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Research Article

Efficient synthesis of [^{11}C]befloxatone, a selective radioligand for the *in vivo* imaging of MAO-A density using PET

F. Dollé[1,*], Y. Bramoullé[1], F. Hinnen[1], S. Demphel[1], P. George[2] and M. Bottlaender[1]

[1] *Département de Recherche Médicale, Service Hospitalier Frédéric Joliot, CEA 4 place du Général Leclerc - F-91401 Orsay, France*

[2] *Synthélabo Recherche - 31 Avenue Paul Vaillant Couturier - F-92200 Bagneux, France*

Summary

Carbon-11 labelled befloxatone ((5R)-5-(methoxymethyl)-3-[4-[(3R)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]-2-oxazolidinone) is a reversible and selective monoamine oxidase-A (MAO-A) inhibitor and appears to be a new potent PET tracer for the *in vivo* imaging of MAO-A density. In this paper, the radiosynthesis of befloxatone was investigated and orientated towards the preparation of multi milliCuries of radiotracer. Typically, using no-carrier-added [^{11}C]phosgene, 150–300 mCi (5.55–11.10 GBq) of [^{11}C]befloxatone was obtained within 20 min of radiosynthesis (including HPLC purification) with specific radioactivities ranging from 500 to 2000 mCi/μmol (18.5–74.0 GBq/μmol). The high efficiency of these radiosyntheses allows for multi-injection protocols and kinetic approaches for absolute quantification of the tracer.

Key Words: befloxatone; carbon-11; positron emission tomography; PET; MAO; monoamine oxidase

*Correspondence to: F. Dollé, Département de Recherche Médicale, Service Hospitalier Frédéric Joliot, CEA 4 place du Général Leclerc - F-91401 Orsay, France. E-mail: dolle@dsvidf.cea.fr

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Introduction

Monoamine oxidases (MAOs) catalyze the oxidative deamination of endogenous neurotransmitters as well as amines from exogenous sources.[1] Two isoforms of MAO have been described, MAO-A and MAO-B. They differ by their substrate selectivity and by their inhibitor sensitivity[2–8] and are both important for neurotransmitter regulation.[9] Fluctuations in functional MAO activity may be associated with human diseases such as Parkinson's- and Alzheimer's disease, depression, and certain psychiatric disorders[10,11]. [^{11}C]Deprenyl and deuterium-substituted [^{11}C]deprenyl have been established as the radioligands of choice for assessing MAO-B activity with positron emission tomography (PET), a high-resolution, sensitive, non-invasive and quantitative imaging technique.[12–19] Carbon-11-labelled radiotracers have also been developed for MAO-A PET studies such as the two irreversible inhibitors [^{11}C]clorgyline and its deuteriated derivative.[20–25]

Befloxatone (**1**, (5R)-5-(methoxymethyl)-3-[4-[(3R)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]-2-oxazolidinone) is an oxazolidinone derivative belonging to a new generation of reversible and selective MAO-A inhibitors.[26–31] It inhibited selectively and competitively MAO-A in human-and rat brain, heart, liver and duodenum homogenates (Ki values ranging from 1.9 to 3.6 nM for MAO-A) and displayed a high activity in tests involving MAO-A in rodents (ED_{50} of about 0.1–0.2 mg/kg p.o. or 0.07 mg/kg i.v.). Based on the biochemical and pharmacological characteristics of this drug, befloxatone has been labelled with carbon-11 and appears to be an excellent candidate for the *in vivo* imaging of MAO-A density using PET.[32,33].

In this paper, we present an efficient radiosynthesis of [^{11}C]Befloxatone ([^{11}C]-**1**) and the experimental procedures used for the preparation of hundreds of milliCuries of [^{11}C]-**1** in order to satisfy the multi-injection protocols and kinetic approaches[34] used in our PET studies for absolute quantification of this radiotracer.

Results and discussion

Befloxatone (**1**) was labelled with carbon-11 ($t_{1/2}$:20.4 min) using no-carrier-added [^{11}C]phosgene and the corresponding ring-opened precursor **2** ((R)-l-methoxy-3-[[4-[(3R)-4,4,4-trifluoro-3-hydroxybutoxy]-phenyl]amino]-2-propanol). [^{11}C]Phosgene ([^{11}C]$COCl_2$) was

synthesized from cyclotron-produced [^{11}C]methane ([^{11}C]CH_4) via [^{11}C]carbon tetrachloride ([^{11}C]CCl_4) using either of two different published processes.[35,36]

In both processes, [^{11}C]CH_4 was separated from the target contents, trapped and concentrated on Porapak-Q.

Process A

$$[^{11}C]CH_4 \xrightarrow[380^\circ C]{Cl_2(10\ mL),\ CuCl_2 \text{ on pumice stone}} [^{11}C]CCl_4 \xrightarrow[290-315^\circ C]{\text{Fe filings}} [^{11}C]COCl_2$$

vector gas : nitrogen containing 2% of oxygen

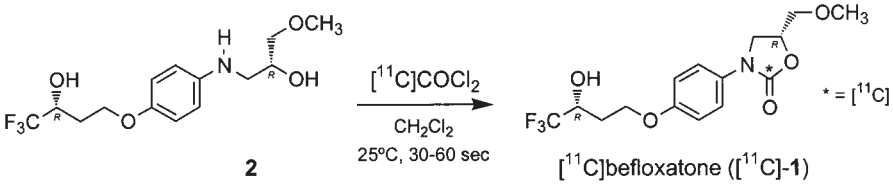
In process A, [^{11}C]CH_4 was carried off by a flow of helium gas and mixed with 10 ml of chlorine. The mixture was then swept away by a flow of nitrogen containing 2% of oxygen and passed through a glass U-tube containing pumice stone, impregnated with $CuCl_2$, at a temperature of 380°C.[35] The on-line synthesized [^{11}C]CCl_4 was continuously swept away using the same nitrogen/oxygen as vector gas and passed through a glass U-tube containing iron filings at a temperature of 290–310°C, giving [^{11}C]$COCl_2$.

Process B

$$[^{11}C]CH_4 \xrightarrow[510^\circ C]{Cl_2(3\ mL)} [^{11}C]CCl_4 \xrightarrow[290-315^\circ C]{\text{Fe filings}} [^{11}C]COCl_2$$

vector gas : helium

In process B, [^{11}C]CH_4 was swept by a flow of helium gas and mixed with only 3 ml of chlorine. The mixture was then carried through an empty linear horizontal glass tube at a temperature of 510°C using the same vector gas.[36] The on-line synthesized [^{11}C]CCl_4 was continuously swept away by the same helium vector gas and passed through a glass U-tube containing iron filings at a temperature of 290–310°C, giving [^{11}C]$COCl_2$. In process B, oxygen is therefore not intentionally added to the system. Both processes gave [^{11}C]$COCl_2$ in 9–10 min radiosynthesis time and 31–54% decay-corrected radiochemical yield ($n = 10$), based on starting [^{11}C]CH_4.



2 [^{11}C]befloxatone ([^{11}C]-**1**)

[^{11}C]$COCl_2$ was trapped (bubbling through) at room temperature in CH_2Cl_2 (250 µl) containing the labelling precursor **2** (0.5–1.5 mg). The cyclization reaction of [^{11}C]phosgene was fast (instanteneously) and almost quantitative. [^{11}C]Befloxatone ([^{11}C]-**1**) was formed in 90–95% radiochemical yield. Typically, starting from a 1.2 Ci (44.4 GBq) [^{11}C]CH_4 production batch, 150–300 mCi (5.55–11.10 GBq) of [^{11}C]befloxatone ([^{11}C]-**1**) with a radiochemical- and chemical purity of more than 99% were routinely obtained within 20 min of radiosynthesis, including HPLC purification. The total decay-corrected radiochemical yield of [^{11}C]befloxatone ([^{11}C]-**1**), based on starting [^{11}C]CH_4, was 27–50% ($n = 10$) whichever the synthetic process of [^{11}C]phosgene employed. The specific radioactivities measured at the end of the radiosynthesis were 500–2000 Ci/µmol (18.5–74.0 GBq/µmol). Again, whichever the synthetic process used, no significant differences could be observed in terms of associated specific radioactivities obtained.

Formulation of labelled product for i.v. injection was effected as follows: (1) HPLC solvent removal by evaporation; (2) taking up the residue in 5 ml of physiological saline containing 10% of ethanol; (3) sterile filtration. The solution for injection was a clear and colorless solution and its pH was between 4.5 and 8.5. The radiosynthesized [^{11}C]befloxatone co-elutes with an authentic sample of befloxatone. The preparation was found to be $>98\%$ chemically and radiochemically pure, as demonstrated by HPLC analysis. The preparation was free from starting labelling precursor and was shown to be chemically and radiochemically stable for at least 120 min. Administration to animals were done within 15 min after end of synthesis. Post-release control results (sterility and endotoxine tests for example) were in accordance with our in-house radiopharmaceutical quality assurance standards.

Conclusion

In this paper, the radiosynthesis of carbon-11-labelled befloxatone ([^{11}C]-**1**), a new potent PET tracer for the *in vivo* imaging of MAO-A density was investigated and orientated towards the preparation of multi-milliCuries of radiotracer using [^{11}C]phosgene. The high efficiency of these radiosyntheses allows for multi-injection protocols and kinetic approaches for absolute quantification of the tracer.

Experimental section

General

Chemicals were purchased from Aldrich-, Fluka- or Sigma France and were used without further purification. Befloxatone (**1**, (5R)-5-(methoxymethyl)-3-[4-[(3R)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]-2-oxazolidinone) and its precursor for labeling (**2**, (R)-l-methoxy-3-[[4-[(3R)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]amino]-2-propanol) were synthesized by Synthélabo Recherche (31 Avenue Paul Vaillant Couturier – F-92200 Bagneux – France). Pumice stone impregnated with $CuCl_2$ was prepared as follows: To 65 g of $CuCl_2$ dissolved in 56 ml of ultrapure water were added 40 g of pumice stone (Merck). The mixture was stirred at room temperature for 20 h, filtered and dried for 2 h at 110°C. Another 40 g of pumice stone were added to the preparation to give the final catalyst used in the chlorination process of [^{11}C]methane. HPLCs (Equipment: Waters- or Shimadzu systems): [HPLC A]: Equipment: system equipped with a Waters 510 pump, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a Geiger–Muller counter (effluent monitoring for radioactivity); column: semipreparative SiO_2, Lichrosphere Si_{60}, Merck (250 × 10 mm), 7 μm; conditions: isocratic elution with CH_2Cl_2/EtOAc: 70/30 [v:v], flow rate: 8 ml/min; temperature: RT, absorbance detection at $\lambda = 254$ nm.[HPLC B]: Equipment: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18 microcolumn, Waters (50 × 4.6 mm), 5 μm; conditions: isocratic elution with solvent A/solvent B:30/70 [v:v] [solvent A: H_2O containing Low-UV PIC® B7 reagent (% by weight: methanol (18–22%), heptane sulfonic acid – sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3, Waters), 20 ml for 1000 ml; solvent B: H_2O/CH_3CN: 50/50 [v:v] containing Low-UV PIC® B7 reagent, 20 ml for 1000 ml], flow rate: 2.0 ml/min, temperature: 30°C, absorbance detection at $\lambda = 240$ nm.

Preparation of [^{11}C]CH_4

[^{11}C]CH_4 was produced by irradiation of a target consisting of an ultrapure Air Liquide 95/5 mixture of N_2/H_2 with a 20 MeV proton beam (30 μA) via the ^{14}N[p,α]^{11}C nuclear reaction on a CGR-MeV 520

cyclotron (54000 μC in 30 min). On average, about 1.20 Ci or 44.40 GBq (EOB) of [^{11}C]CH_4 is routinely obtained in our laboratory for a 30 μA, 30 min (54 000 μC) irradiation. At the end of the bombardment, the target contents were transferred to the 5-cm-lead shielded hot cell dedicated to the radiosynthesis of the tracer and passed firstly through an empty tube (stainless steel coil, 500 mm length, 4 mm internal diameter, cooled at −186°C using liquid argon) in order to remove traces of ammonia (produced during the irradiation) and secondly through a guard of P_2O_5 (glass tube, 70 mm length, 3 mm internal diameter) in order to remove residual moisture. [^{11}C]CH_4 was then separated from the target gas by trapping in a copper-U-tube (150 mm length, 4 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186°C (liquid argon). [^{11}C]CH_4 was released from the trap by warming the copper-U-tube to room temperature (hot air) and swept away by a flow of helium gas (40 ml/min). [^{11}C]CH_4 was then passed through a guard of P_2O_5 (glass tube, 70 mm length, 10 mm internal diameter) and concentrated in a second copper U-tube (150 mm length, 2 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186°C (liquid argon). On average, about 1.20 Ci or 44.40 GBq (EOB) of [^{11}C]CH_4 is routinely produced for a 30 μA, 30 min (54 000 μC) irradiation and then transferred and concentrated in 4–5 min using the process described above.

Preparation of [^{11}C]CCl_4

Procedure using the $CuCl_2$ catalyst. [^{11}C]CH_4 was released from the trap by warming the latter to room temperature and swept (15 ml/min) in a volume of 1–2 ml of helium into a gas mixing chamber containing 10 ml of chlorine (99.99%, Air Liquide). Then, using nitrogen/oxygen (98/2) as vector gas (15 ml/min), the [^{11}C]CH_4–chlorine mixture was passed through a glass U-tube (200 mm length, 6 mm internal diameter) containing 3 g of pumice stone impregnated with $CuCl_2$ (see above for the preparation of this catalyst) at a temperature of 380°C converting it into [^{11}C]CCl_4.

Procedure not using the $CuCl_2$ catalyst. [^{11}C]CH_4 was released from the trap by warming the latter to room temperature and swept (15 ml/min) in a volume of 1–2 ml of helium into a gas mixing chamber containing 3 ml of chlorine (99.99%, Air Liquide). Using the same helium as vector gas (15 ml/min), the [^{11}C]CH_4–chlorine mixture was passed through an

empty horizontal glass tube (215 mm length, 7 mm internal diameter) at a temperature of 510°C converting it into [^{11}C]CCl_4.

Preparation of [^{11}C]$COCl_2$

The on-line synthesized [^{11}C]CCl_4 was then passed through a glass U-tube (200 mm length, 4 mm internal diameter) containing 1.5 g of iron filings (Telar 57, Weber) at a temperature of 290–310°C (using the [^{11}C]CCl_4 vector gas) converting it into [^{11}C]$COCl_2$. The on-line synthesized [^{11}C]$COCl_2$ was then passed through a trap of antimony-trap (glass tube, 70 mm length, 3 mm internal diameter, containing a $\frac{2}{1}$ ratio [v/v] of antimony (400 mg) and glass beads (1 mm diameter)) in order to remove the excess of chlorine.

*Preparation of [^{11}C]befloxatone ([^{11}C]-**1**)*

The on-line synthesized [^{11}C]$COCl_2$ was trapped (bubbling through) at room temperature in a reaction vessel containing 0.5–1.5 mg of the labelling precursor (**2**, (R)-l-methoxy-3-[[4-[(3R)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]amino]-2-propanol, 1.5–4.6 μmol) dissolved in 250 μl of CH_2Cl_2. Trapping of [^{11}C]$COCl_2$ was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the reaction mixture was diluted with 1.0 ml of CH_2Cl_2 containing 2% of Et_2NH [v:v] and injected onto the column. (HPLC A; Rt:(**1**):6.0 to 6.5 min; (**2**): > 30 min).

*Formulation of [^{11}C]befloxatone ([^{11}C]-**1**)*

Formulation of labelled product for i.v. injection was effected as follows: The HPLC-collected fraction containing [^{11}C]befloxatone ([^{11}C]-**1**) was concentrated to dryness (using a rotavapor, water bath temperature: 40–60°C or using a helium gas stream, oil bath temperature: 70–80°C). The residue was taken up in 10 ml of physiological saline containing 10% of ethanol and the resulting solution was filtered on a 0.22 μm FG Millex vented filter (activated beforehand with 1 ml of ethanol followed by a rinse with 1 ml of physiological saline).

*Quality control of [^{11}C]befloxatone ([^{11}C]-**1**)*

The radiopharmaceutical preparation is a clear and colorless solution and its pH is between 5 and 7. As demonstrated by HPLC analysis (HPLC B), the radiolabelled product was found to be >99% radiochemically pure and also co-eluted with a sample of authentic befloxatone (1) (HPLC B; retention time: 2.20 min). The preparation was shown to be free of non-radioactive precursor and radiochemically stable for at least 100 min. Specific radioactivity was calculated from three consecutive HPLC analyses and determined as follows: The area of the UV absorbance peak corresponding to the radiolabelled product was measured (integrated) on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance. The first injection in PET experiments was done within 15 min after the end of synthesis.

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References

1. Singer TP, Ramsay RA, *FASEB J* 1995; **9**: 605–610.
2. O'Carroll A-M, Fowler JC, Phillips JP, Tobbia I, Tipton KF. *Naunyn-Schmiedeberg's Arch Pharmacol* 1983; **322**: 198–202.
3. Fowler CJ, Tipton KF. *J Pharm Pharmacol* 1984; **36**: 111–115.
4. Johnston JP. *Biochem Pharmacol* 1968; **17**: 1286–1297.
5. Knoll J, Maygar K, *Adv Biochem Psychopharmacol* 1972; **5**: 393–406.
6. Youdim MBH, Finberg JPM. Monoamine oxidase inhibitor antidepressants. In *Psychopharmacology 2. Part 1. Preclinical Psychopharmacology*, Grahame-Smith DG (ed.), Elsevier: Amsterdam, 1985; 35–70.
7. Stronlin-Benedetti M, Dostert P. *Trends Pharmacol Sci* 1985; **6**: 246–251.
8. Youdim MBH, Finberg JPM, Tipton KF. Monoamine oxidases. In *Catecholamines I. Handbook of Experimental Psychopharmacology*, vol 10, Trendelenburg U, Weiner N (eds), Springer: Berlin, 1988; 119–192.
9. Fowler CJ, Oreland L, Callingham BA. *Pharm Pharmacol* 1981; **33**: 341–399.
10. Strolin-Benedetti M, Dostert P, *Biochem Pharmacol* 1989; **38**, 555–561.

11. Brunner HG, Nelen M, Breakefield XO, Ropers HH, Van Oost BA. *Science* 1993; **262**: 578.
12. MacGregor RR, Halldin C, Fowler JS, Wolf AP, Arnett CD, Langstrom B and Alexoff D, *Biochem Pharmacol* 1985; **34**: 3207–3210.
13. Arnett CD, Fowler JS, MacGregor RR, Schlyer DJ, Wolf AP, Langstrom B and Halldin C, *J Neurochem* 1987; **49**: 522–527.
14. Fowler JS, MacGregor RR, Wolf AP, Arnett CD, Dewey SL, Schlyer D, Christman D, Logan J, Smith M, Sachs H, Aquilonius M, Bjurling P, Halldin C, Hartvig P, Leenders KL, Lundqvist H, Oreland L, Stalnacke C-G and Langström B, *Science* 1987; **235**: 481–485.
15. MacGregor RR, Fowler JS, Wolf AP. *Label Compds Radiopharm* 1988; **25**: 1–9.
16. Fowler JS, Wang GJ, Logan J, Xie S, Volkow ND, MacGregor RR, Schlyer DJ, Pappas N, Alexoff DL, Patlak C and Wolf AP, *J Nucl Med* 1995; **36**: 1255–1262.
17. Fowler JS, Volkow ND, Wang G-J, Pappas N, Logan J, MacGregor RR, Alexoff D, Shea C, Wolf AP, Warner D, Zezulkova I and Cilento R, *Nature* 1996; **379**: 733–736.
18. Fowler JS, Volkow ND, Wang G-J, Logan J, Pappas N, Shea C and MacGregor RR, *Neurobiol Aging* 1997; **18**: 431–435.
19. Fowler JS, Volkow ND, Wang GJ, Pappas N, Shea C, MacGregor RR, Logan J, *Adv Pharmacol* 1998; **42**: 304–307.
20. Fowler JS, Ding YS, Logan J, MacGregor RR, Shea C, Garza V, Gimi R, Vilkow ND, Wang GJ, Schlyer D, Ferrieri RA, Gatley SJ, Alexoff DL, Carter P, King P and Pappas N, *Nucl Med Biol* 2001; **28**: 779–785.
21. Fowler JS, MacGregor RR, Wolf AP, Arnett CD, Dewey SL, Schlyer D, Christman DR, Logan J, Smith M, Sachs H, Aquilonius SM, Bjurling P, Halldin C, Hartvig P, Leenders KL, Lundquist H, Oreland L, Stalnacke CG and Langström B, *Science* 1987; **235**: 481–485.
22. Fowler JS, Volkow ND, Wang GJ, Pappas N, Logan J, Shea C, Alexoff DL, MacGregor RR, Schlyer D, Zezulkova I and Wolf AP, *Proc Natl Acad Sci USA* 1996; **93**: 14065–14069.
23. Logan J, Fowler JS, Ding YS, Franceschi D, Wang GJ, Volkov ND, Felder C and Alexoff D, *J. Cereb Blood Flow Metab* 2002; **22**: 1367–1376.
24. Fowler JS, Logan J, Ding YS, Franceschi D, Wang GJ, Volkow ND, Pappas N, Schlyer D, Gatley JS, Alexoff D, Felder C, Biegon A and Zhu W, *J Neurochem* 2001; **79**: 1039–1046.
25. MacGregor RR, Halldin C, Fowler JS, Wolf AP, Arnett CD, Langström B and Alexoff D, *Biochem Pharmacol* 1985; **34**: 3207–3210.
26. Curet O, Damoiseau G, Labaune JP, Rovei V, Jarreau FX. *J Neural Transm* 1994; **41**: 349–355.

27. Rovei V, Caille D, Curet O, Ego D, Jarreau FX. *J Neural Transm* 1994; **41**: 339–347.
28. Curet O, Damoiseau G, Aubin N, Sontag N, Rovei V, Jarreau FX. *J Pharmacol Exp Ther* 1996; **77**: 253–264.
29. Caille D, Bergis OE, Fankhauser C, Gardes A, Adam R, Charieras T, Grosset A, Rovei V and Jarreau FX, *J Pharmacol Exp Ther* 1996; **277**: 265–277.
30. Haddjeri N, De Montigny C, Curet O, Blier P. *Eur J Pharmacol* 1998; **343**: 179–192.
31. Wouters J, Moureau F, Evrard G, Koenig JJ, Jegham S, George P and Durant F, *Bioorg Med Chem* 1999; **7**: 1683–1693.
32. Bottlaender M, Dollé F, Guenther I, Roumenov D, Fuseau C, Bramoullé Y, Curet O, Jegham S, Pinquier JL, George P and Valette H, *J Pharmacol Exp Ther* 2003; **305**: 467–473.
33. Dollé F, Valette H, Bramoullé Y, Guenther I, Fuseau C, Coulon C, Lartizien C, Jegham S, Curet O, Pinqier JL, Gegore P and Bottlaender M, *Bioorg Med Chem Lett* 2003; **13**: 1771–1775.
34. Delforge J, Pappata S, Millet P, Samson B, Bendriem B, Jobert A, Crouzel C and Syrota A, *J Cereb Blood Flow Metab* 1995; **15**: 284–300.
35. Landais P, Crouzel C. *Appl Radiat Isot* 1987; **38**: 297–300.
36. Link JM, Krohn KA. *J Label Compds Radiopharm* 1997; **40**: 306–308.

