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Research Article

Efficient synthesis and formulation of (*R*)-(−)-[^{11}C]Deprenyl, a selective radioligand for the quantification of MAO-B activity using PET

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Summary

Carbon-11 labeled (*R*)-(−)-Deprenyl is the tracer of reference for the quantification of monoamine oxidase (MAO)-B activity with PET. In this paper, its radiosynthesis is re-investigated and oriented towards the preparation of multi-milliCuries of radiotracer. Typically, using no-carrier-added [^{11}C]methyl triflate as the alkylating agent, 140–190 mCi (5.1–7.0 GBq) of (*R*)-(−)-[^{11}C]Deprenyl was obtained within 30 min of radiosynthesis (including HPLC purification and formulation) with specific radioactivities ranging from 0.8 to 1.2 Ci/μmol (29.6–44.4 GBq/μmol). The high efficiency of these radiosyntheses allows for multi-injection protocols and kinetic approaches for absolute quantification of the tracer. Copyright © 2002 John Wiley & Sons, Ltd.

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

Introduction

Monoamine oxidase (MAO) is responsible for the oxidative deamination of endogenous neurotransmitter amines as well as amines from

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exogenous sources.[1] Both forms, MAO-A and MAO-B, which are identified by their inhibitor sensitivity and/or by their substrate selectivity,[2,3] are important for neurotransmitter regulation.[4] Fluctuations in functional MAO activity may be associated with human diseases such as Parkinson's disease, depression, and certain psychiatric disorders.[5] It has previously been demonstrated that (*R*)-(−)-[^{11}C]Deprenyl (L-[^{11}C]Deprenyl, [^{11}C]-**1**) is a useful tracer for assessing MAO-B activity with positron emission tomography (PET), a high-resolution, sensitive and non-invasive imaging technique.[6–9] Deuterium substituted [^{11}C]L-deprenyl has also been synthesized.[10] It shows improved kinetics for quantification particularly in regions of high MAO-B concentration and has been used for pharmacological studies and studies of aging.[11–13]

Mathematical compartmental ligand-transporter models using a kinetic approach based on a multi-injection protocol can be used in order to analyze and fit the PET time–concentration curves.[14] A typical multi-injection protocol requires the preparation at a given time of large amounts of labelled tracer.

In the course of our PET program, we were confronted with an increasing demand for (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) and we have therefore re-investigated its radiosynthesis and oriented our procedures towards the preparation of hundreds of milliCuries of radiotracer.

Results and discussion

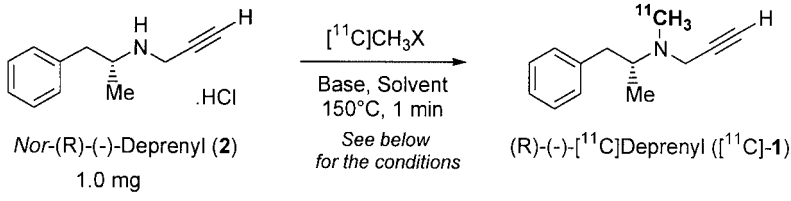
Radiochemistry

(*R*)-(−)-Deprenyl (**1**) was labelled with carbon-11 at its methyl tertiary-amine function from the corresponding secondary-amine precursor **2** and the highly efficient methylation reagent [^{11}C]methyl triflate.

[^{11}C]Methyl triflate was prepared according to a literature procedure from [^{11}C]methyl iodide using silver triflate.[15] [^{11}C]Methyl iodide was prepared from [^{11}C]carbon dioxide using the well-known two step, one pot protocol, consisting of the trapping of [^{11}C]CO_2 and conversion into [^{11}C]methanol ($LiAlH_4$) followed by iodination using aqueous HI giving [^{11}C]methyl iodide.[16]

$$[^{11}C]CO_2 \xrightarrow[\text{then } H_2O]{LiAlH_4/THF} [^{11}C]CH_3OH \xrightarrow{\text{aq. HI}} [^{11}C]CH_3I \xrightarrow[150-200^{\circ}C]{\text{AgOTf}} [^{11}C]CH_3OTf$$

On average, about 650 mCi (24.1 GBq) of [^{11}C]CH_3OTf or [^{11}C]CH_3I is routinely obtained in our laboratory in 7–8 min after the end of the bombardment (EOB) in 70% decay-corrected yield, based on starting [^{11}C]CO_2 (Table 1).



Nor-(R)-(-)-Deprenyl (**2**)
1.0 mg

(R)-(-)-[^{11}C]Deprenyl ([^{11}C]-**1**)

Table 1

[^{11}C]CH_3-X	Solvent	Base (eq.)	Yield[a]
CH_3-I	Acetonitrile[b]	TMBA (0.5–3)	<5%
CH_3-OTf	Acetonitrile	TMBA (0.5–3)	15%
CH_3-OTf	Acetonitrile	Aqueous NaOH (0.5–3)	15%[c]
CH_3-OTf	Acetone	TMBA (1.5–2)	25%
CH_3-OTf	DMF	TMBA (1.5–2)[d]	25%
CH_3-OTf	Acetone	Aqueous NaOH (2)	60%[e]

[a] Decay-corrected, based on [^{11}C]methyl iodide or triflate.
[b] Or acetone.
[c] 25% yield was observed when 5 eq. of base was used.
[d] Or aqueous NaOH.
[e] Occasionally up to 265 mCi were obtained (75% yield).

Conditions: (1) trapping at room temperature of the [^{11}C]methyl iodide or [^{11}C]methyl triflate in 250–300 µl of the solvent containing 1.0 mg of precursor (**2**, hydrochloride, 4.8 µmol) and the base; (2) heating the reaction mixture at 150°C for 1–3 min; (3) taking up the crude with 0.5 ml of the HPLC mobile phase (when acetone was used as the solvent, the reaction mixture was first concentrated to dryness at 150°C using a helium stream); and (4) HPLC purification.

Reaction of the desmethyl-compound **2** hydrochloride with [^{11}C]methyl iodide, employing the standard conditions that have so far been used in our laboratory for the routine radiosynthesis of several radiotracers, yielded (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) in unusally low yield. The conditions used were the following: (1) trapping at room temperature of the [^{11}C]methyl iodide in 300 µl of acetone or acetonitrile containing 1.0 mg of precursor (**2**, hydrochloride, 4.8 µmol) and 1–5 µl of a 3 M solution of TMBA (benzyltrimethylammonium hydroxide) in EtOH (3.0–15.0 µmol, 0.6–3.0 eq.); (2) heating the reaction mixture at 150°C for 1–3 min; (3) concentration to dryness of the reaction mixture (at 150°C using a helium stream); (4) taking up the crude with 0.5 ml of the HPLC mobile phase and (5) HPLC purification.

From an average production batch of 840 mCi (at EOB) of [^{11}C]CH_3I, only 5–15 mCi of (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) could be synthesized in 25 min after EOB (Yield, decay-corrected and based on [^{11}C]methyl iodide: $<5\%$).

Using [^{11}C]methyl triflate as the alkylating agent and 1.0 mg (4.8 μmol) of precursor **2** at 150°C for 1 min in acetonitrile containing TMBA, immediately led to an increase in the radiochemical yield. The conditions used were similar to those described above and up to 15% yield (decay corrected and based on [^{11}C]methyl triflate) was observed. The use of another base aqueous NaOH, did not improve the yield except when higher base over precursor ratio was used. When 5 equivalents of aqueous NaOH were present in the reaction mixture, a 25% yield (decay corrected and based on [^{11}C]methyl triflate) could be observed using similar conditions to those described above. The use of acetone as the solvent and only 1.5–2.0 equivalents of TMBA as the base gave similar results (25% decay-corrected yield, based on [^{11}C]methyl triflate). The use of DMF as the solvent led to an immediate decrease in the radiochemical yield (10%), also when NaOH was used as the base.

The best results were obtained with [^{11}C]methyl triflate as the alkylating agent and 1.0 mg (4.8 μmol) of precursor **2** at 150°C for about 3 min in acetone containing 1.9 eq. of aqueous NaOH. The conditions used were the following: (1) trapping at room temperature of the [^{11}C]methyl triflate in 250 μl of acetone containing 1.0 mg of precursor (**2**, hydrochloride, 4.8 μmol) and 3 μl of a 3 M solution of NaOH in water (9.0 μmol, 1.9 eq.); (2) heating the reaction mixture at 150°C for 1 min; (3) concentration to dryness of the reaction mixture (at 150°C, using a helium stream for usually 2 min); (4) taking up the crude with 0.5 ml of the HPLC mobile phase and (5) HPLC purification.

From an average production batch of [^{11}C]CH_3OTf (840 mCi at EOB), 165–225 mCi of (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) could be synthesized in 25 min after EOB, corresponding to a 60% decay-corrected yield (based on [^{11}C]methyl triflate).

Note that when less equivalents of base were used (1.3 or 1.6 base over precursor ratio), the observed yield was systematically lower (35 and 25%, respectively). The use of a large increase of base equivalents (3–5 eq.) also gave lower yields (40 and 35%, respectively). When higher amounts of precursor were used (up to 5.0 mg), the final yield was not really affected but the HPLC separation of the radiotracer (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) and the precursor **2** was not successful

in all batches, leading to rejection of the radiopharmaceutical preparation by our *quality control* unit.

Formulation and quality control

Formulation of (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) for i.v. injection was effected as follows: the HPLC-collected fraction containing [^{11}C]-**1** was diluted with water and the resulting solution was passed through a C18 Sep-pak cartridge. The cartridge was then washed twice with water, partially dried with nitrogen and finally eluted with ethanol, the solution being then sterile-filtered and diluted with physiological saline.

The radiopharmaceutical preparation is a clear and colourless solution and its pH is between 5 and 7. As demonstrated by HPLC analysis, the radiopharmaceutical preparation was found to be $>95\%$ chemically and $>99\%$ radiochemically pure and was radiochemically stable for at least 60 min. Typically, 140–190 mCi (5.1–7.0 GBq) of (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) were obtained within 30 min of radiosynthesis (including HPLC purification and formulation) with specific radioactivities ranging from 0.8 to 1.2 Ci/μmol (29.6–44.4 GBq/μmol).

Experimental

General

Chemicals were purchased from standard commercial sources (Aldrich, Fluka or Sigma, France) and were used without further purification unless stated otherwise. (*R*)-(−)-Deprenyl (L-Deprenyl, (*R*)-(−)-*N*-α-dimethyl-*N*-2-propynyl-benzeneethanamine, (1) was purchased as analytical standard from RBI France. HPLCs: HPLC A: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481/486 UV-multiwavelength detectors; column: semipreparative SymmetryPrep® C-18, Waters (300 × 7.8 mm); porosity: 7 μm; conditions: isocratic elution with: 50 mM aqueous NaH_2PO_4/CH_3CN: 85/15 (v:v); flow rate: 7.0 ml/min; temperature: RT; UV detection at λ: 210 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, Waters (4.6 × 50 mm, microcolumn); porosity: 5 μm; conditions:

isocratic elution with solvA/solvB: 55/45 (v:v) [solvA: H_2O containing Low-UV PIC® B7 reagent (Waters), 20 ml for 1000 ml; solvB: H_2O/CH_3CN:50/50 (v:v) 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]; flow rate: 2.0 ml/min; temperature: 30°C; UV detection at λ: 214 nm. Radiosyntheses were performed in a 5-cm-lead shielded confinement.

Preparation of [^{11}C]CO_2

[^{11}C]CO_2 was produced by irradiation of an ultrapure N60 Air Liquide N_2 target with a 20 MeV proton beam (30 μA) via the $^{14}N[p,\alpha]^{11}C$ nuclear reaction on a CGR-MeV 520 cyclotron (54 000 μC in 30 min). 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 first through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove moisture. [^{11}C]CO_2 was then separated from the target gas by trapping in an empty stainless-steel coil [1500 mm length, 0.51 mm internal diameter), cooled at −186°C using liquid argon. On average, about 1.20 Ci or 44.40 GBq (EOB) of [^{11}C]CO_2 is routinely obtained in our laboratory for a 30 μA, 30 min (54 000 μC) irradiation.

Preparation of [^{11}C]CH_3I and [^{11}C]CH_3OTf

[^{11}C]CO_2 was released from the trap by simply raising the stainless-steel coil to room temperature, swept away by a flow of nitrogen gas (40 ml/min) and trapped at −10°C (EtOH–ice bath) into 55 μl of THF containing 5 μl of 1.0 M THF solution of lithium aluminium hydride. Concentration to dryness (evaporation of solvent at 165°C using a stream of nitrogen) followed by hydrolysis (100 μl of deionized water) of the formed aluminum complex afforded [^{11}C]CH_3OH, which was distilled using a flow of nitrogen gas into 1 ml of an aqueous 57% HI solution (heating block at 165°C). The [^{11}C]CH_3I thus synthesized was continuously swept away by a flow of nitrogen gas, passed through a combined 1/1 (v:v) soda lime/P_2O_5-guard (35 mm length each, 3 mm internal diameter) and converted into [^{11}C]CH_3OTf by passing trough a glass column (33 cm length, 5 mm internal diameter), heated at 200°C and containing silver-triflate-impregnated graphitized carbon (200 mg).

About 650 mCi (24.1 GBq) of [^{11}C]CH_3I or [^{11}C]CH_3OTf is routinely obtained in our laboratory in 7–8 min after EOB in 70% decay-corrected yield, based on starting [^{11}C]CO_2.

*Preparation of (R)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**)*

[^{11}C]CH_3I or [^{11}C]CH_3OTf, carried by a flow of nitrogen gas, was trapped (bubbling through) at 0°C (EtOH–ice bath) in a reaction vessel containing 0.5–5.0 mg of nor-(*R*)-(−)-deprenyl hydocholoride (**2**, 2.38–23.84 μmol) dissolved in 250–300 μl of the solvent used (CH_3CN, DMF or acetone) and 0.5–5 eq. of the base used (for TMBA, a 1 M solution of TMBA in EtOH; for NaOH, a 1, 3 or 5 M solution of NaOH in water). Trapping of [^{11}C]CH_3I or [^{11}C]CH_3OTf was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the reaction vessel was then isolated, heated at 150°C using a heating block for 1–3 min. When acetone was used as the solvent, the reaction mixture was first concentrated to dryness (at 150°C, using a helium stream), then the crude was taken up with 0.5 ml of the HPLC mobile phase and was injected onto the column. When CH_3CN or DMF was used, the reaction vessel was cooled (EtOH–ice bath) and the reaction mixture was then diluted with 0.5 ml of the HPLC mobile phase and was injected onto the column. (HPLC A; R_t: (**1**): 8.5–9.5 min; (2): 5.0–6.5 min).

Optimal conditions

[^{11}C]CH_3OTf, carried by a flow of nitrogen gas, was trapped (bubbling through) at 0°C (EtOH–ice bath) in a reaction vessel containing 1.0 mg of nor-(*R*)-(−)-deprenyl hydrochloride (**2**, 4.77 μmol) dissolved in acetone (250 μl) containing 3 μl of a 3 M aqueous NaOH solution (9 μmol of base, 1.96 eq.). The reaction vessel was then isolated and heated at 150°C for 1 min. The reaction mixture was then concentrated to dryness (at 150°C, using a helium stream for usually 2 min), then the crude was taken up with 0.5 ml of the HPLC mobile phase and was injected onto the column.

*Formulation of (R)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**)*

Formulation of labelled product for i.v. injection was effected as follows: The HPLC-collected fraction containing (*R*)-(−)-[^{11}C]Deprenyl

([^{11}C]-**1**) was diluted with water (50 ml). The resulting solution was passed through a C18 Sep-pak cartridge (Waters). The cartridge was washed twice with 5 ml of water and partially dried for 10 s by applying a nitrogen stream. The carbon-11 labelled tracer was eluted with 2 ml of EtOH (< 5% of the total radioactivity was left on the cartridge) and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to lower the EtOH concentration below 10%. This whole process was performed by the chemist using a remote-controlled dedicated home-made device based on a literature procedure.[17]

Quality control of (R)-(−)-[^{11}C]Deprenyl ([^{11}C]-1)

The radiopharmaceutical preparation is a clear and colourless 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 (*R*)-(−)-Deprenyl (**1**) (HPLC B; retention time: 2.1 min). The preparation was shown to be free of non-radioactive precursor and radiochemically stable for at least 60 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.

Conclusion

In this paper, the radiosynthesis of carbon-11-labelled (*R*)-(−)-Deprenyl ([^{11}C]-**1**), the PET tracer of reference for the quantification of MAO-B activity, was re-investigated and oriented towards the preparation of multi milliCuries of radiotracer using [^{11}C]methyltriflate as an alkylating agent. Large amounts of (*R*)-(−)-[^{11}C]Deprenyl (typically, 140–190 mCi (5.1–7.0 GBq)) of (*R*)-(−)-[^{11}C]Deprenyl ([^{11}C]-**1**) were obtained within 30 min of radiosynthesis (including HPLC purification and formulation) with specific radioactivities ranging from 0.8 to 1.2 Ci/μmol (29.6–44.4 GBq/μmol). The high efficiency of these radiosyntheses allows for multi-injection protocols and kinetic approaches for absolute quantification of the tracer.

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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. *Pharm Pharmacol* 1984; **36**: 111–115.
4. Fowler CJ, Oreland L, Callingham BA. *Pharm Pharmacol* 1981; **33**: 341–399.
5. Strolin-Benedetti M, Dostert P. *Biochem Pharmacol* 1989; **38**: 555–561.
6. MacGregor RR, Halldin C, Fowler JS, *et al. Biochem Pharmacol* 1985; **34**: 3207–3210.
7. Arnett CD, Fowler JS, MacGregor RR, *et al. Neurochem* 1987; **49**: 522–527.
8. Fowler JS, MacGregor RR, Wolf AP, *et al. Science* 1987; **235**: 481–485.
9. MacGregor RR, Fowler JS, Wolf AP. *J Label Compd Radiopharm* 1988; **25**: 1–9.
10. Fowler JS, Wang GJ, Logan J, *et al. J Nucl Med* 1995; **36**: 1255–1262.
11. Fowler JS, Volkow ND, Wang G-J, *et al. Nature* 1996; **379**: 733–736.
12. Fowler JS, Volkow ND, Wang G-J, *et al. Neurobiol Aging* 1997; **18**: 431–435.
13. Fowler JS, Volkow ND, Wang GJ, *et al.* Visualization of monoamine oxidase in human brain. *Adv Pharmacol* 1998; **42**: 304–307.
14. Delforge J, Pappata S, Miller P, *et al. J Cereb Blood Flow Metab* 1995; **15**: 284–300.
15. Jewett DM. *Appl Radiat Isot* 1992; **43**: 1383–1385.
16. Crouzel C, Langström B, Pike VW, Coenen HH. *Appl Radiat Isot* 1987; **38**: 601–603.
17. Lemaire C, Plenevaux A, Aerts J, *et al. J Label Compd Radiopharm* 1999; **42**: 63–75.

