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6-[^{18}F]Fluoro-*L*-DOPA by Radiofluorodestannylation : A Short and Simple Synthesis of a New Labelling Precursor

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Summary

This paper describes a short and simple synthesis of a new fully protected stannylated precursor, namely *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester, for the preparation of 6-[^{18}F]fluoro-*L*-DOPA, used routinely in our Positron Emission Tomography program on neurodegenerative diseases as a tracer of the cerebral dopamine metabolism. The chemical pathway described for the total synthesis of our labelling precursor uses a straightforward protection sequence. This 4-step chemical synthesis allows the rapid preparation of several grammes of pure material in good overall yield. Regioselective radiofluorodestannylation using [^{18}F]fluorine ([^{18}F]F_2, cyclotron-produced isotope, half-life : 110 min) gave pure 6-[^{18}F]fluoro-*L*-DOPA (**8**) in good radiochemical yield (26% decay-corrected, based on starting [^{18}F]fluorine recovered from the target) in 45-50 min after the End of Bombardment. The product was found to be >99% chemically, radiochemically and enantiomerically pure.

Key Words : 6-[^{18}F]fluoro-*L*-DOPA, *L*-DOPA, fluorine-18, F-18, 18F, labelling precursor.

Introduction

Recently we have been confronted with an increasing demand of 6-[^{18}F]fluoro-*L*-DOPA (**8**) in the course of our routine Positron Emission Tomography (PET) program on neurodegenerative diseases using this tracer as a probe of the cerebral dopamine metabolism.

In our laboratory, for a long time we synthesized fluorine-18 (cyclotron-produced, half-life : 110 min) labelled fluoro-*L*-DOPA (**8**) using the well-known and widely used regioselective fluorodemercuration[1-4] reaction. This fluorodemetallation procedure, compared to the electrophilic direct, poorly regioselective fluorination of *L*-DOPA[5-7] or fully protected *L*-DOPA[8-10] derivatives as well as to the nucleophilic[11-14] enantioselective multistep radiosynthesis of the tracer, was preferred for its easy production, its easy purification by semipreparative HPLC and its simple routine semi-automated production implement.

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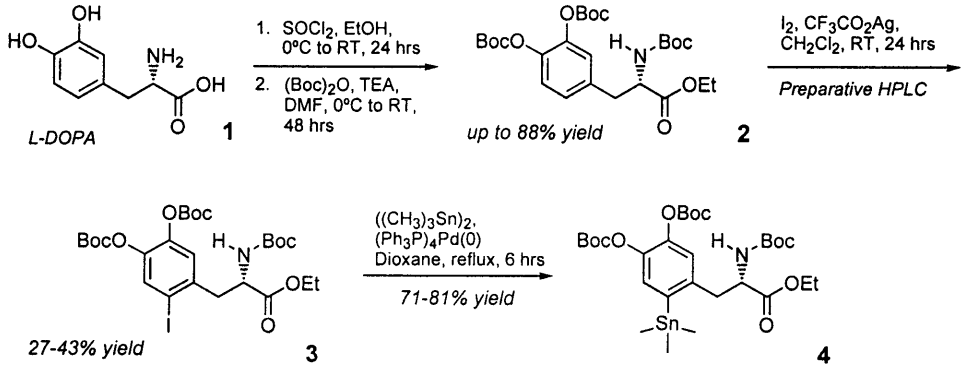
In order to increase our production capability, we prepared according to the literature[15] the protected stannyl-derivative **5**, namely *N*-(formyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester. Undoubtly, the radiochemical yield for the preparation of 6-[^{18}F]fluoro-*L*-DOPA using this labelling precursor and simple [^{18}F]fluorine has at least been doubled compared to our previously used fluorodemercuration procedure. Around 25 mCi or 0.93 GBq of radiochromatographically pure tracer were obtained at 45-50 min after EOB (HPLC purification included) for a 12 μA, 50 min (36000 μC) irradiation using the ^{20}Ne (d,α) ^{18}F nuclear reaction. The decay-corrected radiochemical yield, based on starting [^{18}F]fluorine recovered from the target, was 24%. However, we found the described synthesis[15] of the corresponding labelling precursor to be too long (8 steps) and the overall yield unreproducible.

In this paper, we describe a short and simple synthesis of a new, fully protected stannylated precursor for the preparation of 6-[^{18}F]fluoro-*L*-DOPA, *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester.

Results and Discussion

Chemistry

The preparation of our new tin precursor **4** is described in the scheme below.



Scheme 1 : Preparation of *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester (**4**)

Fully protected *L*-DOPA **2** was synthesized in excellent yield in a two step procedure starting from commercially available enantiomerically pure *L*-DOPA (**1**). Esterification of the carboxylic acid function using $SOCl_2$ at 0°C in EtOH for 24 hours followed by the simultaneous protection of both the phenolic and amino function using *tert*-butyl dicarbonate in DMF containing TEA at room temperature for 48 hours gave compound **2** in excellent yield (up to 88%).

Iodination with trifluoroacetyl hypoiodide, generated *in situ* fron I_2 (1.5 eq) and CF_3CO_2Ag (1.5 eq), in CH_2Cl_2 for 24 hours at room temperature, gave exclusively the monoiodo derivative **3**. Using these experimental conditions, the isolated yield of pure **3** (preparative HPLC purification) was 27 to 43%, based on converted starting material (25 to 20% was recovered). At this stage, various changes have been tried for increasing this moderate yield without success (CH_2Cl_2 was replaced by $CHCl_3$ or CCl_4, various I_2 and/or CF_3CO_2Ag ratios and batches, different reaction concentrations and temperatures). All these reactions were followed by TLC and HPLC, independently worked up and purified by HPLC and we observed that total consumption of starting material in the reaction did not correspond to the best isolated yields. These observations were similar for the iodination step of the published stannylated precursor **5** (see below). However, as described in the literature[15], only the 6-regioisomer could be detected. This was also confirmed in our case by analysis of the corresponding ^{1}H NMR spectrum, showing only two singlets at δ : 7.13 ppm and 7.72 ppm for the H-2 and H-5 signals respectively. The lack of coupling attests that both protons are *para* to each other, leaving as only possible position for the iodine atom the 6-one.

Finally, reaction of the iodo derivative **3** with hexamethylditin and *tetrakis*-triphenylphosphine palladium (0) in refluxing dry dioxane for 6 hours gave *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester (**4**) in 71 to 81% yield. The ^{1}H NMR spectrum of **4** showed the characteristic side-bands of a tin-proton interaction with measured coupling constants of $J^3_{Sn\text{-}H}$: 47.2 Hz (δ : 7.27, H-5), $J^4_{Sn\text{-}H}$: 15.8 Hz (δ : 7.09, H-2) and $J^2_{Sn\text{-}H}$: 54.0 Hz (δ : 0.37, $SnMe_3$). The ^{13}C NMR spectrum also showed distinctive tin satellites with coupling constants varying from 41 Hz ($J^2_{Sn\text{-}C}$, δ : 130.6, CH-5 or $J^3_{Sn\text{-}C}$, δ : 123.8, CH-2) to 339 Hz ($J^1_{Sn\text{-}C}$, δ : -7.78, $SnMe_3$). The particular pattern for the tin isotopes was also observed by mass spectrometry (DCI/NH_4^+ : $C_{29}H_{47}NO_{10}Sn$: 703, 704, 705, 706, 707, 709, 711 [M + NH_4^+] for Sn isotopes of 116, 117, 118, 119, 120, 122 and 124 respectively).

Using a straightforward protection sequence, the number of chemical steps for the total synthesis of our labelling precursor **4** dropped to 4 with respect to literature[15], allowing the rapid preparation of several grammes of pure material in good yield.

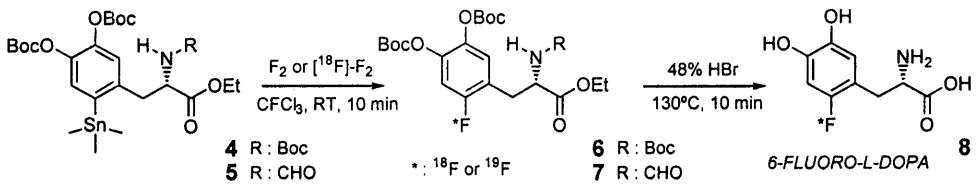
N-(formyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester (**5**) has also been prepared according to the 8-step process described in the literature[15]. Analytical data was in accordance with those already published (see experimental section). In our hands, the yield of the described regioselective iodination step was variable and never were we able to reach the reported 75% yield. Again, the various changes as described above have been tried for increasing our yield (15 to 25% in this case) without success. We also did not see any yield increases when the iodination process was performed on either the dimethoxy or the di-*tert*-butoxycarbonyl protected derivatives of *L*-DOPA.

As a reference, the 6-fluoro protected *L*-DOPA derivative **7** was synthesized from **5** using fluorine at room temperature in $CFCl_3$ in 38% yield. The reaction proceeded almost instantaneously.

Both ^{1}H and ^{13}C NMR showed characteristic proton-fluorine or carbon-fluorine interaction : ^{1}H NMR : coupling constants of 6.9 Hz (J^{4}_{F-H}, δ : 7.07) and 9.9 Hz (J^{3}_{F-H}, δ : 7.02) ; ^{13}C NMR : J_{F-C} : 247 Hz, 7 Hz, 15 Hz, 30 Hz for δ : 158.5, 142.5, 121.7, 111.0 respectively.

Radiochemistry

Regioselective radiofluorodestannylation was performed using either labelling precursor **4** or **5** (0.18 mmol) and [^{18}F]fluorine in $CFCl_3$ at room temperature for 10 minutes (Scheme 2). Deprotection using 48% aq HBr at 130°C for 10 minutes, followed by neutralization with aq NaOH and HPLC purification gave pure 6-[^{18}F]fluoro-*L*-DOPA (**8**) in good radiochemical yield.



Scheme 2 : Preparation of 6-[^{18}F]fluoro-*L*-DOPA (6-[^{18}F]fluoro-3,4-dihydroxy-L-phenylalanine, **8**)

In both procedures, 23-27 mCi (0.85-1.00 GBq) of radiochromatographically pure 6-[^{18}F]fluoro-*L*-DOPA (**8**) were obtained at 45-50 min after the End of Bombardment (EOB) with specific radioactivities of 0.12 mCi/μmol (4.44 MBq/μmol); yield : 26% decay-corrected, based on starting [^{18}F]fluorine recovered from the target (theoretical maximum yield in this radiodestannylation reaction is 50%, the other 50% of the activity is lost with the trimethyltin moiety). The product was found in both procedures to be >99% chemically and radiochemically pure, as demonstrated by analytical HPLC analysis. Only one enantiomer could be detected by chiral HPLC analysis, which suggets that no racemization occurs during the synthesis processes.

Conclusion

This paper described a short and simple synthesis of a new fully protected stannylated precursor, namely *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester for the preparation of 6-[^{18}F]fluoro-*L*-DOPA. The chemical pathway described for the total synthesis of our labelling preçursor uses a straightforward protection sequence. This 4-step chemical synthesis allows the rapid preparation of several grammes of pure material in good overall yield. Regioselective radiofluorodestannylation using [^{18}F]fluorine gave chemically, radiochemically and enantiomerically pure 6-[^{18}F]fluoro-*L*-DOPA (**8**) in good radiochemical yield (26% decay-corrected, based on starting [^{18}F]fluorine recovered from the target) in 45-50 min after the End of Bombardment.

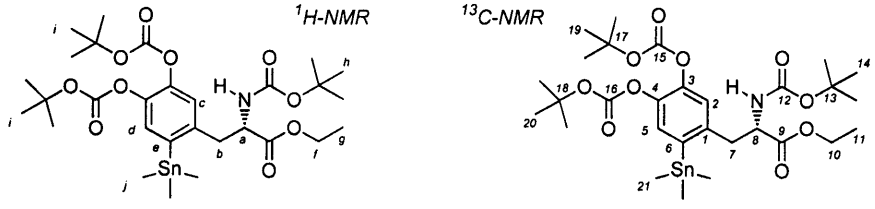
Experimental

General

Chemicals including *L*-DOPA (**1**) were purchased from Aldrich, Fluka or Sigma France and were used without further purification. Racemic 6-fluoro-*DL*-DOPA ((+/-)-**8**) was purchased as a standard from RBI France.

TLC were run on pre-coated plates of silicagel 60F254 (Merck). The compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by iodine staining and/or (3) by dipping the TLC-plates in a 1% ninhydrin solution in ethanol (or 1% aqueous $KMnO_4$) and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyzer. Flash chromatography was conducted on silicagel 63-200 μm (Merck) at 0.3 bars (Ar). Analytical and semipreparative HPLC were run on Waters systems equipped with a 6000A or 510 pumps, 440 UV or 481 & 486 UV-multiwavelength detectors ; the eluent was also monitored for radioactivity with a Geiger-Müller counter. Preparative HPLC was run on a Waters Prep LC 3000 system equipped with a 600E controller and a 481 UV-multiwavelength detector.

NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated solvents ($CDCl_3$, δ = 7.26 ppm ; CD_2Cl_2, δ = 5.32 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuteriated solvents ($CDCl_3$, δ = 77.1 ppm ; CD_2Cl_2, δ = 53.8 ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield either from TMS (s, d, t, dd, b for singlet, doublet, triplet, doublet of doublet and broad respectively ; *, °, #, & : interchangeable assignments). The nomenclature used for the ^{1}H and ^{13}C NMR attribution is given below :



The mass spectra (MS), DCI/NH_4^+ were measured on a Nermag R10-10 apparatus.

Air- or moisture sensitive reactions were conducted in heat gun-dried glassware, under an inert atmosphere and with freshly distilled solvents.

[^{18}F]fluorine was produced on a CGR-MeV 520 cyclotron by irradiation of a 0.42% F_2 in Ne target (volume : 144 mL, 35 x 150 mm) at 11 bar* (water-cooled Monel (Ni65/Cu33/Fe2) target-holder equipped with a He-cooled 25 μm waspaloy window supported with a 50 μm stainless steel foil) using a 14.5 MeV deuteron beam [^{20}Ne(d,α)^{18}F]. An irradiation was routinely preceded by two pre-irradiations of 7200 μC. On average, about 120-130 mCi (4.44-4.81 GBq) of [^{18}F]F_2 is routinely obtained in our laboratory at the End Of Bombardment for a 12 μA, 50 min (36000 μC) irradiation.

* composed of 2.5 bar of 2% F_2 in Ne (Air Liquide, France) and 8.5 bar of pure Ne (N48, Air Liquide, France).

Chemistry

N-(tert-Butoxycarbonyl)-3,4-di(tert-butoxycarbonyloxy)-L-phenylalanine ethyl ester (2)

First step : To a solution of 3,4-dihydroxy-*L*-phenylalanine (**1**, *L*-DOPA) (25.0 g, MW : 197.19, 127 mmol) in EtOH (150 mL), cooled to 0°C, were carefully added dropwise (in 15 min) 45 mL of $SOCl_2$ (5 eq, MW : 118.97, d : 1.63). The mixture was stirred at 0°C for 2 h and at room temperature overnight. After concentration, the residue was taken up in 500 mL of EtOAc, washed with 10% aq Na_2CO_3, water, brine and dried over $MgSO_4$. Concentration gave 25.2-28.0 g of crude 3,4-dihydroxy-*L*-phenylalanine ethyl ester as a dense oil (88-98%) which was used without further purification. Rf (EtOAc/MeOH/TEA : 80/20/1) : 0.45 ; ^{1}H NMR ($CDCl_3$, 298.0K) : δ : 6.80-6.60 (b, 1H, *H-d**) ; 6.65 (s, 1H, *H-c*) ; 6.45-6.35 (bd, 1H, *H-e**) ; 4.15 (q, J : 7.3 Hz, 2H, *H-f*) ; 3.70 (bs, 1H, *H-a*) ; 3.00-2.60 (ABX system, looks like m & b, 2H, *H-b*) ; 1.26 (t, J : 7.3 Hz, 3H, *H-g*) ; ^{13}C NMR ($CDCl_3$, 298.0K) : δ : 174.2 [C-9] ; 145.1 [C-3*] ; 144.2 [C-4*] ; 127.9 [C-1] ; 120.8 [CH-6°] ; 116.8 [CH-2°] ; 115.9 [CH-5°] ; 61.4 [CH_2-10] ; 55.0 [CH-8] ; 39.3 [CH_2-7] ; 14.1 [CH_3-11].

Second step : To a solution of 3,4-dihydroxy-*L*-phenylalanine ethyl ester (20.0 g, MW : 225.24, 89 mmol) in DMF (50 mL), cooled to 0°C, were added 40 mL of triethylamine (3.3 eq, MW : 101.19, d : 0.73, 293 mmol). To this solution, 72 mL of di-*tert*-butyl dicarbonate (3.5 eq, MW : 218.25, d : 0.949, 311.5 mmol) in 50 mL of DMF were added dropwise and the mixture was stirred first at 0°C for 2 h and then 48 h at room temperature. After dilution with EtOAc (250 mL), the solution was washed with water, brine, dried over $MgSO_4$ and concentrated to dryness. The residue was chromatographed on silica gel (eluent : CH_2Cl_2/EtOAc : 95/5 to 90/10) to give 39.8-42.1 g of *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-*L*-phenylalanine ethyl ester (**2**) as an oily residue (85-90%). Rf (Heptane/EtOAc : 90/10) : 0.20 ; (CH_2Cl_2/EtOAc : 95/5) : 0.25 ; ^{1}H NMR (CD_2Cl_2, 298.0K) : δ : 7.16 (d, J : 8.1 Hz, 1H, *H-d**) ; 7.04 (s, 1H, *H-c*) ; 7.03 (d, J : 8.1 Hz, 1H, *H-e**) ; 5.15 (bd, 1H, *N-H*) ; 4.52 (bm, 1H, *H-a*) ; 4.15 (q, J : 7.2 Hz, 2H, *H-f*) ; 3.07 (ABX system, 2H, *H-b*) ; 1.52 (s, 18H, *H-i*) ; 1.41 (s, 9H, *H-h*) ; 1.23 (t, J : 7.2 Hz, 3H, *H-g*) ; ^{13}C NMR (CD_2Cl_2, 298.0K) : δ : 171.8 [C-9] ; 155.3 [C-12] ; 151.0 [2C-15,16] ; 142.8 [C-3*] ; 141.9 [C-4*] ; 135.6 [C-1] ; 127.6 [CH-6°] ; 124.3 [CH-2°] ; 123.2 [CH-5°] ; 84.0 [2C-17,18] ; 80.0 [C-13] ; 61.8 [CH_2-10] ; 54.8 [CH-8] ; 37.8 [CH_2-7] ; 28.5 [CH_3-19$^{\#}$] : 27.7 [CH_3-20$^{\#}$] ; 27.5 [CH_3-14] ; 14.3 [CH_3-11].

N-(tert-Butoxycarbonyl)-3,4-di(tert-butoxycarbonyloxy)-6-iodo-L-phenylalanine ethyl ester (3)

To a solution of *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-*L*-phenylalanine ethyl ester (**2**) (1.6 g, MW : 525.59, 3 mmol) in CH_2Cl_2 (70 mL) at room temperature were added 995 mg of CF_3CO_2Ag (1.5 eq, MW : 220.88, 4.5 mmol) and 1.15 g of I_2 (1.5 eq, MW : 253.81, 4.5 mmol). The mixture was stirred at room temperature for 24 h and then filtered. The filtrate was then diluted with 100 mL of CH_2Cl_2, washed with aq 1.0 M $Na_2S_2O_3$, water, brine, dried over $MgSO_4$ and finally concentrated to dryness. The residue was filtered/chromatographed on silica gel (eluent : Heptane/EtOAc : 90/10 to 85/15) and then purified by preparative HPLC (preparative Merck LiChrosorb Si60 (250 x 25 mm) ; Porosity : 7 μm ; Eluent : Heptane/AcOEt : 85/15 ; Flow rate :

35 mL/min ; Temperature : RT ; UV detection at λ : 254 nm ; Retention time : (**3**) : 6-7 min & (**2**) : 9-10 min). 390-585 mg of pure *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-iodo-*L*-phenylalanine ethyl ester (**3**) were obtained as a foam (20-30%). Rf (Heptane/EtOAc : 85/15) : 0.20 ; (CH_2Cl_2/EtOAc : 95/5) : 0.35 ; ^{1}H NMR (CD_2Cl_2, 298.0K) : δ : 7.72 (s, 1H, *H-d*) ; 7.13 (s, 1H, *H-c*) ; 5.18 (bd, 1H, *N-H*) ; 4.58 (bm, 1H, *H-a*) ; 4.17 (q, J : 7.2 Hz, 2H, *H-f*) ; 3.20 (ABX system, looks like dd & bm, 2H, *H-b*) ; 1.53 (s, 18H, *H-i*) ; 1.41 (s, 9H, *H-h*) ; 1.24 (t, J : 7.2 Hz, 3H, *H-g*) ; ^{13}C NMR (CD_2Cl_2, 298.0K) : δ : 171.8 [C-9] ; 155.3 [C-12] ; 150.6 [2C-15,16] ; 143.0 [C-3*] ; 141.9 [C-4*] ; 138.8 [C-1] ; 133.8 [CH-5°] ; 124.7 [CH-2°] ; 95.5 [C-6] ; 84.5 [C-17$^{\&}$] ; 84.4 [C-18$^{\&}$] ; 80.1 [C-13] ; 62.0 [CH_2-10] ; 53.9 [CH-8] ; 42.8 [CH_2-7] ; 28.5 [CH_3-19$^{\#}$] : 27.5 [CH_3-20$^{\#}$] ; 27.5 [CH_3-14] ; 14.2 [CH_3-11].

N-(tert-Butoxycarbonyl)-3,4-di(tert-butoxycarbonyloxy)-6-trimethylstannyl-L-phenylalanine ethyl ester (4)

To a solution of *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-iodo-*L*-phenylalanine ethyl ester (**3**) (1.7 g, MW : 651.48, 2.6 mmol) in anhydrous 1,4-dioxane (30 mL) at room temperature were added 150 mg of *tetrakis* triphenylphosphine palladium (0) (0.05 eq, MW : 1155.58, 0.13 mmol) and 1.0 mL of hexamethylditin. The mixture was refluxed for 6 h, cooled to room temperature and then filtered. The filtrate was then diluted with 100 mL of EtOAc, washed with water and brine, dried over $MgSO_4$ and concentrated to dryness. The residue was chromatographed on silica gel (eluent : Heptane/EtOAc : 95/5 to 85/15) to give 1.3-1.45 g of *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester (**4**) as a white powder (71-81%). Rf (Heptane/EtOAc : 85/15) : 0.30 ; ^{1}H NMR (CD_2Cl_2, 298.0K) : δ : 7.27 (s, with tin satellites $J^3_{Sn\text{-}H}$: 47.2 Hz, 1H, *H-d*) ; 7.09 (s, with tin satellites $J^4_{Sn\text{-}H}$: 15.8 Hz, 1H, *H-c*) ; 5.02 (bd, 1H, *N-H*) ; 4.45 (bm, 1H, *H-a*) ; 4.15 (q, J : 7.2 Hz, 2H, *H-f*) ; 3.10 (ABX system, looks like dd & bm, 2H, *H-b*) ; 1.53 (s, 18H, *H-i*) ; 1.37 (s, 9H, *H-h*) ; 1.21 (t, J : 7.2 Hz, 3H, *H-g*) ; 0.37 (s, with tin satellites $J^2_{Sn\text{-}H}$: 54.0 Hz, 1H, *H-j*) ; ^{13}C NMR (CD_2Cl_2, 298.0K) : δ : 172.3 [C-9] ; 155.4 [C-12] ; 151.1 [C-15$^{\&}$] ; 151.0 [C-16$^{\&}$] ; 143.0 [C-3*] ; 142.3 [C-4*] ; 142.1 [C-1*] ; 141.3 [C-6*] ; 130.6 [CH-5, s, with tin satellites $J^2_{Sn\text{-}C}$: 43 Hz] ; 123.8 [CH-2, s, with tin satellites $J^3_{Sn\text{-}C}$: 41 Hz] ; 84.0 [2C-17,18] ; 80.2 [C-13] ; 61.9 [CH_2-10] ; 54.2 [CH-8] ; 40.8 [CH_2-7] ; 28.3 [CH_3-19$^{\#}$] : 27.8 [CH_3-20$^{\#}$] ; 27.8 [CH_3-14] ; 14.3 [CH_3-11] ; -7.78 [CH_3-21, s, with tin satellites $J^1_{Sn\text{-}C}$: 339 Hz] ; MS (DCI/NH_4^+) : $C_{29}H_{47}NO_{10}Sn$: 703, 704, 705, 706, 707, 709, 711 [M + NH_4^+] for Sn isotopes of 116, 117, 118, 119, 120, 122 and 124 respectively.

N-(Formyl)-3,4-di(tert-butoxycarbonyloxy)-6-trimethylstannyl-L-phenylalanine ethyl ester (5)

This radiolabelling precursor has been prepared from *L*-DOPA (**1**) according to literature[15]. Analytical data were in accordance with those already published. Rf (Heptane/EtOAc : 50/50) : 0.45 ; ^{1}H NMR (CD_2Cl_2, 298.0K) : δ : 8.03 (s, 1H) ; 7.27 (s, with tin satellites $J^3_{Sn\text{-}H}$: 44.1 Hz, 1H) ; 7.09 (s, with tin satellites $J^4_{Sn\text{-}H}$: 13.9 Hz, 1H) ; 6.40 (bd, 1H) ; 4.75 (bm, 1H) ; 4.15 (q, J : 7.2 Hz, 2H) ; 3.12 (ABX system, looks like two dd, 2H) ; 1.52 (s, 18H) ; 1.20 (t, J : 7.2 Hz, 3H) ; 0.37 (s, with tin satellites $J^2_{Sn\text{-}H}$: 53.9 Hz, 1H) ; ^{13}C NMR (CD_2Cl_2, 298.0K) : δ : 171.5 [C] ; 161.1 [CH]

; 151.2 [C] ; 151.0 [C] ; 143.0 [C] ; 142.1 [C] ; 142.0 [C] ; 141.3 [C] ; 130.7 [CH, s, with tin satellites J^2_{Sn-C} : 40 Hz] ; 123.8 [CH, s, with tin satellites J^3_{Sn-C} : 38 Hz] ; 84.1 [2C] ; 62.1 [CH_2] ; 52.2 [CH] ; 40.5 [CH_2] ; 27.8 [$2CH_3$] ; 14.2 [CH_3] ; -7.78 [CH_3, s, with tin satellites J^1_{Sn-C} : 345 Hz] ; MS (DCI/NH_4^+) : $C_{25}H_{39}NO_9Sn$: 631, 632, 633, 634, 635, 637, 639 [M + NH_4^+] ; 614, 615, 616, 617, 618, 620, 622 [M + H^+] for Sn isotopes of 116, 117, 118, 119, 120, 122 and 124 respectively.

N-(Formyl)-3,4-di(tert-butoxycarbonyloxy)-6-fluoro-L-phenylalanine ethyl ester (7)

2% fluorine in Ne was slowly bubbled into a solution of *N*-(formyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester (**5**, 90 mg, MW : 616.28, 0.15 mmol) in $CFCl_3$ (20 mL) at room temperature. The reaction was monitored by TLC : after a period of 2-3 min, the starting material (Rf : 0.45 in Heptane/EtOAc : 50/50) was completely consumed and only one spot could be detected (Rf : 0.22, same eluent). The solution was then diluted with 20 mL of CH_2Cl_2, washed twice with 1.0 M aq $Na_2S_2O_3$, water, brine, dried over $MgSO_4$ and finally concentrated to dryness. The residue was purified by semipreparative HPLC (SiO_2 Lichrosorb Merck (250 x 10 mm) ; Porosity : 7 µm ; Eluent : CH_2Cl_2/MeOH : 99/1 ; Flow rate : 5 mL/min ; Temperature : RT ; UV detection at λ : 254 nm ; Retention time : 5.5-6.0 min) to give 28.9 mg of pure *N*-(formyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-fluoro-*L*-phenylalanine ethyl ester (**7**) as an oil (38%). Rf (Heptane/EtOAc : 50/50) : 0.22 ; ^{1}H NMR (CD_2Cl_2, 298.0K) : δ : 8.08 (s, 1H) ; 7.07 (d, J^4_{F-H} : 6.9 Hz, 1H) ; 7.02 (d, J^3_{F-H} : 9.9 Hz, 1H) ; 6.41 (bd, 1H) ; 4.76 (bm, 1H) ; 4.16 (q, J : 7.3 Hz, 2H) ; 3.18 (ABX system, looks like two dd, 2H) ; 1.52 (s, 18H) ; 1.20 (t, J : 7.3 Hz, 3H) ; ^{13}C NMR (CD_2Cl_2, 298.0K) : δ : 171.1 [C] ; 161.1 [CH] ; 158.5 [C, d, J_{F-C} : 247 Hz] ; 151.0 [C] ; 150.6 [C] ; 142.5 [C, d, J_{F-C} : 7 Hz] ; 139.0 [C] ; 125.7 [C] ; 121.7 [CH, d, J_{F-C} : 15 Hz] ; 111.0 [CH, d, J_{F-C} : 30 Hz] ; 84.7 [C] ; 84.4 [C] ; 62.3 [CH_2] ; 51.4 [CH] ; 31.4 [CH_2] ; 27.7 [$2CH_3$] ; 14.2 [CH_3].

Radiochemistry : 6-[^{18}F]fluoro-3,4-dihydroxy-L-phenylalanine or 6-[^{18}F]fluoro-L-DOPA (8)

The remote, semiautomated radiosynthesis of 6-[^{18}F]fluoro-*L*-DOPA (**8**) used a slightly modified literature procedure[15].

***a** - From N-(formyl)-3,4-di(tert-butoxycarbonyloxy)-6-trimethylstannyl-L-phenylalanine ethyl ester (5).* Radiolabelled [^{18}F]fluorine was released from the target holder and bubbled into a solution of *N*-(formyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester (**5**, 110 mg, MW : 616.28, 0.18 mmol) in $CFCl_3$ (20 mL) at room temperature over a period of 10 min (this time included one purge of the target-holder with pure Ne). The solution was then diluted with 5 mL of CH_2Cl_2 and transferred to a chromatography column (0.7 cm of internal diameter) packed with finely powdered $Na_2S_2O_3$ (2.0-2.5 cm high, top of the column) and 63-200 µm silica gel (9-10 cm high, bottom of the column). The column was washed with 5 to 10 mL of CH_2Cl_2. The $CFCl_3$/CH_2Cl_2 fraction as well as the CH_2Cl_2 one, only showed one labelled TLC-spot (Rf : Heptane/EtOAc : 30/70 : 0.90) and were discarded (6-10% decay-corrected, from the total

activity). The radiolabelled fully protected fluoro-DOPA derivative was eluted from the column with 20 mL of Et_2O (one labelled TLC-spot, Rf : Heptane/EtOAc : 30/70 : 0.65, co-elution with authentic synthesized unlabelled reference compound **7**; at this stage, 45-55%, decay-corrected, of the total activity stayed on the column). The solvents were evaporated till dryness at the rotary evaporator at room temperature and under reduced pressure. The residue was diluted with 2.0 mL of 48% aq HBr and heated at 130°C for 10 min. After cooling to room temperature, the mixture was neutralized with 1.7 mL of 3N aq NaOH, filtered (Millex HV 0.45 μm, Millipore, rinsed with 1.0 mL of the HPLC mobile phase, see below) and the resulting solution was injected onto the semipreparative HPLC column. 23-27 mCi (0.85-1.00 GBq) of radiochromatographically pure 6-[^{18}F]fluoro-3,4-dihydroxy-*L*-phenylalanine (6-[^{18}F]fluoro-*L*-DOPA, **8**) were obtained at 45-50 min after EOB (HPLC purification included) with specific radioactivities of 0.12 mCi/μmol (4.44 MBq/μmol) for a 12 μA, 50 min (36000 μC) irradiation of a 0.42% F_2/Ne target (11 bars) with a 14.5 MeV deuteron beam; yield : 26% decay-corrected, based on starting [^{18}F]fluorine.

Semipreparative purification : Column : semipreparative Whatman Partisil ODS3 (500 x 9.4 mm) ; Porosity : 10 μm ; Eluent : H_2O/AcOH : 999/1 ; Flow rate : 6.0 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : 11-14 min.

Formulation of the radiolabelled 6-fluoro-DOPA for i.v. injection was effected as follows : (1) HPLC solvent removal by evaporation; (2) taking up the residue in 0.9% saline; (3) filtration on a 0.22 μm Millipore filter into a sterile multidose vial.

b *- From N-(tert-butoxycarbonyl)-3,4-di(tert-butoxycarbonyloxy)-6-trimethylstannyl-L-phenyl alanine ethyl ester (**4**).* The procedure described above was used without modification with *N*-(*tert*-butoxycarbonyl)-3,4-di(*tert*-butoxycarbonyloxy)-6-trimethylstannyl-*L*-phenylalanine ethyl ester (**4**, 130 mg, MW : 701.42, 0.18 mmol) as the radiolabelling precursor to give comparable quantities of 6-[^{18}F]fluoro-*L*-DOPA ([^{18}F]-**8**), with similar specific activities for the same synthesis time.

Quality control : In both procedures, the product was found to be >99% chemically and radiochemically pure, as demonstrated by analytical HPLC analysis. Only one enantiomer could be detected by chiral HPLC analysis, which suggests that no racemization occurs during the synthesis processes.

Determination of the chemical and radiochemical purity : Column : analytical Whatman Partisil ODS3 (250 x 4.6 mm) ; Porosity : 10 μm ; Eluent : H_2O/AcOH : 999/1 ; Flow rate : 1.6 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : 5.5-6.5 min.

Determination of the enantiomeric purity : Column : analytical Astec Chirobiotic (250 x 4.6 mm) ; Porosity : 5 μm ; Eluent : EtOH/H_2O : 75/25 ; Flow rate : 1.0 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : 6.5-7.0 min. Racemic : 6.5-7.0 min & 13.5-14.0 min.

Tin contamination measurements : The final products after decay of ^{18}F-isotope were analysed by inductively coupled plasma spectrometry for organic and ion tin contamination and found to be 1.5-2.5 ppm total.

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References

1. Luxen A., Barrio J.R., Bida G.T. and Satyamurthy N. - *J. Label. Compounds Radiopharm.* 23: 1066 (1986).
2. Luxen A. and Barrio J.R. - *Tetrahedron Letters* 29: 1501 (1988).
3. Adam M.J. and Jivan S. - *Appl. Radiat. Isot.* 39: 1203 (1988).
4. Luxen A., Perlmutter M., Bida G.T., Van Moaffert G., Cook J.S., Satyamurthy N., Phelps M.E. and Barrio J.R. - *Appl. Radiat. Isot.* 41: 275 (1990).
5. Firnau G., Chirakal R. and Garnett E.S. - *J. Nucl. Med.* 25: 1228 (1984).
6. Chirakal R., Firnau G. and Garnett E.S. - *J. Nucl. Med.* 27: 417 (1986).
7. Luxen A., Guillaume M., Melega W.P., Pike V.W., Solin O. and Wagner R. - *Nucl. Med Biol.* 19: 149 (1992).
8. Adam M.J., Grierson J.R., Ruth T.J. and Divan S. - *Appl. Radiat. Isot.* 37: 877 (1986).
9. Adam M.J., Ruth T.J., Grierson J.R., Abeysekera B. and Pate B.D. - *J. Nucl. Med.* 27: 1462 (1986).
10. Coenen H.H., Franken K., Kling P. and Stöcklin G. - *Appl. Radiat. Isot.* 39: 1243 (1988).
11. Lemaire C., Guillaume M., Cantineau R. and Christiaens L. - *J. Nucl. Med.* 31: 1247 (1990).
12. Ding Y.S., Shiue C.-Y., Fowler J.S., Wolf A.P. and Plenevaux A. - *J. Fluorine Chem.* 48: 189 (1990).
13. Lemaire C., Guillaume M., Cantineau R., Plenevaux A. and Christiaens L. - *Appl. Radiat. Isot.* 42: 629 (1991).
14. Lemaire C., Plenevaux A., Cantineau R., Christiaens L., Guillaume M. and Comar D. - *Appl. Radiat. Isot.* 44: 737 (1993).
15. Namavari M., Bishop A., Satyamurthy N., Bida G. and Barrio J.R. - *Appl. Radiat. Isot.* 43(8): 989-996 (1992).

