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Articles

Synthesis and in Vivo Evaluation of Fluorine-18 and Iodine-123 Labeled 2β-Carbo(2-fluoroethoxy)-3β-(4′-((*Z*)-2-iodoethenyl)phenyl)nortropane as a Candidate Serotonin Transporter Imaging Agent

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2β-Carbo(2-fluoroethoxy)-3β-(4′-((*Z*)-2-iodoethenyl)phenyl)nortropane (βFE*p*ZIENT, **1**) was synthesized as a serotonin transporter (SERT) imaging agent for both positron emission tomography (PET) and single photon emission computerized tomography (SPECT). The binding affinity of **1** to human monoamine transporters showed a high affinity for the SERT (K_i = 0.08 nM) with respect to the dopamine transporter (DAT) (K_i = 13 nM) and the norepinephrine transporter (NET) (K_i = 28 nM). In vivo biodistribution and blocking studies performed in male rats demonstrated that [^{123}I]**1** was selective and specific for SERT. In vivo microPET brain imaging studies in an anesthetized monkey with [^{18}F]**1** showed high uptake in the diencephalon and brainstem with peak uptake achieved at 120 min. A chase study with (*R*,*S*)-citalopram·HBr displaced [^{18}F]**1** radioactivity from all SERT-rich brain regions. A chase study with the DAT ligand 2β-carbophenoxy-3β-(4-chlorophenyl)tropane (**9**, RTI-113) failed to displace [^{18}F]**1**, indicating that [^{18}F]**1** is specific to the SERT. The in vivo evaluation of [^{18}F]**1** indicates that this radiotracer is a good candidate for mapping and quantifying CNS SERT.

Introduction

The neuronal uptake of serotonin from the extracellular space into the presynaptic neuron is regulated by the serotonin transporter (SERT). The SERT proteins reside in high density on the cell bodies of the medial and dorsal raphe nuclei in the brainstem and the terminals of the serotonergic neurons located mainly in the hypothalamus, thalamus, striatum, and cerebral cortex.[1−3] The SERT has attracted considerable attention in recent years because of its involvement in the pathophysiology of neuropsychiatric disorders.[4−6] The in vivo imaging of the SERT with positron emission tomography (PET) or single photon emission computerized tomography (SPECT) has been a major target of neuroimaging research because of the potential use of SERT imaging agents for further understanding of neuropsychiatric disorders.

We recently reported the synthesis and in vitro characterization of the candidate SERT ligand 2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane (*p*ZIENT)[7,8] labeled with ^{123}I or ^{11}C. This compound possesses high affinity and selectivity for the human SERT (K_i = 0.04 nM) with affinities for the human dopamine transporter (DAT) and norepinephrine transporter (NET) being 375- and 150-fold lower, respectively, than for SERT.[7,8] Incorporation of ^{18}F into 2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane would provide a positron emitting tracer with a longer half-life (109.8 min vs 20.4 min for ^{11}C) and higher spatial resolution than [^{11}C]2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane. A competitive bind-

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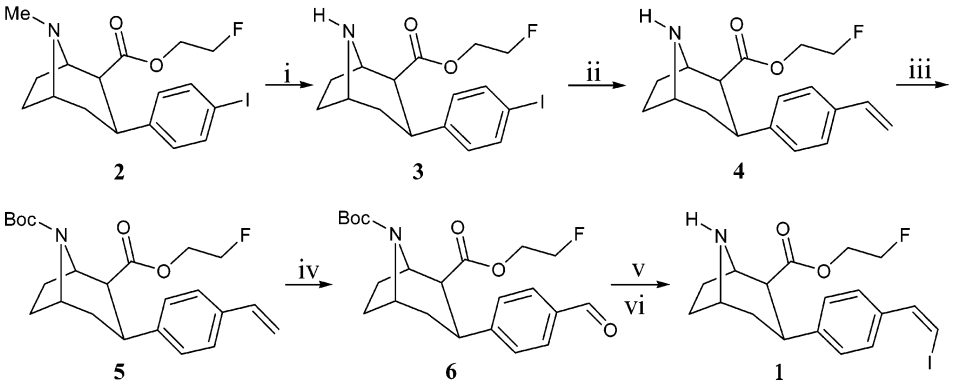
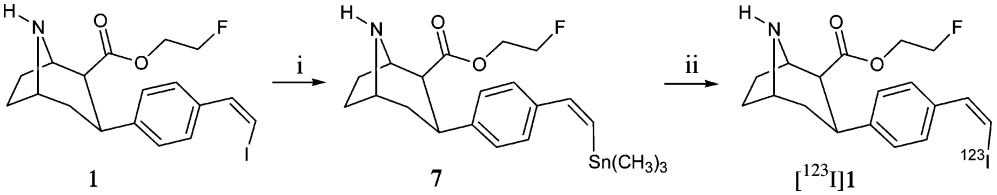
Scheme 1[a]



[a] Reagents: (i) Troc-Cl, toluene, then Zn, AcOH; (ii) *n*-$Bu_3SnCH{=}CH_2$, $Pd(PPh_3)_4$, toluene; (iii) Boc_2O, Et_3N, CH_2Cl_2; (iv) OsO_4, $NaIO_4$, THF/H_2O; (v) Ph_3PCH_2I, $((CH_3)_3Si)_2NNa$, THF; (vi) TFA, CH_2Cl_2.

Scheme 2[a]



[a] Reagents: (i) $(CH_3)_3Sn{-}Sn(CH_3)_3$, $Pd(PPh_3)_4$, THF; (ii) Na[^{123}I]I, H_2O_2.

ing study for DAT affinity of the 2β-carboethoxy analogue of cocaine demonstrated similar DAT binding affinity as cocaine, IC_{50} = 102 and 130 nM for cocaine and the 2β-carboethoxy analogue, respectively.[9] These results suggest that substitution of 2′-fluoroethyl at the 2β-carbomethoxy of 2β-carbomethoxy-3β-[4′-((*Z*)-2-iodoethenyl)phenyl]nortropane will not increase DAT affinity and may lead to high affinity and selective ligands for the SERT that would enable labeling with fluorine-18.

We describe here the synthesis and monoamine transporter binding evaluation of 2β-carbo(2-fluoroethoxy)-3β-(4′-((*Z*)-2-iodoethenyl)phenyl)nortropane (βFE*p*ZIENT, **1**). We also report the radiolabeling of [^{123}I]**1**, its ex vivo brain biodistribution in rats, and SPECT imaging in nonhuman primates as well as the radiolabeling of [^{18}F]**1** and its evaluation in nonhuman primates as a PET imaging agent for the SERT.

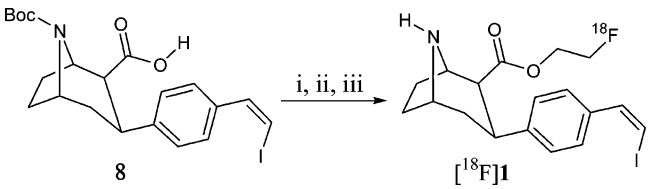
Chemistry

The synthesis of **1** is shown in Scheme 1. The fluoroethyl ester of 2β-carbomethoxy-3β-(4′-iodophenyl)tropane (**2**, β-CIT[10,11]) was demethylated by reaction with 2,2,2-trichloroethyl chloroformate followed by a zinc acetic acid reduction to give **3**. The vinyl group of **4** was introduced via a palladium-mediated coupling reaction using tributyl(vinyl)tin and tetrakis(triphenylphosphine)palladium as catalyst. After protection of the secondary amine with a *tert*-butoxycarbonyl group to give **5**, the exocyclic double bond was cleaved with OsO_4/$NaIO_4$ to afford **6**. Treatment of **6** with the in situ formed methylenetriphenylphosphonium ylide followed by removal of the Boc group with TFA afforded **1**.

Radiochemistry

The radiosynthesis of [^{123}I]**1** is shown in Scheme 2. Palladium-catalyzed coupling of **1** with hexamethylditin afforded the radiolabeling precursor **7**. Vinyltin **7** was then treated with $^{123}I^+$ obtained in situ via oxidation of no-carrier-added Na^{123}I. Starting from 17.6 mCi of Na^{123}I, the radiosynthesis afforded 9.35 mCi (nondecay-corrected) of [^{123}I]**1** in approximately 140 min, which corresponds to a nondecay-corrected yield of 53%.

Scheme 3[a]



[a] Reagents: (i) Bu_4NOH, CH_3CN; (ii) 2-[^{18}F]FEtOBs, CH_3CN; (iii) TFA, then NH_4OH.

Table 1. In Vitro Evaluation of **1** in Competition Assays with Human Monoamine Transporters

	K_i (nM)[a]			SERT selectivity	
compd	hSERT	hDAT	hNET	DAT/SERT	NET/SERT
1	0.08 ± 0.01	13 ± 2	28 ± 5	∼162	∼350

[a] Average value of two determinations ± the standard deviation (each determination performed in triplicate).

The chemical and radiochemical purity of the radiolabeled product was greater than 99% with a specific activity at the time of injection of 3.8 Ci/μmol.

The radiosynthesis of [^{18}F]**1** is shown in Scheme 3. *N*-Boc acid **8**[8] was O-alkylated with 2-[^{18}F]fluoroethylbrosylate[12] followed by acid hydrolysis of the Boc group and purification by semipreparative, reverse-phase HPLC to afford [^{18}F]**1** in an average (n = 4) radiochemical yield of 8% (decay-corrected from EOB[a]). Analytical HPLC demonstrated that the radiolabeled product was over 99% radiochemically pure with a specific activity of 8 Ci/μmol at the time of injection. The total synthesis time was approximately 2 h from EOB.

In Vitro Competition Assays

In vitro competition binding assays were performed with **1** (Table 1) in HEK-293 cells stably expressing the transfected human SERT, DAT, or NET according to a previously reported

[a] Abbreviations: EOB, end of bombardment; SUV, standard uptake value; TAC, time−activity curve; HRRT, high-resolution research tomograph.

Table 2. Brain Distribution of Radioactivity in Male Sprague-Dawley Rats after iv Injection of [^{123}I]**1**[a]

	5 min	30 min	60 min	120 min
thyroid	0.06 ± 0.01	0.27 ± 0.18	0.56 ± 0.02	1.25 ± 0.25
hypothalamus	0.30 ± 0.08	0.16 ± 0.05	0.16 ± 0.02	0.10 ± 0.02
pons	0.25 ± 0.05	0.11 ± 0.04	0.13 ± 0.02	0.07 ± 0.02
cerebellum	0.24 ± 0.04	0.10 ± 0.04	0.09 ± 0.01	0.04 ± 0.01
pre-frontal cortex	0.36 ± 0.09	0.16 ± 0.04	0.15 ± 0.01	0.07 ± 0.02
occipital cortex	0.40 ± 0.12	0.16 ± 0.05	0.15 ± 0.01	0.07 ± 0.02
striatum	0.33 ± 0.08	0.17 ± 0.06	0.16 ± 0.01	0.08 ± 0.02
hippocampus	0.30 ± 0.05	0.13 ± 0.04	0.15 ± 0.01	0.08 ± 0.02

[a] Values are reported as the mean percent of total injected dose per gram (% ID/g) of tissue ± standard deviation ($n = 5$ for all time points).

procedure.[13] Similar to that of the previously reported 3β-[4′-((*Z*)-2-haloethenyl)phenyl]nortropanes and tropanes,[13] **1** showed a high affinity for the hSERT. The affinities of **1** for the hDAT and hNET were ~162 and ~350-fold lower, respectively, than for the hSERT, which indicates that although the methyl ester was substituted by a 2-fluoroethyl ester, **1** retains a high affinity and selectivity for the hSERT versus the hDAT and hNET.

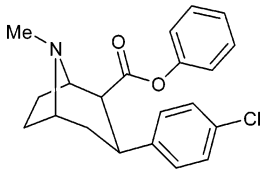
Lipophilicity

The lipophilicity of **1** was determined in order to predict its ability to cross the blood−brain barrier (BBB). Polar molecules ($\log P < 0$) usually have limited brain distribution as a consequence of their low permeability at the BBB. However, although an increasing lipophilicity leads to an enhanced BBB diffusion, a higher lipophilicity also leads to an increase in certain organ uptake such as the liver and an increase in the affinity for metabolic enzymes. It can also lead to a higher binding to proteins such as P-glycoprotein (PGP), which acts as an efflux pump to keep undesired molecules out of the brain. Therefore, a suitable lipophilicity is necessary to achieve a good brain delivery. The central nervous system (CNS) penetrance of radioligands has been reported to be optimum when $\log P_{7.4} = 2.0-3.5$.[14] The octanol/water partition coefficient of **1** was measured according to a previously reported procedure[15] and determined to be $\log P_{7.4} = 1.80$.

Rodent Brain Distribution Studies

The distribution of radioactivity expressed as the mean percent of total injected dose per gram (% ID/g) of tissue in male Sprague-Dawley rats at 5, 30, 60, and 120 min after injection of [^{123}I]**1** is shown in Table 2. The level of accumulation of radioactivity in different parts of the brain at the first time point (5 min) ranged from 0.24% dose/g in the cerebellum to 0.40% dose/g in the occipital cortex, resulting in an initial brain to blood ratio of ~2. These results indicate that [^{123}I]**1** achieves an acceptable brain penetrance and a heterogeneous CNS distribution. The uptake of radioactivity in the thyroid was initially low (0.06% dose/g at 5 min) but gradually increased to 1.25% dose/g at 120 min, thus demonstrating a slow in vivo deiodination. The regional distribution of radioactivity over time showed a constant washout of [^{123}I]**1** from the brain and a good retention in regions rich in SERT sites such as the hypothalamus when compared to the cerebellum, a region with negligible SERT sites.[16] At 120 min the highest ratios of radioactivity uptake relative to the cerebellum were seen in the hypothalamus (2.5:1), hippocampus and the striatum (2.0:1) followed by the cortices and pons (1.75:1). This result is in agreement with the expected regional distribution of SERT protein in rat brain. The high accumulation of activity in areas with a high density of SERT sites and the gradual decrease of [^{123}I]**1** concentration in the brain over the course of the experiment demonstrated that [^{123}I]**1** has potentially favorable tracer kinetic properties for in vivo imaging of the SERT and, subsequently, occupancy determination studies.

To assess the in vivo selectivity of [^{123}I]**1** for the SERT versus the DAT and the NET, a series of blocking studies were performed in which specific monoamine transporter ligands were administered to challenge the [^{123}I]**1** binding. The competing monoamine transporters were (*R,S*)-citalopram·HBr (4 and 6 mg/kg body weight, SERT ligand), 2β-carbophenoxy-3β-(4-chlorophenyl)tropane (**9**, RTI-113)[17] (6 mg/kg, DAT ligand), reboxetine (6 mg/kg, NET ligand), or saline as a control. The blockers were administered intravenously 15 min prior to injection of [^{123}I]**1**. The rats were sacrificed 60 min after injection of [^{123}I]**1**, and the different parts of the brain were dissected and counted for radioactivity (Table 3). In the cerebellum, which was used as the reference tissue because its level of SERT sites is negligible, no significant differences were found between the citalopram, **9**, reboxetine, or saline groups, while the citalopram group was significantly different from the **9**, reboxetine, or saline groups in the regions known to have high to moderate levels of SERT expression (hypothalamus, pons, hippocampus, striatum, cortices). Following administration of citalopram, the % ID/g in these regions was lower than following administration of **9**, reboxetine, or saline, proving that the [^{123}I]**1** uptake was blockable and therefore related to binding to SERT sites. Moreover, there was a trend toward a significantly lower % ID/g in the SERT rich regions when a higher dose of citalopram was administered. In contrast, [^{123}I]**1** uptake in all of the brain regions was identical between the **9**, reboxetine, and saline groups and significantly higher in those groups than in the citalopram groups, suggesting that [^{123}I]**1** has no appreciable DAT or NET binding in the rat brain.



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Nonhuman Primate SPECT Study

An in vivo SPECT imaging study in a rhesus monkey was performed using [^{123}I]**1**. The images were acquired from 35 to 239 min after injection using a dual-headed γ camera in order to determine the kinetics of [^{123}I]**1** binding for the brain regions of interest. The time−activity curves (TACs) obtained after injection of 10 mCi of [^{123}I]**1** are presented in Figure 1. The highest uptake of activity occurred in the diencephalon, a region encompassing the hypothalamus and thalamus, and in the striatum, whereas lower levels of uptake were observed in the occipital cortex and cerebellum, which is in agreement with the distribution and relative density of SERT sites. These promising findings prompted us to investigate the radiosynthesis and in vivo evaluation of **1** labeled with a positron emitting radioisotope because of the higher spatial and temporal resolution obtainable with PET.

Nonhuman Primate microPET Studies

The regional CNS distribution of [^{18}F]**1** in a cynomolgus monkey was evaluated using a Concorde microPET P4 instrument. Sagittal, transversal, and coronal images were acquired for a period of 235 min following administration of [^{18}F]**1** and are depicted in Figure 2. The TACs generated from these images by manually drawing the brain regions of interest are presented in Figure 3. The accumulation of [^{18}F]**1** was consistent with

Table 3. Brain Distribution of Radioactivity in Sprague-Dawley Rats 60 min after iv Injection of [^{123}I]**1** in the Presence of Monoamine Transporter Ligands[a]

	saline[b]	citalopram (4 mg/kg)[c]	citalopram (6 mg/kg)[c]	**9** (6 mg/kg)[c]	reboxetine (6 mg/kg)[c]
hypothalamus	0.27 ± 0.02	0.21 ± 0.02	0.18 ± 0.09	0.32 ± 0.09	0.26 ± 0.06
pons	0.22 ± 0.04	0.18 ± 0.02	0.14 ± 0.03	0.22 ± 0.04	0.20 ± 0.04
cerebellum	0.12 ± 0.04	0.14 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.13 ± 0.01
pre-frontal cortex	0.26 ± 0.03	0.21 ± 0.04	0.17 ± 0.05	0.28 ± 0.07	0.28 ± 0.06
occipital cortex	0.24 ± 0.03	0.21 ± 0.04	0.16 ± 0.04	0.24 ± 0.05	0.23 ± 0.04
striatum	0.25 ± 0.03	0.20 ± 0.04	0.15 ± 0.04	0.26 ± 0.05	0.26 ± 0.06
hippocampus	0.24 ± 0.02	0.18 ± 0.03	0.16 ± 0.04	0.25 ± 0.06	0.23 ± 0.04

[a] Values are reported as the mean percent of total injected dose per gram (% ID/g) of tissue ± standard deviation. The monoamine transporter ligands were given 15 min prior to [^{123}I] **1**. [b] $n = 3$. [c] $n = 4$.

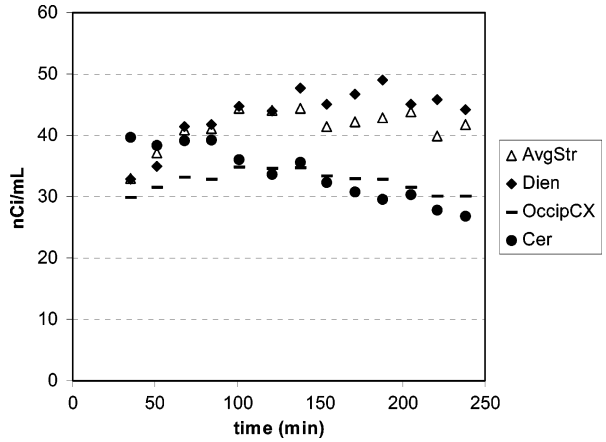

Figure 1. TACs for brain regions of interest after injection of [^{123}I]**1** in a cynomolgus monkey.

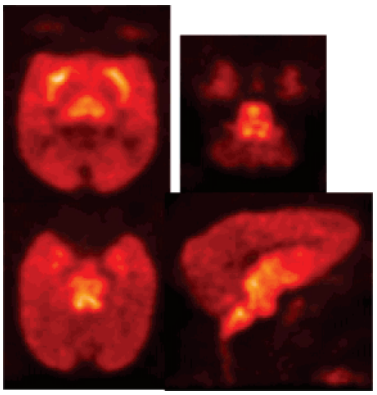

Figure 2. The microPET images (summed 0−235 min) obtained after injection of [^{18}F]**1** (5.2 mCi) into a cynomolgus monkey.

the distribution of the SERT in the brain with the highest uptake occurring in the midbrain followed by the putamen, thalamus, medulla, and pons and at lesser levels the caudate and cortices, with the cerebellum exhibiting the lowest uptake and retention of the radioligand. The midbrain showed the highest uptake of radioactivity with peak uptake achieved at about 120 min after administration of the radiotracer. The level of radioactivity over time in the other regions showed peak uptake after about 90 min followed by a minor but steady washout. At 235 min after injection, the tissue to cerebellum ratios were 4.4, 3.8, 3.3, 3.3, and 3.0 for the midbrain, putamen, thalamus medulla, and pons, respectively, and significantly lower in the caudate, frontal cortex, and occipital cortex with ratios versus cerebellum of 2.6, 1.5, and 1.6, respectively.

To demonstrate that the uptake and retention of [^{18}F]**1** in the different brain regions (except the cerebellum) was related to selective SERT binding, a pharmacological dose of (*R*/*S*)-citalopram·HBr (1.5 mg/kg) was administered at 2 h after injection of [^{18}F]**1**. Marked changes in the brain regions were

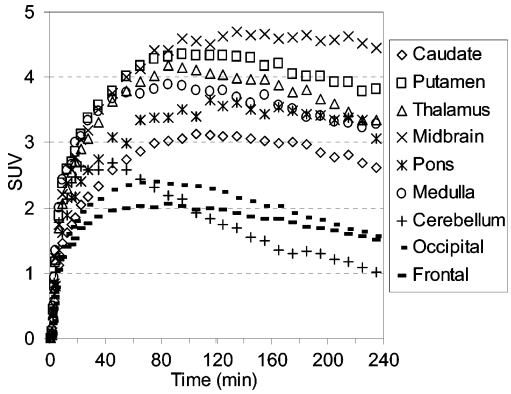

Figure 3. Baseline TACs for brain regions of interest obtained after injection of [^{18}F]**1** into a cynomolgus monkey.

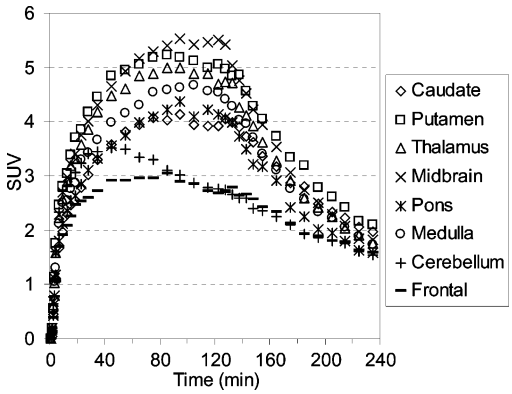

Figure 4. TACs obtained after injection of [^{18}F]**1** into a cynomolgus monkey with (*R*/*S*)-citalopram·HBr (1.5 mg/kg) administered at 120 min.

observed (Figure 4). There was a significant decrease of the radioactivity retention in all regions where SERT is concentrated, with percentages of displacement of 67%, 64%, 62%, 62%, 58%, 50%, and 42% from midbrain, thalamus, pons, medulla, putamen, caudate, and frontal cortex, respectively, at 235 min after injection. Two hours after administration of (*R*/*S*)-citalopram·HBr, the radioactivity uptake in these regions reached levels similar to that of the cerebellum showing an apparent saturable signal. This competition experiment established that this radioligand uptake is directly related to binding to SERT sites and is selective for the SERT.

A displacement study using the DAT ligand **9** was also performed. This study aimed at assessing the specificity of [^{18}F]-**1** binding for the DAT. In this experiment, a dose of 0.3 mg/kg of **9** was administered 2 h after injection of the radioactive tracer. The resulting TACs (Figure 5) show what appears to be an appreciable displacement of radioactivity from the caudate and putamen, but a decrease in radioactivity is also observed in the thalamus, medulla, and pons but not in the midbrain. Compari-

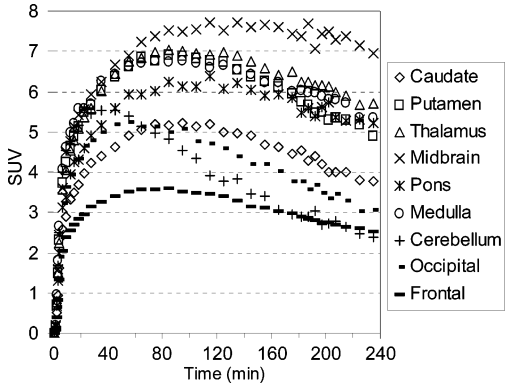

Figure 5. TACs obtained after injection of [^{18}F]**1** into a cynomolgus monkey with **9** (0.3 mg/kg) administered at 120 min after injection (45 s infusion).

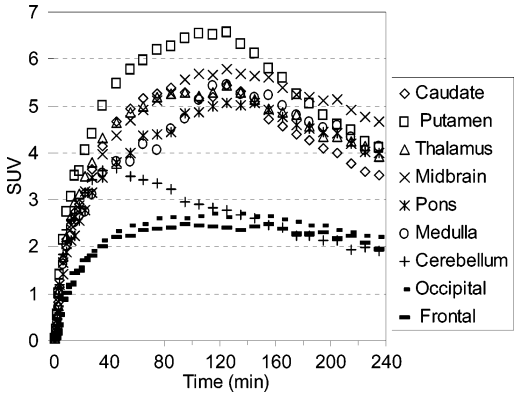

Figure 6. TACs obtained after injection of [^{18}F]**1** into a cynomolgus monkey with (±)-reboxetine·mesylate (1.5 mg/kg) administered at 120 min after injection.

son to the baseline TACs in Figure 3 suggests that this decrease in radioactivity is actually a result of washout and not displacement by **9**.

A chase study was also performed with the NET ligand (±)-reboxetine·mesylate to determine if [^{18}F]**1** binds to the NET (Figure 6). The TACs in Figure 6 show that there is displacement of the tracer from all the areas of the brain with a high SERT density. Reboxetine has a high affinity for the NET but also an appreciable affinity for the SERT,[7,18] and this is presumably the cause of the observed displacement. The cerebellum contains a high NET density, but the slope of the cerebellum TAC in Figure 6 does not change after the administration of (±)-reboxetine·mesylate, thus providing further evidence that [^{18}F]**1** is being displaced from the SERT.

PET Imaging in an Awake Nonhuman Primate

Recent work performed using [^{18}F]FECNT, a selective DAT radioligand, demonstrated that 1% isoflurane anesthesia increases radiotracer binding to DAT-rich regions, consistent with an increase in plasma membrane expressed DAT.[19] To address the possibility of isoflurane influencing the kinetics and/or binding sites of [^{18}F]**1**, a PET study was performed in an awake rhesus monkey using a high-resolution research tomograph (HRRT). Cynomolgus and rhesus both are macaque monkeys with very similar physiology and neurochemistry. They are frequently used interchangeably in behavioral, physiological, and pharmacological studies, and therefore, there is no reason to assume that the uptake kinetics would differ between cynomolgus and rhesus.

The PET images were acquired for 120 min and coregistered with composite MRI images of several rhesus monkeys to accurately identify the regions of interest (Figure 7). The kinetics of [^{18}F]**1** (Figure 8) were not significantly altered compared to the anesthetized monkey studies. In both awake and anesthetized monkeys, [^{18}F]**1** showed prolonged retention in every part of brain over the course of the imaging session with significant washout only observed in the cerebellum.

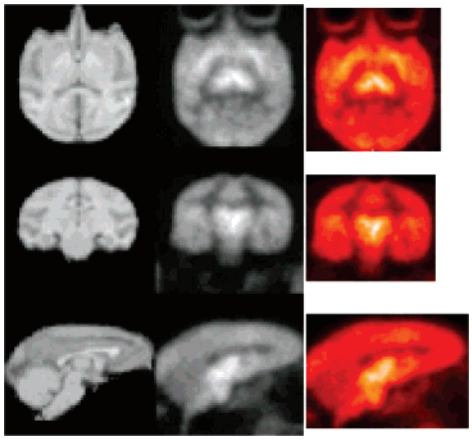

Figure 7. HRRT PET images (right and center) obtained by injection of [^{18}F]**1** into an awake rhesus monkey and composite MRIs of several rhesus monkeys (left).

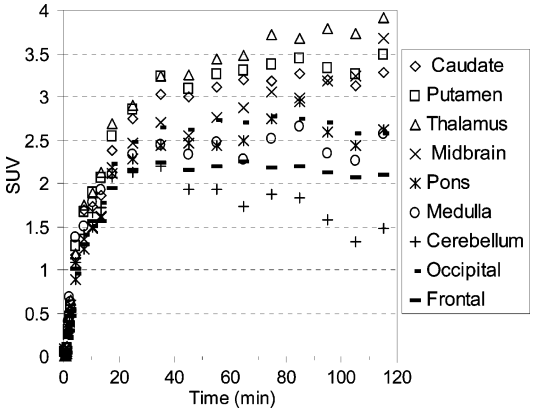

Figure 8. HRRT TACs obtained after injection of [^{18}F]**1** into an awake rhesus monkey.

Conclusion

The SERT ligand 2β-carbo(2-fluoroethoxy)-3β-(4′-((*Z*)-2-iodoethenyl)phenyl)nortropane (βFE*p*ZIENT, **1**) has been synthesized, radiolabeled with both fluorine-18 and iodine-123, and evaluated in rats and in anesthetized and awake nonhuman primates. Competition binding assays in cells stably expressing the human monoamine transporters demonstrated that **1** has a high affinity for the SERT with **1** showing significantly higher selectivity for SERT over DAT and NET, 162 to 1 and 350 to 1, respectively. Biodistribution studies in rats showed that [^{123}I]-**1** uptake occurred specifically in regions rich in SERT. In vivo microPET imaging studies in anesthetized monkeys demonstrated that [^{18}F]**1** exhibited high uptake in the midbrain, putamen, thalamus, and brainstem, regions rich in SERT. In vivo PET imaging studies in awake monkeys demonstrated that [^{18}F]**1** regional brain uptake and kinetics were not significantly altered. These results suggest that anesthesia does not influence the binding potential of [^{18}F]**1** at the SERT. Finally, the high CNS SERT rich region to cerebellar ratios in awake and anesthetized nonhuman primates support the candidacy of [^{18}F]-**1** for further study as a radioligand for in vivo quantitation of SERT sites by PET in humans.

Experimental Section

All reagents used were obtained from commercially available sources. Solvents used in reactions were purchased from Aldrich, while solvents for chromatography were obtained from VWR. ^{1}H NMR spectra were recorded on a Varian spectrometer at 300, 400, or 600 MHz and referenced to the NMR solvent or internal TMS. Mass spectra were determined on a VG 70-S double-focusing mass spectrometer using high-resolution electron ionization (EI) or a JEOL JMS-SX102 double-focusing mass spectrometer using fast atom bombardment (FAB). Silica gel column chromatography was performed using Merck silica gel 60 (40−63 μm particle size). Thin layer chromatography (TLC) was performed using 250 μm layers of F-254 silica on aluminum plates obtained from Whatman.

All animal experiments were carried out according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) and Radiation Safety Committee of Emory University.

2β-Carbo(2-fluoroethoxy)-3β-(4′-iodophenyl)nortropane (3). To a suspension of tropane **2** (0.55 g, 1.3 mmol) in toluene (20 mL) was added 2,2,2-trichloroethyl chloroformate (1 mL). The reaction mixture was refluxed for 6 h. Then the volatiles were evaporated. The residue was dissolved in acetic acid (14 mL). Zinc powder (0.46 g, 7 mmol) and water (3 drops) were added. The reaction mix was stirred at room temperature for 60 h. Zinc was filtered. The filtrate was poured into a mix of ice and water (100 mL) and basified with concentrated ammonium hydroxide. Extraction with dichloromethane and purification by flash chromatography (dichloromethane/methanol, 9/1) afforded a white-yellowish solid (0.34 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61−1.80 (m, 3 H), 1.97−2.05 (m, 1 H), 2.08−2.16 (m, 1 H), 2.33−2.40 (m, 1 H), 2.54 (s br, 1 H), 2.81−2.82 (m, 1 H), 3.18−3.23 (m, 1 H), 3.75−3.77 (m, 1 H), 3.92−4.20 (m, 2.5 H), 4.26−4.32 (m, 1 H), 4.40−4.44 (m, 0.5 H), 6.96 (d, J = 8.4 Hz, 2 H), 7.59 (d, J = 8.4 Hz, 2 H).

2β-Carbo(2-fluoroethoxy)-3β-(4′-ethenyl)phenyl)nortropane (4). A solution of tropane **3** (0.32 g, 0.79 mmol) in toluene (16 mL) was degassed by passing argon through it for 1 min. Tributylvinyltin (0.35 mL, 1.2 mmol) and tetrakis(triphenylphosphine)palladium (55 mg, 0.05 mmol) were added. The reaction mixture was heated at 100 °C for 8 h. The solvent was evaporated and the residue was purified by flash chromatography (dichloromethane/methanol, 94/6) to give a yellow oil (0.21 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.81 (m, 3 H), 1.98−2.05 (m, 1 H), 2.08−2.17 (m, 1 H), 2.37−2.44 (m, 1 H), 2.80−2.84 (m, 2 H), 3.23−3.29 (m, 1 H), 3.74−3.76 (m, 2 H), 3.88−4.14 (m, 2.5 H), 4.19−4.26 (m, 1 H), 4.34−4.39 (m, 0.5 H), 5.19 (d, J = 10.8 Hz, 1 H), 5.70 (d, J = 17.6 Hz, 1 H), 6.66 (dd, J = 17.6, 10.8 Hz, 1 H), 7.16 (d, J = 8.4 Hz, 2 H), 7.32 (d, J = 8.4 Hz, 2 H).

***N*-(*tert*-Butoxycarbonyl)-2β-carbo(2-fluoroethoxy)-3β-(4′-ethenyl)phenyl)nortropane (5).** To a suspension of tropane **4** (0.21 g, 0.69 mmol) in dichloromethane (6 mL) was added triethylamine (0.29 mL) followed by a solution of di-*tert*-butyl dicarbonate (0.23 g, 1.08 mmol) in dichloromethane (2.5 mL). The reaction mixture was stirred at room temperature for 3 h. The volatiles were evaporated under reduced pressure and the residue was purified by flash chromatography (hexane/ethyl acetate, 75/25) to afford a colorless oil (0.25 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 and 1.44 (s, 9 H), 1.65−1.75 (m, 2 H), 1.82−1.86 (m, 1 H), 1.89−2.19 (m, 2 H), 2.71−2.78 (m, 1 H), 2.94−2.96 (m, 1 H), 3.22−3.28 (m, 1 H), 4.00−4.10 (m, 2 H), 4.20−4.69 (m, 4 H), 5.17 (d, J = 10.8 Hz, 1 H), 5.68 (d, J = 17.6 Hz, 1 H), 6.64 (dd, J = 10.8 Hz, 17.2 Hz, 1 H), 7.19 (d, J = 8.4 Hz, 2 H), 7.30 (d, J = 8.4 H, 2 H).

***N*-(*tert*-Butoxycarbonyl)-2β-carbo(2-fluoroethoxy)-3β-(4′-formylphenyl)nortropane (6).** To a solution of vinyltropane **5** (0.25 g, 0.62 mmol) in a mixture of tetrahydrofuran and water (50/50) was added osmium tetroxide (2 mg). Then sodium periodate (0.265 g, 1.24 mmol) was added portionwise over a 20 min period. The reaction mixture was stirred at room temperature for 3 h. The solution was extracted with dichloromethane and the residue obtained after evaporation was purified by flash chromatography (hexane/ethyl acetate, 70/30) to give a colorless oil (0.225 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (br s, 9 H), 1.72−1.77 (m, 2 H), 1.84−1.90 (m, 1 H), 2.01−2.22 (m, 2 H), 2.75−2.81 (m, 1 H), 3.00−3.09 (m, 1 H), 3.31−3.37 (m, 1 H), 4.01−4.19 (m, 2 H), 4.22−4.78 (m, 4 H), 7.41 (d, J = 8.4 Hz, 2 H), 7.79 (d, J = 8.4 Hz, 2 H), 9.95 (s, 1 H).

2β-Carbo(2-fluoroethoxy)-3β-(4′-((*Z*)-2-iodoethenyl)phenyl)-nortropane (1). To a suspension of methyltriphenylphosphonium iodide (192 mg, 0.36 mmol) in 3 mL of dry THF was added sodium bis(trimethylsilyl)amide (0.345 mL, 1 M in THF, 0.35 mmol). The resulting mixture was stirred at room temperature for 5 min. The reaction mixture was then cooled to −78 °C, and the aldehyde **6** (70 mg, 0.17 mmol) in 1.5 mL of THF was added dropwise. After completion of the addition, the reaction mixture was allowed to warm to room temperature over a period of 1 h and stirred for 30 min. The resulting mixture was then quenched with a saturated solution of ammonium chloride. The solution was extracted with dichloromethane and the residue obtained after evaporation was purified by flash chromatography (hexane/ethyl acetate, 75/25) to give a colorless oil. The oil obtained was dissolved in dichloromethane (2.5 mL), the solution was cooled to 0 °C, and trifluoroacetic acid (0.25 mL) was added dropwise. The reaction mixture was stirred for 45 min at 25 °C. The solvent and reagent were evaporated under reduced pressure. The residue was dissolved in dichloromethane and washed with ammonium hydroxide (5% w/w). Purification by HPLC (Waters, Novapak C_{18} semipreparative column, 25 mm × 100 mm, 22.5% H_2O in methanol + 0.1% Et_3N, 6 mL/min, t_R = 21 min) gave a thick oil (17.5 mg, 53%): TLC R_f = 0.16 (silica, 20:75:5 v/v/v hexane/EtOAc/NEt_3); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (d, 2 H, J = 9.0 Hz), 7.27 (d, 1 H, J = 8.7 Hz), 7.22 (d, 2 H, J = 9.0 Hz), 6.52 (d, 1 H, J = 8.4 Hz), 4.32 (dddd, 1 H, $^2J_{HF}$ = 46.9 Hz, $^2J_{HH}$ = 10.7 Hz, $^3J_{HH}$ = 5.2 Hz, $^3J_{HH}$ = 2.4 Hz), 4.18 (dddd, 1 H, $^2J_{HF}$ = 47.7 Hz, $^2J_{HH}$ = 10.7 Hz, $^3J_{HH}$ = 6.7 Hz, $^3J_{HH}$ = 2.4 Hz), 4.09 (dddd, 1 H, $^3J_{HF}$ = 26.2 Hz, $^2J_{HH}$ = 12.9 Hz, $^3J_{HH}$ = 6.7 Hz, $^3J_{HH}$ = 2.4 Hz), 3.96 (dddd, 1 H, $^3J_{HF}$ = 30.5 Hz, $^2J_{HH}$ = 12.9 Hz, $^3J_{HH}$ = 5.2 Hz, $^3J_{HH}$ = 2.4 Hz), 3.76 (m, 2 H), 3.29 (dt, 1 H, J = 13.2 Hz, J = 5.4 Hz), 2.85 (m, 1 H), 2.44 (br s, 1 H), 2.42 (td, 1 H, J = 12.9 Hz, J = 3.0 Hz), 2.14 (m, 1 H), 2.03 (m, 1 H), 1.78 (m, 1 H), 1.69 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.26, 142.43, 138.37, 135.22, 128.54, 127.30, 81.32 (d, $^1J_{CF}$ = 170.1 Hz), 79.20, 63.04 (d, $^2J_{CF}$ = 18.7 Hz), 56.49, 53.89, 51.00, 35.54, 33.43, 29.21, 27.77. HRMS (EI) $[M]^+$ Calcd for $C_{18}H_{21}NO_2FI$: 429.0601. Found: 429.0612. HRMS (ESI) $[MH]^+$ Calcd for $C_{18}H_{22}NO_2F^{127}I$: 430.0674. Found: 430.0691. Anal. Calcd for $C_{18}H_{21}FINO_2$: C, 50.36; H, 4.93; N, 3.26. Found: C, 50.17; H, 5.10; N, 3.04 (see Supporting Information).

2β-Carbo(2-fluoroethoxy)-3β-(4′-((*Z*)-2-trimethylstannylethenyl)phenyl)nortropane (7). A solution of **1** (6 mg, 0.014 mmol) in tetrahydrofuran (2 mL) was degassed with argon for 10 min. The tetrakis(triphenylphosphine)palladium (2 mg) and the hexamethylditin (10 mg, 0.028 mmol) were added, and the solution was degassed with argon for 5 min. The flask was sealed and heated at 60 °C overnight. The solvent was removed under reduced pressure. The residue was taken up with a solution of dichloromethane containing 0.2% of triethylamine and deposited on a silica Sep-Pak. Elution with dichloromethane/methanol (98/2 to 93/7) + 0.2% triethylamine followed by purification by HPLC (Waters, X-terra RP_{18}, 7.8 mm × 100 mm, 70% methanol in H_2O + 0.1% Et_3N, 2 mL/min, t_R = 10.8 min) afforded a yellow oil (3 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.071 (m, 9 H), 1.76−1.90 (m, 3 H), 2.28−2.47 (m, 2 H), 2.54−2.60 (m, 1 H), 2.92−2.94 (m, 1 H), 3.30−3.36 (m, 1 H), 3.87−4.41 (m, 6 H), 6.18 (d, J = 13.6 Hz, 1 H), 7.14−7.21 (m, 4 H), 7.51 (d, J = 13.6 Hz, 1 H). HRMS (ESI) $[MH]^+$ Calcd for $C_{21}H_{31}O_2NF^{120}Sn$: 468.1355. Found: 468.1355.

2-[^{18}F]Fluoroethylbrosylate. 2-[^{18}F]Fluoroethylbrosylate was prepared in a CPCU as previously reported.[12] The 2-[^{18}F]fluoroethylbrosylate was trapped on a silica Sep-Pak (previously prepped with 10 mL of EtOEt), eluted with EtOEt, transferred to a hot cell under $N_{2(g)}$ pressure, and collected in a V-tube. The V-tube was placed in a 90 °C oil bath, and the EtOEt was evaporated with an $Ar_{(g)}$ flow.

2β-Carbo(2-[^{18}F]fluoroethoxy)-3β-(4′-((*Z*)-2-iodoethenyl)phenyl)nortropane [^{18}F]1. A suspension containing 2.5 mg of acid (**8**) in 0.6 mL of acetonitrile and 1 μL of tetrabutylammonium hydroxide (1 M in methanol) was added to a V-tube containing 2-[^{18}F]fluoroethylbrosylate. The reaction mixture was heated at 85 °C for 14 min and then cooled in a ice bath for 2 min. An amount of 65 μL of trifluoroacetic acid was introduced, and the reaction mix was allowed to stand at room temperature for 18 min. After the mixture was cooled in an ice bath, 0.15 mL of concentrated ammonium hydroxide was added. The reaction mix was diluted with HPLC mobile phase (300 μL) prior to injection onto the semipreparative HPLC column. HPLC purification was performed using a reverse-phase C_{18} column (Waters, NovaPak RP_{18}, 25 mm × 100 mm) at a 7 mL/min flow rate with a buffered mobile phase consisting of 43% water in acetonitrile and containing 1.5 g of ammonium acetate per liter. The fractions eluting at approximately 19 min contained the desired product and were concentrated using a modified solid-phase extraction (SPE) procedure.[13,20] The radiolabeled tropane was eluted with ethanol (1.5 mL) and driven to a sterile empty vial containing 3.5 mL of saline. The resulting solution was transferred under argon pressure through a Millipore filter (pore size of 1.0 μm) followed by a smaller one (pore size of 0.2 μm) to a 30 mL sterile vial containing 10 mL of saline. HPLC analysis (Waters, NovaPak, 3.9 mm × 150 mm) at 1.0 mL/min flow rate with a mobile phase consisting of 75:25:0.1 methanol/water/triethylamine using a UV detector and a radioactivity detector showed the formulated dose to have a radiochemical purity of greater than 99%. The total synthesis time (including purification and formulation) was approximately 120 min from end of bombardment.

2β-Carbo(2-fluoroethoxy)-3β-(4′-((*Z*)-2-[^{123}I]iodoethenyl)-phenyl)nortropane [^{123}I]1. The trimethyltin precursor **7** (100 μg) dissolved in ethanol (0.3 mL) was added to a sealed vial containing no-carrier-added Na[^{123}I]I (MDS Nordion, Inc.) in 0.1 N sodium hydroxide (~140 μL). In rapid succession, an excess of 0.4 N aqueous hydrochloric acid (100 μL) was added followed by 3% hydrogen peroxide (50 μL). The reaction vial was inverted several times to mix the reagents, and the reaction was allowed to proceed at room temperature for 25 min. The reaction was quenched by the addition of aqueous sodium hydrogen sulfate (0.25 g/mL, 50 μL), and the reaction mixture was drawn into a syringe containing an aqueous solution of saturated sodium bicarbonate (0.5 mL). The reaction mix was passed through a C_{18} SepPak (preconditioned with methanol (10 mL)), and elution was done sequentially with water (5, 10, and 5 mL). The radiolabeled product was then eluted from the C_{18} SepPak with 1 mL portions of methanol, which were collected as individual fractions. The methanol fractions containing significant activity (about five total fractions) were combined and evaporated at 65 °C with argon flow over the course of approximately 20 min. The residue was diluted with 0.4 mL of water prior to HPLC. Purification via HPLC was performed using a reverse-phase column (Waters, X-terra RP_{18}, 7.8 mm × 100 mm) at a 3.0 mL/min flow rate with the mobile phase consisting of 40% water in methanol + 0.1% of triethylamine. Under these conditions, the [^{123}I]**1** product had a retention time of approximately 16 min. Fractions containing radioactivity eluting at the expected retention time were combined and subject to SPE formulation as described previously.[13,20] The same analytical HPLC conditions used to assess doses of [^{18}F]**1** were used for [^{123}I]**1**.

Rodent Biodistribution Studies. Tissue distribution studies were performed in male Sprague-Dawley rats (200−250 g) after intravenous injection of [^{123}I]**1** in 0.1−0.2 mL of 10% ethanol/saline. The animals were allowed food and water ad libitum before the experiments. The animals were anesthetized with an intramuscular injection of 0.1 mL/100 g of a 1/1 ketamine (500 mg/mL)/xylazine (20 mg/mL) solution, and cannulas were placed in the tail veins. The animals were killed at the appropriate time points, their tissues were dissected, and selected tissues were weighed and counted along with dose standards in a Packard Cobra II auto-γ counter. The raw counts were decay-corrected to a standard time, and the counts were normalized as the percent of total injected dose per gram of tissue (% ID/g).

Brain tissue distribution studies were performed in male Sprague-Dawley rats (200−250 g) after intravenous injection of ~15 μCi of [^{123}I]**1**. The rats were killed in groups of five at 5, 30, 60, and 120 min after injection of [^{123}I]**1**. The brains of the rats were dissected, and the amount of radioactivity in the various brain regions was measured. In the blocking experiments, the rats were divided into four groups. Each of the groups was given a competing monoamine transporter ligand 15 min prior to the injection of [^{123}I]-**1**. The monoamine transporter ligands used in this study were **9** and reboxetine at doses of 6 mg/kg and (*R*/*S*)-citalopram·HBr at doses of 4 and 6 mg/kg. The rats in each of the four groups were killed 60 min after the injection of [^{123}I]**1**.

Nonhuman Primate SPECT Study. Quantitative brain images were acquired in a male rhesus monkey. The monkey was fasted for 12 h. The animal was initially anesthetized with Telazol (3 mg/kg, im) and maintained on a 1% isofluorane/5% oxygen mixture throughout the imaging procedure. The animal was intubated with assurance of adequate patency of the airway and was placed on a ventilator with arterial blood gases monitored throughout the study to ensure physiologic levels of respiration. The monkey's head was immobilized and positioned in an ADAC Vertex camera, and a slow bolus of radioligand [^{123}I]**1** was injected iv over ~30 s. A dynamic SPECT sequence of scans (16 × 15 min) was obtained to measure regional brain uptake. SPECT images were reconstructed, and the regions of interest were placed over the diencephalon (thalamus and hypothalamus), striatum (caudate, putamen), cerebellum, and occipital cortex. Time−activity curves were estimated by extracting maximal counts from each of the regions of interest for each of the SPECT acquisitions.

Nonhuman Primate microPET Studies. The microPET studies were performed using an adult male cynomolgus monkey. The animal was fasted for 12 h prior to the PET study. The animal was initially anesthetized with an intramuscular injection of Telazol (3 mg/kg), intubated, and then maintained on a 1% isoflurane/95% oxygen gas mixture throughout the imaging session. Respiration was maintained through a mechanical ventilator with measurement of expiratory oxygen and carbon dioxide levels to ensure physiological levels of respiration. [^{18}F]**1** was injected via the antecubital vein over the course of 5 min. Quantitative brain imaging studies employing [^{18}F]**1** were performed using a Concorde microPET P4 system (Knoxville, TN). In each study, the animal was positioned in the scanner with its head immobilized. A transmission scan was obtained with a cobalt-57 point source. Attenuation correction factors were determined by segmenting the resulting transmission image into bone, water, scanner bed, and air and then reprojecting into sinogram space. Emission data acquired were subject to iterative reconstruction (OSEM, 2 iterations, 40 subsets) with no pre- or postfiltering to provide images with an isotropic resolution of 3 mm fwhm. For generation of time-activity curves, regions of interest (ROIs) were drawn manually based on the anatomical landmarks visible in reconstructed PET images using ASIPro software (Concorde, Knoxville, TN). Several imaging studies were performed using [^{18}F]**1** with and without injection of pharmacological doses of monoamine transporter ligands. Emission data were collected in listmode with the microPET continuously for 260 min after injection of [^{18}F]**1** and subsequently binned into short time frames for analysis.

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Supporting Information Available: Elemental analysis data and HPLC purity of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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