Deuterium substituted 2-(2′-((Dimethylamino)methyl)-4′-[^{18}F](fluoropropoxy)phenyl-thio)benzenamine as a serotonin transporter imaging agent

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Abstract

Positron emission tomography (PET) imaging of serotonin transporter (SERT) is useful for studying brain diseases with altered serotonergic function. A deuterated imaging agent, ([^{18}F]2-((2-((bis(methyl-d3)amino)methyl)-4-(3-fluoropropoxy-1,1,2,2,3,3-d6)phenyl)thio)aniline, [^{18}F]D12FPBM, [^{18}F]**1**), was prepared as a new chemical entity (NCE). The deuterated agent, **1**, showed excellent binding affinity to SERT; Ki was 0.086 nM, comparable to the un-deuterated FPBM. In vivo biodistribution studies in rats with [^{18}F]**1** showed good brain uptake (1.09% dose/g at 2 min post-injection), high specific uptake into the hypothalamus (HY) as compared to cerebellum (CB) (HY/CB = 7.55 at 120 min), suggesting a specific localization to SERT binding sites. Regional brain distribution in rats provided clear indication that [^{18}F]**1** concentrated in the hypothalamus, hippocampus, and striatum, areas with a high SERT density. Results indicate that very little D to H substitution effect was found; [^{18}F]FPBM and [^{18}F]**1** showed very similar SERT binding. [^{18}F]**1** might be an excellent candidate for SERT imaging.

Keywords: serotonin transporter, ^{18}F, deuterium, brain, PET imaging agent

Introduction

Recently, FDA has approved a new deuterium containing neurologically active drugs, deuterated tetrabenazine (SD-809, AUSTEDO) for treatment of Tardive Dyskinesia; and D6-dextromethorphan (AVP-786) for treatment of agitation in Patients with Alzheimer's Disease has reached phase III study (Fig. 1). These new deuterated drugs demonstrate benefits of deuterium's kinetic isotope effect on the safety and clearance of drug substances and creation of new chemical entities (NCE) as new drugs through deuterated versions of existing molecules [1, 2].

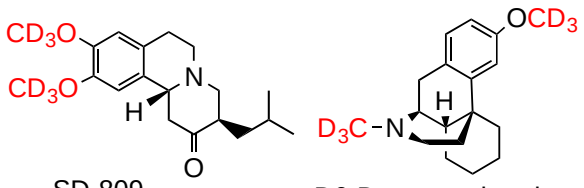


Figure 1. Chemical structures of deuterated tetrabenazine (SD-809, Austedo) and D6-dextromethorphan (AVP-786).

In the past few decades many selective serotonin reuptake inhibitors, SSRIs, such as Fluoxetine, Sertraline, Paroxetine, Escitalopram, etc., specifically target serotonin transporter (SERT) and inhibit serotonin reuptake to the neurons. Consequently, SSRIs are useful in the treatment of depression as well as many other psychiatric conditions by regulating the serotonin concentration in the synapse [3]. Positron emission tomography (PET) imaging for SERT binding sites in the brain with [^{11}C]DASB ($T_{1/2}$ = 20 min) (Fig. 2) has been reported, and its clinical application is well reported in the literature [4-7]. A suitably ^{18}F labeled SERT imaging agent with its longer physical half-life ($T_{1/2}$ = 110 min) is more convenient to manufacture and distribute for probing pathophysiological and therapeutic mechanisms in various psychological diseases [8]. A number of SERT ligands for in vivo imaging (Fig. 2)

have been developed [9-21]. Biphenyl sulfide derivatives showed promising results as in vivo SERT imaging agents (Fig. 2) [22]. This biphenyl sulfide series of imaging agents display one unique advantage over other series, because they are highly selective towards SERT binding and have low affinity to dopamine transporter and norepinephrine transporters [11, 23, 24].

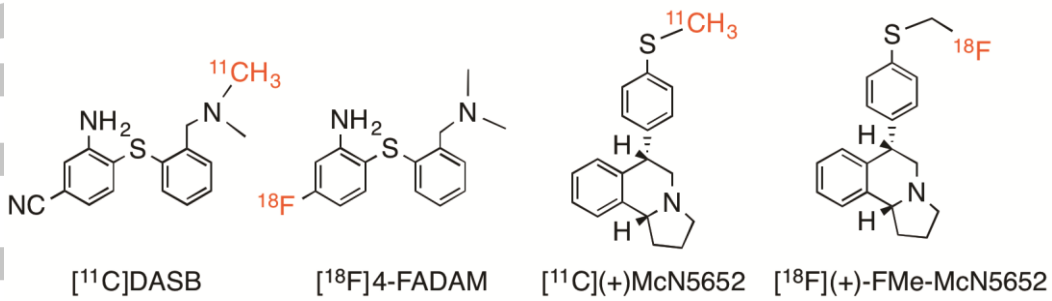


Figure 2. Chemical structures of known imaging agents for serotonin transporters (SERT).

Many reports suggested that substituting deuterium for hydrogen might slow down the in vivo metabolism to reduce the loss of radioactive tracer while maintaining binding capability for a specific enzyme or receptor-binding site [25-27]. The best example of this approach is the use of [^{11}C]-L-deprenyl-D_2 for measurement of the monoamine oxidase B (MAO-B) activity in vivo brain [28, 29]. The ability to modulate drug metabolism through hydrogen to deuterium substitution provides a useful approach in developing novel radiopharmaceuticals by increasing in vivo stability [25]. We intend to apply this strategy in developing new SERT imaging agents. Previously, [^{18}F]FPBM (Fig. 3) was reported with having substitution on the phenyl ring B (compared to [^{18}F]4-FADAM or [^{11}C]DASB) (Fig. 3). [^{18}F]FPBM possesses high selective binding, high brain uptake (0.99% dose/g at 2 min post iv injection), and an excellent in vivo target-to-non-target ratio (7.7 at 120 min post injection) [11, 30-33]. Labeling studies suggested that there is a tendency to produce a vinyl side product through an elimination reaction during the SN2 substitution by the activated [^{18}F]fluoride ion [32]. Through hydrogen to deuterium substitution on the alkyl chain it may also be possible to reduce the elimination reaction. We also reasoned that the N-methyl

groups of this series of biphenyl sulfides are usually removed by a rapid in vivo metabolism [34, 35]. The N-mono-de-methylated or the N,N-di-demethylated biphenyl sulfides might lead to a lower binding affinity towards SERT binding sites [33, 34, 36]. Deuterium on the N-methyl group could also reduce the de-methylation in vivo. However, this approach needs a careful consideration of in vivo kinetics and metabolism, and it is not likely will be universally applicable.

In order to test the hypothesis and evaluate the effects of deuterium (D) for hydrogen (H) substitution as a novel strategy for developing effective SERT imaging agents, we have prepared and evaluated brain uptake and biodistribution in rats between [^{18}F]FPBM and [^{18}F]**1** (Fig. 3). We wanted to prepare and test deuterated ligand as a new chemical entity (NCE) as new drugs. We also want to maximize D to H substitution effect; therefore, deuterium substitutions were added to the fluoropropyl side chain reducing the chances of de-fluorination, which led to the design of **1**. By loading up thedeuterium atoms maximally we hoped to test if the D to H substitution might change the properties of FPBM in its SERT binding and/or in vivo biodistribution targeting the SERT binding sites in the brain. The deuterium effort may also stop, or reduce, the elimination side product during the fluorination reaction as previously reported [32]. Reported herein is the synthesis and evaluation of [^{18}F]**1** and related deuterated derivatives (Fig. 3).

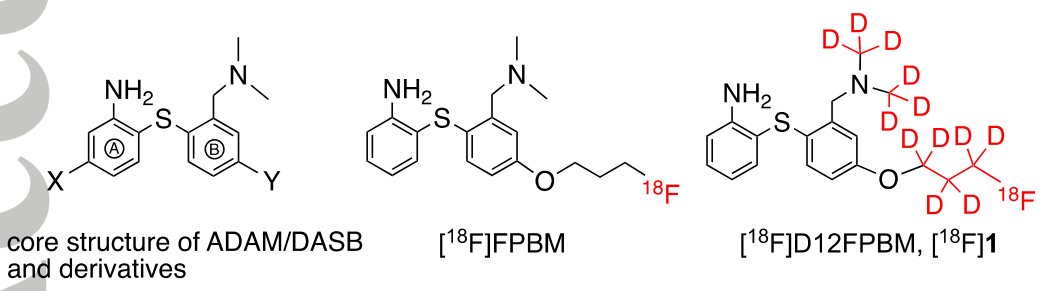


Figure 3. Chemical structures of ^{18}F]FPBM and [^{18}F]D12FPBM ([^{18}F]**1**) and core structure of ADAM/DASB

Methods

1. General

All reagents and solvents were commercial products purchased from Aldrich, Acros, Alfa and Fisher, and were used without further purification, unless otherwise indicated. Solvents were dried through a molecular sieve system (Pure Solve Solvent Purification System; Innovative Technology, Inc.). ^{1}H spectra and ^{13}C NMR were recorded on an Bruker Avance II spectrometer at 400 and 100 MHz, respectively, and referenced to NMR solvents as indicated. Chemical shifts are reported in units of ppm (δ), with a coupling constant, *J*, in Hz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet). High-resolution mass spectrometry (HRMS) data was obtained with an Agilent (Santa Clara, CA) G3250AA LC/MSD TOF system. Thin-layer chromatography (TLC) analyses were performed using Merck (Darmstadt, Germany) silica gel 60 F254 plates. Generally, crude compounds were purified by flash column chromatography (FC) packed with silica gel (Aldrich). [^{18}F]fluoride aqueous solution was obtained from PET/cyclotron of University of Pennsylvania. Solid-phase extraction cartridges (SEP Pak® Light QMA, Oasis® HLB 3cc) were obtained from Waters (Milford, MA, USA).

2 Chemistry

2.1. 2-Bromo-5-methoxy-N,N-dimethyl-d6-benzamide, 3

A mixture of dimethyl-d6-amine hydrochloride (0.53 g, 6.50 mmol) and triethylamine (Et_3N) (1.32 g, 12.99 mmol) was stirred in anhydrous dichloromethane (DCM) (20 mL) at 0 °C, then compound **2** (1.08 g, 4.33 mmol) in anhydrous DCM (15 mL) was added dropwise, the reaction was stirred at room temperature for 5h, then H_2O (30 mL) was added and extracted with DCM (20 mL x 2) two times, the organic layers were combined and dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated in vacuum, purified by flash

chromatography (silica gel) (ethyl acetate (EA)/Hexane, 0% to 60%, vol/vol) to get product **3.** (0.95 g, yield 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 8.3 Hz, 1H), 6.83 – 6.79 (m, 2H), 3.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.16, 139.29, 133.52, 116.55, 112.87, 109.38, 55.59. HRMS calcd. for $C_{10}H_6D_6BrNO_2$ $[M+H]^+$ 264.0506. Found 264.0529.

2.2 2-((2-Aminophenyl)thio)-5-methoxy-N,N-dimethyl-d6-benzamide, 4

A mixture of compound **3** (510 mg, 1.94 mmol), Cu（25 mg, 0.39 mmol), Cu_2O (27 mg, 0.19 mmol), 2-aminothiophenol (364 mg, 2.91 mmol) and Et_3N (1.96 g, 19.40 mmol) was stirred in 2-ethoxyethanol (8 mL) at 130°C for 50 h. The reaction solution was filtered and washed with methanol (20 mL) and DCM (20 mL), the filtrate was evaporated in vacuum, purified by flash chromatography (silica gel) (EA/Hexane, 0% to 60%, vol/vol) to get product **4** (370 mg, yield 62%) as a colorless viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 7.6 Hz, 1H), 7.21 – 7.09 (m, 2H), 6.84 – 6.66 (m, 4H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.71, 148.65, 138.91, 136.72, 132.33, 130.62, 123.43, 118.02, 115.94, 115.80, 115.37, 111.54, 55.47. HRMS calcd. for $C_{16}H_{12}D_6N_2O_2S$ $[M+H]^+$ 309.1544. Found 309.1640.

2.3. 2-((2-Aminophenyl)thio)-5-hydroxy-N,N-dimethyl-d6-benzamide, 5

A mixture of compound **4** (300 mg, 0.97 mmol) was stirred in anhydrous DCM (15 mL) at 0 °C, then 1M BBr_3 in DCM (2.92 mL, 2.92 mmol) was added dropwise under N_2. The reaction mixture was stirred at room temperature over night, when finished, saturated solution of $NaHCO_3$ was added and extracted with DCM (20 mL x 2), the organic layers were combined and dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated in vacuum and purified by flash chromatography (silica gel) (EA/Hexane, 0% to 80%, vol/vol) to get product **5** (200 mg, yield 70%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40

(dd, *J* = 7.6, 1.5 Hz, 1H), 7.18 – 7.14 (m, 1H), 6.89 (d, *J* = 8.6 Hz, 1H), 6.73 – 6.54 (m, 4H), 4.44 (br, 2H). HRMS calcd. for $C_{15}H_{10}D_6N_2O_2S$ $[M+H]^+$ 295.1387. Found 295.1304.

2.4. 4-((2-Aminophenyl)thio)-3-((dimethyl-d6-amino)methyl)phenol, 6

A mixture of compound **5** (200 mg, 0.68 mmol) was stirred in anhydrous THF (4 mL) at room temperature then 1M BH_3 in THF (3.4 mL, 3.40 mmol) was added, the reaction mixture was refluxed for 8h, when finished, the mixture was cooled and 0.5 mL concentrated HCl (0.5 mL) was cautiously added and the solvent was removed in vacuum, and 1M HCl solution (10 mL) was added then refluxed for 1h, cooled to room temperature and adjust pH 8 with saturated solution of Na_2CO_3, extracted with EA (20 mL x 3), the organic layers were combined and dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated in vacuum, purified by flash chromatography (silica gel) (Methanol/EA, 0% to 10%, vol/vol) to get product **6** (135 mg, yield 71%) as a colorless oil. ^{1}H NMR (400 MHz, MeOD) δ 7.16 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.12-7.08 (m, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 2.7 Hz, 1H), 6.78 (dd, *J* = 8.1, 1.3 Hz, 1H), 6.66 – 6.61 (m, 2H), 3.60 (s, 2H). ^{13}C NMR (100 MHz, MeOD) δ 156.32, 148.33, 138.51, 134.51, 131.68, 129.18, 124.90, 117.71, 117.41, 117.33, 115.20, 115.15, 60.85. HRMS calcd. for $C_{15}H_{12}D_6N_2OS$ $[M+H]^+$ 281.1595. Found 281.1210.

2.5. 3-(4-((2-Aminophenyl)thio)-3-[((bis-(methyl-d3)-amino)methyl)phenoxy)propyl-d6] 4-methylbenzenesulfonate, 7

To a solution of [1,1,2,2,3,3-D_6]-propanediol (270 mg, 3.29 mmol) in THF (10 mL) was added NaOH (527 mg, 13.17 mmol) in H_2O (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1h. TsCl (1.88 g, 9.88 mmol) in THF (10 mL) was then added dropwise. The reaction was stirred at room temperature for 24h. H_2O (20 mL) was added and

the mixture was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined and dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated in vacuum, purified by flash chromatography (silica gel) (EA/Hexane, 0% to 60%, vol/vol) to give [1,1,2,2,3,3-D_6]-propane-1,3-diylbis(4-methylbenzenesulfonate) (970 mg, 76%) as a white solid. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.78 – 7.76 (m, 4H), 7.38 – 7.36 (m, 4H), 2.483 (s, 6H), HRMS calcd. for $C_{17}H_{14}D_6O_6S_2$ $[M+H]^+$ 391.1156. Found 391.1140.

A mixture of compound **6** (40 mg, 0.14 mmol) and K_2CO_3 (59 mg, 0.43 mmol) was stirred in anhydrous DMF (3 mL) at 65°C for 1h, [1,1,2,2,3,3-D_6]-propane-1,3-diylbis(4-methylbenzenesulfonate) (67 mg, 0.17 mmol) was added and the mixture was stirred for another 2h, cooled to room temperature and saturated solution of NaCl (12 mL) was added and extracted with EA (15 mL x 3) , the organic layers were combined and dried over $MgSO_4$, filtered and the filtrate was evaporated in vacuum, purified by flash chromatography (silica gel) (MeOH/DCM, 0% to 10%, vol/vol) to get product **7** (38 mg, yield 54%) as a colorless oil. ^{1}H NMR (400 MHz, Acetone) δ 7.76 (d, J = 8.2 Hz, 2H), 7.39 – 7.33 (m, 3H), 7.12 (t, J = 7.6 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 6.87 (d, J = 2.6 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.63 – 6.59 (m, 2H), 5.31 (s, 1H), 3.55 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, Acetone) δ 157.30, 149.78, 144.89, 139.61, 136.24, 133.20, 131.03, 130.03, 129.94, 127.69, 127.34, 116.66, 116.03, 114.80, 113.94, 61.74, 20.62. HRMS calcd. for $C_{25}H_{18}D_{12}N_2O_4S_2$ $[M+H]^+$ 499.2478. Found 499.2432.

2.6. 2-((2-((Bis(methyl-d3)amino)methyl)-4-(3-fluoropropoxy-1,1,2,2,3,3-d6)-phenyl)thio)aniline (D12FPBM, 1)

A mixture of compound **7** (20 mg, 0.04 mmol) was stirred in anhydrous THF (3 mL) at 65 °C then 1M TBAF (tetrabutylammonium fluoride) in THF (0.2 mL, 0.20 mmol) was

added, the reaction mixture was stirred for 3h at 65 °C, then the solvent was evaporated in vacuum, H_2O (8 mL) was added and extracted with EA (15 mL x 3), the organic layers were combined and dried over $MgSO_4$, filtered and the filtrate was evaporated in vacuum, purified by flash chromatography (silica gel) (THF/Hexane, 0% to 60%, vol/vol) to get product D12FPBM**, 1** (9.5 mg, yield 68%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (dd, J = 8.0, 1.6 Hz, 1H), 7.20 – 7.14 (m, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.90 (d, J = 2.8 Hz, 1H), 6.76 – 6.66 (m, 3H), 4.55 (brs, 1H), 3.56 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.34, 148.29, 139.31, 136.33, 130.76, 130.08, 127.22, 118.21, 117.09, 116.39, 115.23, 114.08, 62.22. HRMS calcd. for $C_{18}H_{11}D_{12}FN_2OS$ $[M+H]^+$ 347.2347. Found 347.2484.

3. Radiochemistry

[^{18}F]1 was prepared using a one-step radiochemical reaction described in Scheme 2, [^{18}F]fluoride (1.85 – 2.04 GBq), produced by a cyclotron using the $^{18}O(p,n)^{18}F$ reaction, was trapped on a Light QMA cartridge (preconditioned with 10 mL 1 N $NaHCO_3$ solution and 10 mL water) and then eluted with phase transfer catalyst solution (22.0 mg K_{222} and 40.0 mg K_2CO_3 in 18.4 mL acetonitrile and 3.6 mL water) The eluent was added into a 10 mL test tube and evaporated at 90 °C under a stream of argon. Furthermore, the residue was azeotropically dried twice with 2.0 mL anhydrous ACN at 90 °C under a stream of argon. The precursor, 7 (1, 2, or 3 mg), was dissolved in 1.0 mL anhydrous solvent (acetonitrile). The precursor solution was added into a test tube containing the dried residue prepared above. The mixture was heated at 80 °C for 15 min. Then the reaction mixture was cooled in ice water, and 10 mL water was added. The resulting mixture was loaded on a 3 cm^3 Oasis HLB cartridge (preconditioned with 10 mL ethanol and 10 mL water). The cartridge was flushed with 15 mL water. The crude product was eluted from the cartridge with 1 mL acetonitrile, diluted with 1mL water, purified by Pre-HPLC (Gemini 250 x 4.6 mm,

ACN/water 60/40, 4 mL/min; fraction at 21min was collected. The solution was diluted with 30 mL water and pushed through 3 cm^3 Oasis HLB cartridge (preconditioned with 10 mL ethanol and 10 mL water), washed with 3mL water and the dose was eluted with 1 mL EtOH (0.38 GBq), and analyzed by injecting into a HPLC system. The HPLC for radiochemical reaction studies was equipped with a gamma ray radiodetector and a UV/Vis detector [Agilent 1200 series system; Ascentis C18, 150 x 4.6 mm, 5 µm; mobile phase: ACN/10mM AFB 50/50, 1 mL/min. [^{18}F]**1** = 4.323 min.] (Representative HPLC profiles are included in supplemental information).

4. In vitro binding assay

To determine binding affinity to SERT, membrane homogenates of LLCPK1 cells stably transfected with SERT was mixed with buffer, 0.1 nM [^{125}I] IDAM [37] and a range of 10 concentrations of the compound. Nonspecific binding was defined with 2.5 µ M citalopram. Incubation was carried out for 1 h at room temperature, and the bound ligand was collected on glass fiber filters presoaked with 1% polyethylenimine and counted in a gamma counter. Results of competition experiments were subjected to nonlinear regression analysis.

5. Biodistribution study in rats

While under isoflurane anesthesia, 0.74 MBq of radiotracer was injected into each rat via the femoral vein. The rats were then sacrificed at the selected time point. Organs and brain regions of interest were dissected, weighed and counted for radioactivity. The percent dose per organ or percent dose per gram was calculated by dividing the tissue counts by the initial dose counts and then by the weight of the tissue sample. The initial dose sample was prepared by taking a (1 ml) aliquot of an injection dose diluted in a (100 ml) volumetric flask. The initial dose sample was then counted right after the tissue sample. Cerebellum, which

contains low levels of SERT, was used as the nontarget region when calculating the target to non-target ratio of the brain regions.

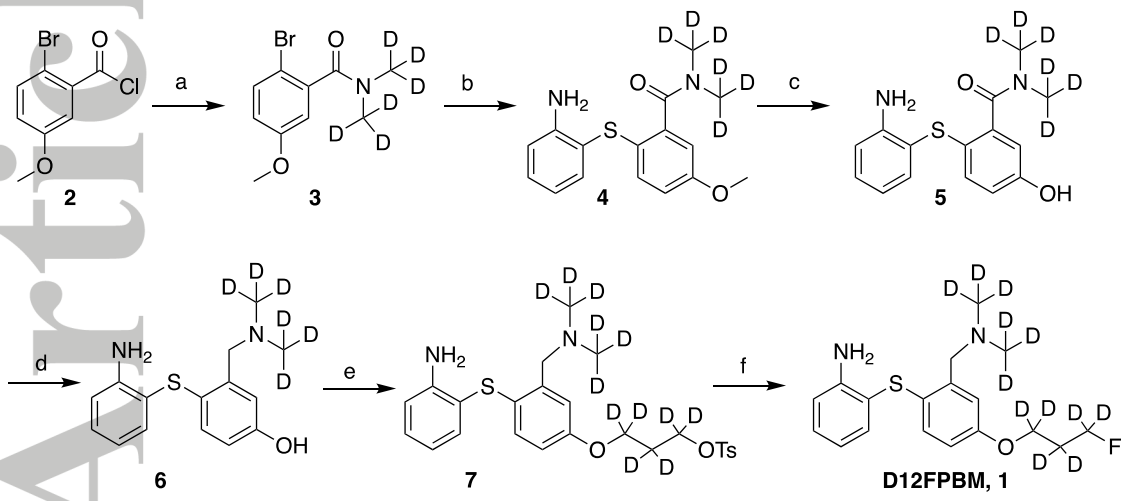
6. MicroPET imaging study in rats

Dynamic microPET imaging was performed using the Philips Mosaic Animal PET (A-PET) imaging system. Rat was anesthetized with 1.5% isoflurane in oxygen, positioned on the bed of the microPET gantry, and fixed near the center of the scanner. Isoflurane anesthesia was continued throughout the study. Rat was injected intravenously via the tail vein with 49 MBq of [^{18}F]**1**. The scan duration was 120 min from the time of injection. Scans were obtained in the frame sequence of 5 min. The data were histogrammed into 24 consecutive frames, and the images were reconstructed. Images were analyzed with AMIDE (http://amide.sourceforge.net/). Frames were summed to manually draw regions of interest (ROIs). ROIs were drawn manually from three brain regions (midbrain, striatum and cerebellum), and time–activity curves for the ROIs were obtained. Regions of interest (ROIs) were identified according to the stereotaxic atlas [38].

Results and Discussion

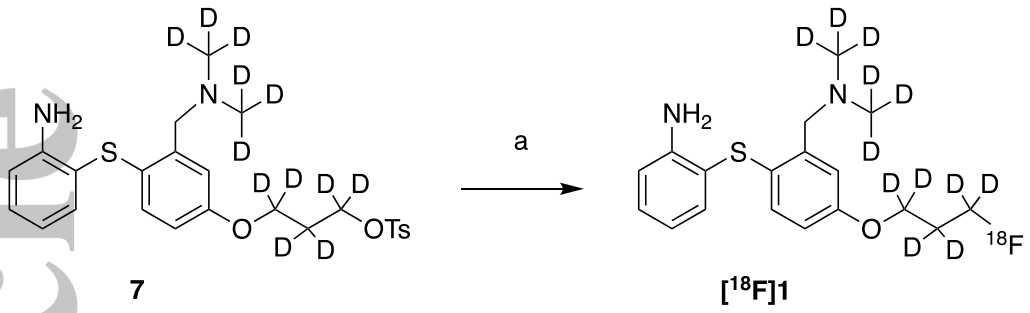
Chemical synthesis. Synthesis of deuterated compound, **1**, is illustrated in scheme 1. The reaction scheme was similar to that reported previously for the nondeuterated FPBM with minor modifications [24]. First dimethyl-d6-amine was reacted with 2-bromo-5-methoxybenzoyl chloride, **2**, to give the amide compound 3 in 83% yield. A copper and copper (I) oxide catalyzed reaction between **3** and 2-aminothiophenol produced desired compound **4** in 62% yield. The methyl protecting group of **4** was selectively removed by BBr_3 to give **5** in 70% yield. Compound **5** was then treated with diborane in THF and provided **6** in 71% yield. Compound **6** was coupled with

[1,1,2,2,3,3-D6]-propane-1,3-diylbis(4-methylbenzenesulfonate) to yield **7** in 54% yield. Subsequently, the O-tosylated compound **7** was converted to the fluoride compound **1** with TBAF in 68% yield.



Scheme 1. Reagents and conditions: (a) Dimethyl-d6-amine hydrochloride , Et_3N, DCM, 0 °C to RT, 5 h. (b) Cu, Cu_2O, 2-aminothiophenol, Et_3N, 2-ethoxyethanol, 130 °C for 50 h. (c) 1M BBr_3, DCM, 0 °C to RT, over night. (d) 1M BH_3 in THF, THF, reflux, 8 h. (e) K_2CO_3, DMF, [1,1,2,2,3,3-D_6]-propane-1,3-diylbis(4-methylbenzenesulfonate), 65 °C, 3 h. (f) 1M TBAF in THF, THF, 65 ºC, 3 h.

Radiolabeling. The ^{18}F labeling reaction was accomplished by using a one step labeling reaction (scheme 2). The labeling reaction was carried out by reacting precursor, **7**, with activated ^{18}F fluoride (in the presence of Kryptofix 222 and potassium carbonate) in acetonitrile at 80°C for 15 min, which gave [^{18}F]**1**. The crude mixture was purified by HPLC. Fractions of eluent containing the desired compound, [^{18}F]**1**, were collected, evaporated and re-constituted in saline. The procedure took 60 - 80 min, and the molar activity was 23.6 GBq/mmol (the radiochemical purity was > 95% and radiochemical yield was 32% (decay corrected). The HPLC profiles for the "hot" and "cold" (**1** and [^{18}F]**1**)are included in the supplemental information.



Scheme 2. Reagents and conditions: (a) $^{18}F^{-}/K_{222}$, K_2CO_3, ACN, 80 °C, 15 min

In vitro binding assay. Binding affinity study using membrane homogenates of LLCPK1 cells stably transfected with SERT and [^{125}I]IDAM [37] showed that the "cold" deuterated agent, **1**, displayed very comparable binding affinity towards SERT binding sites as compared to the corresponding non-deuterated agent FPBM (K_i = 0.086 and 0.052 nM for **1** and FPBM, respectively) (Table 1). The deuterated agent exhibited the same excellent binding to SERT binding sites. We have also prepared and tested the mono- and di-demethylated derivatives of the FPBM and **1,** including **8**, **9**, **10** and **11**. Results of the binding studies suggest that N-methyl groups are important for SERT binding affinity. Mono-demethylated and dimethylated derivatives showed significantly reduced affinity; while the corresponding deuterated or non-deuterated derivatives (**8** vs **10** and **9** vs **11**) display no differences in the binding affinity (Table 1). In vitro binding study showed that there is no change in binding affinity after H to D substitution.

[Insert table 1]

In vivo biodistribution in rats. Biodistribution study in rats after an i.v. injection of [^{18}F]**1**, showed that the new deuterated agent penetrated the blood-brain-barrier and localized in the regions, where the concentration of serotonin transporter binding sites are high, i.e., hypothalamus, hippocampus and striatum regions (Table 2). The regional distribution of deuterated, [^{18}F]**1**, in the brain is very comparable to that of the non-deuterated [^{18}F]FPBM, reported previously. The brain uptake of [^{18}F]**1**, at 120 min post iv injection showed a value

very similar to that reported for [^{18}F]FPBM reported previously [30]. In vivo biodistribution in rats showed no differences between [^{18}F]**1**, and [^{18}F]FPBM (Table 2). The results suggested there is no change in in vivo behavior in rats between these two tracers; and therefore, the in vivo metabolism between these two agents would be very comparable. We did not investigate this issue on in vivo metabolism further.

[Insert table 2]

In vivo microPET imaging study

Binding of [^{18}F]**1** to SERT in the brain was confirmed in vivo by performing microPET imaging in rats. MicroPET images of normal rat brain at 0-120 min after tail vein injection of [^{18}F]**1** are shown in Fig. 4. A high accumulation of radioactivity was observed in midbrain (hippocampus and hypothalamus) and striatum, the sites known to have the highest concentrations of SERT. Harderian glands near the eyes also showed uptake of [^{18}F]**1.** Nonspecific binding in Harderian glands (HG) is commonly observed in rats and mice for other PET agents [39].

Time–radioactivity curves [^{18}F]**1** (Fig. 5a) for the midbrain, striatum and cerebellum and ratios of target regions to cerebellum (Fig. 5b) following tail vein injection of [^{18}F]**1** are shown in Fig 5. After the injection of [^{18}F]**1** the radioactivity was rapidly taken up by the brain, with the midbrain exhibiting maximal activity around 25 min. The egress of radioactivity from brain regions was also very rapid, with good differentiation of the midbrain from the cerebellum visible 15 min after injection. The midbrain exhibited the highest uptake level and good retention of the radioligand. The midbrain to cerebellum ratio (MB/CB) of radioactivity was 3.0 at 1hr and it increased to 3.5 at 2hr post-injection. The PET images of [^{18}F]**1** obtained were very similar to those previously reported for [^{18}F]FPBM in rats [30].

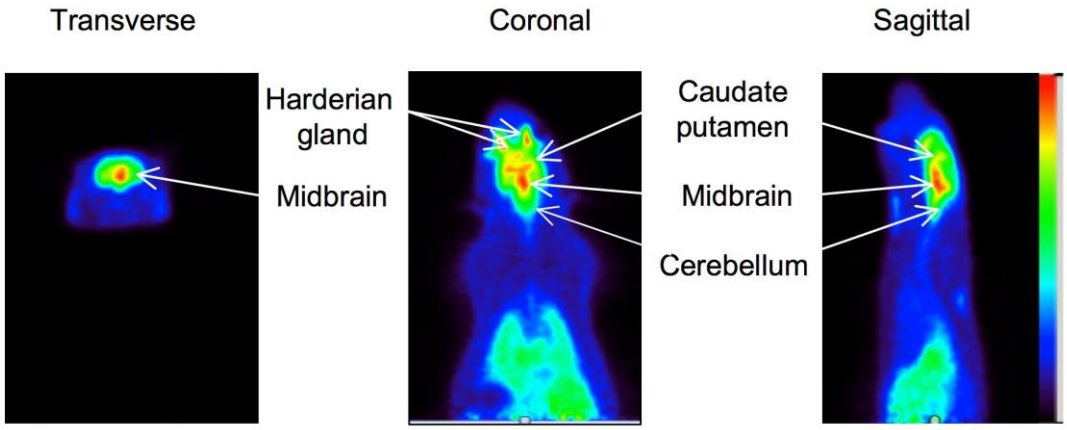


Figure 4. Transverse, coronal and sagittal sections of microPET images of [^{18}F]**1** in the brain of normal rat. Images were generated by the summation of data collected for 120 min after radiotracer injection. Highest concentration of [^{18}F]**1** was localized in midbrain region inside the rat brain.

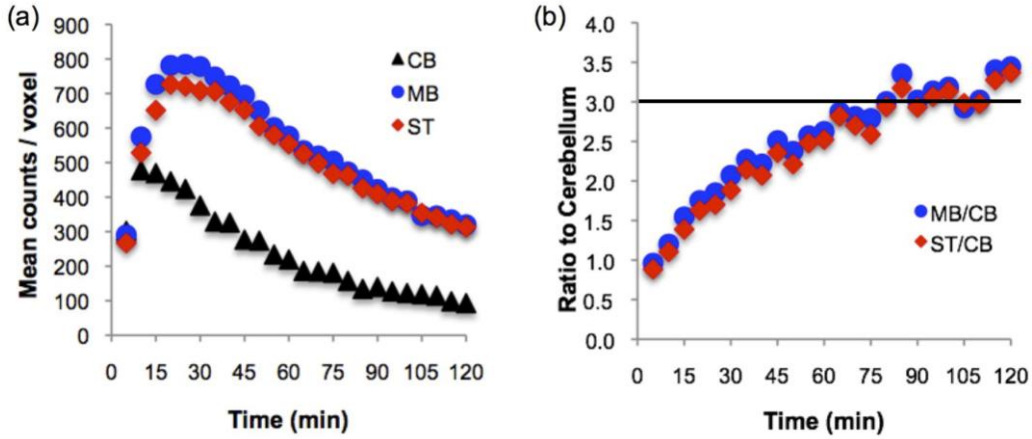


Figure 5. Representative data of time–activity curves of [^{18}F]**1**, (a) in the brains of normal rat, expressed as the mean count/voxel, for striatum (ST), midbrain (MB) and cerebellum (CB). Ratios of target regions to cerebellum of [^{18}F]**1**, are shown in (b). PET data were collected for 120 min. The curves suggest that the ratio of uptake of midbrain

area/cerebellum area peaked between 60 to 120 min, and the data are very similar to reported earlier for [^{18}F]FPBM [30].

In this paper we have reported results of comparison of [^{18}F]**1**, and [^{18}F]FPBM, which show almost identical in vitro and in vivo binding properties for SERT. In this particular case there is no advantage in substituting hydrogen with deuterium; it only produced deuterated agent with equal potency. Using the strategy of D to H substitution to create new drugs does not always lead to success; it will only work when certain criteria in changing in vivo metabolism were met. It is sobering to observe that the lack of differences on in vivo kinetics in the rat brain between [^{18}F]**1**, and [^{18}F]FPBM. Both deuterated and non-deuterated agents appeared to target SERT binding sites in the hypothalamus, hippocampus and striatum regions of the brain with equal effectiveness. It is likely due to the fact that in vivo N-demethylation, which reduces the SERT binding affinity, reported for commonly prescribed antidepressants, is related to a long term dosing after days or months. The ^{18}F labeled PET imaging agents, such as [^{18}F]**1**, require a faster kinetics in vivo (within two to four hours due to the physical decay of ^{18}F); therefore, the imaging agent may not have enough time in the blood circulation to show differences in its in vivo metabolism (N-demethylation) as expected. Future efforts on developing deuterated imaging agents may need to carefully consider the in vivo pharmacokinetics in order to take advantage of the isotope effects in [^{18}F]fluorine radiochemistry and in vivo metabolism.

Conclusion

The comparison of [^{18}F]**1**, and [^{18}F]FPBM showed no differences by in vivo and in vitro testing. The deuterated compound, [^{18}F]**1**, which is a new chemical entity, can be useful

for PET imaging of SERT binding in the brain for patients before and after taking serotonin reuptake inhibitors (SSRIs) – a direct method for monitoring the drug effects in the brain.

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Abbreviations Used

DASB: (N,N-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine, (+)-McN5652: trans-1,2,3,5,6,10-<beta>-hexahydro-6-[4-(methylthio)phenyl-[pyrrolo-[2,1-<alpha>]isoquinoline, FADAM: *N*,*N*-dimethyl-2-(2-amino-4-fluorophenylthio)- benzylamine, FPBM: 2-(2'-((dimethylamino)methyl)-4'-(3-fluoropropoxy)phenylthio)benzenamine, D12FPBM: 2-((2-((bis(methyl-d3)-amino)methyl)-4-(3-fluoropropoxy-1,1,2,2,3,3-d6)phenyl)thio)aniline, PET: positron emission tomography, SPECT: single photon emission computed tomography, SERT: serotonin transporter, SSRI: selective serotonin reuptake inhibitor,

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References

1. Meanwell NA. Synopsis of some recent tactical application of bioisosteres in drug design. J Med Chem 2011;54:2529-91.

2. Gant TG. Using deuterium in drug discovery: leaving the label in the drug. J Med Chem 2014;57:3595-611.

3. Bousman CA, Forbes M, Jayaram M, Eyre H, Reynolds CF, Berk M, et al. Antidepressant prescribing in the precision medicine era: a prescriber's primer on pharmacogenetic tools. BMC Psychiatry 2017;17:60.

4. Houle S, Ginovart N, Hussey D, Meyer JH, and Wilson AA. Imaging the serotonin transporter with positron emission tomography: Initial human studies with [^{11}C]DAPP and [^{11}C]DASB. Eur J Nucl Med 2000;27:1719-22.

5. Baldinger P, Kranz GS, Haeusler D, Savli M, Spies M, Philippe C, et al. Regional differences in SERT occupancy after acute and prolonged SSRI intake investigated by brain PET. Neuroimage 2014;88:252-62.

6. Frankle WG, Slifstein M, Gunn RN, Huang Y, Hwang D-R, Darr EA, et al. Estimation of serotonin transporter parameters with 11C-DASB in healthy humans: reproducibility and comparison of methods. J Nucl Med 2006;47:815-26.

7. Barrett FS, Workman CI, Sair HI, Savonenko AV, Kraut MA, Sodums DJ, et al. Association between serotonin denervation and resting-state functional connectivity in mild cognitive impairment. Hum Brain Mapp 2017.

8. Spies M, Knudsen GM, Lanzenberger R, and Kasper S. The serotonin transporter in psychiatric disorders: insights from PET imaging. Lancet Psychiatry 2015;2:743-55.

9. Oya S, Choi S, Kung M, and Kung H.
5-Chloro-2-(2'-((dimethylamino)methyl)-4'-iodophenylthio)benzenamine: a new serotonin transporter ligand. Nucl Med Biol 2007;34:129-39.

10. Kung H, Newman S, Choi S, Oya S, Hou C, Zhuang Z, et al.
2-(2-(dimethylaminomethyl)phenoxy)-5-iodophenylamine: an improved serotonin transporter imaging agent. J Med Chem 2004;47:5258-64.

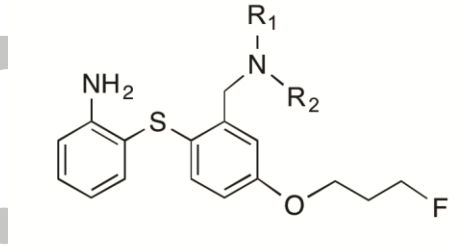
11. Wang JL, Parhi AK, Oya S, Lieberman B, Kung MP, and Kung HF.
2-(2'-((Dimethylamino)methyl)-4'-(3-[(18)F]fluoropropoxy)-phenylthio)benzenamine for positron emission tomography imaging of serotonin transporters. Nucl Med Biol 2008;35:447-58.

12. Wang JL, Deutsch EC, Oya S, and Kung HF. FlipADAM: a potential new SPECT imaging agent for the serotonin transporter. Nucl Med Biol 2010;37:577-86.
13. Huang Y, Zheng MQ, and Gerdes JM. Development of effective PET and SPECT imaging agents for the serotonin transporter: has a twenty-year journey reached its destination? Curr Top Med Chem 2010;10:1499-526.
14. Mavel S, Meheux N, Guilloteau D, and Emond P. Synthesis and in vitro evaluation of fluorinated diphenyloxide derivatives and sulfur analogs as serotonin transporter ligands. Bioorg Med Chem 2010;18:236-41.
15. Jarkas N, Voll RJ, Williams L, and Goodman MM. Validation of two fluoro-analogues of N,N-dimethyl-2-(2'-amino-4'-hydroxymethyl-phenylthio)benzylamine as serotonin transporter imaging agents using microPET. Nucl Med Biol 2010;37:593-603.
16. Kang HH, Wang CH, Chen HC, Li IH, Cheng CY, Liu RS, et al. Investigating the effects of noise-induced hearing loss on serotonin transporters in rat brain using 4-[^{18}F]-ADAM/small animal PET. Neuroimage 2012.
17. Huang YY, Huang WS, Ma KH, Chou TK, Kuo YY, Cheng CY, et al. Synthesis and comparison of 4-[^{18}F]F-ADAM, 2-[^{18}F]F-ADAM, N-Desmethyl-4-[^{18}F]F-ADAM and [^{18}F]F-AFM as serotonin transporter imaging agents. Appl Radiat Isot 2012;70:2298-307.
18. Chen YA, Huang WS, Lin YS, Cheng CY, Liu RS, Wang SJ, et al. Characterization of 4-[^{18}F]-ADAM as an imaging agent for SERT in non-human primate brain using PET: a dynamic study. Nucl Med Biol 2012;39:279-85.
19. Hesse S, Brust P, Mading P, Becker GA, Patt M, Seese A, et al. Imaging of the brain serotonin transporters (SERT) with (18)F-labelled fluoromethyl-McN5652 and PET in humans. Eur J Nucl Med Mol Imaging 2012;39:1001-11.
20. Paterson LM, Kornum BR, Nutt DJ, Pike VW, and Knudsen GM. 5-HT radioligands for human brain imaging with PET and SPECT. Med Res Rev 2013;33:54-111.
21. Huang WS, Huang SY, Ho PS, Ma KH, Huang YY, Yeh CB, et al. PET imaging of the brain serotonin transporters (SERT) with N,N-dimethyl-2-(2-amino-4-[^{18}F]fluorophenylthio)benzylamine (4-[^{18}F]-ADAM) in humans: a preliminary study. Eur J Nucl Med Mol Imaging 2013;40:115-24.
22. Stehouwer JS and Goodman MM. (11) C and (18) F PET radioligands for the serotonin transporter (SERT). J Labelled Comp Radiopharm 2013;56:114-9.
23. Oya S, Kung M, Acton P, Mu M, Hou C, and Kung H. A new single-photon emission computed tomography imaging agent for serotonin transporters: [^{123}I]IDAM,

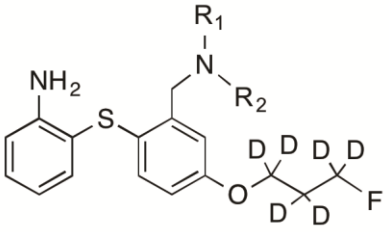
5-iodo-2-((2-((dimethylamino)methyl)phenyl)thio)benzyl alcohol. J Med Chem 1999;42:333-5.
24. Parhi A, Wang J, Oya S, Choi S, Kung M, and Kung H. 2-(2'-((Dimethylamino)methyl)-4'-(fluoroalkoxy)-phenylthio)benzenamine Derivatives as Serotonin Transporter Imaging Agents. J Med Chem 2007;50:6673-84.
25. Kuchar M and Mamat C. Methods to Increase the Metabolic Stability of ^{18}F-Radiotracers. Molecules 2015;20:16186-220.
26. Nag S, Fazio P, Lehmann L, Kettschau G, Heinrich T, Thiele A, et al. In Vivo and In Vitro Characterization of a Novel MAO-B Inhibitor Radioligand, ^{18}F-Labeled Deuterated Fluorodeprenyl. J Nucl Med 2016;57:315-20.
27. Challapalli A, Sharma R, Hallett WA, Kozlowski K, Carroll L, Brickute D, et al. Biodistribution and radiation dosimetry of deuterium-substituted 18F-fluoromethyl-[1, 2-2H4]choline in healthy volunteers. J Nucl Med 2014;55:256-63.
28. Fowler JS, Wang GJ, Logan J, Xie S, Volkow ND, MacGregor RR, et al. Selective reduction of radiotracer trapping by deuterium substitution: comparison of carbon-11-L-deprenyl and carbon-11-deprenyl-D_2 for MAO B mapping. J Nucl Med 1995;36:1255-62.
29. Arakawa R, Stenkrona P, Takano A, Nag S, Maior RS, and Halldin C. Test-retest reproducibility of [^{11}C]-L-deprenyl-D2 binding to MAO-B in the human brain. EJNMMI Res 2017;7:54.
30. Wang J, Parhi A, Oya S, Lieberman B, and Kung H. In vivo characterization of a series of ^{18}F-diaryl sulfides (^{18}F-2-(2'-((dimethylamino)methyl)-4'-(fluoroalkoxy)phenylthio)benzenamine) for PET imaging of the serotonin transporter. J Nucl Med 2009;50:1509-17.
31. Wang J, Oya S, Parhi A, Lieberman B, Ploessl K, Hou C, et al. In vivo studies of the SERT-selective [^{18}F]FPBM and VMAT2-selective [^{18}F]AV-133 radiotracers in a rat model of Parkinson's disease. Nucl Med Biol 2010;37:479-86.
32. Zhu L, Li G, Choi SR, Plossl K, Chan P, Qiao H, et al. An improved preparation of [^{18}F]FPBM: A potential serotonin transporter (SERT) imaging agent. Nucl Med Biol 2013;40:974-9.
33. Qiao H, Zhang Y, Wu Z, Zhu L, Choi SR, Ploessl K, et al. One-step preparation of [^{18}F]FPBM for PET imaging of serotonin transporter (SERT) in the brain. Nucl Med Biol 2016;43:470-7.

34. Wilson AA, Ginovart N, Schmidt M, Meyer JH, Threlkeld PG, and Houle S. Novel radiotracers for imaging the serotonin transporter by positron emission tomography: synthesis, radiosynthesis, and in vitro and ex vivo evaluation of ^{11}C-labeled 2-(phenylthio)araalkylamines. J Med Chem 2000;43:3103-10.
35. Szabo Z, McCann UD, Wilson AA, Scheffel U, Owonikoko T, Mathews WB, et al. Comparison of (+)-^{11}C-McN5652 and ^{11}C-DASB as Serotonin Transporter Radioligands Under Various Experimental Conditions. J Nucl Med 2002;43:678-92.
36. Parhi A, Kung M, Ploessl K, and Kung H. Synthesis of Fluorescent Probes based on Stilbenes and Diphenylacetylenes targeting beta-Amyloid Plaques. Tetrahedron Lett 2008;49:3395-9.
37. Kung MP, Hou C, Oya S, Mu M, Acton PD, and Kung HF. Characterization of [^{123}I]IDAM as a novel single-photon emission tomography tracer for serotonin transporters. Eur J Nucl Med 1999;26:844-53.
38. Paxinos G and Watson C. The Rat Brain In Stereotaxic Coordinates, New York: Academic Press,1986.
39. Kim M, Woo SK, Yu JW, Lee YJ, Kim KM, Kang JH, et al. Effect of Harderian adenectomy on the statistical analyses of mouse brain imaging using positron emission tomography. J Vet Sci 2014;15:157-61.

Table 1. Comparison of binding affinity of FPBM, **1** and related N-demethylated derivatives**, 8**, **9**, **10** and **11 (**K_i, nM ± SD, n = 3)



FPBM $R_1 = R_2 = CH_3$
Mono-demethyl FPBM, **8** $R_1 = CH_3$; $R_2 = H$
Di-demethyl FPBM, **9** $R_1 = R_2 = H$



D12FPBM, **1** $R_1 = R_2 = CD_3$
Mono-demethyl D9FPBM, **10** $R_1 = CD_3$; $R_2 = H$
Di-demethyl D6FPBM, **11** $R_1 = R_2 = H$

Un-deuterated	K_i (nM ± SD)	Deuterated	K_i (nM ± SD)
FPBM	0.052 ± 0.007	D12FPBM, **1**	0.086 ± 0.029
[+]Mono-demethyl-FPBM, **8**	5.26 ± 0.66	[+]Mono-demethyl-D9FPBM, **10**	3.05 ± 0.81
[+]Di-demethyl-FPBM, **9**	39.3 ± 11.9	[+]Di-demethyl-D6FPBM, **11**	44.9 ± 2.5

***** Using membrane homogenates of LLCPK1 cells stably transfected with SERT and [^{125}I]IDAM [37]. [+]Detail information on the synthesis of compounds **8, 9, 10** and **11** is available in the supplemental information.

Table 2. Biodistribution of [^{18}F]**1**, in normal rats after an i.v. injection (% dose/g, Avg ± SD of n=3).

	2 min	30 min	60 min	120 min
Blood	0.25±0.04	0.16±0.03	0.10±0.02	0.06±0.00
Heart	1.19±0.18	0.18±0.02	0.09±0.01	0.05±0.01
Muscle	0.14±0.02	0.09±0.02	0.07±0.01	0.03±0.01
Lung	9.56±2.29	1.86±0.42	0.85±0.11	0.45±0.10
Kidney	3.02±0.16	1.79±0.38	1.29±0.29	0.83±0.39
Spleen	1.48±0.31	0.88±0.05	0.46±0.10	0.21±0.04
Pancreas	1.13±0.02	0.27±0.06	0.19±0.02	0.13±0.07
Liver	0.74±0.16	0.30±0.05	0.22±0.02	0.16±0.01
Skin	0.22±0.04	0.19±0.03	0.16±0.01	0.10±0.02
Bone	0.35±0.06	0.19±0.01	0.16±0.02	0.13±0.01
Brain	1.09±0.06	0.81±0.04	0.54±0.05	0.31±0.03

Regional brain distribution (% dose/g, Avg ± SD of n=3) for [^{18}F]**1**,

	2min	30min	60min	120min
Cerebellum	0.94±0.07	0.37±0.02	0.16±0.01	0.08±0.01
Hypothalamus	1.08±0.13	1.11±0.17	0.86±0.06	0.58±0.06
Hippocampus	0.90±0.06	0.76±0.11	0.56±0.05	0.30±0.04
Cortex	1.34±0.18	0.67±0.10	0.43±0.05	0.21±0.03
Striatum	1.07±0.10	0.90±0.14	0.64±0.07	0.36±0.04
Remainder	1.12±0.08	0.90±0.03	0.61±0.06	0.37±0.04

Region to cerebellum ratio for [^{18}F]**1**,

	2 min	30 min	60 min	120 min
Hypothalamus	1.17±0.21	2.99±0.46	5.32±0.45	7.55±0.82
Hippocampus	0.97±0.13	2.04±0.27	3.45±0.42	3.85±0.28
Cortex	1.44±0.27	1.81±0.30	2.68±0.35	2.67±0.50
Striatum	1.15±0.18	2.43±0.38	3.96±0.62	4.69±1.26
Remainder	1.21±0.13	2.42±0.13	3.77±0.43	4.74±0.71

Region to cerebellum ratio for [^{18}F]FPBM (Reported previously [30])

Organ	2 min	30 min	60 min	120 min
Hypothalamus	1.14±0.16	3.50±0.67	4.69±1.76	7.67±2.60
Hippocampus	0.88±0.11	2.26±0.44	2.82±1.06	3.87±1.00
Cortex	1.03±0.13	2.87±0.64	4.26±1.58	4.53±1.33
Striatum	0.90±0.10	2.58±0.59	3.19±1.23	4.67±1.51
Remainder	0.98±0.14	2.54±0.40	3.11±1.22	4.49±1.29

Deuterium substituted 2-(2′-((Dimethylamino)methyl)-4′-[^{18}F](fluoropropoxy)phenyl-thio)benzenamine as a serotonin transporter imaging agent

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