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Research Article

Synthesis and PET evaluation of (*R*)-[*S*-methyl-^{11}C]thionisoxetine, a candidate radioligand for imaging brain norepinephrine transporters

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Summary

Introduction: (*R*)-3-(2-(methylthio)phenoxy)-*N*-methyl-3-phenylpropan-1-amine [(*R*)–thionisoxetine; **1**] is a potent inhibitor of the norepinephrine transporter (NET). We aimed to label **1** with carbon-11 ($t_{1/2}$ = 20.4 min) for evaluation as a radioligand for imaging NET in living brain with positron emission tomography (PET).

Methods: Methyl 3-(2-((*R*)-3-(methylamino)-1-phenylpropoxy)phenylthio)-propanoate (MPPP) and **1** were each prepared from *o*-hydroxythiophenol in three steps. Treatment of MPPP with potassium *t*-butoxide and [^{11}C]methyl iodide in tetrahydrofuran gave [*S*-methyl-^{11}C]thionisoxetine ([^{11}C]**1**), which was purified with HPLC. The distribution of radioactivity in brain after intravenous injection of [^{11}C]**1** into cynomolgus monkey was followed with PET and the appearance of radiometabolites in plasma monitored with radio-HPLC.

Results: [^{11}C]**1** was obtained in high yield from [^{11}C]methyl iodide. Of the radioactivity injected into monkey, 2.4% entered brain. Ratios of radioactivity in thalamus, mesencephalon, occipital cortex and caudate to that in cerebellum at 93 min were 1.3, 1.2, 1.2 and 1.1, respectively. The radioactivity in plasma corresponding to unchanged radioligand decreased to 53% at 45 min, with the remainder represented by hydrophilic radiometabolites.

Conclusions: MPPP is an effective precursor for ^{11}C-methylation to [^{11}C]**1**, suggesting that the *S*-γ-propionic acid methyl ester protecting group may have wider

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value in the ^{11}C-labeling of aryl methyl sulfides. However, the relatively low ratios of radioactivity to the cerebellum together with an unexpected accumulation of radioactivity in the caudate, makes [^{11}C]**1** an unpromising NET radioligand.

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Key Words: norepinephrine transporter; PET; monkey; radioligand; carbon-11

Introduction

Although the norepinephrine transporter (NET) has long been recognized as a target in the treatment of several neuropsychiatric disorders,[1–5] the detailed mapping of NET in the human brain, including the determination of regional concentrations (B_{max}), has been hindered through the lack of an effective radioligand. [^{3}H]nisoxetine,[6] after initial studies with rodent tissue,[7,8] appeared to be suitable for imaging NET in human brain *in vitro*. Nevertheless, only the binding of this radioligand to the two highest NET density regions in *post mortem* human brain, the locus coeruleus and the raphe nuclei, has been described.[1,9] Furthermore, *ex vivo* studies with [^{11}C]nisoxetine (^{11}C, $t_{1/2} = 20.4$ min) in mice[10] and positron emission tomography (PET) measurements with its eutomer, (*R*)-[^{11}C]nisoxetine, in baboons[11] have failed to show a useful NET-specific signal.

Among several other evaluated radioligands,[11–21] analogs of reboxetine (Edronax®) so far show the best properties for imaging primate brain NET *in vivo*. These include the *O*-methyl, *O*-fluoromethyl and *O*-fluoroethyl analogs, namely (*S,S*)-[^{11}C]MeNER,[18–20] (*S,S*)-[^{18}F]FMeNER-D$_2$ [21] and (*S,S*)-[^{18}F]FRB-D$_4$ [11], respectively. In human studies with (*S,S*)-[^{11}C]MeNER, measurements of NET suffered from slow pharmacokinetics rendering the PET data vulnerable to noise.[22] Test-retest variabilities were therefore too large (17–34%) for assessing discrete changes in NET inhibition following reboxetine treatment. (*S,S*)-[^{18}F]FMeNER-D$_2$ gives a signal of similar magnitude to that of (*S,S*)-[^{11}C]MeNER in primates and, because of the longer half-life of fluorine-18 ($t_{1/2}$ = 109.8 min), also allows PET data to be acquired at the time of specific binding equilibrium, an asset to biomathematical modeling of the PET data and derivation of NET-radioligand binding parameters.[23] Although (*S,S*)-[^{18}F]FMeNER-D$_2$ could be used to image NETs in the locus coeruleus on *post mortem* human brain hemispheres *in vitro*, the radioligand also failed to detect NET in low density regions.[24] Therefore, (*S,S*)-[^{18}F]FMeNER-D$_2$ may have limited sensitivity for investigations of regional NET densities in human subjects with PET.

We aim to develop a more sensitive radioligand for PET imaging of NET across the entire living human brain. (*R*)-thionisoxetine (**1**; Figure 1) is

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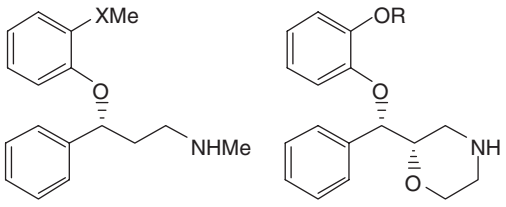

Figure 1. Structures of the NET inhibitors, (*R*)-thionisoxetine (*X* = S; 1), (*R*)-nisoxetine (X = O; 2), (*S*,*S*)-MeNER (*R* = Me) and (*S*,*S*)-FMeNER-D$_2$ (*R* = FCD$_2$)

reportedly about three-fold more potent ($IC_{50,}$ 1.9 nM) than (*R*)-nisoxetine (IC_{50}, 5.8 nM, 2; Figure 1).[25] Such an increase in binding affinity, though modest, may be critical for achieving sensitivity for imaging NET with PET, since on theoretical grounds, binding potential (BP) is inversely proportional to binding affinity. **1** is also 68-fold selective for binding to NET versus the serotonin transporter (SERT).[25] Due to its very close structural similarity to the highly NET-selective **2**,[26] **1** is also not expected to have any significant affinity towards the dopamine transporter (DAT) or any other transporter or neurotransmitter. By inspection, the lipophilicity of **1** is expected to be only slightly greater than that of **2**. On this basis, we considered **1** to be worthy of labeling with carbon-11 and evaluation as a new PET radioligand for NET.

1 appeared amenable to labeling through either *N*- or *S*-methylation with, for example, [^{11}C]methyl iodide. We chose to synthesize a precursor for *S*-^{11}C-methylation, since such a precursor might also allow preparation of the *S*-[^{18}F]fluoromethyl analog of **1**. *S*-[^{18}F]fluoromethyl analogs are known to be chemically stable, as exemplified by [^{18}F]FMcN5652,[27] whereas few *N*-fluoromethyl compounds are stable enough to survive isolation.[28] A longer-lived radioligand might be valuable if the ^{11}C-labeled ligand showed unacceptably slow pharmacokinetics *in vivo*. Given the susceptibility of thiols to oxidize, we aimed to prepare an *S*-protected precursor that might be stored and then deprotected *in situ* for radiomethylation with [^{11}C]methyl iodide, such as, for example, an *S*-acetyl ester.[29] We further aimed to evaluate [^{11}C]**1** as a radioligand for brain NET in cynomolgus monkey with PET.

Results and discussion

Chemistry

We were interested to obtain an *S*-protected precursor that would be stable to storage and directly useful for preparing [^{11}C]**1**. In view of a previous report, in which an *S*-acetyl ester was used as a precursor for *S*-^{11}C-methylation,[29] we attempted to protect the thiol group of the starting material,

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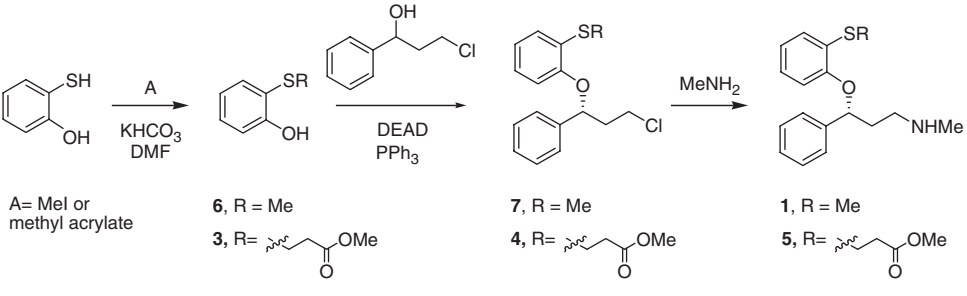
o-hydroxy-thiophenol, as a thioester by treatment with acetyl chloride or acetic anhydride. However, consistent with a previous report,[30] very low regioselectivity was observed under all attempted conditions. In contrast, we found methylation and benzylation of *o*-hydroxy-thiophenol to be thiol-selective. We therefore re-directed our attention to removal of *S*-methyl, *S*-benzyl and *S*-*p*-methoxybenzyl groups from analogs of **1** with sodium thiomethoxide, sodium metal in ethanol or mercury(II) trifluoroacetate.[31–33] However, these reactions failed to proceed cleanly, as previously observed in the particular case of demethylations of some aryl methyl sulfides with sodium thiomethoxide.[34]

Aryl sulfides may be protected as their sulfanyl γ-propionic acid methyl esters and deprotected by β-elimination under basic conditions.[35] We therefore decided to explore the Michael addition of methyl acrylate to *o*-hydroxy-thiophenol as the first step in the synthesis of **1**. Alkylation of *o*-hydroxy-thiophenol with methyl acrylate was found to be highly thiol-selective; for example, a four-fold molar excess of methyl acrylate gave only *S*-alkylation. To obtain enantiomerically pure **1** and **5**, commercially available (*S*)-3-chloro-1-phenylpropan-1-ol was first coupled with the respective *o*-hydroxy-thiophenol derivative, under Mitsunobu conditions. This type of reaction proceeds with complete inversion of configuration.[36, 37] Treatment of the chloro products with methylamine gave the enantiomerically pure free bases of **1** and **5** in 19 and 7% overall yields, respectively (Scheme 1). The synthetic sequence for **1** was similar to that described earlier for related compounds.[36]



Scheme 1. Synthesis of (*R*)-thionisoxetine (1) and its sulfanyl γ-propionic acid methyl ester precursor (MPPP, 5)

Radiochemistry

[^{11}C]**1** was obtained in >99% decay-corrected radiochemical yield (as assessed by analytical radio-HPLC of the radiomethylation mixture) from the intermediate thiol **8** and [^{11}C]methyl iodide (Scheme 2) within 30 min from

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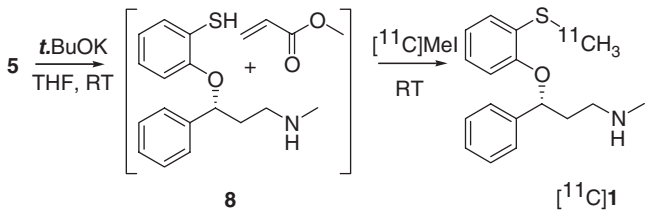

Scheme 2. Radiolabeling of 1 via alkylation of the intermediate thiol (8) formed *in situ* by β-elimination of the γ-propionic acid methyl ester moiety in 5

the end of radionuclide production and with a radiochemical purity exceeding 99%. Base-promoted *in situ* β-elimination of the sulfanyl γ-propionic acid methyl ester moiety in **5** to generate the free thiolate ion (**8**) was thus effective for accomplishing ^{11}C-methylation. The high radiochemical yield of [^{11}C]**1** indicates a wider potential utility for this protective group in the radiosyntheses of other *S*-[methyl-^{11}C] or *S*-[^{18}F]fluoromethyl radiotracers. The specific radioactivity of [^{11}C]**1** was 8.2 Ci/μmol (304 GBq/μmol) at the time of administration to monkey. Precursor (**5**) was absent from the product formulation, which was radiochemically stable for at least 90 min.

c Log P calculations

Both neutral and ionized species of **1** were calculated to be more lipophilic than **2**. The *c Log P* values of **1** and **2** were 3.94 and 3.52, respectively, and the *c Log D* values were 1.47 and 1.02, respectively.

PET measurements

After injection of [^{11}C]**1** into cynomolgus monkey (1.38 mCi; 51 MBq; 0.06 μg carrier), radioactivity readily entered brain (maximally 2.4% of injected dose at 14 min after injection) with a regional distribution approximately matching that expected for NET; however, radioactivity also accumulated in striatum (caudate), a region shown to be low in NET density in rat,[8] cat[38] and human.[39] This accumulation is most likely non-specific, but it cannot be ruled out that [^{11}C]**1** binds to another non-identified binding site. The rank order for radioactivity accumulation in different brain regions was: thalamus > mesencephalon ~ occipital cortex > caudate > cerebellum (Figure 2). Cerebellum, has a NET density similar to striatum in these species,[8,38,39] Also, the cerebellum shows radioactivity accumulation similar to striatum in PET measurements with (*S,S*)-[^{11}C]MeNER in cynomolgus monkeys (Schou *et al.*, unpublished data). Therefore, in this study we decided to use cerebellum as an alternative reference region. Ratios of radioactivity concentration in

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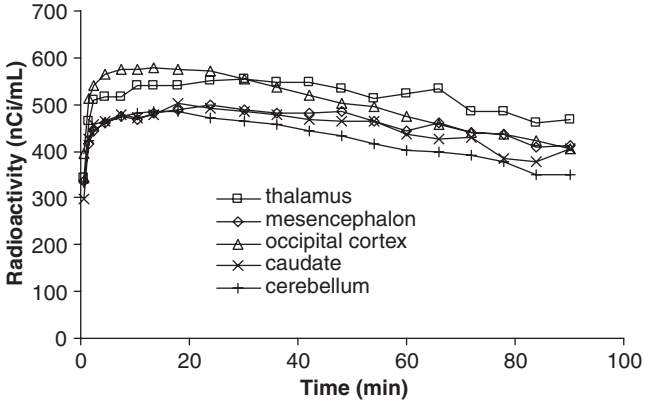

Figure 2. Regional distribution of radioactivity in brain following intravenous injection of [^{11}C]1 into a cynomolgus monkey

thalamus, mesencephalon and occipital cortex to that in caudate were 1.15, 1.0 and 1.0 at 93 min, respectively. Corresponding ratios to cerebellum were 1.3, 1.2 and 1.2. The ratio of caudate to cerebellum radioactivity at 93 min was 1.1. The maximal ratio of radioactivity in any ROI to the cerebellum in PET measurements with [^{11}C]**1** was not greater than 1.3 (for thalamus). This ratio is lower than that observed with the NET radioligands, (*S,S*)-[^{11}C]MeNER and (*S,S*)-[^{18}F]FMeNER-D_2, which show thalamus to striatum ratios of about 1.5–1.6.[19, 21] This lower ratio, together with the accumulation of radioactivity in the striatum, therefore severely limits the utility of [^{11}C]**1** as a PET radioligand for NET. Since with [^{11}C]**1** there was no sign of wash-out of radioactivity from the striatum nor any sign that specific binding in thalamus increased with time, we expended no effort in preparing the *S*-[^{18}F]fluoromethyl analog of this radioligand.

Ex vivo studies with (*S,S*)-[^{11}C]MeNER in the rodent brain gave a signal to noise ratio (hypothalamus to striatum) of about 2.5,[20] whereas the corresponding ratio in non-human primates was about 1.5–1.6.[19] It has been reported that the density of NETs in human cortex is about nine times lower than in rodents.[40] Although this may not be the case for all brain regions, a new structural class of radioligands displaying very high affinity for NET will probably be required for detailed mapping of NET throughout the entire primate brain.

Analysis of radiometabolites in plasma

The overall recovery of radioactivity from the analytical procedure was between 88 and 91%. After injection of [^{11}C]**1** into monkey, unchanged radioligand ($t_{R,}$ 5.6 min) represented 95% of the radioactivity in plasma at 4 min, 70% at 15 min, 40% at 30 min and 53% at 45 min. Radiometabolites of

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[^{11}C]**1** in monkey plasma were all highly hydrophilic based on their lack of retention on the reverse phase HPLC column (i.e. elution at the void volume) under the employed conditions. It is thus unlikely, although not impossible, that radiometabolites cross the blood–brain barrier and contribute to brain radioactivity.

Conclusions

The thiol moiety of *o*-hydroxy-thiophenol, a starting material in the synthesis of **1** and [^{11}C]**1**, was regioselectively protected by reaction with methyl acrylate. This protective group was removable *in situ* under mild conditions during ^{11}C-methylation of precursor to [^{11}C]**1** in high radiochemical yield. Following intravenous injection of [^{11}C]**1** into cynomolgus monkey, there was uptake of radioactivity into NET-rich regions, but only to a slightly greater extent than into NET-poor regions. Hence, [^{11}C]**1** is inferior to existing PET radioligands, such as (*S,S*)-[^{11}C]MeNER or (*S,S*)-[^{18}F]FMeNER-D$_2$, for the study of NET. Nevertheless, the protecting group applied in the synthesis of [^{11}C]**1** may find wider useful application in the labeling of other aryl alkyl sulfides.

Experimental

Chemistry

Materials and general procedures. Diethyl azodicarboxylate (DEAD) (40% in toluene) was obtained from TCI (USA). All other reagents and solvents (e.g. *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF)), including anhydrous solvents, were obtained from Sigma-Aldrich (USA) and used without further purification. Reactions were carried out under a dry inert atmosphere with dry solvents, unless otherwise stated. Thin layer chromatography was performed on silica gel layers (60 F254; Merck) with compound detection under UV light (λ = 254 nm). Flash column chromatography was performed on silica gel (60 Å; 70–230 mesh; Aldrich). ^{1}H-NMR (400 MHz) and ^{13}C-NMR spectra (100 MHz) were recorded on a Bruker drx400 spectrometer for solutions in $CDCl_3$ or MeOD-d_4 [both with TMS ($\delta = 0.00$) as internal standard] at 300 K. Mass spectrometry data were acquired on a quadrupole-orthogonal injection time-of-flight (TOF) mass spectrometer (QTOF1, Waters/Micromass Plc, Manchester, UK). Samples were sprayed from gold-plated borosilicate capillaries (Protana AS, Odense, Denmark) using a capillary voltage of +1 kV.

*Methyl 3-(2-hydroxyphenylthio)propanoate (**3**).* To a solution of *o*-hydroxy-thiophenol (4 g; 31.7 mmol) in DMF (30 ml) was added potassium bicarbonate (2.73 g; 31.7 mmol). The solution was stirred at room temperature (RT) for 30 min, after which methyl acrylate (3.1 g; 31.7 mmol) was added. The reaction

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mixture was stirred for 4 h, diluted with water and extracted with ether. The organic layer was dried ($MgSO_4$), solvents were removed *in vacuo* and the crude product purified by flash column chromatography (hexanes-EtOAc; 20: 1, v/v) to yield **3** as a yellow oil (5.17 g; 24.7 mmol, 78%). ^{1}H-NMR ($CDCl_3$): 7.45 (dd, J = 1.6, 7.7 Hz, 1 H), 7.26 (m, 1 H), 7.03 (s, 1 H), 6.99 (dd, J = 1.4, 8.1 Hz, 1 H), 6.86 (dt, J = 1.3, 7.2 Hz, 1 H), 3.68 (s, 3 H), 2.96 (t, J = 7.1 Hz, 2 H), 2.56 (t, J = 7.1 Hz, 2 H). ^{13}C-NMR ($CDCl_3$): 172.2, 157.3, 135.9, 131.2, 120.6, 118.1, 115.3, 51.9, 34.0, 30.8. MS (TOF ES+): 213.08 [analytically (M + 1)] 212.05 (calculated.)

*Methyl 3-(2-((R)-3-chloro-1-phenylpropoxy)phenylthio)propanoate (**4**).* To a solution of (*S*)-3-chloro-1-phenylpropan-1-ol (3.9 g, 22.6 mmol) and **3** (2.4 g; 11.3 mmol) in THF (75 ml) at 0°C was added triphenyl phosphine (5.9 g; 22.6 mmol) and DEAD (3.9 g; 22.6 mmol). The reaction mixture was allowed to attain RT and was stirred overnight, after which solvents were removed *in vacuo*. The sticky crude product was purified by flash column chromatography (hexanes–EtOAc; 10: 1, v/v) to yield **4** as a thick yellow oil (1.4 g; 3.5 mmol; 31%). ^{1}H-NMR ($CDCl_3$): 7.22–7.40 (6 H), 6.97 (dq, J = 0.8, 1.7, 7.7 Hz, 1 H), 6.84 (dd, J = 1.2, 7.5 Hz, 1 H), 6.66 (dd, J = 1.1, 8.3 Hz, 1 H), 5.42 (m, 1 H), 3.89 (m, 1 H), 3.68 (s, 3 H), 3.60 (m, 1 H), 3.18 (m, J = 1.1 Hz, 2 H), 2.67 (t, J = 7.5 Hz, 2 H), 2.51 (m, 1 H), 2.23 (m, 1 H). ^{13}C-NMR ($CDCl_3$): 172.3, 156.8, 142.1, 131.2, 120.6, 118.1, 115.3, 51.9, 34.0, 30.8. MS (TOF ES+): 365.14 [analytically (M + 1)] (calculated) 364.09.

*Methyl 3-(2-((R)-3-(methylamino)-1-phenylpropoxy)phenylthio)propanoate (MPPP, **5**).* A stirred solution of **4** (1.0 g; 2.74 mmol) and methylamine (10 ml; 2 M in THF) was heated at 90°C in a sealed vessel for 4 h, after which solvents were evaporated off. The crude product was dissolved in hydrochloric acid (0.1 M) and washed with dichloromethane. The aqueous phase was then basified with potassium hydroxide pellets and extracted with ether. Purification with flash column chromatography (CH_2Cl_2–MeOH–NH_4OH; 9:1:0.1 by vol.) gave **5** as a sticky white wax (300 mg; 0.82 mmol; 30%). ^{1}H-NMR (MeOD-d_4): 7.17–7.41 (6 H), 6.97 (m, 1 H), 6.83 (m, 1 H), 6.63 (m, 1 H), 5.33 (m, 1 H), 3.68 (s, 3 H), 3.21 (m, 2 H), 2.85 (m, 2 H), 2.67 (t, J = 7.5 Hz, 2 H), 2.46 (s, 3 H), 2.2 (m, 2 H). ^{13}C-NMR (MeOD-d_4): 172.3, 156.8, 142.1, 131.2, 120.6, 118.1, 115.3, 51.9, 34.0, 30.8. MS (TOF ES+): 360.19 [analytically (M + 1)] 359.16 (calculated). $[\alpha]_D$ = 84.0° (*c.* = 5, MeOH).

*2-(Methylthio)phenol (**6**).* Aryl methyl sulfide **6** was obtained from *o*-hydroxy-thiophenol (2.15 g; 17.0 mmol), potassium bicarbonate (1.35 g; 17.0 mmol) and methyl iodide (2.42 g; 17.0 mmol) by the same method as for **3**. Purification with flash column chromatography ($CHCl_3$) gave **6** as a yellow liquid (1.8 g; 12.9 mmol, 76%). ^{1}H-NMR ($CDCl_3$): 7.47 (dd, J = 1.6, 7.7 Hz,

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1 H), 7.24 (m, 1 H), 6.98 (dd, J = 1.3, 8.3 Hz, 1 H), 6.87 (dt, J = 1.3, 7.54 Hz, 1 H), 6.67 (s, 1 H), 2.31 (s, 3 H). ^{13}C-NMR ($CDCl_3$): 154.2, 132.7, 128.6, 119.1, 112.4, 17.3. MS (TOF ES+): 141.18 [analytically (M + 1)] 140.03 (calculated).

*(2-((R)-3-chloro-1-phenylpropoxy)phenyl)(methyl)sulfane (**7**).* Compound **7** was obtained from **6** (1.00 g; 7.1 mmol), (*S*)-3-chloro-1-phenylpropan-1-ol (2.44 g; 14.3 mmol), triphenyl phosphine (3.75 g, 14.3 mmol) and DEAD (2.44 g; 14.3 mmol) under Mitsunobu conditions similar to those described for **4**. Purification with flash column chromatography (hexanes–EtOAc; 10: 1, v/v), gave **7** as a thick colorless oil (1.0 g; 3.4 mmol; 48%). ^{1}H-NMR ($CDCl_3$): 7.18–7.38 (5 H), 7.05 (m, 1 H), 6.86 (m, 1 H), 6.61 (m, 1 H), 5.40 (m, 1 H), 3.87 (m, 1 H), 3.87 (m, 1 H), 3.61 (m, 1 H), 2.47 (m, 1 H), 2.37 (s, 3 H), 2.19 (m, 1 H). ^{13}C-NMR ($CDCl_3$): 172.8, 156.5, 142.4, 132.8, 129.2, 128.6, 128.1, 127.7, 126.6, 125.4, 125.4, 125.1, 121.9, 112.8, 80.1, 52.2, 48.5, 34.9, 37.5, 36.4, 29.1. MS (TOF ES+): 293.23 [analytical (M + 1)] 292.07 (calculated).

*(R)-3-(2-(Methylthio)phenoxy)-N-methyl-3-phenylpropan-1-amine ((R)-thionisoxetine, **1**).* **7** (900 mg; 3.1 mmol) was heated with methylamine (10 ml; 2 M in THF) as described for the preparation of **5**. Purification by flash column chromatography (CH_2Cl_2–MeOH–NH_4OH; 9: 1: 0.1 by vol.) gave **8** as a sticky brown oil (350 mg; 1.2 mmol, 51%). ^{1}H-NMR (MeOD-d_4): 7.16–7.39 (5 H), 7.07 (m, 1 H), 6.86 (m, 1 H), 6.59 (m, 1 H), 5.32 (m, 1 H), 3.61 (s, 1 H), 2.83 (m, 2 H), 2.42 (s, 3 H), 2.40 (s, 3 H), 2.24 (m, 1 H), 2.10 (m, 1 H). ^{13}C-NMR (MeOD-d_4): 172.3, 156.0, 141.1, 130.0, 128.7, 128.6, 127.4, 127.2, 126.0, 125.8, 125.8, 123.9, 121.2, 113.7, 79.2, 51.9, 48.1, 37.8, 36.5, 35.9, 27.2. MS (TOF ES+): 288.16 [analytically (M + 1)] 287.13 (calculated). $[\alpha]_D$ = 80.7° (*c.* = 0.5, MeOH).

Radiochemistry

Materials and general procedures. [^{11}C]Methane was produced at the Karolinska Hospital with a PETtrace cyclotron (GE Medical Sciences) using 16 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction on nitrogen containing hydrogen (10%). [^{11}C]methane was isolated from the target gas on a cooled [N_2 (l)] Porapak Q trap and subsequently converted into [^{11}C]methyl iodide by radical iodination in a recirculation system.[41]

Preparative HPLC was performed using a reverse phase μ-Bondapak C-18 column (300 × 7.8 mm, 10 μm; waters) eluted with mobile phase system A (MeCN–aq. HCO_2NH_4, (0.1 M), 35:65 v/v). The column outlet was connected with an UV absorbance detector (λ = 254 nm) in series with a GM-tube for radiation detection.

The radiochemical purity of [^{11}C]**1** was determined by reverse phase HPLC equipped with a μ-Bondapak C-18 column (300 × 3.9 mm; 10 μm; Waters) and

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an absorbance detector ($\lambda = 254$) in series with a β-flow detector (Beckman) for radiation detection. The HPLC column was eluted with mobile phase system B (MeCN–aq. H_3PO_4 (10 mM); 35:65 v/v). The retention time of **1** was 5 min.

The specific radioactivity was determined by HPLC using a μ-Bondapak C-18 column (300 × 3.9 mm; 10 μm; Waters) eluted with mobile phase system C (MeCN–H_3PO_4 (50 mM); 35:65 v/v). The level of non-radioactive precursor (**5**) present in the formulated solution was determined under the same conditions.

(R)-[S-methyl-^{11}C]-3-(2-(methylthio)phenoxy)-N-methyl-3-phenylpropan-1-amine. To a suspension of **5** (1 mg, 2.8 μmol) in THF (400 μl) at RT was added potassium *t*-butoxide (6μl; 1.0 M in THF). The reaction vial was sealed, mixed and kept at RT for 15–45 min until [^{11}C]methyl iodide was delivered to the vial in a helium stream. After entrapment of [^{11}C]methyl iodide was complete, mobile phase (system A; 600 μl) was added to the crude reaction mixture which was then injected onto the preparative HPLC column. The product fraction which eluted at 9 min was collected and concentrated to dryness. The residue was taken up in sterile disodium phosphate-buffered saline (pH 7.4; 8 ml) and filtered through a sterile Millipore filter (0.22 μm), yielding [^{11}C]**1** in sterile solution free from pyrogens.

c Log P calculations

c Log P and *c Log D* values of **1** and **2** were calculated with Pallas 3.0 for Windows software (CompuDrug International Inc., San Fransisco CA, USA).

PET measurements

The PET experimental procedure was similar to that employed for other radioligands. An ECAT EXACT HR PET system (Siemens) was run in 3D mode. The spatial resolution is about 3.8 mm full width half maximum. Images were displayed as 47 brain sections with a separation of 3.3 mm.[42]

One cynomolgus monkey (4900 g) was supplied by the National Institute for Infectious Disease Control (Solna, Stockholm). The study was approved by the Animal Ethics Committee of Northern Stockholm. The cynomolgus monkey was anaesthetized treated as previously described before and during the PET measurement,[19,43] in which the monkey was injected with [^{11}C]**1** (1.38 mCi; 51 MBq) as a bolus into the left sural vein. Radioactivity in brain was measured according to a pre-programmed sequence of frames during 93 min.

Regions of interest (ROI's) (cerebellum, caudate, mesencephalon, occipital cortex, thalamus and whole brain) were delineated as previously described in detail elsewhere.[19] Caudate, which is a region almost devoid of NET,[1,38,39]

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was initially used as a reference region for free radioligand concentration and non-specific binding in brain. The cerebellum was subsequently used as an alternative reference region, since it has also often been reported to have low NET density.[1, 38, 39] Binding ratios were thus calculated by dividing radioactivity in a given ROI with radioactivity in the caudate or cerebellum. To calculate specific binding, radioactivity in a reference region was subtracted from the radioactivity in the ROI.

Analysis of radiometabolites in plasma

The HPLC method used to determine the percentages of radioactivity in monkey plasma corresponding to unchanged radioligand and radiometabolites at set times during the course of a PET experiment was adapted from a method established for other PET radioligands.[19, 44]

Mixtures of phosphoric acid (10 mM) (*D*) and acetonitrile (*E*) were used as the mobile phase at 6.0 ml/min in the following elution program; 0–5.0 min, (*D*/*E*) 80/20–30/70; 5.0–7.5 min, (*D*/*E*) 30/70–80/20; 7.5–10 min (*D*/*E*) 80/20 isocratic. The radioactive peak having a retention time corresponding to **1** was integrated and its area expressed as a percentage of the sum of the areas of all detected radioactive peaks (decay-corrected).

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