Direct synthesis of 3-fluoro-α-fluoromethyl-*p*-tyrosine

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Abstract

3-Fluoro-α-fluoromethyl-*p*-tyrosine was synthesized directly by the reaction of acetyl hypofluorite with α-fluoromethyl-*p*-tyrosine. α-Fluoromethyl-*p*-tyrosine was prepared by a four-step reaction while acetyl hypofluorite was prepared from elemental fluorine. Products were characterized by mass spectrometry, multinuclear (^{1}H, ^{13}C and ^{19}F) NMR spectroscopy and HPLC. 3-[^{18}F]-Fluoro-α-fluoromethyl-*p*-tyrosine, a potential imaging agent for dopamine neurons, was subsequently prepared from cyclotron-produced [^{18}F]acetyl hypofluorite with an average radiochemical yield of 19.3 ± 1.7%.

Introduction

The visualizing of dopamine nerve terminals by positron emission tomography (PET) has been useful in the study of neurological diseases such as Parkinson's disease, a disease which involves the degeneration of nigrostriatal dopamine pathway (Firnau *et al.* [1]). The PET tracers currently used for imaging brain dopamine nerve terminals are 6-fluoro-L-Dopa (6-FD) [1], fluoro-*m*-tyrosine (FMT) [2–4] and fluoro-β-fluoromethylene-*m*-tyrosine (F-FMMT) [5]. These L-Dopa-based tracer compounds enter the dopamine metabolic pathway at the decarboxylation step involving the enzyme L-aromatic amino acid decarboxylase (L-AAAD). This decarboxylation step, however, is fast and not rate-determining in the biosynthesis of dopamine. The control of dopamine biosynthesis involves the step prior to decarboxylation – hydroxylation of *p*-tyrosine by the enzyme tyrosine hydroxylase to form L-Dopa [6]. Since dopamine synthesis is coupled to tyrosine hydroxylase activity, which in turn is known to be tightly coupled to neuronal activity [7], a tracer technique which can estimate tyrosine hydroxylase activity may be more useful in the assessment of dopamine neuronal activity in man compared to tracer techniques based on L-AAAD activity.

α-Fluoromethyl-*p*-tyrosine (FMPT), a recently developed compound [8], may be a potential tracer for tyrosine hydroxylase activity. *In vivo* and *in vitro* studies have demonstrated that FMPT is a selective L-AAAD inhibitor for sites where active catecholamine synthesis occurs [8]. Since FMPT has to be converted by tyrosine hydroxylase to α-monofluoromethyl-L-Dopa, a potent L-AAAD inhibitor [9], the extent of such L-AAAD inhibition is related to tyrosine hydroxylase activity and thus to catecholamine neuronal activity.

In this paper we report the *de novo* preparation of DL-FMPT via a four-step reaction scheme involving the fluoromethylation of a protected derivative of *p*-tyrosine adapting the method of Bey *et al.* [10]. 3-Fluoro-FMPT was subsequently prepared by the direct reaction of acetylhypofluorite and free FMPT. This method was then applied to the preparation of [^{18}F]-labelled 3-*F*-FMPT for evaluation as PET imaging agent for dopamine nerve terminals.

Results and discussion

The four-step synthesis of FMPT is shown in Scheme 1. The first step involved the protection of the amine group in the hydrochloride of the methyl ester of *p*-tyrosine (**1**) using benzaldehyde to give **2**. This was followed by reaction with lithium diisopropylamide (LDA) and fluorochloromethane affording **3**. Consecutive deprotection with 2 N HCl and HBr gave **4** and then the desired starting material FMPT (**5**).

Fluorination of **5** dissolved in a 1:1 glacial acetic acid/trifluoroacetic acid mixture with acetyl hypofluorite (AcOF) gave 3-*F*-FMPT (**6**). Multinuclear (^{1}H, ^{13}C and ^{19}F) NMR spectroscopy (Tables 1 and 2) confirmed the structure of the products **5** and **6** as FMPT and 3-*F*-FMPT, respectively. Fluorinations at the 3-position of *p*-tyrosine have been reported previously by other

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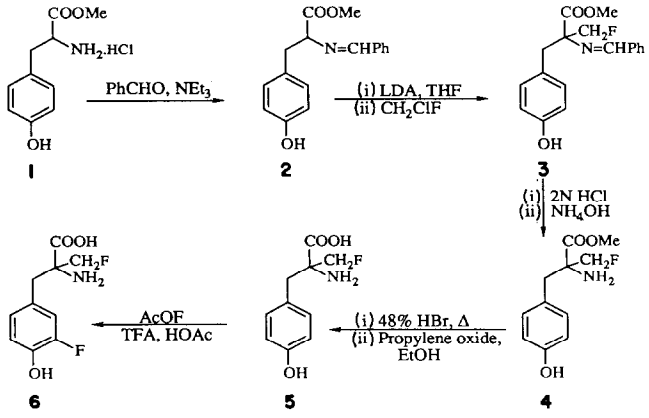


Scheme 1.

workers using either elemental fluorine or acetyl hypofluorite [11, 12].

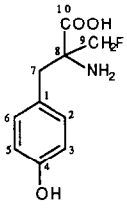
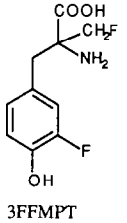
Subsequent direct radiofluorination of DL-FMPT (**5**) with [^{18}F]acetyl hypofluorite gave [^{18}F]3-*F*-DL-FMPT (**6**) with an average radiochemical yield of 19.3 ± 1.7% (n = 3) of HPLC purified product based on starting [^{18}F]acetyl hypofluorite and decay corrected to the end of bombardment.

[^{18}F]3-*F*-FMPT is the first PET imaging agent targeted towards tyrosine hydroxylase, an important enzyme in catecholamine biosynthesis. Our efforts are currently directed toward biochemical studies both *in vitro* and *in vivo* aimed at validating [^{18}F]3-*F*-FMPT as a tracer for tyrosine hydroxylase activity in the non-invasive estimation of dopamine neuronal activity. Preliminary results of these studies have been reported [13].

Experimental

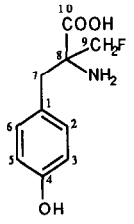
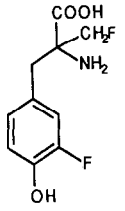
^{1}H NMR and ^{19}F NMR spectra were recorded at 400 MHz and 376.5 MHz, respectively, using a Bruker AM-400 WB spectrometer, while ^{13}C NMR spectra were recorded at 125.76 MHz on a Bruker AM-500 instrument. For proton spectra recorded in $CDCl_3$ or $CDCl_3$ + DMSO, TMS was used as an external standard, while for those recorded in D_2O + DCl, DSS was used as the external standard. For ^{13}C and ^{19}F NMR, respectively, TMS and trifluoroacetic acid were used as the external standards. In the case of ^{13}C NMR analysis, the multiplicities arising from C–H couplings observed in off-resonance spectra were in agreement with the proposed structures. High-performance liquid chromatography (HPLC) was carried out using a Gilson liquid chromatograph with the variable UV detector set at 254 nm. An Econosil reverse-phase C-18 150 × 4.6 mm, 3 μ column and an Econosil reverse-phase C-18 250 × 10 mm, 10 μ column were used, respectively, for analytical and semi-preparative purposes. The mobile phase consisted of a 0.02 M solution of NaOAc (pH adjusted to 3.5 using conc. HCl). The high-resolution mass spectrum was obtained on a Kratos MS-80RFA with DS55/DS90 instrument using the EI method. Melting points given are uncorrected.

TABLE 1. ^{1}H and ^{19}F NMR data for FMPT (**5**) and 3-fluoro-FMPT (**6**)

Compound	^{1}H NMR spectroscopy[a] δ (ppm)		^{19}F NMR spectroscopy[b] δ (ppm)
	Multiplicity	Assignment	
 FMPT	2.93, d, $^2J_{HH}$ = 15.3 Hz	H-7a	−149.8 (F-9)
	3.16, d, $^2J_{HH}$ = 15.3 Hz	H-7b	
	4.68, dd, $^2J_{HF}$ = 47.0 Hz, $^2J_{HH}$ = 10.6 Hz	H-9a	
	5.03, dd, $^2J_{HF}$ = 44.6 Hz, $^2J_{HH}$ = 10.6 Hz	H-9b	
	6.75, d, $^3J_{HH}$ = 8.2 Hz	H-3 and H-5	
	7.11, d, $^3J_{HH}$ = 8.2 Hz	H-2 and H-6	
 3FFMPT	2.99, d, $^2J_{HH}$ = 15.3 Hz	H-7a	−149.8 (F-9)
	3.24, d, $^2J_{HH}$ = 15.3 Hz	H-7b	−58.8 (F-3)
	4.54–5.64, (masked by solvent peak)	H-9a and H-9b	
	6.87, dd, $^3J_{HH}$ = 8.2 Hz; $^4J_{HH}$ = 2.0 Hz	H-6	
	6.94, dd, $^4J_{HF}$ = 8.2 Hz; $^3J_{HH}$ = 8.2 Hz	H-5	
	7.02, dd, $^3J_{HF}$ = 11.7 Hz; $^4J_{HH}$ = 2.0 Hz	H-2	

[a]In D_2O with DSS as external standard.
[b]In D_2O with trifluoroacetic acid as external standard.

TABLE 2. ^{13}C chemical shift values for FMPT (5) and 3-fluoro-FMPT (6)

Compound	Carbon number	Experimental shift[a,b] δ (ppm)	Calculated shift[c] δ (ppm)
	C-1	125.9	
	C-2	134.3	
	C-3	118.8	
	C-4	158.3	
	C-5	118.8	
	C-6	134.3	
	C-7	38.7	
	C-8	67.0	
	C-9	86.4 ($^1J_{CF}$ = 174.8 Hz)	
FMPT	C-10	172.8	
	C-1	127.0	127.3
	C-2	120.5	121.4
	C-3	154.0 ($^1J_{CF}$ = 244.1 Hz)	153.6
	C-4	146.0	145.4
	C-5	121.0	120.2
	C-6	129.2	129.8
	C-7	38.6	
	C-8	67.1	
	C-9	85.6 ($^1J_{CF}$ = 175.7 Hz)	
3FFMPT	C-10	173.0	

[a]In D_2O with TMS in $CDCl_3$ as external standard.
[b]The C—H couplings observed in off-resonance are in agreement with the assignment.
[c]Using the fluorine chemical shift substituent constants given in R.M. Silverstein, G.C. Bassler and T.C. Morrill, *Spectrometric Identification of Organic Compounds*, 4th edn., John Wiley & Sons, New York, 1980, p. 265.

Methyl N-*benzylidene*-p-*tyrosinate (2)*

To a mixture of *p*-tyrosine methyl ester hydrochloride (**1**) (46.3 g, 0.2 mol), benzaldehyde (21.2 g, 0.2 mol) and CH_2Cl_2 (200 ml), kept in a 500 ml round-bottom flask, cooled in an ice bath, was added (using a pressure equalizer) a solution of triethylamine (20.2 g, 0.2 mol) in CH_2Cl_2 (50 ml) under magnetic stirring. After the addition was completed, the cooling bath was removed and the stirring of the mixture continued overnight. Solvent was then removed and anhydrous ether (250 ml) added to the residue. The precipitated triethyl amine hydrochloride was filtered off and the filtrate washed with water (5×100 ml), then brine (100 ml) and dried ($MgSO_4$). Solvent was then removed to yield the crude product (55.0 g, 97%). ^1H NMR spectroscopy showed the product to be almost pure **2**. ^1H NMR ($CDCl_3$) δ: 3.07 (1H, dd, H-7a, $^2J_{H7a-H7b}$ = 13.6 Hz and $^3J_{H7a-H8}$ = 8.6 Hz); 3.28 (1H, dd, H-7b, $^2J_{H7a-H7b}$ = 13.7 Hz and $^3J_{H7b-H8}$ = 5.2 Hz); 3.73 (3H, s, $COOCH_3$); 4.12 (1H, dd, H-8, $^3J_{H8-H7a}$ = 8.9 Hz and $^3J_{H8-H7b}$ = 5.0 Hz); 6.69 (2H, d, Ar-H2 and Ar-H6, *J* = 8.8 Hz); 7.02 (2H, d, Ar-H3 and Ar-H5, *J* = 8.2 Hz); 7.35–7.45 (3H, m, Ar-H3′, Ar-H4′ and Ar-H5′); 7.69 (2H, d, Ar-H2′ and Ar-H6′, *J* = 7.4 Hz); and 7.92 (1H, s, N=CH) ppm.

Methyl-α-fluoromethyl-N-*benzylidine*-p-*tyrosinate (3)*

To a mixture of LDA (2.0 M solution in heptane/THF/PhEt, 10 ml, 20 mmol) and THF (15 ml) kept at −78 °C (Dry Ice/acetone bath) was dropwise added a solution of **2** (2.83 g, 10 mmol) in hexamethyl phosphoramide (HMPA) (10 ml) and THF (15 ml) precooled to −78 °C. The mixture immediately turned deep reddish brown in color. This mixture was stirred for 30 min at this temperature. Meanwhile 4 ml CH_2ClF (PCR Research Chemicals, Gainesville, FL) was collected at −78 °C and dissolved in 10 ml precooled THF. This solution was then added to the substrate mixture at −78 °C. The mixture was allowed to gradually warm up to room temperature and stirring was continued overnight. Most of the THF was removed under reduced pressure at room temperature. Water (100 ml) was added to the residue and the mixture extracted with ether (4×50 ml). The combined organic layers were washed with water (3×100 ml) and dried (Na_2SO_4). Removal of solvent under reduced pressure gave the crude residue **3** (2 g) which was used in the next step without further purification.

Methyl-α-fluoromethyl-p-*tyrosinate (4)*

The above crude **3** (2 g) was first dissolved in ether (10 ml) and then 2 N aqueous HCl (15 ml) was added. The mixture was vigorously stirred at room temperature for 3 h. The aqueous layer was separated, washed with ether (2×10 ml) and evaporated *in vacuo*. The residue was neutralised with conc. NH_4OH and then passed through a short column of silica gel (hexane/ethyl

acetate, gradient elution) to give a pale yellow solid which was further purified by crystallizing from chloroform ethyl acetate to give **4** as a pure white crystalline solid. Yield, 0.57 g (25% overall), m.p. 155–156 °C. ^{1}H NMR ($CDCl_3$ + DMSO) δ: 2.62 (1H, d, H-7a, $^2J_{H7a-H7b}$ = 13.1 Hz); 2.91 (1H, d, H-7b, $^2J_{H7b-H7a}$ = 13.5 Hz); 3.67 (3H, s, $COOCH_3$); 4.31 (1H, dd, H-10a, $^2J_{H10a-F}$ = 47.2 Hz and $^2J_{H10a-H10b}$ = 8.7 Hz); 4.65 (1H, dd, H-10b, $^2J_{H10b-F}$ = 46.5 Hz and $^2J_{H10b-H10a}$ = 8.5 Hz); 6.73 (2H, d, Ar-H2 and Ar-H6, J = 7.43 Hz); and 6.90 (2H, d, Ar-H3 and Ar-H5, J = 7.48 Hz) ppm. High-resolution mass spectrometry: parent m/e = 227.0965 (calculated for $C_{11}H_{14}O_3NF$, 227.0958).

*α-Fluoromethyl-*p*-tyrosine, FMPT (5)*

α-Fluoromethyl-*p*-tyrosine methyl ester (**4**) (200 mg) was dissolved in 48% aqueous HBr (10 ml). The mixture was heated at reflux under a nitrogen atmosphere for 24 h, after which time HBr was evaporated under reduced pressure and the residue dissolved in ethanol (10 ml). To this ethanolic solution was added, with stirring, propylene oxide (0.5 ml) and the mixture left standing in the refrigerator overnight. Volatiles were evaporated under reduced pressure and the residue was chromatographed over a short column of silica gel and eluted with ethyl acetate. α-Fluoromethyl-*p*-tyrosine (**5**) was obtained as a white powdery solid. Yield, 150 mg (80%) m.p. > 230 °C (dec.). The results of a multinuclear (^{1}H, ^{13}C and ^{19}F) NMR analysis of the product are shown in Tables 1 and 2. High-resolution mass spectrometry: $(M+H)^+$ m/e = 214.0883 (calculated for $C_{10}H_{12}O_3NF$ + H, 214.0879).

*3-Fluoro-α-fluoromethyl-*p*-tyrosine (6)*

Through a solution of **5** (42.6 mg, 200 μmol) in acetic acid (3 ml) and trifluoroacetic acid (3 ml) was passed acetyl hypofluorite (1% in argon, prepared by passing a solution of 1% F_2 in argon through a stainless-steel column packed with 1:1 KOAc/HOAc, a method originally developed by Jewett *et al.* [14]). After 400 μmol of acetylhypofluorite had passed, the reaction was stopped and the solvent removed by blowing helium. The residue was purified by semi-preparative HPLC (flow rate 5 ml min^{-1}). The starting material was eluted at 13.0 min and the product, 3-fluoro-FMPT, eluted at 17.1 min. The product fraction was evaporated to dryness *in vacuo* at ambient temperature. Water (3 ml) was added to the residue. The insoluble, powdery, white solid collected by filtration was found to be pure **2**. Yield obtained was 16.2 mg (35%). The results of multinuclear (^{1}H, ^{13}C and ^{19}F) NMR analysis are shown in Tables and 2. High-resolution mass spectrometry: parent m/e = 231.0705 (calculated for $C_{10}H_{11}O_3NF_2$, 231.0707).

*3-[^{18}F]Fluoro-α-fluoromethyl-*p*-tyrosine (6)*

[^{18}F]-Fluorine gas, produced in a CTI RDS 11 cyclotron using a previously reported procedure [15], was converted on-line to [^{18}F]acetyl hypofluorite by the method of Jewett *et al.* [14]. To 10 mg DL-FMPT (**5**) in 5 ml 1:1 trifluoroacetic acid/acetic acid was bubbled 80 μmol [^{18}F]acetyl hypofluorite. This was followed by solvent evaporation with a stream of helium gas. The residue was then dissolved in the HPLC mobile phase (0.02 M NaOAc, pH 3.5) and injected directly into a calibrated HPLC system with a semi-preparative reversed-phase column and dual detectors (UV = 254 nm with a pair of radiation detectors in coincidence). The product (retention time, 13.6 min) was easily separated from the starting material (retention time, 10.1 min). The preparation time needed from the end of trapping [^{18}F]acetyl hypofluorite to the finished ready-to-inject HPLC-purified product was about 45 min.

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