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Synthesis and C-11 labeling of three potent norepinephrine transporter selective ligands ((*R*)-nisoxetine, lortalamine, and oxaprotiline) for comparative PET studies in baboons

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Abstract—Three potent antidepressants, (*R*)-nisoxetine, lortalamine, and oxaprotiline, with high affinity and high selectivity for the norepinephrine transporter (NET) were synthesized and radiolabeled with C-11 via [^{11}C]methylation. The reference compounds and their corresponding normethyl precursors were synthesized via multi-step synthetic approaches. The radiochemical syntheses were performed by simple alkylation of the corresponding normethyl precursors with no-carrier-added [^{11}C]CH_3I in DMF. After HPLC purification, (*R*)-[N–$^{11}CH_3$]nisoxetine, [^{11}C]lortalamine, and [^{11}C]oxaprotiline were obtained in 63–97% radiochemical yields, whereas (*R*)-[O–$^{11}CH_3$]nisoxetine was obtained in 23–29% radiochemical yields due to substantial formation of the undesired *N*-[^{11}C]methylated byproduct (64–70%). These C-11 labeled tracers allowed us to carry out comparative studies of NET in baboons with positron emission tomography (PET) and evaluate their potential as PET tracers for imaging brain NET.
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1. Introduction

The norepinephrine transporter (NET) is a member of a large family of Na^+/Cl^--dependent transporters, and is located at the pre-synaptic neurons of nerve endings.[1] Its functions include the removal of norepinephrine (NE) from the synaptic cleft to terminate the action of NE and avoid overstimulation and the recycling of NE into the intracellular vesicle for re-release.[2] Down regulation of the density of NET in the brain possibly resulting from low levels of NE has been associated with major depression.[3,4] Therapeutic agents with high affinity and selectivity toward NET have been developed to treat depressive illness.[5–7] Since other neurotransmitter transporters, including dopamine and serotonin transporters (DAT and SERT), have also been shown to be involved in major depression,[8,9] suitable radiotracers capable of mapping specific transporter systems in vivo would help to tease out the roles of individual transporters (NET, DAT, and SERT) underlying depressive illness. Furthermore, these target-specific radiotracers would facilitate the development of therapeutic agents for depressive illness, optimize the therapeutic dosage, and monitor the efficacy of treatment.

Potent NET-selective antidepressants have been the molecular models for the development of PET tracers for imaging the brain NET. Van Dort et al.[10] reported the synthesis of ^{11}C-labeled tricyclic antidepressants, desipramine and 2-hydroxydesipramine, but the in vivo evaluation was not included. McConathy et al.[11] reported the preparation of [^{11}C]talopram and [^{11}C]talsupram. Although both compounds displayed high affinity and selectivity for the human NET in vitro, the biodistribution studies in rats showed that the brain uptake of these two C-11 labeled tracers was low, which diminished their potential application for imaging brain NET. Our initial effort was focused on radiolabeled analogs of reboxetine (Fig. 1), a potent and NET-selective antidepressant, since they have high specificity and selectivity toward NET,[12] and reasonable calculated log *P* values (Table 1). We have developed synthetic strategies to prepare the precursor, followed by chiral resolution, and selective C-11 methylation to obtain a C-11 labeled methyl analog of reboxetine, (*S*,*S*)-[^{11}C]MRB (Fig. 1).[17] We and other research groups demonstrated its specific binding to NET in rats,[18] monkeys,[19] and baboons.[20] Recently (*S*,*S*)-[^{11}C]MRB has been used to

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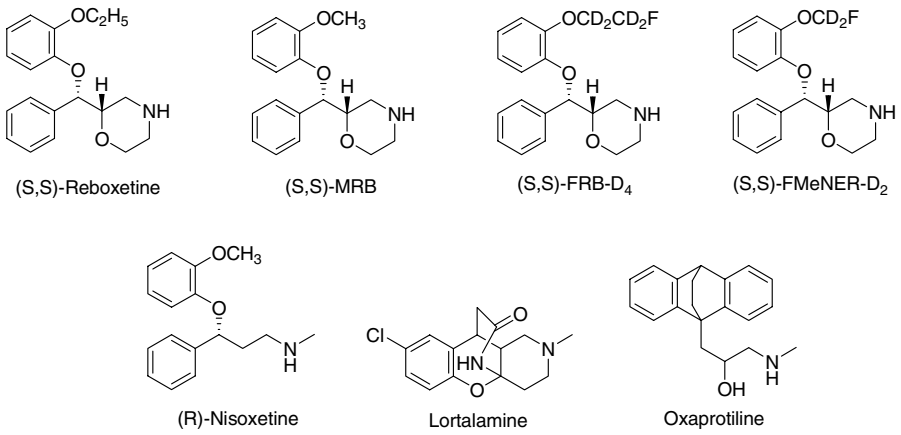


Figure 1. Chemical structures of selective NET ligands.

Table 1. NET inhibitors: lipophilicity and their affinity (nM) and selectivity for DAT, SERT, and NET

NET inhibitors	DAT	SERT	NET	DAT/NET	SERT/NET	CS Log P[f]
Reboxetine[a]	>10,000	1070	8.2	>1000	130	1.01
MRB[a]	>10,000	310	2.5	>4000	125	1.13
Nisoxetine[b]	360	1000	1	360	1000	1.74
Lortalamine[c]	>10,000	>100,000	<1[d]	>10,000	>100,000	2.08
Oxaprotiline[e]	4340 ± 30	3900 ± 100	4.9 ± 0.2	890	800	3.50

[a] IC_{50}.[12]
[b] K_i.[13]
[c] IC_{50}.[14]
[d] Lortalamine is a potent NET inhibitor with a potency higher than desipramine (fivefold).[14,15]
[e] K_d.[16]
[f] Lipophilicity was calculated as CS log P using the ChemSilico LLC (Tewksbury, MA) family of property prediction software (CSPredict).

assess the NET occupancy by therapeutic doses of reboxetine in humans.[21] The syntheses of F-18 labeled reboxetine analogs, including (*S*,*S*)-[^{18}F]FMeNER-D_2 and (*S*,*S*)-[^{18}F]FRB-D_4 (Fig. 1), were reported by Schou et al.[22] and our group,[23] respectively, and their potential values as NET radioligands were demonstrated in monkeys and baboons.

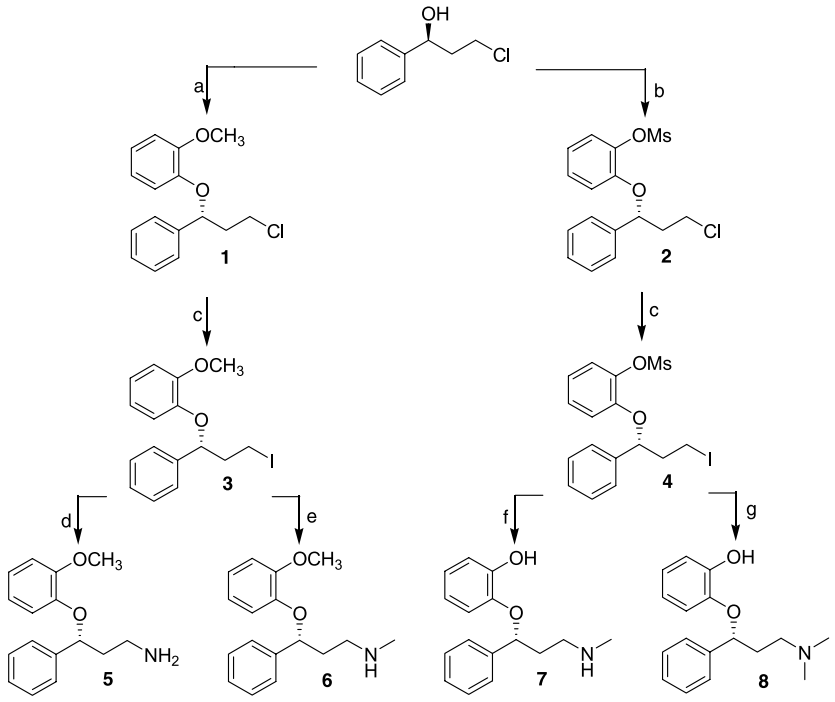
With promising results from radiolabeled reboxetine analogs in hand, we searched other potential NET-selective antidepressants for comparative PET studies. Nisoxetine is a potent and selective NET inhibitor.[13] [^{3}H]Nisoxetine has been used as the gold standard for the in vitro mapping of NET, and it has a structure similar to that of reboxetine (Fig. 1). Previously, nisoxetine has been C-11 labeled at the amino nitrogen as a *R*/*S* racemic mixture, and evaluated for its potential to study brain NET using a rodent model.[24] Although the biodistribution study of (*R*/*S*)-[N–^{11}CH_3]nisoxetine in mice showed only modest specific binding, we think C-11 labeled nisoxetine deserves further investigation since its ultimate value as a PET imaging agent has never been explored. In our study, we proposed to label the more potent (*R*)-enantiomer (IC_{50} (nM): (*R*)-nisoxetine, 5.8; (*S*)-nisoxetine, 18)[25] and label it at two different positions, namely (*R*)-[O–^{11}CH_3]nisoxetine and (*R*)-[N–^{11}CH_3]nisoxetine, to further evaluate its potential as a PET tracer to study NET system using a primate model. Since the metabolic profile for the labeled *N*- versus *O*-methyl compounds may be different, labeling nisoxetine at different positions may provide useful information. Two other potent NET-selective antidepressants, lortalamine[14] and oxaprotiline (Fig. 1),[16] with reasonable calculated log P values (Table 1) and structures different from those of reboxetine and nisoxetine, were also selected for comparative PET studies.

In this article, we report the synthesis of precursors ((*R*)-*N*-nornisoxetine, (*R*)-*O*-nornisoxetine, norlortalamine, and noroxaprotiline), and their C-11 methylation. Results of comparative PET studies of these tracers in baboon brain will be published separately.

2. Results and discussion

2.1. Synthesis of reference compounds and precursors

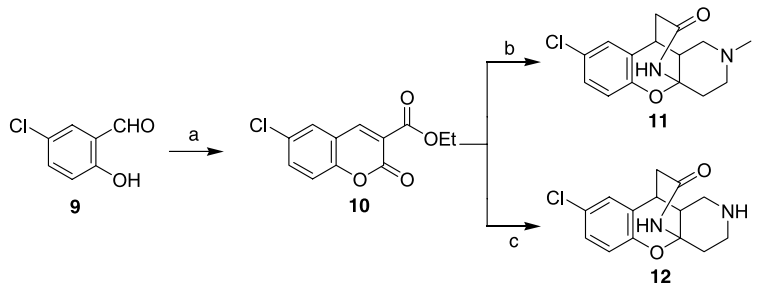
The syntheses of the reference compound, (*R*)-nisoxetine **6**, as well as two [^{11}C]methylation precursors, (*R*)-*N*-nornisoxetine **5** and (*R*)-*O*-nornisoxetine **7**, and a potential byproduct during [^{11}C]*O*-methylation ((*R*)-*N*,*N*-dimethyl-*O*-nornisoxetine **8**) are depicted in Scheme 1 by starting from the coupling of (*S*)-3-chloro-1-phenyl-1-propanol with 2-substituted phenols. The reaction of (*S*)-3-chloro-1-phenyl-1-propanol with guaiacol and 2-mesyloxyphenol under Mitsunobu conditions[26] afforded (*R*)-1-chloro-3-(2-methoxyphenoxy)-3-phenylpropane **1** and (*R*)-1-chloro-3-(2-mesyloxyphenoxy)-3-phenylpropane **2** in 90% and 62% yields, respectively. The (*S*)



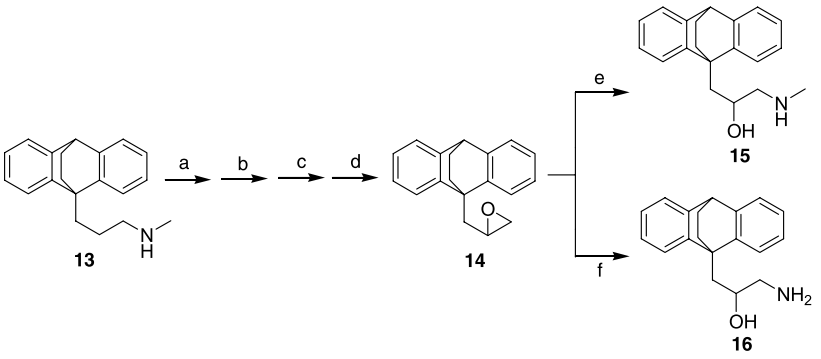
Scheme 1. Syntheses of (*R*)-enantiomers of *N*-nornisoxetine **5**, nisoxetine **6**, *O*-nornisoxetine **7**, and *N*,*N*-dimethyl-*O*-nornisoxetine **8**. Reagents: (a) diethyl azodicarboxylate, PPh_3, guaiacol, Et_2O; (b) diethyl azodicarboxylate, PPh_3, 2-mesyloxyphenol, Et_2O; (c) NaI, acetone; (d) NH_4OH, MeOH; (e) CH_3NH_2, THF; (f) (1) CH_3NH_2, THF; (2) NaOH, MeOH; (g) (1) $(CH_3)_2NH$, THF; (2) NaOH, MeOH.

configuration of the benzylic carbon in (*S*)-3-chloro-1-phenyl-1-propanol was inverted after Mitsunobu coupling reaction to furnish the (*R*) form of aryl benzyl ethers, as demonstrated by Srebnik et al.[27] and Liu et al.[28] Compounds **1** and **2** were then converted into their corresponding iodo-counterparts ((*R*)-1-iodo-3-(2-methoxyphenoxy)-3-phenylpropane **3** and (*R*)-1-iodo-3-(2-mesyloxyphenoxy)-3-phenylpropane **4**) in quantitative yields after overnight refluxing with excess NaI in acetone. The treatment of **3** with methanolic ammonium hydroxide at room temperature afforded (*R*)-*N*-nornisoxetine **5** in 88% yield, whereas (*R*)-nisoxetine **6** was obtained in 79% after the treatment of **3** with methylamine at room temperature. (*R*)-Nisoxetine **6** has been synthesized previously directly from compound **1** under reflux at 130 °C in the presence of excess methylamine.[27,28] However, the interconversion of chloride to iodide provided a better leaving group, iodide ion, and therefore harsh reaction conditions used for the nucleophilic substitution reaction could be avoided. *O*-Nornisoxetine **7** and *N*,*N*-dimethyl-*O*-nornisoxetine **8** were obtained by the treatment of **4** with excess methylamine and dimethylamine, respectively, at room temperature followed by the removal of the mesyl protecting group with aqueous sodium hydroxide.

Lortalamine **11** and norlortalamine **12** were synthesized from ethyl 6-chloro-2-oxo-2*H*-1-benzopyran-3-carboxylate **10** in a one-pot two-step procedure as shown in Scheme 2. Compound **10** was prepared from 5-chlorosalicylaldehyde **9** and diethyl malonate via Knoevenagel condensation and transesterification according to the published procedure.[29] The reaction of **10** with 1-methyl-4-piperidone and ammonium acetate in ethanol generated an intermediate β-keto ester[30] that was subsequently decarboxylated to yield lortalamine in 53% yield. Norlortalamine was synthesized following a similar procedure using 1-Boc-4-piperidone. Previously, norlortalamine was prepared via a similar procedure using 1-benzyl-4-piperidone, followed by *N*-debenzylation



Scheme 2. Syntheses of lortalamine **11** and norlortalamine **12**. Reagents: (a) diethyl malonate, piperidine, acetic acid, ethanol; (b) (1) 1-methyl-4-piperidone, ammonium acetate, ethanol, (2) HCl; (c) (1) 1-Boc-4-piperidone, ammonium acetate, ethanol, (2) HCl.



Scheme 3. Syntheses of oxaprotiline **15** and noroxaprotiline **16**. Reagents: (a) HCOH, $NaBH_3CN$, H_2O, acetic acid, CH_3CN; (b) CH_3I, AcOEt; (c) (1) Ag_2O, CH_3OH, H_2O, (2) 160 °C, reduced pressure; (d) peracetic acid, CH_2Cl_2, Na_2CO_3; (e) CH_3NH_2, THF; (f) NH_4OH, MeOH.

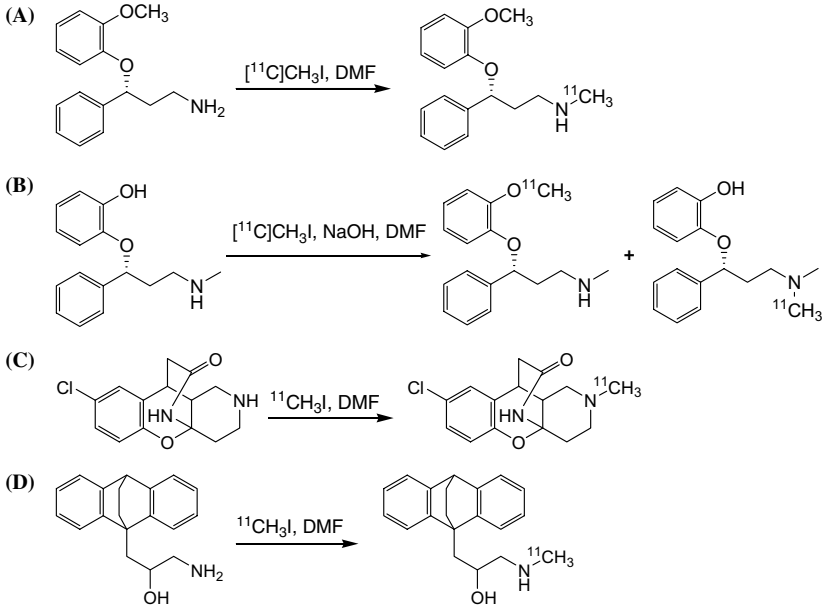
with benzyl chloroformate and acid hydrolysis.[31] The use of 1-Boc-4-piperidone for the synthesis of norlortalamine provided a much simpler procedure since the Boc protecting group was simultaneously removed during acid decarboxylation.

The synthesis of oxaprotiline **15** and noroxaprotiline **16** is depicted in Scheme 3. 9-(2,3-Epoxypropyl)-9,10-dihydro-9,10-ethanoanthracene **14** was prepared from maprotiline **13** via reductive methylation, formation of alkyl trimethyl quaternary ammonium salt, Hofmann elimination, and epoxidation according to literature procedures.[32] The treatment of **14** with excess methylamine or ammonium hydroxide yielded oxaprotiline (83%) and noroxaprotiline (56%), respectively. Racemic [^{11}C]oxaprotiline was then synthesized and subjected to initial evaluation in baboons with PET. Our PET studies in baboon brain showed that the distribution did not match with the known NET distribution, which is consistent with a bio-distribution study in mice reported previously as an abstract.[33]

2.2. Radiolabeling

The radiochemical syntheses of (*R*)-[*N*-$^{11}CH_3$]nisoxetine, (*R*)-[O–$^{11}CH_3$]nisoxetine, [^{11}C]lortalamine, and [^{11}C]oxaprotiline by simple alkylation of the normethyl precursors with no-carrier-added [^{11}C]CH_3I are shown in Scheme 4, and the results are listed in Table 2. Simple *N*-[^{11}C]methylation of (*R*)-*N*-nornisoxetine, norlortalamine, and noroxaprotiline in DMF provided (*R*)-[N–$^{11}CH_3$]nisoxetine, [^{11}C]lortalamine, and [^{11}C]oxaprotiline in high yields (63–97%, EOB). On the contrary, the radiochemical yields of (*R*)-[O–$^{11}CH_3$]nisoxetine were relatively lower (23–29%, EOB) due to substantial formation of the undesired *N*-[^{11}C]methylated byproduct, [^{11}C]*N*,*N*-dimethyl-*O*-nornisoxetine, in 64–70% (EOB). The radiochemical purities of C-11 labeled (*R*)-nisoxetine, lortalamine, and oxaprotiline were >99% and the specific activities ranged from 1.7 to 3.7 Ci/μmol.

The structure of (*R*)-nornisoxetine is very similar to that of (*S*,*S*)-desethylreboxetine. However, when we



Scheme 4. Radiochemical syntheses of (A) (*R*)-[N–$^{11}CH_3$]nisoxetine; (B) [O–$^{11}CH_3$]nisoxetine; (C) [^{11}C]lortalamine; and (D) [^{11}C]oxaprotiline.

Table 2. Synthesis times, radiochemical yields, radiochemical purities, and specific activities of C-11 labeled (*R*)-nisoxetine, lortalamine, and oxaprotiline

Radiotracers	Synthesis time (min)	Radiochemical yield (%) at EOB	Radiochemical purity (%)	Specific activity (Ci/μmol) at EOB
(*R*)-[N–$^{11}CH_3$]Nisoxetine	39	63–72	>99	1.7–3.7
(*R*)-[O–$^{11}CH_3$]Nisoxetine	42	23–29[a]	>99	2.1–2.5
[^{11}C]Lortalamine	32	85–97	>99	2.2–3.1
[^{11}C]Oxaprotiline	36	67–78	>99	1.8–3.0

[a] Low radiochemical yields were due to substantial formation (64–70%) of *N*-[^{11}C]methylated byproduct, (*R*)-[^{11}C]*N*,*N*-dimethyl-*O*-nisoxetine.

prepared (*S*,*S*)-[^{11}C]MRB by [^{11}C]methylation of desethylreboxetine at the phenolic oxygen under the same condition that we used for the preparation of (*R*)-[O–$^{11}CH_3$]nisoxetine, high radiochemical yields (>60%) of (*S*,*S*)-[^{11}C]MRB were obtained without substantial formation of *N*-[^{11}C]methylated byproduct (<10%).[17] The difference of product yields and the amount of *N*-[^{11}C]methylated byproduct between preparation of (*R*)-[O–$^{11}CH_3$]nisoxetine and (*S*,*S*)-[^{11}C]MRB was probably due to different characteristics of the secondary amino nitrogen in each structure. Compared with that of (*R*)-nornisoxetine, the secondary amino nitrogen of (*S*,*S*)-desethylreboxetine was possibly less reactive due to limited flexibility from ring restriction, and therefore, less *N*-[^{11}C]methylated byproduct was formed during the radiosynthesis.

3. Conclusion

C-11 labeled NET-selective antidepressants, including (*R*)-[O–$^{11}CH_3$]nisoxetine, (*R*)-[N–$^{11}CH_3$]nisoxetine, [^{11}C]-lortalamine, and [^{11}C]oxaprotiline, were prepared in moderate to high radiochemical yields with high radiochemical purity and specific activity. These NET-selective radiotracers allow us to carry out comparative PET studies, and evaluate their potential as PET radiotracers for imaging brain NET using a nonhuman primate model.

4. Experimental

4.1. General methods

2-Mesyloxyphenol,[17] ethyl 6-chloro-2-oxo-2*H*-1-benzopyran-3-carboxylate **10**,[29] and 9-(2,3-epoxypropyl)-9,10-dihydro-9,10-ethanoanthracene **14**[32] were prepared according to the published procedures. All other chemicals were purchased from the Aldrich Chemical Company (Milwaukee, WI) and were used as received without further purification. Melting points were taken on a Fisher–Johns melting point apparatus (Fisher Scientific Co., Pittsburgh, PA) and were uncorrected. NMR spectra were recorded in $CDCl_3$ solution (unless specified) using a Bruker Avance 400 MHz NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) (Bruker Instruments Inc., Billerica, MA), and were reported in parts per million downfield from internal tetramethylsilane. The central peak of $CDCl_3$ signal at 77.0 ppm was used as the ^{13}C NMR reference. High resolution mass spectrometry (HRMS) experiments were performed at UCR Mass Spectrometry Facility (Riverside, CA) on a VG 7070 high resolution mass spectrometer. Radioactivity was measured in a Capintec CRC-712MV radioisotope calibrator (Capintec Inc., Ramsey, NJ). [^{11}C]CH_3I was prepared from [^{11}C]CO_2 in an automated PETtrace MeI Microlab (GE Medical Systems, Milwaukee, WI). [^{11}C]CO_2 was produced by the BNL JSW 1710 cyclotron via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using 100 ppm O_2 in N_2 as the target gas. The [^{11}C]CO_2 was first transformed into [^{11}C]CH_4 through Ni catalyzed hydrogenation, followed by the reaction with I_2 to give the no-carrier-added [^{11}C]CH_3I.

4.2. (*R*)-1-Chloro-3-(2-methoxyphenoxy)-3-phenylpropane (1)

This compound was synthesized via a modified literature procedure.[27,28] To a solution of (*S*)-3-chloro-1-phenyl-1-propanol (170 mg, 1 mmol), guaiacol (220 μL, 2 mmol), and triphenylphosphine (393 mg, 1.5 mmol) in ether (10 mL) was added diethyl azodicarboxylate (263 μL, 1.5 mmol) dropwise at −10 to −15 °C. After completion of addition, the mixture was stirred at −10 °C for 5 h. The mixture was then concentrated and purified by chromatography on silica gel eluting with 85:15 hexane/ethyl acetate to give the title compound **1** as a colorless oil (248 mg, 90%). ^{1}H NMR: δ 2.17–2.24 (m, 1H), 2.51–2.58 (m, 1H), 3.61–3.67 (m, 1H), 3.86–3.92 (m, 1H), 3.87 (s, 3H), 5.33 (dd, J = 8.7, 4.3 Hz, 1H), 6.70–6.74 (m, 2H), 6.80–6.90 (m, 2H), 7.24–7.29 (m, 1H), 7.30–7.36 (m, 2H), 7.37–7.41 (m, 2H); ^{13}C NMR: δ 41.3, 41.5, 55.9, 78.5, 112.0, 116.6, 120.6, 121.8, 126.0, 127.8, 128.6, 141.0, 147.4, 150.1. HRMS (DCI/NH_3) m/z calcd for $C_{16}H_{21}ClNO_2$ (MNH_4^+), 294.1260; found 294.1273.

4.3. (*R*)-1-Chloro-3-(2-mesyloxyphenoxy)-3-phenylpropane (2)

To a solution of (*S*)-3-chloro-1-phenyl-1-propanol (170 mg, 1 mmol), 2-mesyloxyphenol (376 mg, 2 mmol), and triphenylphosphine (393 mg, 1.5 mmol) in ether (10 mL) was added diethyl azodicarboxylate (263 μL, 1.5 mmol) dropwise at −10 to −15 °C. After completion of addition, the mixture was stirred at −10 °C for 5 h. The mixture was then concentrated and purified by chromatography on silica gel eluting with 70:30 hexane/ethyl acetate to give the title compound **2** as a colorless oil (211 mg, 62%). ^{1}H NMR: δ 2.28–2.34 (m, 1H), 2.53–2.59 (m, 1H), 3.25 (s, 3H), 3.57–3.62 (m, 1H), 3.82–3.88 (m, 1H), 5.48 (dd, J = 8.0, 5.0 Hz, 1H), 6.86 (dd, J = 8.3, 1.4 Hz, 1H), 6.93 (td, J = 7.9, 1.5 Hz,

1H), 7.10 (td, $J = 7.7$, 1.7 Hz, 1H), 7.27–7.36 (m, 2H), 7.37–7.46 (m, 4H); ^{13}C NMR: δ 38.2, 40.9, 41.0, 78.0, 115.9, 121.3, 124.0, 125.9, 128.0, 128.3, 128.9, 138.7, 139.6, 149.8. HRMS (DEI) *m*/*z* calcd for $C_{16}H_{17}ClO_4S$ (M^+), 340.0536; found 340.0526.

4.4. (*R*)-1-Iodo-3-(2-methoxyphenoxy)-3-phenylpropane (3)

A solution of **1** (237 mg, 0.86 mmol) and sodium iodide (20 g, 133 mmol) in acetone (50 mL) was refluxed for 16 h. After cooling, the solvent was removed under reduced pressure. Water (50 mL) was added to the residue and the mixture was extracted with ethyl acetate (2×50 mL). The combined ethyl acetate extract was washed with water (2×50 mL), dried over sodium sulfate, and concentrated to yield the title compound **3** as a colorless oil (314 mg, 99%). ^{1}H NMR: δ 2.30–2.38 (m, 1H), 2.57–2.66 (m, 1H), 3.31–3.36 (m, 1H), 3.47–3.53 (m, 1H), 3.90 (s, 3H), 5.25 (dd, $J = 8.4$, 4.3 Hz, 1H), 6.73–6.80 (m, 2H), 6.88–6.97 (m, 2H), 7.29–7.46 (m, 5H); ^{13}C NMR: δ 2.6, 42.2, 55.9, 81.4, 112.0, 116.7, 120.6, 121.8, 126.0, 127.8, 128.6, 140.8, 147.4, 150.2. HRMS (DEI) *m*/*z* calcd for $C_{16}H_{17}IO_2$ (M^+), 368.0273; found 368.0260.

4.5. (*R*)-1-Iodo-3-(2-mesyloxyphenoxy)-3-phenylpropane (4)

A solution of **2** (197 mg, 0.58 mmol) and sodium iodide (20 g, 133 mmol) in acetone (50 mL) was refluxed for 16 h. After cooling, the mixture was worked up by the same procedure as for compound **3** to yield the title compound **4** as a pale yellow oil (251 mg, 100%). ^{1}H NMR: δ 2.35–2.41 (m, 1H), 2.53–2.60 (m, 1H), 3.21–3.25 (m, 1H), 3.24 (s, 3H), 3.39–3.44 (m, 1H), 5.35 (dd, $J = 7.6$, 5.0 Hz, 1H), 6.87 (dd, $J = 8.3$, 1.4 Hz, 1H), 6.93 (td, $J = 7.8$, 1.5 Hz, 1H), 7.11 (td, $J = 7.9$, 1.6 Hz, 1H), 7.28–7.47 (m, 6H); ^{13}C NMR: δ 1.5, 38.3, 41.7, 80.9, 115.9, 121.3, 124.1, 125.9, 128.0, 128.3, 128.9, 138.7, 139.4, 149.8. HRMS (DCI/NH_3) *m*/*z* calcd for $C_{16}H_{21}INO_4S$ (MNH_4^+), 450.0235; found 450.0221.

4.6. (*R*)-3-(2-Methoxyphenoxy)-3-phenyl-1-propylamine ((*R*)-*N*-nornisoxetine, 5)

A solution of **3** (134 mg, 0.36 mmol) and aqueous ammonium hydroxide (28 wt. %, 12 mL) in methanol (18 mL) was stirred at room temperature for 2 days. After removing the solvent under reduced pressure, water (20 mL) was added to the residue and the pH was adjusted to 2 with concentrated HCl. The mixture was washed with ether (2×20 mL) and the ether fractions discarded. The pH of the aqueous layer was adjusted to 12 with 25% sodium hydroxide and then extracted with ether (2×20 mL). The combined ethereal fractions were washed with water (2×20 mL), dried over sodium sulfate, and concentrated to give the title compound **5** as a pale yellow oil (82 mg, 88%). ^{1}H NMR: δ 1.61 (br, 2H), 1.94–2.02 (m, 1H), 2.18–2.25 (m, 1H), 2.94 (m, 2H), 3.87 (s, 3H), 5.22 (dd, $J = 8.5$, 4.4 Hz, 1H), 6.65–6.73 (m, 2H), 6.80–6.88 (m, 2H), 7.20–7.38 (m, 5H); ^{13}C NMR: δ 39.0, 42.2, 55.8, 80.2, 111.9, 116.3, 120.5, 121.4, 125.9, 127.4, 128.4, 141.9, 147.5, 150.0. HRMS (DEI) *m*/*z* calcd for $C_{16}H_{20}NO_2$ (MH^+), 258.1494; found 258.1488.

4.7. (*R*)-*N*-Methyl-3-(2-methoxyphenoxy)-3-phenyl-1-propylamine ((*R*)-nisoxetine, 6)

A solution of **3** (268 mg, 0.73 mmol) and aqueous methylamine (40 wt. %, 10 mL) in THF (5 mL) was stirred at room temperature for 3 h. After removing the solvent under reduced pressure, the residue was worked up by the same procedure as for compound **5** to give the title compound **6** as a pale yellow oil (156 mg, 79%). ^{1}H NMR: δ 1.43 (br, 1H), 2.00–2.08 (m, 1H), 2.21–2.29 (m, 1H), 2.43 (s, 3H), 2.73–2.86 (m, 2H), 3.88 (s, 3H), 5.20 (dd, $J = 8.4$, 4.7 Hz, 1H), 6.64–6.72 (m, 2H), 6.80–6.88 (m, 2H), 7.23–7.40 (m, 5H); ^{13}C NMR: δ 36.3, 38.2, 48.6, 55.9, 80.6, 112.0, 116.3, 120.6, 121.4, 125.9, 127.4, 128.4, 141.8, 147.5, 150.0. HRMS (DEI) *m*/*z* calcd for $C_{17}H_{22}NO_2$ (MH^+), 272.1650; found 272.1641.

4.8. (*R*)-*N*-Methyl-3-(2-hydroxyphenoxy)-3-phenyl-1-propylamine ((*R*)-*O*-nornisoxetine, 7)

A solution of **4** (136 mg, 0.32 mmol) and aqueous methylamine (40 wt. %, 10 mL) in THF (5 mL) was stirred at room temperature for 3 h. After removing the solvent under reduced pressure, methanol (10 mL) and sodium hydroxide (2.5 N, 8 mL) were added to the residue and the mixture was stirred at room temperature for 24 h. The mixture was neutralized with concentrated HCl and then concentrated under reduced pressure. Water (20 mL) was added to the residue and the pH was adjusted to 2 with concentrated HCl. The mixture was washed with ether (2×20 mL) and the ether fractions discarded. The pH of the aqueous layer was adjusted to 8 with saturated aqueous sodium bicarbonate solution and then extracted with ether (2×20 mL). The combined ethereal fractions were washed with water (2×20 mL), dried over sodium sulfate, and concentrated to give the title compound **7** as a pale yellow solid (64 mg, 80%): mp 125–128 °C. ^{1}H NMR: δ 1.85–1.95 (m, 1H), 2.20–2.30 (m, 1H), 2.54 (s, 3H), 2.85–2.95 (m, 1H), 3.00–3.10 (m, 1H), 4.77 (dd, $J = 10.4$, 1.8 Hz, 1H), 6.38–6.49 (m, 2H), 6.87–6.92 (m, 2H), 7.29–7.42 (m, 5H); ^{13}C NMR: δ 35.8, 37.0, 49.9, 85.1, 117.1, 118.2, 122.4, 124.8, 126.6, 127.8, 128.3, 142.4, 146.0, 151.1. HRMS (DEI) *m*/*z* calcd for $C_{16}H_{19}NO_2$ (M^+), 257.1416; found 257.1412.

4.9. (*R*)-*N*,*N*-Dimethyl-3-(2-hydroxyphenoxy)-3-phenyl-1-propylamine ((*R*)-*N*,*N*-dimethyl-*O*-nornisoxetine, 8)

A solution of **4** (98 mg, 0.23 mmol) and dimethylamine (2 M in THF, 12 mL) was stirred at room temperature for 3 h. After removing the solvent under reduced pressure, the residue was worked up by the same procedure as for compound **7** to give the title compound **8** as a pale yellow solid (60 mg, 97%): mp 78–82 °C. ^{1}H NMR: δ 1.78–1.86 (m, 1H), 2.31–2.38 (m, 1H), 2.42 (s, 6H), 2.64–2.79 (m, 2H), 4.73 (dd, $J = 10.7$, 2.2 Hz, 1H), 6.35–6.50 (m, 2H), 6.87–6.92 (m, 2H), 7.27–7.37 (m, 5H); ^{13}C NMR: δ 34.8, 44.7, 57.0, 84.3, 117.1, 118.2,

122.7, 124.9, 126.8, 127.9, 128.3, 142.3, 145.7, 151.1. HRMS (DEI) *m/z* calcd for $C_{17}H_{21}NO_2$ (M^+), 271.1572; found 271.1576.

4.10. (+/−)-8-Chloro-1,2,3,4,10,10a-hexahydro-2-methyl-4a,10-(iminoethano)-4a*H*-[1]benzopyrano[3,2-*c*]pyridin-12-one (lortalamine, 11)

The following procedure was modified from the literature procedure.[31] A solution of ethyl 6-chloro-2-oxo-2*H*-1-benzopyran-3-carboxylate **10** (1.26 g, 5 mmol), 1-methyl-4-piperidone (566 mg, 5 mmol), and ammonium acetate (771 mg, 10 mmol) in ethanol (12 mL) was refluxed for 8 h. After cooling to ambient temperature, the volatile solvent was removed under reduced pressure. Concentrated HCl (10 mL) was added to the residue, and the mixture was refluxed for 30 min. The mixture was then basified to pH 12 with NaOH (25%) and extracted with chloroform (2 × 70 mL). The combined chloroform fractions were washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude product was purified by recrystallization from ethanol to yield the title compound **11** as a white crystalline solid (770 mg, 53%); mp 248–249 °C (literature[31] 245 °C). ^{1}H NMR: δ 1.80 (t, J = 11.5 Hz, 1H), 1.94–2.09 (m, 2H), 2.27 (s, 3H), 2.28–2.37 (m, 2H), 2.58 (dd, J = 17.3, 1.6 Hz, 1H), 2.70 (dd, J = 11.3, 3.9 Hz, 1H), 2.74–2.83 (m, 2H), 2.87–2.90 (m, 1H), 6.75 (d, J = 8.7 Hz, 1H), 6.94 (br, 1H), 7.04 (d, J = 2.5 Hz), 7.12 (dd, J = 8.7, 2.5 Hz, 1H); ^{13}C NMR: δ 32.6, 36.1, 37.4, 41.9, 45.6, 50.8, 54.6, 81.1, 118.8, 124.7, 126.4, 128.6, 128.7, 148.4, 170.8. HRMS (DEI) *m/z* calcd for $C_{15}H_{17}ClN_2O_2$ (M^+), 292.0979; found 292.0970.

4.11. (+/−)-8-Chloro-1,2,3,4,10,10a-hexahydro-4a,10-(iminoethano)-4a*H*-[1]benzopyrano[3,2-c]pyridin-12-one (norlortalamine, 12)

To a solution of ethyl 6-chloro-2-oxo-2*H*-1-benzopyran-3-carboxylate **10** (878 mg, 3 mmol) and 1-Boc-4-piperidone (597 mg, 3 mmol) in ethanol (100 mL) was added ammonium acetate (462 mg, 6 mmol). The mixture was refluxed for 15 h. After cooling to ambient temperature, volatile solvent was removed under reduced pressure. Concentrated HCl (8 mL) was added to the residue and the mixture was refluxed for 30 min. The mixture was then basified to pH 12 with NaOH (25%) and extracted with methylene chloride (2 × 75 mL). The combined methylene chloride fractions were washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude product was purified by recrystallization from methanol to yield the title compound **12** as a white solid (206 mg, 25%); mp 258–260 °C (literature[31] 252–254 °C). ^{1}H NMR: δ 1.81–1.95 (m, 2H), 2.04–2.09 (m, 1H), 2.17–2.23 (m, 1H), 2.42 (t, J = 12.0 Hz, 1H), 2.59 (dd, J = 17.4, 1.4 Hz, 1H), 2.80 (dd, J = 17.4, 5.1 Hz, 1H), 2.87–3.09 (m, 4H), 6.63 (br, 1H), 6.77 (d, J = 8.7 Hz), 7.05 (d, J = 2.5 Hz), 7.14 (dd, J = 8.7, 2.5 Hz, 1H); ^{13}C NMR: δ 32.5, 36.9, 38.7, 41.8, 42.0, 45.6, 81.9, 118.8, 124.6, 126.4, 128.7, 128.8, 148.4, 170.5. HRMS (DEI) *m/z* calcd for $C_{14}H_{15}ClN_2O_2$ (M^+), 278.0822; found 278.0818.

4.12. 9-(2-Hydroxy-3-methylaminopropyl)-9,10-dihydro-9,10-ethanoanthracene (oxaprotiline, 15)

A solution of 9-(2,3-epoxypropyl)-9,10-dihydro-9,10-ethanoanthracene **14** (135 mg, 0.51 mmol) and aqueous methylamine (40 wt. %, 10 mL) in THF (5 mL) was stirred at room temperature for 24 h. After removing the solvent under reduced pressure, HCl (2 N, 30 mL) was added to the residue. The resulting solution was washed with ether (2 × 30 mL) and the ether fractions were discarded. The pH of the aqueous layer was adjusted to 12 with NaOH (50%) and then extracted with ether (30 mL). The phases were separated, and the ethereal fraction was washed with water (2 × 30 mL), dried over magnesium sulfate, and concentrated under reduced pressure. The residue was then transformed into its HCl salt to give 140 mg of white solid (83%); mp 243–244 °C (literature[34] 237–239 °C). ^{1}H NMR (D_2O): δ 1.59–1.68 (m, 1H), 1.72–1.89 (m, 3H), 2.69 (dd, J = 14.4, 6.8 Hz, 1H), 2.78–2.85 (m, 1H), 2.84 (s, 3H), 3.35 (dd, J = 12.5, 10.1 Hz, 1H), 3.49 (dd, J = 12.5, 2.5 Hz, 1H), 4.44 (br, 1H), 4.46–4.54 (m, 1H), 7.14–7.26 (m, 5H), 7.38–7.44 (m, 3H); ^{13}C NMR (D_2O): δ 26.9, 29.2, 32.7, 35.7, 43.5, 44.2, 54.9, 64.2, 120.8, 120.9, 123.6, 123.9, 125.6, 125.7, 125.9, 144.6, 144.9, 145.0, 145.2. HRMS (DEI) *m/z* calcd for $C_{20}H_{23}NO$ (M^+), 293.1780; found 293.1790.

4.13. 9-(2-Hydroxy-3-aminopropyl)-9,10-dihydro-9,10-ethanoanthracene (noroxaprotiline, 16)

A solution of 9-(2,3-epoxypropyl)-9,10-dihydro-9,10-ethanoanthracene **14** (450 mg, 1.72 mmol) and aqueous ammonium hydroxide (28 wt. %, 12 mL) in methanol (30 mL) was stirred at room temperature for 2 days. After removing the solvent under reduced pressure, the residue was recrystallized from ethyl acetate to give the title compound **14** as a white solid (270 mg, 56%); mp 183–184 °C (literature[34] 176–177 °C). ^{1}H NMR: δ 1.61–1.90 (m, 4H), 2.05 (br, 3H), 2.55–2.65 (m, 2H), 2.79 (dd, J = 12.4, 9.3 Hz, 1H), 3.20 (dd, J = 12.4, 3.1 Hz, 1H), 4.05–4.12 (m, 1H), 4.28 (br, 1H), 7.05–7.15 (m, 5H), 7.23–7.29 (m, 2H), 7.41 (d, J = 7.1 Hz, 1H); ^{13}C NMR: δ 27.8, 30.1, 36.5, 44.4, 44.5, 48.9, 68.9, 120.9, 121.3, 123.2, 123.4, 125.1, 125.2, 125.3, 144.7, 145.1, 145.3. HRMS (DEI) *m/z* calcd for $C_{19}H_{21}NO$ (M^+), 279.1623; found 279.1619.

4.14. Radiosynthesis of (*R*)-[N–^{11}CH$_3$]nisoxetine

A V-shaped three-necked glass vessel containing 1 mg (3.9 μmol) of (*R*)-*N*-nornisoxetine **5** in DMF (0.25 mL) was dipped into a dry ice/acetonitrile bath 10 min before the release of [^{11}C]CH_3I from the GE PETtrace MeI Microlab. [^{11}C]CH_3I was transferred into the V-shaped vessel using Ar as the carrier gas. After the radioactivity trapped inside the vessel reached its maximum as monitored by an NaI detector, the vessel was sealed and heated in an oil bath at 100 °C for 7 min. At the end of reaction, water (1 mL) was added to the reaction mixture, and the product was separated using a Phenomenex Luna C-18 semipreparative column (250 mm × 10 mm, 5 μm). This column was connected to a Knauer HPLC system (Sonntek Inc., Woodcliff Lake, NJ) equipped with a

model K-500 pump, a model 87 variable wavelength monitor (set at 254 nm), an NaI radioactivity detector and two Hewlett–Packard 3390A integrators, and was eluted with 250:250:500:8:3.2 acetonitrile/methanol/water/triethylamine/acetic acid at a flow rate of 6 mL/min. The fraction containing (*R*)-[N–$^{11}CH_3$]nisoxetine was collected from 14.5 to 15.9 min after injection. The collected eluate was rotary evaporated, and then coevaporated with acetonitrile. The residue was dissolved in sterile water (4 mL) and passed through a 0.22 μm Millipore filter into a sterile vial to make the final injectate for baboon studies with PET.

4.15. Radiosynthesis of (*R*)-[O–$^{11}CH_3$]nisoxetine

(*R*)-[O–$^{11}CH_3$]nisoxetine was prepared by using a similar radiosynthetic procedure as for the preparation of (*R*)-[N–$^{11}CH_3$]nisoxetine by starting with 1 mg (3.9 μmol) of (*R*)-*O*-nornisoxetine **7** and NaOH (5 N, 9 μL) in DMF (0.2 mL). After 10 min incubation at 100 °C, the reaction mixture was worked up by the same procedure described above.

4.16. Radiosynthesis of [^{11}C]lortalamine

[^{11}C]Lortalamine was prepared by using a similar radiosynthetic procedure as for the preparation of (*R*)-[N–$^{11}CH_3$]nisoxetine by starting with 1 mg (3.6 μmol) of norlortalamine **12** in DMF (0.25 mL). After 5 min incubation at 100 °C, the reaction mixture was worked up by the same procedure described above. HPLC solvent was 30:70 CH_3CN/0.2 M $HCOONH_4$ at a flow rate of 5.0 mL/min. The fraction containing [^{11}C]lortalamine was collected from 9.4 to 11.9 min after injection.

4.17. Radiosynthesis of [^{11}C]oxaprotiline

[^{11}C]Oxaprotiline was prepared by using a similar radiosynthetic procedure as for the preparation of (*R*)-[N–$^{11}CH_3$]nisoxetine by starting with 0.8 mg (2.9 μmol) of noroxaprotiline **16** in DMF (0.25 mL). After 5 min incubation at 100 °C, the reaction mixture was worked up by the same procedure described above. HPLC solvent was 35:65 CH_3CN–0.1 M $HCOONH_4$ at a flow rate of 5.0 mL/min. The fraction containing [^{11}C]oxaprotiline was collected from 14.8 to 16.2 min after injection.

4.18. Quality control of purified C-11 labeled (*R*)-nisoxetine, lortalamine, and oxaprotiline

The specific activities of C-11 labeled (*R*)-nisoxetine, lortalamine, and oxaprotiline were determined by the UV absorbance of the radioactive peaks as compared with standard curves of unlabeled reference compounds. The radiochemical purities were determined by an analytical radio-HPLC system and a TLC system in the presence of the unlabeled reference compound as a carrier. The analytical radio-HPLC system consisted of a Phenomenex Luna C-18 analytical column (250 mm × 4.6 mm, 5 μm), a Knauer model K-1001 pump, a Knauer model K-1500 solvent organizer, a Knauer model K2501 UV detector (254 nm), an NaI radioactivity detector and two Hewlett–Packard 3390A integrators. The analytical column was eluted with 250:250:500:8:3.2 acetonitrile/methanol/water/triethylamine/acetic acid at 1.5 mL/min for [^{11}C]nisoxetine, 25:75 acetonitrile/0.2 M ammonium formate at 1 mL/min for [^{11}C]lortalamine, and 35:65 acetonitrile/0.05 M ammonium formate at 1.4 mL/min for [^{11}C]oxaprotiline. The retention times of nisoxetine, lortalamine, and oxaprotiline were 9.21, 8.21, and 6.37 min, respectively. For TLC analysis, Macherey–Nagel polygram sil G/UV_{254} plastic-back TLC plate was used. The TLC plate was developed with 10:0.3 methanol/ammonium hydroxide for [^{11}C]lortalamine and 10:0.5 methanol/ammonium hydroxide for both [^{11}C]nisoxetine and [^{11}C]oxaprotiline. The developed TLC plate was scanned by using a Bioscan System 200 imaging scanner. The R_fs of nisoxetine, lortalamine, and oxaprotiline were 0.37, 0.34, and 0.30, respectively.

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