 20%, from the reaction mixture can be achieved perfectly by HPLC on the Whatman preparative ODS column. The optimized HPLC conditions, previously reported, led to a retention time of 17 min for the fluoroamino acid. The first HPLC step is necessary in order to achieve the quantitative resolution of the D and L enantiomers in the second chiral purification. This separation was performed on the analytical chiral column prepared by chemical coupling of L-proline on a normal silica gel column (*18*). The eluent was the usual aqueous buffer containing 160 mg of Cu II ions/liter. In such conditions, a 1-ml injected sample was perfectly resolved. D and L isomers peaked, as shown in Figure 3, at 4.5 and 9 min, respectively, with a nearly quantitative radiochemical yield of 50%. Each isomer was collected in 3 ml and 6 ml volumes, respectively.

The radioactive peak corresponding to 6-[^{18}F]fluoro-L-dopa showed the same retention time as an authentic inactive sample prepared according to a preparative scale synthesis of carrier-added 6-[^{18}F]fluoro-L-dopa synthesized by the acetylhypofluorite method (*9*) and characterized by ^{1}H and ^{19}F NMR analysis. This reference compound showed the same retention time on the ODS and the chiral column as the final n.c.a. product.

The enantiomeric purity was checked by HPLC using the same chiral column and was authenticated as better than 99% of the L form.

The following figures were derived from a series of successive preparations:

^{18}F production parameters:
- 18 MeV protons (10 μA, 1 hr).
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- 15% enriched ^{18}O-H_2O.
- 105 mCi (EOB) ^{18}F-.

Synthesis radiochemical parameters:
- Total synthesis time for the L form: 100 min.
- Radiochemical yield EOB corrected: 10%.
- EOS available activity in the L form: 5-6 mCi.

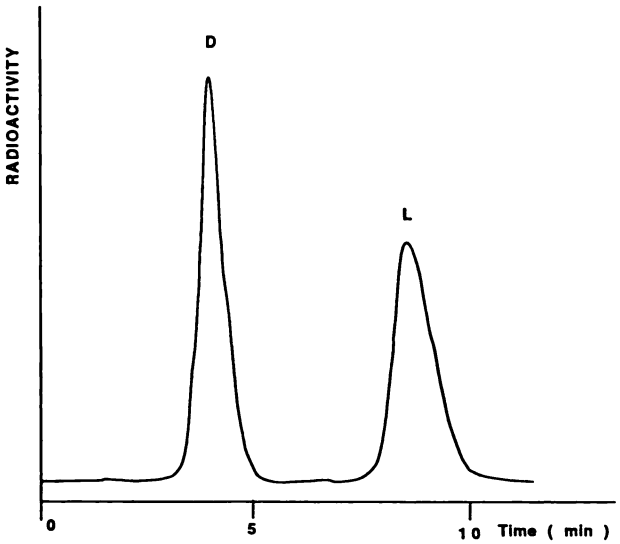

FIGURE 3
Typical radiochromatographic resolution of 6-(^{18}F)-fluoro-D,L-dopa on a chiral column.

In conclusion, this synthesis offers several advantages in comparison with the methods described to date for the preparation of 6-[^{18}F]fluoro-L-dopa. This n.c.a. labeling method led to a 100% regiospecificity (6 isomer only). Both the D and L isomers are obtained in equal amounts from this method. The simplicity of the two main successive steps will facilitate a remotely operated synthesis requiring minimum handling of radioactivity and allowing routine preparations.

The nonsterospecificity of this radiochemical synthesis required a chiral HPLC separation that was the most time- and yield- (50%) consuming step of the method. A direct asymmetric synthesis appears to be one of the ideal methods which could solve the stereospecificity problem of the n.c.a. fast preparation of 6-[^{18}F]fluoro-L-dopa. Our work in this field is in progress.

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