SYNTHESIS OF A DOPAMINE UPTAKE INHIBITOR FOR PET STUDIES: 1-[1-(2-BENZO(b)THIOPHENYL)CYCLOHEXYL] -4-(2-[^{18}F]FLUOROETHYL) PIPERAZINE.

LOUSTAU-THEN I.[1,2], PONCHANT M.*[1], KAMENKA J.M.[2], CROUZEL C.[1].

[1]Service Hospitalier Frédéric-Joliot, D.R.M., C.E.A., 4 place du Général Leclerc, 91406 Orsay (FRANCE).

[2] CNRS UPR 9008-INSERM U 249, 8 rue de l'Ecole Normale, 34053 Montpellier Cedex 1 (FRANCE).

ABSTRACT

The BTCP derivative 1-[1-(2-benzo(b)thiophenyl) cyclohexyl]-4-(2-hydroxyethyl) piperazine **2** showed, *in vitro*, high affinity and selectivity for the Dopamine transporter. In order to evaluate the potential of such a compound as an imaging tool for studying the dopaminergic system by Positron Emission Tomography (PET) the cold fluoroethyl BTCP piperazine **6** was synthesized. After checking the biological activity of the cold compound **6** , the [^{18}F] analogue **7** was synthesized. The radiosynthesis was carried out by the nucleophilic substitution of 1-[1-(2-benzo(b)thiophenyl) cyclohexyl]-4-(2-chloroethyl) piperazine **5** with cyclotron-produced n.c.a. $^{18}F^-$, obtained by the (p,n) reaction on ^{18}O enriched water.

KEYWORDS:

^{18}F, PET, Dopamine transporter, reuptake inhibitor,

BTCP or 1-[1-(2-benzo(b)thiophenyl)cyclohexyl] piperidine

Fluoroethyl BTCP piperazine or 1-[1-(2-benzo(b)thiophenyl)cyclohexyl] -4-(2-fluoroethyl) piperazine

* Author to whom correspondence should be addressed.

CCC 0362–4803/96/030299–10

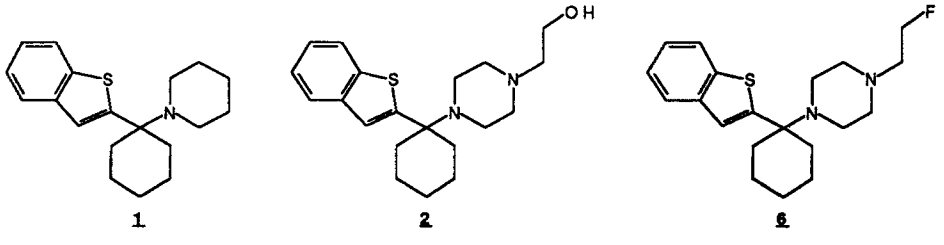
Received 10 October 1995
Revised 23 November 1995

INTRODUCTION

Degeneration or functional alterations in dopaminergic neurons are known to be involved in neurological diseases such as Parkinson's or in psychiatric disorders such as schizophrenia (1,2). The use of Positron Emission Tomography (PET) and specific radiotracers provides a unique way of examining the rate of progression of these alteration of the dopamine system *in vivo* (3,4). As part of our programm to develop new radiotracers selective for the dopaminergic neurons we have been interested in various compounds belonging to the family of BTCP (scheme 1) which display affinity for the dopamine transporter *in vitro* and in vivo (11).

Our first approach with 3-[^{18}F]fluoromethyl BTCP (5) demonstrated a limited interest in this compound due to a high lipophylicity. More recently several other BTCP-piperazinyl derivatives have shown affinity for the dopamine transporter *in vitro* (6). Among them, the 4-hydroxyethylpiperazine analogue **2** (Scheme 1) displays high affinity and selectivity for the dopamine transporter. Such results, a lower lipophylicity of this compound as compared to the 3-[^{18}F]fluoromethyl BTCP analogue (5) together with the possibility of replacing the hydroxyl group by a fluor atom rendered this compound an attractive candidate for a radiotracer. This report describes the synthesis of the non-radioactive fluoro compound **6** and the radiochemical synthesis of the ^{18}F analogue **7**.

Scheme 1 : Analogues of BTCP.



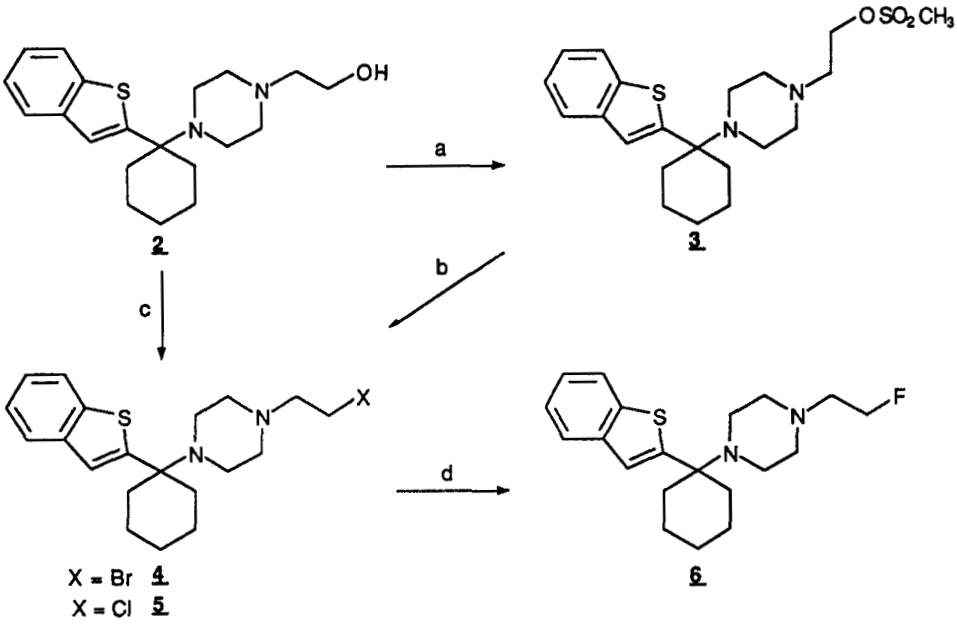
1 **2** **6**

CHEMISTRY

The 4-hydroxyethylpiperazine-BTCP **2** was synthesized as described by Coderc *et al* (6). The brominated derivative **4** was the first precursor chosen for the synthesis of 4-[^{18}F]fluoroethylpiperazine-BTCP **7**. However, this compound could not be obtained

from the mesylate **3** by reaction with lithium bromide in acetone possibly because neither lithium bromide nor compound **3** were enough soluble in acetone. On the contrary, reaction of **3** with lithium bromide in methylene chloride gave a mixture of the brominated and chlorinated compounds **4** and **5** (yield 56% and 44% respectively). Since the only chlorine source was methylene chloride, we suspected that there had been an exchange between bromine and chlorine in the solvent. A purification of the crude material by chromatography on silica gel showed that the brominated derivative **4** was easily degraded into alcohol **2**.

Scheme 2 : Synthesis of 4-halogeno piperazine BTCP.



a) CH_3SO_2Cl, Et_3N, CH_2Cl_2 b) LiBr, CH_2Cl_2

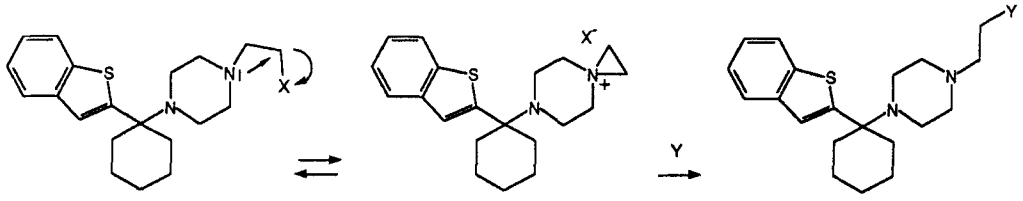
c) Ph_3P - CCl_4, CH_3CN d) KF, K_{222}, CH_3CN

Therefore, we synthesized 4-fluoroethylpiperazine-BTCP **6** from the mixture of both halogenated derivatives **4** and **5** . The reaction was carried out with potassium fluoride and cryptand (Kryptofix K_{222}) in acetonitrile (5,9). As 4-fluoroethylpiperazine-BTCP **6** was obtained easily we choose to synthesize the radiotracer **7** from the chlorinated derivative **5**. Compound **5** could be obtained in

good yield from alcohol **2** by reaction with triphenylphosphine and carbon tetrachloride in acetonitrile (Scheme 2) in good yield. However, **5** too was easily degraded into alcohol **2** during the purification by chromatography on alumina.

The behaviour of these β-haloamines may be due to the participation effect of nitrogen which cause chlorine to leave by aliphatic chain cyclisation giving an aziridinium ion (7, 8) (Scheme 3) which may react with a nucleophilic agent Y, such as F^- or $-OH^-$.

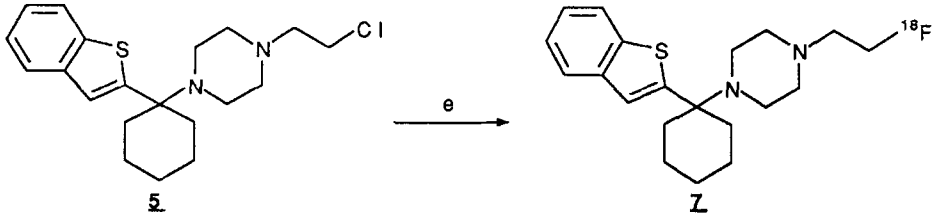
Scheme 3 : Behaviour of the β-haloamines.



X = Br, Cl

1-[1-(2-benzo(b)thiophenyl)-cyclohexyl]-4-(2-[^{18}F]fluoroethyl) piperazine **7** was synthesized by nucleophilic substitution of **5** with $^{18}F^-$ fluoride ion (Scheme 4). The radiosynthesis and the purification of **7** were tried out using $^{18}F^-$ produced by the (^{3}He,p) reaction, with 30 MeV protons on water . For the ultimate assay at high specific activity, $^{18}F^-$ was produced by the (p,n) reaction, with 16 MeV protons on 80% enriched ^{18}O water.

Scheme 4: Radiosynthesis of N-[^{18}F]fluoroethylpiperazine-BTCP.



5 **7**

e) $K^{18}F$, K_{222}, DMSO

[^{18}F] KF / K_{222} solution was prepared classicaly (5,9). Then, this solution was evaporated to dryness under a stream of nitrogen. After dissolution of [^{18}F] KF/K_{222} in dimethylsulfoxide, the mixture was added into a vial containing the precursor **5** and was heated for 15 minutes at 140°C. The crude material was then transferred onto a C_{18} Sep Pak cartridge in order to separate ionic compounds (among which unreacted [^{18}F] KF) from organic compounds. An optimization of this reaction was necessary due to the behaviour of β-chloroamine **5** . Table 1 presents the different conditions that were tested in order to improve the fluorine-18 incorporation rate.

Table 1: Parameter studies for radiolabelling of 5.

Compound 5 (μmol)	K_{222} (μmol)	K_2CO_3 (μmol)	Water evaporation temperature (°C)	^{18}F incorporation %[a]
2,7	19	8	140	14
2,7	50	21	140	15
5,5	53	31	140	34
6	58	33	140	37
6,6	56	33	120	43
6	58	33	120	60[b]

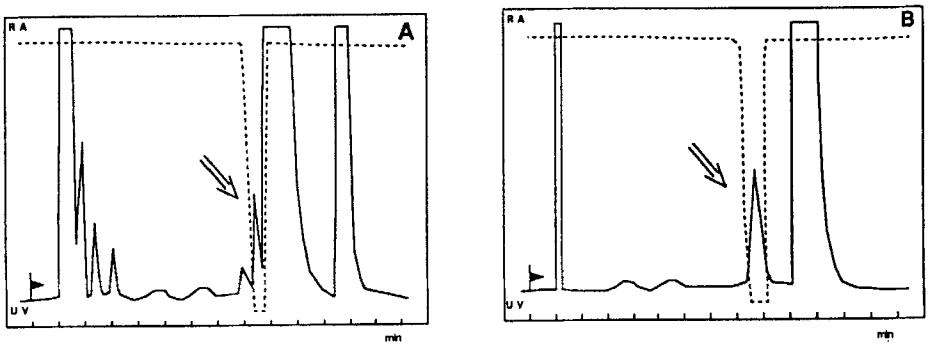
a: decay-corrected and calculated in comparison with the activity obtained at the end of irradiation.

b: [$^{18}F^-$] produced from [^{18}O] H_2O.

A large amount of non radioactive impurities was formed during the reaction. An elution on a silica Sep Pak cartridge, a HPLC purification on a normal phase then followed by a second one on a reverse phase (Figure 1) were necessary to isolate 4-[^{18}F]fluoroethylpiperazine-BTCP **7**. However, the radiotracer was degraded during the evaporation of the last purification eluant (water-isopropanol-trifluoroacetic acid). When heated in an acidic medium, compound **7** gave 1-(2-benzo(b)thiophenyl) cyclohexene. To avoid such problem, the collected fraction was basified with a saturated solution of Na_2CO_3. The mixture was then transferred onto a C_{18} Sep Pak cartridge from which compound **7** was eluted with a methylene chloride-methanol

mixture. Using this treatment, the ultimate assay yielded 22mCi (814MBq) of compound 7 after 125min with a specific radioactivity of 2.5 Ci/μmol (92.5GBq/μmol). After evaporation of the organic solvents and formulation in aqueous salts the stability of the radioactive compound 7 was checked by analytical HPLC. No degradation was observed after 150min (time necessary for a biodistribution study).

Figure 1: Chromatograms of HPLC purifications of 4-[^{18}F]fluoroethyl piperazine-BTCP 8.



A :normal phase HPLC purification using dichloromethane/ethyl acetate/triethylamine (97/3/0.05 : v/v/v). Flow rate = 5ml/min, retention time = 9.4min.

B : reversed phase HPLC purification using water/isopropanol/trifluoroacetic acid (86/14/0.05 : v/v/v). Flow rate = 5ml/min, retention time = 16.6min.

CONCLUSION

The behaviour of β-haloamines led us to carry out the synthesis of the radiotracer 7 by nucleophilic substitution of a chlorinated derivative with $^{18}F^-$. Ammonium ion formation makes fluorine ion attack easier but favours β-chloroamine degradation. On the other hand, it seems that β-fluoroamines give the ammonium intermediate far less easily. The adjustment of radiosynthesis parameters led to a good fluorine-18 incorporation rate into the precursor. The drastic conditions of the reaction in DMSO as well as radiotracer instability in an acid medium made the purification of 7 rather complex. However, the radiotracer could be obtained with a radiochemical

yield of 49.6% with good specific radioactivity. Biodistribution studies in the rodents are now in progress to confirm the potential use of this compound as a PET radiotracer for the dopamine transporter.

EXPERIMENTAL

Commercial liquids were distilled under an inert atmosphere and solids were dried over P_2O_5 under vacuum (0.5 mm Hg). Diethyl ether was distilled over sodium with benzophenone and stored under nitrogen, over sodium wires. Methylene chloride and acetonitrile were distilled over P_2O_5 and stored under argon. Dimethylsulfoxide was distilled over CaH_2 and stored under argon. Kryptofix K222 was purchased from Merck.

^{1}H NMR spectra were recorded on a Brucker Ac 300 and ^{13}C NMR spectra on a Brucker Ac 200 or Brucker Ac 300. Mass spectra were obtained using a Nermag R10-10 instrument at 70 eV and GC-MS spectra with a Hewlett-Packard CPV 5890 instrument at 90 eV (column: OV1, l= 30 m, d= 0,53 mm).

HPLC purifications were performed using a Waters system (Waters 510 pump) on a Hibar Si 60 column (25 cm, 10 mm O.D., 7 mm) with UV detection at 254 nm (Waters 441) and a Whatman Partisil 10 C_8 column (25 cm; 9,4 mm O.D.) with UV detection at 260 nm (Waters 481).

1-[1-(2-benzo(b)thiophenyl)cyclohexyl]-4-[2-(methylsulfonyloxy)ethyl] piperazine: 3

Mesyl chloride (78 µl; 1 mmol) was added to a solution of **2** (274 mg; 0.79 mmol) and triethylamine (140 µl; 1 mmol) in 10 ml of methylene chloride, cooled at 0°C. The mixture was stirred for 30 minutes at 0°C and 2 hours at ambient tamperature. The crude material was washed with water; the organic layer was dried over sodium sulfate and concentrated under vacuum to give 330 mg (98% yield) of **3** which was used without further purification.

M.S. (DCI/NH_3): (M+1) = 423.

1-[1-(2-benzo(b)thiophenyl)cyclohexyl]-4-(2-bromoethyl)piperazine 4 and 1-[1-(2-benzo(b)thiophenyl)cyclohexyl]-4-(2-chloroethyl) piperazine 5.

To a solution of **3** (300 mg; 0.71 mmol) in 20 ml of methylene chloride were added 185 mg (2.13 mmol) of lithium bromide. The mixture was refluxed for 20 hours. The crude material was washed with water and the organic layer was dried over sodium sulfate, then concentrated under vacuum. The mass spectroscopy analysis (DCI/NH_3) of the solid obtained showed that it was a mixture of **4** (56%) and **5** (44%). A purification by chromatography on silica gel using hexane/dichloromethane (70/30; v/v) gave 28 mg (11%) of **2** and 163 mg of a mixture containing 49% of **4** and 51% of **5**.

M.S. (DCI/NH_3): compound **5** : (M+1) = 407.

compound **6** : (M+1) = 363.

1-[1-(2-benzo(b)thiophenyl)cyclohexyl]-4-(2-fluoroethyl)piperazine: 6

To a solution of above mixture of compounds **4** and **5** (159 mg; 0.41 mmol) in 25 ml of acetonitrile were added 250 mg (0.66 mmol) of Kryptofix K_{222} and 39 mg (0.67 mmol) of potassium fluoride. The solution was refluxed for 20 hours. The mixture was acidified with 1.2N HCl and washed with diethyl ether. The aqueous layer was basified with NH_4OH 20% and extracted with methylene chloride. Then, the organic layer was dried over sodium sulfate and concentrated under vacuum. After purification by chromatography on silica gel using dichloromethane/ethanol (98/2; v/v), 97 mg (68% yield) of **6** were obtained. The hydrochloride salt was prepared with a solution of hydrogen chloride in diethyl ether.

M.S. (DCI/NH_3): (M+1) = 347.

^{13}C NMR (75,47 MHz; $CDCl_3$): 22.49 (cyclohexane C_3); 26.08 (cyclohexane C_4); 35.43 (cyclohexane C_2); 45.23 (piperazine C_2); 54.66 (piperazine C_3); 58.25 (d; $\underline{CH_2}CH_2F$; J_{C-F} = 20 Hz); 60.52 (cyclohexane C_1); 82.05 (d; $CH_2\underline{CH_2}F$; J_{C-F} = 167 Hz); 121.31 - 122.00 - 123.18 - 123.79 - 124.01 (benzothiophene CH); 139.09 - 139.81 - 147.51 (benzothiophene quaternary C).

^{1}H NMR (300 MHz; $CDCl_3$): 1.8 - 2.1 (m, 4H, CH_2 piperazine); 2.2 - 2.5 (m, 4H, CH_2 piperazine); 2.79 (s large, 10H, CH_2 cyclohexane); 2,.86 (d t, 2H, $\underline{CH_2}CH_2F$; $^{1,3}J_{H-F}$ = 28 Hz, $^{1,3}J_{H-H}$ = 5 Hz); 4.73 (d t, 2H, $CH_2\underline{CH_2}F$; $^{1,2}J_{H-F}$ = 47 Hz, $^{1,3}J_{H-H}$ = 5 Hz); 7,4 - 7.7 (m, 3H, CH benzothiophene); 7.8 - 8.1 (m, 2H, CH benzothiophene).

1-[1-(2-benzo(b)thiophenyl)cyclohexyl]-4-(2-chloroethyl) piperazine 5.

To a solution of **2** (296 mg; 0.86 mmol) in 15 ml of acetonitrile were added 320 mg (1.22 mmol) of triphenylphosphine and 335 µl (3.47 mmol) of carbon tetrachloride. The mixture was stirred for 24 hours at ambient temperature and concentrated under vacuum. According to mass spectroscopy and HPLC analysis, the ratio between compound **5** and the alcohol **2** in the crude material was 97/3 and the only other product detected was triphenylphosphine oxyde. A purification by chromatography on alumina using hexane/dichloromethane (70/30; v/v) gave 22 mg of **2** and 265 mg of **5**.

M.S. (DCI/NH_3): (M+1) = 363.

^{13}C NMR (50,3 MHz; $CDCl_3$): 22.21 (cyclohexane C_3); 25.88 (cyclohexane C_4); 35.22 (cyclohexane C_2); 40.81 ($\underline{CH_2}CH_2Cl$); 44.99 (piperazine C_2); 54.07 (piperazine C_3); 59.72 - 60.21 ($CH_2\underline{CH_2}Cl$, cyclohexane C_1); 120.92 - 121.74 - 122.94 - 123.57 - 123.76 (benzothiophene CH); 138.82 - 139.53 - 147.39 (benzothiophene quaternary C).

1-[1-(2-benzo(b)thiophenyl)cyclohexyl]-4-(2-[^{18}F]fluoroethyl) piperazine: 7

419 mCi of n.c.a. $K^{18}F$ / K_{222} (K_{222}: 58 mmol; K_2CO_3: 33 mmol) in 0.6 ml of dry DMSO was added to 6 µmol (2.2 mg) of **6** in a pyrex vial. After fitted with a septum plug, the vial was heated at 140°C for 15 minutes. The mixture was then transferred onto a C_{18} Sep Pak cartridge. After a wash with water, the organic compounds were eluted with methylene chloride(with triethylamine 0.1%). The solution was transferred onto a silica Sep Pak cartridge and an elution with dichloromethane/acetonitrile (85/15 : v/v) gave a fraction containing the radioactive compound **7**. The fraction was then purified by HPLC on a silica column using dichloromethane/ethyl acetate/triethylamine (97/3/0.05 : v/v/v). The radioactive compound **7** was collected and about a quarter of the solution was purified by HPLC on a C_8 column using

water/isopropanol/trifluoroacetic acid (86/14/0.05 : v/v/v). The collected product fraction was then basified with a saturated solution of Na_2CO_3 and transferred onto a C_{18} Sep Pak cartridge. After a wash with water, compound **7** was eluted with dichloromethane/methanol (90/10 : v/v). Thus, after 125 minutes from the end of irradiation, 22 mCi (814MBq) of chemically and radiochemically pure **7** were obtained with a specific radioactivity of 2.5 Ci/μmol (92.5GBq/μmol).

REFERENCES

1. Agid Y., Javoy-Agid F. and Ruberg M. - Movement Disorders, Butterworths, London, 166 (1987).

2. Kish S.J., Shannack K. and Hornykiewicz O. - New Engl. J. Med., 318: 876 (1988)

3. Homykiewicz O. - Pharmacol. Rev., 18: 925 (1966).

4. Frost J.J. and Wagner H.N. - Quantitative Imaging : neuroreceptors, neurotransmitters and enzymes, Raven Press, Newyork (1990).

5. Ponchant M., Varastet M., Hantraye P. Chicheportiche R. Kamenka J.M. and Crouzel C. - Nucl. Med. Biol., 10: 727 (1993).

6. Coderc E., Martin-Fardon R., Vignon J. and Kamenka J.M. - Eur. J. Med. Chem. 28: 893 (1993).

7. Barton D. and Ollis W.D. - Heterocyclic compounds, Ed. SAMMES P.G., Pergamon press 4: 119 (1979).

8. Chuchani G. - The chemistry of the amino group, Ed. S. PATAI, 230,1971.

9. Ponchant M., Kamenka J.M. and Crouzel C. - J.L.C.R., 11: 955 (1992).

10. Kilbourn M.R., Jerabek P.A. and Welch M.J. - Int. J. Appl. Radiat. Isot., 36: 327 (1985).

11. Maurice T., Vignon J., Kamenka J.M. and Chicheportiche R. - J. Neurochem., 56: 553 (1991).