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Synthesis and *in Vivo* Evaluation of a Novel PET Radiotracer for Imaging of Synaptic Vesicle Glycoprotein 2A (SV2A) in Nonhuman Primates

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Abstract

Structural disruption and alterations of synapses are associated with many brain disorders including Alzheimer's disease, epilepsy, depression, and schizophrenia. We have previously developed the PET radiotracer ¹¹C-UCB-J for imaging and quantification of synaptic vesicle glycoprotein 2A (SV2A) and synaptic density in nonhuman primates and humans. Here we report the synthesis of a novel radiotracer ¹⁸F-SDM-8 and its *in vivo* evaluation in rhesus monkeys. The *in vitro* binding assay of SDM-8 showed high SV2A binding affinity ($K_i = 0.58$ nM). ¹⁸F-SDM-8 was prepared in high molar activity (241.7 MBq/nmol) and radiochemical purity (>98%). In the brain, ¹⁸F-SDM-8 displayed very high uptake with peak standardized uptake value (SVU) greater than 8 and fast and reversible kinetics. A displacement study with levetiracetam and blocking studies with UCB-J and levetiracetam demonstrated its binding reversibility and specificity toward SV2A. Regional binding potential values were calculated and ranged from 0.8 in the brainstem to 4.5 in the cingulate cortex. By comparing to ¹¹C-UCB-J, ¹⁸F-SDM-8 displayed the same attractive imaging properties: very high brain uptake, appropriate tissue kinetics, and high levels of specific binding. Given the longer half-life of F-18 and the feasibility for central production and multisite distribution, ¹⁸F-SDM-8 holds promise as an excellent radiotracer for SV2A and as a biomarker for synaptic density measurement in neurodegenerative diseases and psychiatric disorders.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Keywords

^{18}F -SDM-8; UCB-J; synaptic density; SV2A; PET; nonhuman primates

INTRODUCTION

Structural disruption and alterations of synapses are associated with many neurodegenerative disorders including Alzheimer's disease (AD),^{1,2} epilepsy,^{3,4} Parkinson's disease (PD),⁵ and autism spectrum disorders,⁶ as well as psychiatric diseases such as depression⁷ and schizophrenia.^{8,9} An imbalance between inhibitory and excitatory synaptic transmission in the brain may explain the pathogenic mechanism of epilepsy.¹⁰ Animal models of epilepsy also demonstrate changes in synaptic proteins during epileptogenesis.¹¹ In PD, the impairment of two forms of synaptic plasticity, long-term depression, and long-term potentiation in the nucleus striatum could account for the onset and progression of motor and cognitive symptoms of the disease.¹² Studies showed that disturbance in synaptic signaling in addition to dendritic spine abnormalities are closely related to cognitive deficits, and these abnormalities progress from preclinical to clinical AD.¹³ Synaptic loss, especially in the frontal cortex and hippocampus regions, is believed to be a distinctive hallmark of AD and precedes the appearance of β -amyloid plaques and tau protein tangles.¹⁴ Furthermore, downregulation of several presynaptic and postsynaptic proteins is observed in the brain of AD patients, suggesting substantial synaptic alterations.¹⁵ Previously, evaluation of synaptic density or its change has been limited to examination of brain tissue obtained from surgery or autopsy. Therefore, a method for *in vivo* evaluation of presynaptic proteins would enable the measurement of synaptic changes in living patients.

Synaptic vesicles are crucial to neurotransmission from pre- to postsynaptic terminals, propagating nerve impulses between neurons. Synaptic vesicle glycoprotein 2 (SV2) is one of the membrane proteins on the synaptic vesicles of all vertebrates with three isoforms: SV2A, SV2B, and SV2C. SV2A is the most abundant and ubiquitously expressed throughout the brain, with high concentrations in cortical regions.¹⁶

In collaboration with UCB Pharma, we have previously developed ^{11}C -UCB-J (Figure 1) as a novel positron emission tomography (PET) radiotracer for *in vivo* imaging and quantification of SV2A in nonhuman primates and humans.^{17–19} Further, ^{11}C -UCB-J was validated as an *in vivo* biomarker for synaptic density.¹⁸ Preliminary studies have indicated the broad utility of this SV2A radiotracer as a biomarker in many CNS diseases.²⁰ For example, SV2A PET evaluation in patients with temporal lobe epilepsy showed marked reduction in ^{11}C -UCB-J uptake in the seizure onset zone (SOZ), thus indicating its potential use in the presurgery identification of SOZ in these patients.¹⁸ Compared to healthy controls, patients with PD showed a global reduction in ^{11}C -UCB-J uptake, with the most significant decrease in the substantia nigra region.²¹ Most significantly, SV2A PET with ^{11}C -UCB-J, for the first time, detected reduced synaptic density in living patients with AD and mild cognitive impairment (MCI): ^{11}C -UCB-J binding potential in the hippocampus was found to be ~41% lower in AD/MCI than cognitively normal subjects.²²

With the potential wide application of SV2A PET as a diagnostic tool in multiple brain disorders, ^{11}C -UCB-J, with the short 20 min radioactive half-life of its ^{11}C -label, cannot be transported to off-sites for imaging applications but has to be produced and used on-site. Therefore, we set out to develop an ^{18}F -labeled SV2A radiotracer with comparable or improved imaging characteristics for multicenter studies and potential clinical diagnostic application. Toward this goal, we pursued two parallel strategies. First, we attempted to label UCB-J with the ^{18}F -radionuclide (radioactive half-life of 110 min), given that this molecule contains three fluorine atoms on the benzene ring. However, our initial efforts to prepare ^{18}F -UCB-J experienced some difficulties, as radiolabeling of one of the three adjacent fluorine atoms on the phenyl moiety turned out to be challenging. Although we were able to prepare sufficient ^{18}F -UCB-J to carry out *in vivo* evaluation in rhesus monkey and the results were as compelling as those with ^{11}C -UCB-J, the radiochemical yield and molar activity were quite low;²³ hence, ^{18}F -UCB-J was considered to be too difficult to produce for clinical translation. The second strategy was to prepare a library of UCB-J analogs for a structure–activity relationship (SAR) study to seek other candidates for ^{18}F -labeling by removing one or two fluorine atoms on the phenyl moiety (Figure 1). As previously reported,²⁴ the pyrrolidinone acetamide scaffold is essential for SV2A binding affinity. Our preliminary results indicated the necessity of the methyl group on the pyridine fragment to maintain good SV2A binding affinity, as switching to either fluorine (UCB-H) or hydrogen would noticeably reduce SV2A binding affinity *in vitro* and tracer specific binding *in vivo* (*vide infra*).

In this report, we describe the synthesis of a difluoro-analog of UCB-J (named SDM-8), its ^{18}F -radiolabeling, and comprehensive *in vivo* evaluation in rhesus monkeys.²⁵

RESULTS AND DISCUSSION

Chemistry and Radiochemistry.

As summarized in Scheme 1, following the procedures of Mercier et al.²⁶ with some modifications, SDM-8 ((R)-**13**) was prepared from 3,5-difluorobenzaldehyde in four steps in an overall yield of 18%, requiring only a single flash column purification in the last step, in addition to chiral resolution of the racemic product. 3,5-Difluorobenzaldehyde (**1**)

underwent subsequent Wittig reaction with carbethoxymethylene phosphorus ylide, Michael addition with nitromethane, and reduction with iron powder under mildly acidic condition followed by *in situ* cyclization with saturated NaOH solution to afford the pyrrolidin-2-one intermediate (**10**). Coupling with 4-(chloromethyl)-3-methylpyridine gave the racemic compound (**13**), which was resolved with a chiral HPLC column to afford the two enantiomers, SDM-8 ((*R*)-**13**) and (*S*)-**13**, with greater than 99% enantiomeric excess (*ee*).

We have previously used iodonium ylide as precursor for ^{18}F -fluorination to successfully prepare the kappa opioid receptor radiotracer ^{18}F -LY2459989 in good radiochemical yield and molar activity.²⁷ Thus, we first adopted the same chemistry for ^{18}F -SDM-8. Synthesis of the racemic iodo-compound (**14**) started with 3-fluoro-5-iodobenzaldehyde (**2**) and followed similar procedures described above for the synthesis of racemic compound **13**. Conversion to the iodonium ylide precursor (**16**) followed the previously reported procedures.²⁸ The overall yield was about 20%.

The recently reported ^{18}F -radiolabeling procedures²⁹ (Scheme 2) were tested with the iodonium ylide precursor (**16**) and tetraethylammonium bicarbonate (TEAB) as the base in *N,N*-dimethylformamide (DMF). Preliminary results indicated that radiolabeling required heating at 150 °C, while simulation with SDM-8 under the radiolabeling conditions demonstrated a gradual racemization when heating at above 120 °C. As a result, ^{18}F -fluorination was performed with the racemic iodonium precursor (**16**), followed by sequential HPLC purification, first on a reverse phase C18 column, then a reverse phase chiral column to separate the two enantiomers. This led to a loss of more than 75% of the radioactivity during the purification. Total synthesis time was ~120 min including purification and formulation. The radiochemical yield of racemic product was 2% (HPLC incorporation yield, decay-uncorrected). ^{18}F -SDM-8 was produced in >99% radio-chemical purity and enantiomeric purity. Isolated radio-chemical yield was <1% (decay-uncorrected), and molar activity at the end of synthesis (EOS) was moderate (mean of 25.6 MBq/nmol, *n* = 2).

Recent developments in radiofluorination methodologies using iodonium, sulfonium, boron, or tin precursors^{30–33} provide new ways to synthesize radiotracers that are otherwise inaccessible through conventional radiofluorination approaches. Thus, we further carried out a screening of fluorination precursors including the boronic ester (**17**) and trimethyltin (**18**) compounds. These precursors were initially prepared from the iodo- intermediate (**14**) and tested (Schemes 1 and 2). By employing the procedures reported by Preshlock et al.,³³ the racemic boronic ester precursor (**17**) produced the radiolabeled product in an improved radio-chemical yield (11% at 150 °C or 6% at 110 °C, HPLC incorporation yield, decay-uncorrected) compared to the iodonium ylide precursor (**16**, 2%). Radiolabeling of the trimethyltin precursor (**18**) was more successful, and could be efficiently achieved following the procedures of Makaravage et al. at 110 °C,³² a temperature known to cause no racemization of the product. Hence, we proceeded to prepare enantiomerically pure trimethyltin precursor for the radiosynthesis of ^{18}F -SDM-8. For both economic consideration and relatively higher stability, we then prepared the bromo intermediate (**15**), which underwent chiral resolution to yield both pure enantiomers (>99% *ee*) for conversion to the trimethyltin compounds. With the enantiopure trimethyltin precursor ((*R*)-**18**), ^{18}F -

SDM-8 was produced in >98% radiochemical and enantiomeric purity, high molar activity (mean of 241.7 MBq/nmol, EOS, $n = 4$), and much improved radiochemical yield (mean of 24%, HPLC incorporation yield, decay-uncorrected). The isolated yield in one radiosynthesis was measured to be 19% (decay-uncorrected); in other cases, only a portion of the product was collected to conform to local radiation safety rules. Total synthesis time was reduced to ~95 min.

Radioligand Competition Binding Assays *in Vitro*.

The two enantiomers ((*R*)-**13** and (*S*)-**13**) were assayed for their *in vitro* binding affinities to SV2A using rat brain homogenates and the radioligand ^{18}F -SDM-2.³⁴ The inhibition constant (K_i) value was calculated at 0.58 nM for SDM-8 ((*R*)-**13**), compared to 0.27 nM for UCB-J. The other enantiomer ((*S*)-**13**) displayed much lower binding affinity ($K_i = 116$ nM).

PET Imaging Experiments in Rhesus Monkeys.

Injection Parameters.—A total of seven PET scans were performed in three different monkeys. Injected activity was 122.6 ± 59.8 MBq, with injected mass of 0.58 ± 0.54 μg .

Plasma Analysis.—Metabolism rate of ^{18}F -SDM-8 was moderate (Figure 2), with $42 \pm 13\%$ of parent radiotracer remaining at 30 min after injection, which further decreased to $27 \pm 5\%$ and $23 \pm 5\%$, respectively, at 60 and 90 min ($n = 5$). After the administration of ^{18}F -SDM-8, parent radioactivity level in plasma displayed a quick rise to peak and a sharp decline phase within 5 min, then a slow decrease afterward. Plasma free fraction (f_p) of ^{18}F -SDM-8 was high, at $43 \pm 2\%$ ($n = 5$). The measured log P value was 2.32, which fits in the range of PET radioligands predicted to have good permeability through the blood–brain barrier.³⁵

Brain Analysis.—PET images and regional time-activity curves (TACs) of ^{18}F -SDM-8 from baseline, displacement, and blocking scans are shown in Figures 3 and 4. In the monkey brain, ^{18}F -SDM-8 exhibited very high uptake and a heterogeneous distribution (Figure 3). High concentrations of the radiotracer were observed in the various gray matter regions, with the lowest uptake seen in the white matter region of centrum semiovale. In the displacement scan, administration of the SV2A-selective anticonvulsant levetiracetam (LEV, 30 mg/kg) at 90 min after tracer injection led to a sharp reduction in uptake, as illustrated by the difference in the summed PET images at 30–45 min (pre-LEV) and 120–140 min (post-LEV). Pretreatment with 0.15 mg/kg of UCB-J brought regional uptake levels across all brain regions to an almost homogeneous pattern, demonstrating blockade of ^{18}F -SDM-8 specific binding. Blocking with LEV at a dose of 30 mg/kg displayed similar effects.

Regional TACs of ^{18}F -SDM-8 baseline scans demonstrated fast and reversible kinetics. Highest tissue uptake levels were in the frontal cortex and putamen, with peak standardized uptake value (SUV) above 8, and lowest in centrum semiovale (SUV < 2). Peak uptake was reached within 30 min postinjection in all brain regions followed by a moderate rate of clearance over time (Figure 4A). In the displacement study, administration of LEV at 90 min post injection resulted in a rapid reduction in ^{18}F -SDM-8 uptake across gray matter regions, indicating competition by the displacing drug for the same binding site (Figure 4B). In both

blocking studies, pretreatment with LEV (30 mg/kg) or UCB-J (0.15 mg/kg) resulted in earlier peak uptake within 10 min and a rapid decrease thereafter with greatly reduced differences in regional activity levels (Figure 4C,D). These results confirmed the reversible and specific binding of ^{18}F -SDM-8 for SV2A.

Similar to the TACs from the blocking scans, a baseline scan with the inactive enantiomer ^{18}F -(*S*)-**19** displayed largely homogeneous brain uptake (Figure 5B), which indicated a lack of specific binding and chiral selectivity of radiotracer binding to SV2A, consistent with its low binding affinity (K_i of 116 nM for the (*S*)-enantiomer vs 0.58 nM for SDM-8).

Regional TACs were processed with the one-tissue compartment (1TC) model to generate binding parameters using the metabolite-corrected plasma activity as input function. The 1TC model produced good fits of the TACs and reliable estimates of regional volumes of distribution (V_T). Listed in Table 1 are regional V_T values, which are highest in cortical regions, followed by caudate, putamen, and thalamus, and lowest in centrum semiovale, consistent with autoradiography and *in vitro* binding results.³⁶ Large reductions in regional V_T values were seen in the LEV and UCB-J blocking scans, while the inactive enantiomer ^{18}F -(*S*)-**19** exhibited low V_T values among the brain regions.

Previous studies with ^{11}C -UCB-J indicated that the centrum semiovale can be used as a reference region in monkey to calculate the nondisplaceable binding potential (BP_{ND}). ^{18}F -SDM-8 BP_{ND} values, calculated from 1TC V_T values, are shown in Table 2. The rank order of BP_{ND} values is cingulate cortex > frontal cortex > insula > temporal cortex > caudate > putamen > hippocampus > cerebellum > amygdala > brainstem.

Pretreatment with LEV or UCB-J significantly reduced regional BP_{ND} values across the monkey brain. Using the occupancy plot, SV2A occupancy was calculated to be 81% with LEV at the dose of 30 mg/kg and 79% with UCB-J at the dose of 0.15 mg/kg, demonstrating the binding specificity of ^{18}F -SDM-8 *in vivo*.

Comparison of ^{18}F -SDM-8 with ^{11}C -UCB-J.—Three baseline PET scans with ^{11}C -UCB-J were performed on the same monkeys for comparison. Example regional TACs are shown in Figure 5A and 5C, demonstrating similar regional brain distribution pattern and tissue kinetics. Plasma measurements for these two radiotracers are also very similar: parent fraction was $42 \pm 13\%$ for ^{18}F -SDM-8 ($n = 5$) versus $47 \pm 12\%$ for ^{11}C -UCB-J ($n = 3$) at 30 min postinjection, while plasma free fraction was the same ($43 \pm 2\%$) for both ^{18}F -SDM-8 ($n = 5$) and ^{11}C -UCB-J ($n = 3$), and lipophilicity was very similar (2.32 for ^{18}F -SDM-8 vs 2.46 for ^{11}C -UCB-J). Specific binding signals, as measured by BP_{ND} values, are also presented in Table 2. The rank order of BP_{ND} values in ROIs was similar between ^{18}F -SDM-8 and ^{11}C -UCB-J in the same monkeys. However, the mean BP_{ND} value of ^{18}F -SDM-8 was 46% higher than that of ^{11}C -UCB-J. Under the same dose of the blocking drug UCB-J, 75% and 79% blocking was achieved in the ^{11}C -UCB-J and ^{18}F -SDM-8 scans, respectively, as they have similar binding affinity *in vitro*.

CONCLUSIONS

We have successfully synthesized ^{18}F -SDM-8 and evaluated its imaging characteristics in rhesus monkey. ^{18}F -SDM-8 displayed the same attractive imaging properties as ^{11}C -UCB-J: very high brain uptake, appropriate tissue kinetics, and good specific binding signals, which are higher than those of ^{11}C -UCB-J. Given the longer half-life of F-18 and feasibility for central production and distribution, ^{18}F -SDM-8 holds promise to be an excellent radiotracer for SV2A and as a biomarker for synaptic density measurement in neurodegenerative diseases and psychiatric disorders. Advancement of this novel radio-tracer to evaluation in humans is currently underway.

METHODS

Chemistry.

General Conditions.—All reagents and solvents were purchased from commercial sources (Sigma-Aldrich, VWR, and Fisher Scientific, etc.) and used without further purification. Proton, carbon, and fluorine nuclear magnetic resonance (^1H , ^{13}C , and ^{19}F NMR) spectra were recorded on an Agilent 400 or 600 MHz spectrometer (A400a, A400c, A600a). Chemical shifts are reported in parts per million (ppm), with either the solvent resonance or tetramethyl silane (TMS) as the internal standard (TMS, 0 ppm; CDCl_3 , 7.26 ppm; $\text{DMSO}-d_6$, 2.49 ppm in ^1H NMR and CDCl_3 , 77.0 ppm; $\text{DMSO}-d_6$, 39.7 ppm in ^{13}C NMR). Multiplicities are indicated as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet); coupling constants J are given in hertz (Hz). High resolution mass spectrometry (HRMS) was performed with a Thermo Scientific LTQ-Orbitrap XL Elite system. All chemicals used in this study were of 95% purity based on HPLC, LC-MS, or NMR.

Ethyl-3-(3,5-difluorophenyl)acrylate (4).—To a solution of 3,5-difluorobenzaldehyde (**1**, 8.30 mL, 75.44 mmol) in anhydrous THF (80.0 mL) under argon and cooled at 0 °C was added dropwise a solution of (carbethoxymethylene) triphenylphosphorane (28.90 g, 82.96 mmol) in anhydrous CH_2Cl_2 (130.0 mL). The reaction mixture was stirred for 1 h at 5 °C and then concentrated *in vacuo*. A mixture of hexanes and diethyl ether (4:1, v/v, 200 mL) was added, and the suspension was stirred for 10 min at room temperature. Filtration of the mixture through a silica gel plug and evaporation of the solvents afforded compound **4** as a white solid (14.30 g, 89%), which was used in the next step of synthesis without further purification. ^1H NMR (CDCl_3 , 400 MHz): δ 7.57 (d, J = 15.96 Hz, 1H), 7.12–6.97 (m, 1H), 6.93–6.73 (m, 1H), 6.42 (d, J = 15.96 Hz, 1H), 4.28 (q, J = 7.13 Hz, 2H), 1.34 (t, J = 7.14 Hz, 3H).

Ethyl-3-(3-fluoro-5-iodophenyl)acrylate (5).—Compound **5** was prepared in procedures similar to those described above for **4**. Yield: Quantitative. ^1H NMR (CDCl_3 , 400 MHz): δ 7.63 (s, 1H), 7.51 (d, J = 15.98 Hz, 1H), 7.42 (d, J = 7.57 Hz, 1H), 7.16 (d, J = 9.23 Hz, 1H), 6.39 (d, J = 16.00 Hz, 1H), 4.25 (q, J = 7.13 Hz, 2H), 1.32 (t, J = 7.11 Hz, 3H).

Ethyl 3-(3-bromo-5-fluorophenyl)acrylate (6).—Compound **6** was prepared in procedures similar to those described above for **4**. Yield: Quantitative. ^1H NMR (CDCl_3 , 400 MHz): δ 7.53 (d, J = 15.98 Hz, 1H), 7.43 (d, J = 1.64 Hz, 1H), 7.27–7.19 (m, 1H), 7.13 (dt, J = 9.11, 1.85 Hz, 1H), 6.40 (d, J = 15.99 Hz, 1H), 4.25 (q, J = 7.12 Hz, 2H), 1.32 (t, J = 7.11 Hz, 3H).

Ethyl 3-(3,5-difluorophenyl)-4-nitrobutanoate (7).—Compound **4** (10.00 g, 47.17 mmol) was dissolved in nitromethane (CH_3NO_2 , 8.20 mL, 153.15 mmol) under argon and cooled at -20°C . 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 7.00 mL, 47.17 mmol) was added, and the reaction mixture was kept stirring for 2 h at -20°C . Deionized (DI) water (30.0 mL) was added, followed by 12 N HCl until the pH of the mixture reached 1. The mixture was extracted with EtOAc (30.0 mL $\times$ 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was used in the next step of synthesis without further purification, and it also can be purified on a silica gel column eluting with 0–15% EtOAc in hexanes to afford compound **7** as a light brown oil/white crystal (M.P. is around room temperature, 13.00 g, quantitative). ^1H NMR (CDCl_3 , 400 MHz): δ 6.78 (d, J = 6.05 Hz, 2H), 6.76–6.70 (m, 1H), 4.72 (dd, J = 12.91, 6.55 Hz, 1H), 4.62 (dd, J = 12.90, 8.22 Hz, 1H), 4.11 (q, J = 6.75 Hz, 2H), 3.98 (quint., J = 7.23 Hz, 1H), 2.81–2.61 (m, 2H), 1.21 (t, J = 7.15 Hz, 3H).

Ethyl 3-(3-fluoro-5-iodophenyl)-4-nitrobutanoate (8).—Compound **8** was prepared in procedures similar to those described above for **7**. The crude product was purified on a silica gel column eluting with 0–15% EtOAc/hexanes to afford compound **8** as a white solid. Yield: 88%. ^1H NMR (CDCl_3 , 400 MHz): δ 7.35 (dd, J = 6.84, 1.61 Hz, 2H), 6.92 (d, J = 9.17 Hz, 1H), 4.70 (dd, J = 12.95, 6.63 Hz, 1H), 4.59 (dd, J = 12.93, 8.16 Hz, 1H), 4.10 (q, J = 7.03 Hz, 2H), 3.92 (quint., J = 7.37 Hz, 1H), 2.77–2.63 (m, 2H), 1.19 (t, J = 7.14 Hz, 3H).

Ethyl 3-(3-bromo-5-fluorophenyl)-4-nitrobutanoate (9).—Compound **9** was prepared in procedures similar to those described above for **7**. Yield: Quantitative. ^1H NMR (CDCl_3 , 400 MHz): δ 7.21–7.18 (m, 1H), 7.19–7.16 (m, 1H), 6.92 (dt, J = 9.08, 1.95 Hz, 1H), 4.73 (dd, J = 12.95, 8.22 Hz, 1H), 4.62 (dd, J = 12.96, 6.60 Hz, 1H), 4.12 (q, J = 7.12 Hz, 2H), 3.96 (quint., J = 7.35 Hz, 1H), 2.81–2.66 (m, 2H), 1.21 (t, J = 7.15 Hz, 3H).

4-(3,5-Difluorophenyl)pyrrolidin-2-one (10).—To a suspension of compound **7** (5.00 g, 18.23 mmol) and iron powder (10.24 g, 183.35 mmol) in a mixture of EtOH and DI water (2:1, v/v, 176.0 mL) was added NH_4Cl (29.50 g, 551.50 mmol). After stirring for 16 h at room temperature, the reaction mixture was adjusted to pH 14 with saturated NaOH solution and extracted with EtOAc (100 mL $\times$ 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to afford compound **5** as a white solid (1.82 g, 51%), which was used in the next step of synthesis without further purification. M.P. = $87\text{--}90^\circ\text{C}$. ^1H NMR (CDCl_3 , 400 MHz): δ 6.78 (d, J = 6.44 Hz, 2H), 6.77–6.63 (m, 1H), 5.90 (s, 1H), 3.80 (t, J = 8.78 Hz, 1H), 3.69 (quint., J = 8.19 Hz, 1H), 3.40 (t, J = 8.28 Hz, 1H), 2.75 (dd, J = 16.91, 8.88 Hz, 1H), 2.45 (dd, J = 16.93, 8.34 Hz, 1H).

4-(3-Fluoro-5-iodophenyl)pyrrolidin-2-one (11).—Compound **11** was prepared in procedures similar to those described above for **10**. Yield: 85%. ^1H NMR (CDCl_3 , 400 MHz): δ 7.37 (s, 1H), 7.33 (d, J = 7.76 Hz, 1H), 6.92 (d, J = 9.06 Hz, 1H), 5.68 (s, 1H), 3.76 (t, J = 8.82 Hz, 1H), 3.63 (p, J = 7.96 Hz, 1H), 3.41–3.33 (m, 1H), 2.72 (dd, J = 16.97, 8.98 Hz, 1H), 2.41 (dd, J = 16.80, 8.32 Hz, 1H).

4-(3-Bromo-5-fluorophenyl)pyrrolidin-2-one (12).—Compound **12** was prepared in procedures similar to those described above for **10**. Yield: 66%. ^1H NMR (CDCl_3 , 400 MHz): δ 7.18 (t, J = 1.6 Hz, 1H), 7.15 (dt, J = 8.0, 2.0 Hz, 1H), 6.90 (dt, J = 9.3, 1.9 Hz, 1H), 5.91 (s, 1H), 3.77 (t, J = 8.9 Hz, 1H), 3.65 (quint, J = 8.2 Hz, 1H), 3.38 (dd, J = 9.5, 6.8 Hz, 1H), 2.73 (dd, J = 16.9, 8.9 Hz, 1H), 2.42 (dd, J = 16.9, 8.3 Hz, 1H).

4-(3,5-Difluorophenyl)-1-((3-methylpyridin-4-yl)methyl)-pyrrolidin-2-one (13).—To a solution of compound **10** (0.20 g, 1.01 mmol) in anhydrous THF (5.0 mL) under argon and cooled to 0 °C was added sodium hydride (NaH, 0.09 g, 2.20 mmol). Tetrabutylammonium iodide (TBAI, 18 mg, 0.05 mmol) and 4-(chloromethyl)-3-methylpyridine hydrochloride (0.21 g, 1.10 mmol) were added after 30 min. The reaction mixture was kept stirring for 16 h at room temperature, then quenched with saturated NaHCO_3 solution (5 mL) and extracted with EtOAc (5 mL $\times$ 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 0–10% EtOH/EtOAc to afford compound **13** as a white solid (238 mg, 78%). M.P. 92–93 °C. ^1H NMR (CDCl_3 , 400 MHz): δ 8.46–8.40 (m, 2H), 7.05 (d, J = 4.97 Hz, 1H), 6.78–6.65 (m, 3H), 4.63 (d, J = 15.45 Hz, 1H), 4.42 (d, J = 15.47 Hz, 1H), 3.67–3.54 (m, 2H), 3.29–3.18 (m, 1H), 2.92 (dd, J = 16.90, 8.66 Hz, 1H), 2.60 (dd, J = 17.01, 8.01 Hz, 1H), 2.32 (s, 3H). ^{19}F NMR (CDCl_3 , 376 MHz, ppm): δ –108.61. ^{13}C NMR (CDCl_3 , 100 MHz, ppm): δ 173.24, 164.70, 162.21 (dd, J = 248.47, 12.92 Hz, 2C, C–F coupling), 151.43, 148.08, 145.79, 142.92, 131.81, 122.57, 109.86 (dd, J = 25.56, 11.61 Hz, 2C, C–F coupling), 102.97 (t, J = 25.23 Hz, 1C, C–F coupling), 53.55, 43.76, 38.18, 37.12, 16.10. HRMS calculated for $\text{C}_{17}\text{H}_{16}\text{F}_2\text{N}_2\text{O}$ ($[\text{M} + \text{H}]^+$) 303.1303; found, 303.1300.

Chiral Separation of Compound 13.—The separation was conducted on a Chiralpak IA preparative HPLC column, eluting with 30/70 EtOH/hexane with 0.1% TEA, the first compound eluting out from the column was the inactive enantiomer ((*S*)-**13**), while the second compound was the active enantiomer ((*R*)-**13**). The *ee.* was determined on an analytical Chiralpak IA column (5 μm , 4.6 $\times$ 150 mm). Mobile phase, 30:70 EtOH/hexane with 0.1% TEA; flow rate, 1.00 mL/min. Both enantiomers showed >99% *ee.* (*R*)-**13**: R_t = 4.91 min. ^1H NMR (CDCl_3 , 400 MHz): δ 8.45–8.40 (m, 2H), 7.04 (d, J = 4.95 Hz, 1H), 6.75–6.68 (m, 3H), 4.62 (d, J = 15.45 Hz, 1H), 4.42 (d, J = 15.45 Hz, 1H), 3.66–3.55 (m, 2H), 3.24 (dd, J = 5.61, 2.62 Hz, 1H), 2.92 (dd, J = 16.92, 8.77 Hz, 1H), 2.60 (dd, J = 16.97, 8.00 Hz, 1H), 2.31 (s, 3H). ^{19}F NMR (CDCl_3 , 376 MHz): δ –108.60. ^{13}C NMR (CDCl_3 , 150 MHz): δ 173.25, 164.27, 162.61 (dd, J = 247.5, 12.80 Hz, 2C, C–F coupling), 151.52, 148.17, 145.78, 142.79, 131.77, 122.54, 109.87 (dd, J = 20.15, 5.24 Hz, 2C, C–F coupling), 102.98 (t, J = 25.19 Hz, C–F coupling), 53.54, 43.76, 38.19, 37.11, 16.11. $[\alpha]_D^{25}$ = 47.6° (c = 0.05, MeOH). HRMS: calculated for $\text{C}_{17}\text{H}_{16}\text{F}_2\text{N}_2\text{O}$ ($[\text{M} + \text{H}]^+$) 303.1303; found, 303.1305.

4-(3-Fluoro-5-iodophenyl)-1-((3-methylpyridin-4-yl)methyl)-pyrrolidin-2-one

(14).—Compound **14** was prepared in procedures similar to those described above for **13**.

Yield: 71%. ¹H NMR (CDCl₃, 400 MHz): δ 8.41 (s, 2H), 7.35–7.24 (m, 2H), 7.02 (d, *J* = 1H), 6.84 (d, *J* = 9.37 Hz, 1H), 4.57 (d, *J* = 15.43 Hz, 1H), 4.42 (d, *J* = 15.43 Hz, 1H), 3.67–3.45 (m, 2H), 3.25–3.16 (m, 1H), 2.89 (dd, *J* = 17.02, 8.79 Hz, 1H), 2.57 (dd, *J* = 17.02, 7.87 Hz, 1H), 2.30 (s, 3H).

4-(3-Bromo-5-fluorophenyl)-1-((3-methylpyridin-4-yl)methyl)-pyrrolidin-2-one

(15).—Compound **15** was prepared in procedures similar to those described above for **13**.

Yield: 62%. ¹H NMR (CDCl₃, 400 MHz): δ 8.47–8.40 (m, 2H), 7.14 (dt, *J* = 7.95, 1.99 Hz, 1H), 7.13–7.09 (m, 1H), 7.04 (d, *J* = 4.97 Hz, 1H), 6.84 (dt, *J* = 9.25, 1.97 Hz, 1H), 4.60 (d, *J* = 15.45 Hz, 1H), 4.43 (d, *J* = 15.45 Hz, 1H), 3.65–3.52 (m, 2H), 3.23 (dd, *J* = 9.11, 6.22 Hz, 1H), 2.91 (dd, *J* = 16.99, 8.79 Hz, 1H), 2.59 (dd, *J* = 17.14, 7.86 Hz, 1H), 2.31 (s, 3H). HRMS calculated for C₁₇H₁₆BrFN₂O ([M + H]⁺) 365.0482; found, 365.0476.

Chiral Separation of Compound 15.—The separation was conducted on a Chiralpak

IA preparative HPLC column, eluting with 100% MeOH, the first compound eluting out from the column was the undesired enantiomer ((*S*)-**15**), while the second compound was the desired enantiomer ((*R*)-**15**). The *ee*. was determined on an analytical Chiralpak IA column (5 μm, 4.6 × 250 mm). Mobile phase, 100% MeOH; flow rate, 1.00 mL/min. Both enantiomers showed >99% *ee*. (*R*)-**15**: *R*_t = 8.89 min. ¹H NMR (CDCl₃, 400 MHz): δ 8.44–8.37 (m, 2H), 7.13 (dt, *J* = 7.98, 2.03 Hz, 1H), 7.10 (s, 1H), 7.02 (d, *J* = 4.96 Hz, 1H), 6.82 (dt, *J* = 9.24, 1.98 Hz, 1H), 4.58 (d, *J* = 15.42 Hz, 1H), 4.41 (d, *J* = 15.45 Hz, 1H), 3.58 (m, 2H), 3.22 (dd, *J* = 9.08, 6.21 Hz, 1H), 2.89 (dd, *J* = 17.05, 8.75 Hz, 1H), 2.57 (dd, *J* = 17.12, 7.84 Hz, 1H), 2.30 (s, 3H). ¹⁹F NMR (CDCl₃, 376 MHz): δ –109.61.

8-((3-Fluoro-5-(1-((3-methylpyridin-4-yl)methyl)-5-oxopyrrolidin-3-yl)phenyl)-λ³-iodaneylidene)-6,10-dioxaspiro[4.5]decane-7,9-dione (16).—To a solution of

compound **14** (316 mg, 0.77 mmol) in CHCl₃ (3 mL) was added trifluoroacetic acid (TFA, 1.8 mL, 23.4 mmol) followed by oxone (380 mg, 1.24 mmol). The reaction mixture was stirred for 2 h until it turned to a white suspension. The volatile contents were removed in vacuo. The residue was suspended in ethanol (3 mL), and 6,10-dioxaspiro[4.5]decane-7,9-dione (SPI-5, 150 mg, 0.88 mmol) was added, followed by 10% Na₂CO₃ until the pH of the mixture reached 10. The reaction mixture was stirred for 2 h, diluted with DI water (2.0 mL), and extracted with CH₂Cl₂ (2.0 mL × 3). The combined organic phase was dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 10–40% EtOH/EtOAc to afford compound **16** as a white solid (190 mg, 43%). M.P. 135–137 °C (decomposition). ¹H NMR (DMSO-*d*₆, 400 MHz): δ 8.36 (s, 2H), 7.56 (s, 1H), 7.47–7.35 (m, 2H), 7.18–7.10 (m, 1H), 4.52 (d, *J* = 15.95 Hz, 1H), 4.33 (d, *J* = 16.04 Hz, 1H), 3.78–3.68 (m, 1H), 3.61 (t, *J* = 8.78 Hz, 1H), 3.25–3.17 (m, 1H), 2.75 (dd, *J* = 16.41, 8.67 Hz, 1H), 2.61–2.48 (m, 1H, overlapped with DMSO-*d*₆ solvent peak), 2.24 (s, 3H), 1.99–1.93 (m, 4H), 1.70–1.60 (m, 4H).

4-(3-Fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)-1-((3-methylpyridin-4-yl)methyl)pyrrolidin-2-one (17).—To a solution of compound **15**

(0.16 g, 0.44 mmol) in anhydrous DMF (3.0 mL) was added bis(pinacolato)diboron (0.17 g, 0.66 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (32 mg, 0.04 mmol), and potassium acetate (0.22 g, 2.22 mmol) under argon. The reaction mixture was degassed and refilled with argon for 3 min and kept stirring at 80 °C for 12 h. The reaction was then quenched with DI water (10 mL) and extracted with EtOAc (10 mL × 3). The combined organic phase was dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 0–10% EtOH/EtOAc to afford compound **17** as a pale yellow solid (65 mg, 36%). M.P. 100–102 °C. ¹H NMR (CDCl₃, 400 MHz): δ 8.49 (s, 2H), 7.40 (s, 1H), 7.37 (dd, *J* = 8.72, 2.61 Hz, 1H), 7.12 (s, 1H), 6.98 (dt, *J* = 9.83, 2.16 Hz, 1H), 4.61 (d, *J* = 15.65 Hz, 1H), 4.41 (d, *J* = 15.60 Hz, 1H), 3.68–3.58 (m, 2H), 3.33–3.25 (m, 1H), 2.91 (dd, *J* = 16.90, 8.49 Hz, 1H), 2.67 (dd, *J* = 16.91, 8.14 Hz, 1H), 2.33 (s, 3H), 1.34 (s, 12H).

(R)-4-(3-Fluoro-5-(trimethylstannyl)phenyl)-1-((3-methylpyridin-4-yl)methyl)pyrrolidine-2-one ((R)-18).—To a solution of compound (*R*)-**15** (0.15 g, 0.41 mmol) in anhydrous toluene (1.5 mL) was added lithium chloride (0.09 g, 2.05 mmol), tetrakis-(triphenylphosphine)palladium(0) (70 mg, 0.06 mmol), and hexamethylditin (0.13 mL, 0.63 mmol) under argon. The reaction mixture was degassed and refilled with argon for 3 min and kept stirring at 105 °C for 1 h. The reaction mixture was diluted with EtOAc (8 mL), passed through celite, and rinsed with EtOAc (4 mL × 2). The filtrate was concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 0–20% EtOH/EtOAc, followed by the trituration with CHCl₃ and hexane to afford the product as a white solid (110 mg, 60%). M.P. 95–97 °C. ¹H NMR (CDCl₃, 400 MHz): δ 8.43–8.34 (m, 2H), 7.15–6.92 (m, 3H), 6.80 (dt, *J* = 10.09, 2.12 Hz, 1H), 4.61 (d, *J* = 15.54 Hz, 1H), 4.39 (d, *J* = 15.55 Hz, 1H), 3.66–3.52 (m, 2H), 3.31–3.19 (m, 1H), 2.90 (dd, *J* = 16.98, 8.83 Hz, 1H), 2.63 (dd, *J* = 17.14, 8.16 Hz, 1H), 2.29 (s, 3H), 0.44–0.10 (m, 9H). ¹⁹F NMR (CDCl₃, 376 MHz): δ –113.16. ¹³C NMR (CDCl₃, 100 MHz): δ 173.73, 164.17, and 161.67 (d, *J* = 250.51 Hz, 1C, C–F coupling), 151.37, 148.06, 146.43 (d, *J* = 2.75 Hz, 1C, C–F coupling), 143.83 (d, *J* = 5.65 Hz, 1C, C–F coupling), 143.05, 131.74, 129.71, 122.44, 121.02 (d, *J* = 17.40 Hz, 1C, C–F coupling), 113.40 (d, *J* = 21.76 Hz, 1C, C–F coupling), 54.08, 43.73, 38.49, 37.09, 16.07, –9.27 (3C). [α]_D²⁵ = 14.4° (c = 0.05, MeOH). HRMS: calculated for C₂₀H₂₅FN₂OSn ([M + H]⁺) 449.1046; found, 449.1037.

Radiochemistry.

H₂¹⁸O was obtained from Huayi Isotopes (Toronto, Canada). Anion exchange Chromafix cartridges (PSHCO₃) were purchased from Macherey-Nagel (Dueringen, Germany). Solid-phase extraction (SPE) cartridges were purchased from Waters Associates (Milford, MA, USA). The HPLC system used for purification of crude product included a Shimadzu LC-20A pump, a Knauer K200 UV detector, and a Bioscan γ-flow detector, with a Luna C18(2) semipreparative column (10 × 250 mm, 10 μm, Phenomenex, Torrance, CA, USA) or ChiralCel OD-H semipreparative column (10 × 250 mm, 5 μm, ChiralCel, West Chester, PA, USA). The HPLC system used for quality control tests was composed of a Shimadzu LC-20A pump, a Shimadzu SPD-M20A PDA or SPD-20A UV detector, a Bioscan γ-flow detector, with a Genesis C18 column (4.6 × 250 mm, 4 μm, Hichrom, Berkshire, UK) eluting with a mobile phase of 34% CH₃CN and 66% 0.1 M ammonium formate solution

with 0.5% acetic acid (AcOH) (pH 4.2) at a flow rate of 2 mL/min for purity check as well as a ChiralCel OD-RH (4.6 × 150 mm, 5 μm, ChiralCel, West Chester, PA, USA) eluting with a mobile phase of 30% CH₃CN and 70% 0.1 M ammonium formate solution with 0.5% AcOH (pH 4.2) at a flow rate of 1 mL/min for chirality check. Identity was confirmed by coinjection of the product with SDM-8 ((*R*)-**13**) and detection of a single UV peak at 261 nm wavelength on the chromatogram.

¹⁸F-Fluoride was produced via the ¹⁸O(p, n)¹⁸F nuclear reaction in a 16.5-MeV GE PETtrace cyclotron (Uppsala, Sweden). The cyclotron produced aqueous ¹⁸F-fluoride solution in ¹⁸O-water was transferred to a reaction V-vial in a lead-shielded hot cell, where the ¹⁸F-fluoride anion was trapped on an anionic exchange resin cartridge (Chromafix-PS-HCO₃).

(*R*)- and (*S*)-4-(3-Fluoro-5-(fluoro-¹⁸F)phenyl)-1-((3-methylpyridin-4-yl)methyl)pyrrolidin-2-one (¹⁸F-SDM-8 and ¹⁸F-(*S*)-19**, from Iodonium Ylide Precursor).**—The produced ¹⁸F was trapped on a Chromafix cartridge, which was activated with DI water (5 mL) before use. The trapped ¹⁸F was eluted out with a solution of TEAB (2.0 mg) in a mixture of DI water (0.3 mL) and MeCN (0.7 mL) and dried azeotropically under argon at 105 °C, which was dried further with MeCN (1.0 mL × 2) at 105 °C. A solution of compound **16** (2.0 mg) in DMF (0.5 mL) was added to the dried ¹⁸F-fluoride-TEAB, and the reaction mixture was heated at 150 °C for 10 min. The reaction mixture was diluted with the HPLC mobile phase (1.5 mL) and loaded onto a semipreparative Luna C18(2) column eluting with a mobile phase of 30% CH₃CN and 70% 0.1 M ammonium formate solution with 0.5% AcOH (pH 4.2) at a flow rate of 5 mL/min. The HPLC fraction containing the product was collected, diluted with DI water (50 mL) and passed through a Waters C18 SepPak cartridge. The cartridge was washed with DI water (10 mL) and dried with air. The trapped radioactive product was eluted from the cartridge with EtOH (1 mL), diluted with DI water (1 mL), and then loaded onto the semipreparative ChiralCel OD-H column eluting with a mobile phase of 30% CH₃CN and 70% 0.1 M ammonium formate solution with 0.5% AcOH (pH 4.2) at a flow rate of 5 mL/min. The first radioactive peak, eluting at 15.1 min, was the (*S*)-enantiomer (¹⁸F-(*S*)-**19**) and the second radioactive peak, eluting at 16.8 min, was the (*R*)-enantiomer (¹⁸F-SDM-8).

The chiral-HPLC fraction with the desired enantiomer was collected, diluted with DI water (50 mL), and passed through a Waters C18 SepPak cartridge. The cartridge was washed with 0.001 M HCl solution (10 mL) and dried with air. The trapped product was recovered by elution with U.S. Pharmacopeial Convention (USP) EtOH (1 mL) followed by USP saline (3 mL). The resulting mixture was then passed through a sterile membrane filter (0.22 μm, Merck Millipore, Ireland) for terminal sterilization and collected in a sterile vial precharged with 7 mL of USP saline and 20 μL of 8.4% NaHCO₃ USP solution to afford a formulated solution ready for administration.

(*R*)-4-(3-Fluoro-5-(fluoro-¹⁸F)phenyl)-1-((3-methylpyridin-4-yl)methyl)pyrrolidin-2-one (¹⁸F-SDM-8, from Trimethyltin Precursor).—The produced ¹⁸F was trapped on a Chromafix cartridge, which was activated with EtOH (5 mL), a solution of potassium triflate (KOTf) in DI water (90 mg/mL, 5 mL), and DI water (5 mL)

before use. The trapped ^{18}F was eluted out with a mixture of a solution of KOTf in DI water (10 mg/mL, 0.45 mL), a solution of K_2CO_3 in DI water (1 mg/mL, 50 μL), and MeCN (0.5 mL), then dried azeotropically under argon at 105 $^\circ\text{C}$, which was dried further with MeCN (1.0 mL $\times$ 2) at 105 $^\circ\text{C}$. A solution of compound (*R*)-**18** (1.7–3 mg) in anhydrous dimethylacetamide (DMA, 0.4 mL) was added to the dried ^{18}F followed by a solution of pyridine in DMA (1M, 0.1 mL) and a solution of copper(II) triflate in DMA (0.2 M, 67 μL), then the reaction mixture was heated at 110 $^\circ\text{C}$ for 20 min. The reaction mixture was diluted with HPLC mobile phase (1.5 mL) and loaded onto a semipreparative Luna C18(2) column eluting with a mobile phase of 25% CH_3CN and 75% 0.1 M ammonium formate solution with 0.5% AcOH (pH 4.2) at a flow rate of 5 mL/min. The procedures for the collection and formulation of ^{18}F -SDM-8 were the same as those described above in the radiosynthesis with iodonium ylide precursor.

Radioligand Competition Binding Assays *in Vitro*.

One Sprague–Dawley rat was euthanized with carbon dioxide inhalation. The brain was harvested, rapidly frozen on dry ice, and then stored at $-80\text{ }^\circ\text{C}$. The whole brain homogenate was obtained following published procedures.³⁷ The final homogenate was suspended in 20 mM Tris–HCl buffer (pH 7.4) containing 250 mM of sucrose at a wet tissue concentration of 100 mg/mL and stored at $-80\text{ }^\circ\text{C}$ until use.

For competition binding experiments, 0.5 mg of the rat brain homogenate in 800 μL of binding buffer (2 mM MgCl_2 in 50 mM Tris–HCl, pH = 7.4) were incubated on a thermoshaker at 37 $^\circ\text{C}$ for 30 min with 100 μL ^{18}F -SDM-2³⁴ and 100 μL of the compound over a range of concentrations (from 0.1 to 100 nM). Nonspecific binding was defined as the residual binding observed in the presence of 1 mM levetiracetam (100 μL). At completion of the incubation period, the membrane-bound radioligand was recovered using rapid filtration through GF/B glass fiber filters (13 mm GD/X, Whatman Inc.), which were presoaked for at least 30 min in 0.5% polyethyleneimine (PEI). The membranes were washed twice with 1 mL of ice-cooled binding buffer. The binding mixtures, filters, and filtrates were counted using calibrated gamma counters (1480/2480 WIZARD; PerkinElmer, Waltham, MA, USA). Values of IC_{50} were calculated using the GraphPad Prism software and converted to inhibition constants (K_i) using the Cheng–Prusoff equation.³⁸

PET Imaging Experiments in Rhesus Monkeys.

PET Scan Procedures.—PET imaging experiments were performed in rhesus monkeys (*Macaca mulatta*) according to a protocol approved by the Yale University Institutional Animal Care and Use Committee. Three monkeys were used in a total of six scans including three 120 min baseline scans with ^{18}F -SDM-8 ($n = 2$) and undesired enantiomer ^{18}F -(*S*)-**19**, respectively, one 180 min ^{18}F -SDM-8 displacement scan with levetiracetam (LEV, 30 mg/kg) given as a 5 min infusion at 90 min post radiotracer injection, and two 120 min ^{18}F -SDM-8 blocking scans with LEV (30 mg/kg) or UCB-J (0.15 mg/kg) administered as a 10 min infusion beginning 10 min before radiotracer injection. Three baseline scans with ^{11}C -UCB-J were also conducted in the same monkeys for comparison with ^{18}F -SDM-8.

Before each scan, each monkey was fasted overnight, and immobilized with ketamine (10 mg/kg, intramuscularly) at least two hours prior to the scan. A venous line was inserted in one limb for administration of radiotracer, displacement and blocking drugs. A catheter was placed in the femoral artery in the other limb for blood sampling. Endotracheal intubation was performed to permit administration of isoflurane (1.5–2.5% in oxygen) to effect. A water-jacket heating pad was used to maintain body temperature. The animal was attached to a physiological monitor, and vital signs (heart rate, blood pressure, respirations, SPO₂, EKG, ETCO₂, and body temperature) were continuously monitored.

Dynamic PET scans were performed on a Focus 220 scanner (Siemens Medical Solutions, Knoxville, TN, USA) with a reconstructed image resolution of approximately 1.5 mm. Before radiotracer injection, a 9 min transmission scan was obtained for attenuation correction. The radiotracer was administered by an infusion pump over 3 min. Emission data were collected in list mode for 120 min and reformatted into 33 successive frames of increasing durations (6 × 10 s, 3 × 1 min, 2 × 2 min, and 22 × 5 min). For the 180 min scan, additional frames (12 × 5 min) were acquired.

Plasma Metabolite Analysis and Input Function Measurement.—Arterial blood samples were collected at preselected time points and assayed for radioactivity in whole blood and plasma gamma counters. Six samples, drawn at 0, 5, 15, 30, 60, and 90 min, were processed and analyzed to measure the radioligand metabolite profile by HPLC using a column-switching method.³⁹ Whole blood samples in EDTA tubes were centrifuged at 2930g at 4 °C for 5 min to separate the plasma. Supernatant plasma was collected and activity in 0.2 mL aliquots was counted on a gamma counter. Plasma samples were then mixed with urea (8 M) to denature plasma protein, filtered with 1.0 μm Whatman 13 mm CD/X syringe filter and loaded onto an automatic column-switching HPLC system connecting a capture column (4.6 × 19 mm) self-packed with Phenomenex Strata-X polymeric SPE sorbent and eluting with 1% MeCN in DI water at 2 mL/min for 4 min. The trapped activity in the capture column was then back flushed and eluted through a Gemini-NX column (4.6 × 250 mm, 5 μm) using 40% MeCN in 0.1 M ammonium formate (pH = 6.4, v/v) at a flow rate of 1.65 mL/min. The eluent fractions were collected with an automated fraction collector (Spectrum Chromatography CF-1). Activity in the whole blood, plasma, filtered plasma-urea mix, filter, and HPLC eluent fractions was all counted with the automatic γ counters. The sample recovery rate, extraction efficiency, and HPLC fraction recovery were monitored. The unmetabolized parent fraction was determined as the ratio of the sum of radioactivity in fractions containing the parent compound to the total amount of radioactivity collected and fitted with an inverted gamma function and corrected for filtration efficiency. The arterial plasma input function was then calculated as the product of total counts in the plasma and interpolated parent fraction at each time point.

Measurement of Radiotracer Free Fraction (f_p) in Plasma.—An ultrafiltration method was used for measuring the unbound portion (free fraction) of ¹⁸F-SDM-8 in plasma as previously described.²⁷ The f_p was determined as the ratio of the radioactivity concentration in the filtrate to the total activity in plasma. Measurements of f_p were performed in triplicate for each scan.

Measurement of Lipophilicity.—Lipophilicity ($\log P$) was determined by the modified method from previously published procedures.²⁷ $\log P$ was calculated as the ratio of decay-corrected radioactivity concentrations in 1-octanol and in phosphate buffered saline (PBS, pH = 7.4, Dulbecco). Six consecutive equilibration procedures were performed until a constant value of $\log P$ was obtained.

Imaging Analysis and Kinetic Modeling.—Representative high-resolution magnetic resonance (MR) images were previously acquired with a Siemens 3T Trio scanner to assist with image coregistration and anatomical localization of regions of interest (ROIs). The MR image was registered to an atlas and to the PET images, as previously described.⁴⁰

PET emission data were corrected for attenuation, scanner normalization, scatter, and randoms, and dynamic images were reconstructed using a Fourier rebinning and filtered back projection algorithm. Using the MR images, the following ROIs were defined: amygdala, brain stem, caudate nucleus, centrum semiovale, cerebellum, cingulate cortex, frontal cortex, globus pallidus, hippocampus, insula, nucleus accumbens, occipital cortex, pons, putamen, substantia nigra, temporal cortex, and thalamus. For each PET scan, radiotracer concentrations over time were measured and regional TACs were generated for the ROIs.

Regional TACs were fitted and analyzed with the 1TC model.⁴¹ Regional distribution volume (V_T , mL/cm³) was calculated from kinetic analysis using the metabolite-corrected arterial plasma concentration as the input function.⁴² In the 1TC model, kinetic parameters K_1 (mL min⁻¹ cm⁻³) and k_2 (min⁻¹) were the rate constants monitoring the ligand transfer into and out of the brain, respectively, and V_T was calculated as K_1/k_2 .

Nondisplaceable binding potential (BP_{ND}) was calculated from regional V_T values using centrum semiovale (CS) as the reference region, that is, $BP_{ND} = (V_{T,ROI} - V_{T,CS}) / V_{T,CS}$.

SV2A occupancy by blocking drugs was obtained from the occupancy plot using the regional V_T values from the baseline scan and V_T difference between baseline and blocking scans.⁴³

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Figure 1.

Chemical structures of example SV2A radiotracers. *, Binding assay performed with human brain tissues as described in ref 26. **, Binding assay performed with rat brain homogenate at Yale PET Center.

Figure 2.
(A) Parent fraction of ^{18}F -SDM-8 in plasma and (B) metabolite-corrected plasma radioactivity over time ($n = 5$, mean $\pm$ SD).

Figure 3. Summed PET SUV images from pre-LEV at 30–45 min (as a baseline, top row) and post-LEV at 120–140 min (middle row*) in a displacement scan with LEV (30 mg/kg) given at 90 min, as well as a blocking scan (bottom row) with LEV (30 mg/kg). *, Scale of post-LEV (middle row) summed PET image is 0–7.

Figure 4.

Regional TACs of ^{18}F -SDM-8 from a (A) baseline scan, (B) displacement scan with levetiracetam (30 mg/kg) given at 90 min and blocking scans with (C) LEV (30 mg/kg), and with (D) UCB-J (0.15 mg/kg).

Figure 5. Regional TACs from baseline scans with (A) ^{18}F -SDM-8, (B) ^{18}F -(S)-19, and (C) ^{11}C -UCB-J.

Scheme 1. Synthesis of SDM-8 and Its Radiolabeling Precursors¹

¹Reagents and conditions: (a) carbethoxymethylene triphenyl phosphorene, THF/CH₂Cl₂, 5 °C, 1 h; (b) DBU, MeNO₂, -20 °C, 2 h; (c) Fe, NH₄Cl, EtOH/H₂O, r.t., 16 h, then sat. NaOH, r.t.; (d) 4-(chloromethyl)-3-methylpyridine hydrochloride, NaH, TBAI, 0 °C to r.t., 16 h; (e) Chiral separation; (f) TFA, oxone, CHCl₃, r.t., 2 h, then 6,10-dioxaspiro[4,5]decane-7,9-dione (SPI-5), Na₂CO₃, EtOH, r.t., 2 h; (g) B₂Pin₂, KOAc, PdCl₂(dppf), DMF, 80 °C, 12 h; (h) Sn₂Me₆, LiCl, Pd(PPh₃)₄, toluene, 105 °C, 1 h.

Scheme 2. Radiosynthesis of ^{18}F -SDM-8, from Racemic Iodonium Ylide (16**) or Enantiopure Trimethyltin Precursor (*(R)*-**18**), and Radiosynthesis of Racemic ^{18}F -**19** from Racemic Boronic Ester Precursor (**17**)¹**

¹Reagents and conditions: (a) ^{18}F /TEAB, DMF, 150 °C, 10 min; (b) chiral separation; (c) $\text{Cu}(\text{OTf})_2$, pyridine, K^{18}F , DMA, 110 °C, 20 min; (d) $\text{Cu}(\text{OTf})_2\text{Py}_4$, ^{18}F /TEAB, DMA, 150 or 110 °C, 20 min.

1TC-Derived Regional Distribution Volume (V_T , mL/cm³) from Baseline Scans of ¹⁸F-SDM-8 ($n = 3$) and ¹⁸F-(S)-19 ($n = 1$), as Well as Blocking Scans of ¹⁸F-SDM-8 with LEV ($n = 1$) and UCB-J ($n = 1$), in Comparison with Those from ¹¹C-UCB-J Baseline Scans ($n = 3$)

Table 1.

region of interest (ROI)	V_T (mL/cm ³)				
	¹⁸ F-SDM-8 baseline	¹⁸ F-SDM-8 blocking w/LEV	¹⁸ F-SDM-8 blocking w/UCB-J	¹⁸ F-(S)-19 baseline	¹¹ C-UCB-J baseline
amygdala	24.8 ± 8.5	6.7	7.1	3.2	25.9 ± 0.4
brainstem	16.3 ± 3.6	8.8	4.5	3.1	23.0 ± 6.1
caudate	35.0 ± 6.3	11.7	7.1	4.1	43.2 ± 8.6
centrum semiovale	8.9 ± 2.0	7.12	4.1	2.9	14.1 ± 4.1
cerebellum	28.9 ± 6.8	8.2	4.5	3.8	36.2 ± 9.4
cingulate cortex	48.5 ± 8.9	14.8	10.9	5.5	56.4 ± 14.6
frontal cortex	47.1 ± 9.1	13.4	10.7	5.4	55.3 ± 11.7
globus pallidus	21.6 ± 4.2	12.1	6.5	3.1	27.4 ± 6.3
hippocampus	30.1 ± 5.6	10.5	7.4	3.3	34.3 ± 5.4
insula	46.1 ± 9.2	15.3	10.0	4.6	53.2 ± 12.4
nucleus accumbens	45.2 ± 7.9	16.5	10.4	4.6	54.5 ± 14.4
occipital cortex	43.7 ± 10.7	13.1	8.6	4.6	51.9 ± 13.9
pons	16.0 ± 3.4	9.0	4.7	2.9	23.2 ± 6.3
putamen	34.6 ± 4.9	14.2	8.4	4.3	42.0 ± 10.3
temporal cortex	42.2 ± 10.0	12.9	8.7	4.2	49.5 ± 13.8
thalamus	34.2 ± 3.0	12.2	8.7	4.3	41.7 ± 6.5

Table 2.

Regional Non-displaceable Binding Potential (BP_{ND}) Values for ^{18}F -SDM-8 Baseline Scans ($n = 3$) and Blocking Scans of ^{18}F -SDM-8 with LEV ($n = 1$) and UCB-J ($n = 1$), in Comparison with Those from ^{11}C -UCB-J Baseline Scans ($n = 3$)

region of interest (ROI)	BP_{ND}			
	^{18}F -SDM-8 baseline	^{11}C -UCB-J baseline	^{18}F -SDM-8 blocking w/ LEV	^{18}F -SDM-8 blocking w/ UCB-J
amygdala	1.9 ± 1.1	1.0 ± 0.7	-0.1	0.6
brainstem	0.8 ± 0.0	0.6 ± 0.1	0.2	0.1
caudate	3.0 ± 0.4	2.1 ± 0.4	0.8	1.0
cerebellum	2.2 ± 0.2	1.6 ± 0.1	0.6	0.6
cingulate cortex	4.5 ± 0.4	3.0 ± 0.5	1.1	1.5
frontal cortex	4.3 ± 0.6	3.0 ± 0.6	0.9	1.4
globus pallidus	1.4 ± 0.3	1.0 ± 0.2	0.7	1.5
hippocampus	2.4 ± 0.4	1.5 ± 0.4	0.5	0.7
insula	4.2 ± 0.3	2.8 ± 0.4	1.2	1.3
nucleus accumbens	4.1 ± 0.4	2.9 ± 0.4	1.3	1.4
occipital cortex	3.9 ± 0.2	2.7 ± 0.2	0.8	1.0
pons	0.8 ± 0.0	0.7 ± 0.1	0.3	0.1
putamen	2.9 ± 0.4	2.0 ± 0.3	1.0	1.0
temporal cortex	3.7 ± 0.3	2.5 ± 0.3	0.8	1.0
thalamus	2.9 ± 0.7	2.1 ± 0.5	0.7	1.0
mean	2.87	1.97		