SYNTHESIS OF [^{18}F]-(S)-FLUOXETINE : A SELECTIVE SEROTONINE UPTAKE INHIBITOR

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SUMMARY

The (S)-N-methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine, an antidepressant with potential applications in the treatment of other illnesses was labelled with fluorine-18 for Positron Emission Tomography studies. The synthesis was accomplished from the [^{18}F]-4-chlorobenzotrifluoride where [^{18}F]-label was introduced via a nucleophilic aliphatic substitution reaction. [^{18}F]-(S)-Fluoxetine was obtained with a radiochemical yield of 9-10% (decay corrected) and a specific radioactivity of 100-150 mCi/μmol (3.70-5.55 GBq/μmol) in a total synthesis time of 150 min. A facile isotopic exchange reaction was desmonstrated; it is expected to reduce the specific activity of the final [^{18}F]-product. The experimental parameters play an important role, which is discussed.

KEY WORDS : Fluoxetine, fluorine-18, PET, antidepressant.

INTRODUCTION

Fluoxetine, N-methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine is a specific serotonin re-uptake inhibitor which is clinically useful as an antidepressant and in the treatment of a variety of human diseases, including anxiety, alcoholism, chronic pain, and eating disorders[1,2]. The mode of action of fluoxetine has been proposed as a selective inhibition of serotonin uptake in presynaptic neurons. Fluoxetine has been labelled with carbon-11 for positron emission tomography (PET) studies, involving the methylation of the nor-methyl amine with [^{11}C] methyl iodide or [^{11}C] formaldehyde[3,4]. However, Parli and Hicks have shown that one of the main routes of metabolism of fluoxetine is a N-demethylation[5]. Synthesis of Fluoxetine with carbon-11 in any other position than the methyl is presently difficult. On the other hand, the half-life of carbon-11 is shorter than that of fluorine-18 (20 min vs. 110 min). For these reasons the radiosynthesis of Fluoxetine labelled with fluorine-18 was undertaken for PET studies of reuptake sites. In the present work, the synthesis

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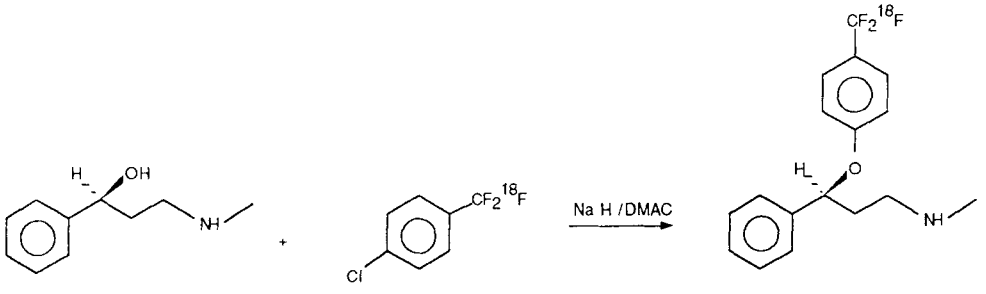
of [^{18}F]-(S)-Fluoxetine **1** (Scheme 1) is divided into two major parts, the preparation of [^{18}F]-4-chloro-α,α,α-trifluorotoluene **2** followed by condensation with the (S)-(-)-3-(methylamino)-1-phenyl-1-propanol **3**.



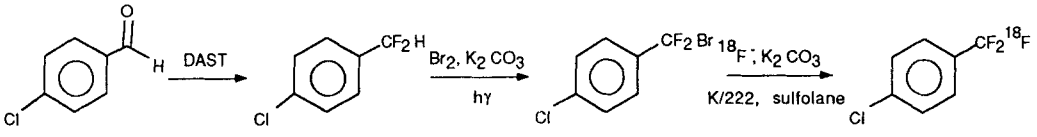
3 **2** **1**

Scheme 1

RESULTS AND DISCUSSION

Synthesis of [^{18}F]-4-chloro-α,α,α-trifluorotoluene 2

4-chlorobenzaldehyde **6** was reacted with diethylaminosulfur trifluoride (DAST) at 60°C to provide 4-chloro-α,α-difluorotoluene **5** in 66 % yield[6] (Scheme 2). α-bromo-4-chloro-α,α-difluorotoluene **4** is then prepared by bromination of the difluoromethyl group according to the reported procedure[7] (72% yield). The nucleophilic aliphatic substitution of bromide of compound **4** with nca [^{18}F]-fluoride gave the expected [^{18}F]-4-chloro-α,α,α-trifluorotoluene **2**.

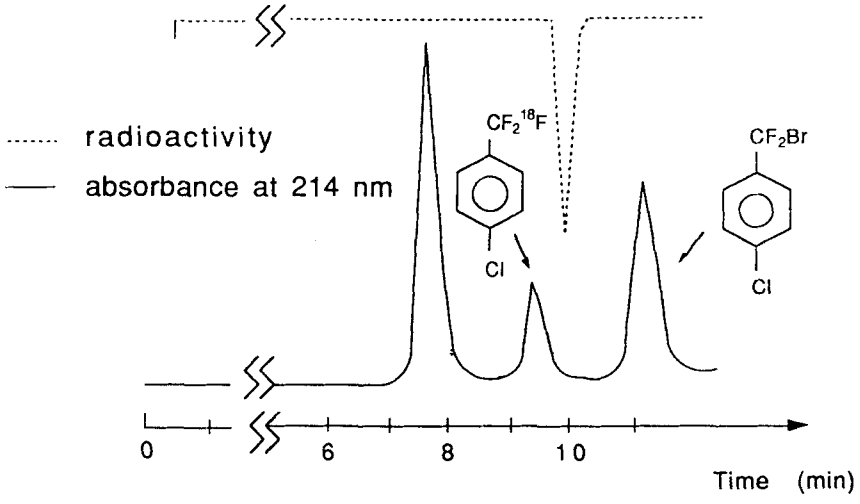


6 **5** **4** **2**

Scheme 2

The first exchange reaction experiments were carried out with [^{18}F]-fluoride produced by irradiation of natural water with ^{3}He (starting activity of 30 mCi). After evaporation of water and drying of the fluoride by acetonitrile evaporation, the fluorination reaction was performed at 160°C for 18 min in a sealed vessel, using Kryptofix 2.2.2. (27 μmol), K_2CO_3 (18 μmol) and α-bromo-4-chloro-α,α-difluorotoluene (4 μmol) in tetramethylene sulfone (0.4 ml). The desired labelled compound **2** was isolated by distillation of the reaction mixture

in reacti-vial containing 2 ml of acetonitrile. Purification by HPLC on a Whatman partisil 9 ODS-2 column with CH_3CN / H_2O (7/3) as the mobile phase and detection at 214 nm, gave [^{18}F]-4-chloro-α,α,α-trifluorotoluene **2** (Figure 1) with a radiochemical yield of 25-30% (corrected for decay). The overall synthesis time is nearly 60 min. Unfortunately, specific activity of labelled compound **2** is low : 4-6 mCi/μmol.



Column : whatman, partisil ODS-2, M9, 10/50
Mobile phase : CH_3CN/H_2O (7/3)
Flow rate : 6 ml/min

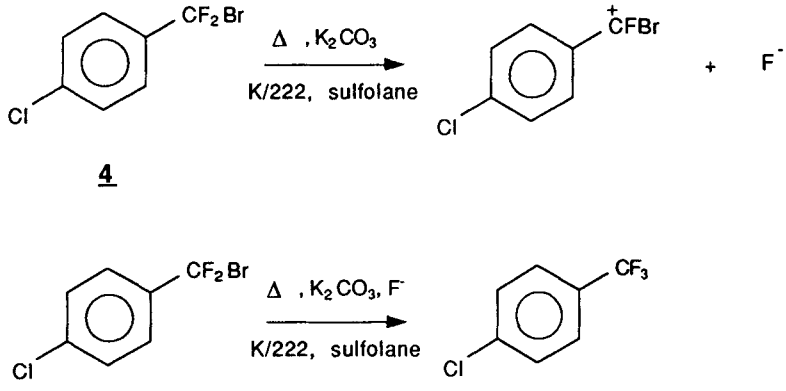
Figure 1 : Separation of [^{18}F]-4-chloro-α,α,α-trifluorotoluene 2 by HPLC

During the preliminary experiments, we made the following observations :

- The reaction performed without fluoride ion (KF), under the same experimental conditions as for the synthesis of the [^{18}F]-compound **2**, gave unlabelled 4-chloro-α,α,α-trifluorotoluene.

- It was found that the parameters influencing the isotopic dilution and the specific radioactivity are the concentration of **4** and the reaction temperature.

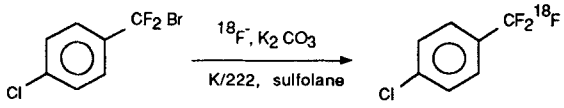
These results can be interpreted by the superconjugation of the α-bromo-4-chloro-α,α-difluorotoluene **4** at high temperature (Scheme 3). The stable fluoride reacts with the excess of **4** to give the unlabelled 4-chloro-α,α,α-trifluorotoluene and an isotopic dilution occurs.



Scheme 3 : Transformation of α-bromo-4-chloro-α,α-difluorotoluene 4 at high temperature

We checked these experimental parameters (Table 1) in the synthesis of the [^{18}F]-compound **2**. There is an important increase in the specific radioactivity when decreasing the temperature or the concentration of **4**. When the reaction is performed at 90°C with a precursor concentration of 2 μmol/ml (Table 1, entry 6), the specific radioactivity of [^{18}F]-compound **2** reaches a maximum of 50 mCi/μmol(1.85 GBq/μmol) starting with 24 mCi (0.89 GBq) of fluoride from natural water target.

In order to obtain the highest specific activity in the synthesis of [^{18}F]-(S)-Fluoxetine **1**, we selected these conditions (Table 1, entry 6) in the preparation of [^{18}F]-4-chloro-α,α,α-trifluorotoluene **2** .



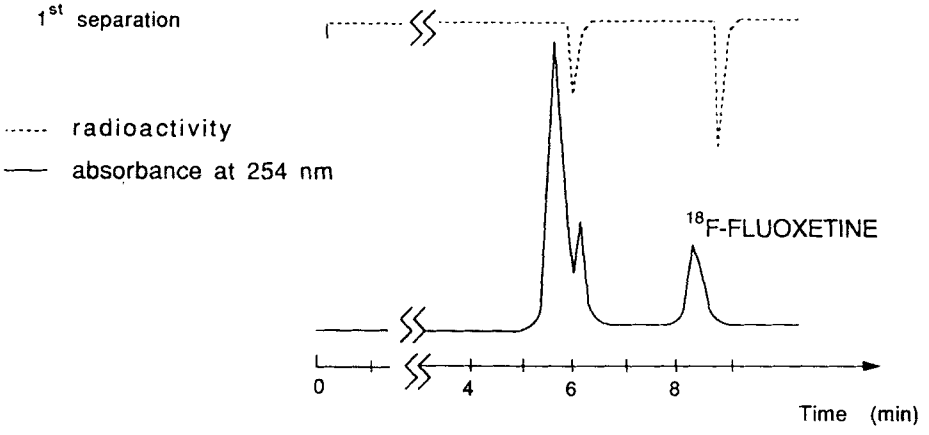
4 **2**

Entry	Starting activity (GBq)	**4** (mg)	Reaction temperature (°C)	Reaction time (min)	Radiochemical yield (% - EOB)	Specific activity of [^{18}F]- **2** (GBq/μmol - EOS)
1	1.30	3	130	10	16	0.41
2	1.18	3	160	10	28	0.22
3	1.41	3	195	10	33	0.19
4	1.15	0.2	160	18	21	1.15
5	1.07	0.2	120	3	22	1.41
6	0.89	0.2	90	4	21	1.85

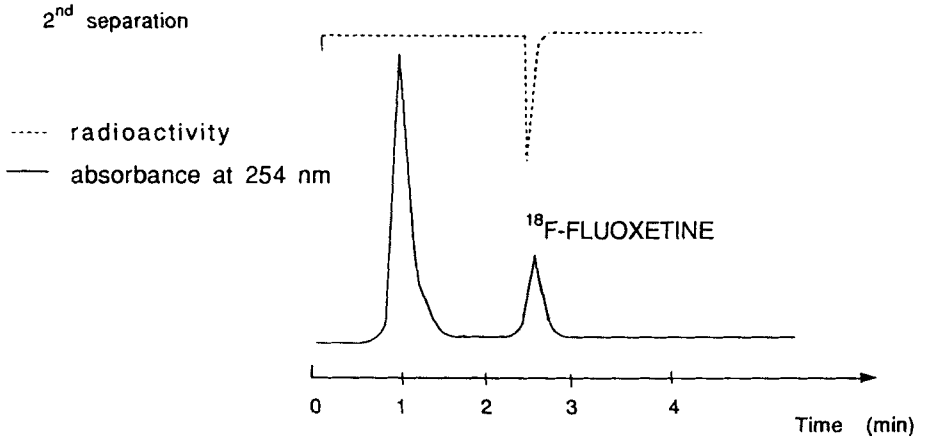
Table 1

Synthesis of [18F]-(S)-Fluoxetine 1

In order to determine the optimal synthesis conditions of [18F]-(S)-fluoxetine 1, preliminary experiments were conducted with [18F]-fluoride produced by irradiation of natural water with 3He (starting activity between 30-50 mCi) and then with [18F]-fluoride prepared by proton bombardment of [18O]-water (starting activity between 300-350 mCi).



Column : Whatman, Partisil 10, M9, 10/50
Mobile phase : $CH_2Cl_2/CH_3OH/NH_4OH$ (150/10/1)
Flow rate : 3 ml/min



Column : Whatman, Partisil 10, 4/25 (analytical column)
Mobile phase : $CH_2Cl_2/CH_3OH/NH_4OH$ (150/10/1)
Flow rate : 3 ml/min

Figure 2 : Purifications of [18F]-(S)-Fluoxetine : 1 by HPLC

[^{18}F]-4-chloro-α,α,α-trifluorotoluene **2** was synthesized according to the experimental procedure finally selected (Table 1, entry 6). The sodium alkoxide of **3**[8] was generated in N,N-dimethylacetamide (DMAC) solution using sodium hydride by reaction at 0°C initially and then, after warming, at 90°C for 30 min (Scheme 1). The labelled compound **2** was then distilled from the reaction mixture in the reacti-vial containing sodium alkoxide **3** at 0°C. The condensation of **2** with the amino alcohol **3** occured when this solution was heated at 120°C for 45 min. The mixture is then transferred onto a C-18 Sep-Pak, then onto a silica gel cartridge. Two HPLC purifications on silica gel columns with CH_2Cl_2/MeOH/NH_4OH (150/10/1 v/v) as elution solvent (Figure 2), gives [^{18}F]-(S)-Fluoxetine **1** with a radiochemical yield of 9-10 % (corrected for decay) and a specific activity of 100-150 mCi/μmol (3.70-5.55 MBq/μmol, EOS). The identity of [^{18}F]-(S)-Fluoxetine **1** was confirmed by comparison of its HPLC retention time with unlabelled (S)-Fluoxetine[8-10]. The overall synthesis time was nearly 150 min.

In summary, a useful synthesis of [^{18}F]-(S)-Fluoxetine **1** has been developed. The labelling method of the trifluoromethyl group from bromodifluoromethyl group by a nucleophilic aliphatic substitution of bromine with [^{18}F]-fluoride requires a good relationship between the reaction temperature of the first step and the concentration of the precursor to obtain a high specific activity.

EXPERIMENTAL

^{1}H-NMR spectra were recorded on a Brucker AM 250 (250 MHz) and a Bruker 200 (200 MHz) with tetramethylsilane as internal standard. Mass spectra were obtained using a Nermag R 10-10 instrument at 70eV. Optical rotations were measured in a 1 ml cell at 20°C (± 2°C) using a Perkin-Elmer Polarimeter 241. Analytical thin layer chromatography was performed on Merck Kieselgel 60 F_{254}. Silica gel 60 (230-400 mesh) was used for column chromatography. Solvents were distilled before use and, if necessary, dried following literature methods.

4-chloro-α,α-difluorotoluene **5**[6]

4-chlorobenzaldehyde **6** (5 mmol) was treated with diethylaminosulfur trifluoride (5 mmol) at 60 °C for 15 min. The reaction mixture was cooled and dissolved in carbon tetrachloride (8ml). The solution was then poured into ice water (10 ml), and the organic layer dried over magnesium sulfate. The solvent was evaporated to give an oil which was distilled (58-59 °C/12 torr) to give 33 mmol (66 % yield) of **5**.

^{1}H-NMR ($CDCl_3$) δ 7.80 (4H, s), 7.00 (1H, t, 59 Hz)

Mass spectrum (EI), m/z (relative intensity): 162 (50 M^+); 143 (14); 127 (100); 112 (14).

α-bromo-4-chloro-α,α-difluorotoluene **4**

Bromination of 4-chloro-α,α-difluorotoluene **5** was performed using bromine in CCl_4 under photolytic conditions (95 % yield), with solid potassium carbonate

added to consume HBr formed in the reaction as described by others[7]. The product **4** was purified by distillation at reduced pressure (90 °C / 18 torr).
Mass spectrum (EI), m/z (relative intensity): 242 (1 M^+); 223 (2); 161 (100); 125 (8).

(S)-(-)-3-(methylamino)-1-phenyl-1-propanol 3.

The synthesis of **3** was performed using the procedure described by Robertson et al.[8] (yield : 85%).
^{1}H-NMR ($CDCl_3$) δ 1.80 (2H, m), 240 (3H, s), 2.90 (2H, m), 4.90 (1H, dd), 7.20-7.50 (5H, m).
$[\alpha]_D$ = - 37 (c 1 %, $CHCl_3$).
Mass spectrum (DCI / NH_3), m/z (relative intensity): 166 (100 MH^+).

Sodium alkoxide of 3

To a solution of (S)-(-)-3-(methylamino)-1-phenyl-1-propanol **3** (11 mg, 0.07 mmol) in dimethylacetamide (3 ml) was added sodium hydride (21 mg of a 60 % dispersion in oil, 0.1 mmol). The reaction was heated at 90 °C for 30 min, and an orange solution resulted.

[18F]-(S)-Fluoxetine 1

[18F]-fluoride was produced by proton bombardment of [18O]-water. After evaporation of water, the fluorination reaction was performed at 120 °C for 4 min in a sealed glass vessel using Kryptofix 2.2.2. (27 µmol), K_2CO_3 (18 µmol) and α-bromo-4-chloro-α,α-difluorotoluene (4 µmol) in tetramethylene sulfone (0.4 ml). [18F]-4-chloro-α,α,α-trifluorotoluene **2** is distilled from the reaction vessel into a second reaction vessel containing the sodium alkoxide of **3** in N,N-dimethylacetamide (DMAC, 3 ml) at 0°C. The condensation of the labelled compound **2** with the amino alcohol **3** is then performed at 120 °C for 45 min. After cooling, water (5 ml) was added to stop the reaction and decompose the excess NaH. The mixture was passed through a C-18 Sep Pak. The Sep Pak was washed with water (2 x 2 ml) and the washings were discarded. The product was eluted with CH_2Cl_2 (4 ml). The organic layer was then transferred onto a silica cartridge. The labelled compound **1** was eluted with CH_2Cl_2 : MeOH (70 : 30). After evaporation of solvent, the residue was diluted with 1 ml of the HPLC mobile phase. Two HPLC purifications were performed on slica gel columns (Figure 2) with CH_2Cl_2/MeOH/NH_4OH (150/10/1 v/v) as elution solvent. The solution collected from HPLC was evaporated and the residue was dissolved in 3 ml of saline. The overall synthesis time was 150 min and the radiochemical yield ranged from 9-10 % (corrected for decay). The specific activity was measured : 100-150 mCi/µmol at the end of the synthesis.

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