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The DAT Ligand [^{18}F]PR17.MZ Mirrors the in vivo Pharmacokinetic Profile of [^{11}C]Cocaine with Significantly Improved Monoamine Transporter Selectivity

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Positron emission tomography (PET) studies of the availability of the dopamine transporter (DAT) provide valuable insight into the presynaptic integrity of dopaminergic neurons in vivo.[1] Noninvasive PET imaging thereby contributes to the understanding of basic neuronal mechanisms in psychiatric diseases and to the routine diagnosis of movement disorders.[2] The development of appropriate positron-emitter-labeled DAT ligands, however, has been complicated by nonspecific binding, low selectivity for the DAT, and slow binding equilibria.[3] Highly specific and selective DAT inhibitors are prone to slow accumulation in DAT-containing brain regions and slow binding equilibrium. These characteristics lead to exhaustively long-lasting PET scans. Conversely, the nonspecific monoamine transporter inhibiting ligand (−)-cocaine has a rapid pharmacokinetic profile and a fast binding equilibrium, resulting in much shorter PET scans. Unfortunately, it suffers from low affinity and a nonselective binding profile with similar affinity to dopamine, serotonin, and noradrenalin transporters.[4] In addition, the corresponding PET ligand (−)-*N*-[^{11}C]cocaine is only available in PET centers equipped with an in-house cyclotron, owing to the isotope's short half-life of 20.3 min. Ideally, a DAT imaging agent for PET would combine moderate affinity, high DAT selectivity, low nonspecific binding, rapid accumulation in the DAT-containing brain regions followed by fast washout, and the advantageous half-life of a fluorine-18 label.

We designed and synthesized a series of cocaine analogues with improved affinity and selectivity profiles relative to (−)-cocaine.[3] These compounds generally allow labeling with carbon-11 as well as fluorine-18. Herein we report our discovery of a potent and selective ^{18}F-labeled DAT ligand that provides cocaine-like pharmacokinetic properties.

PR17.MZ, (1*R*,2*S*,3*S*,5*S*)-methyl-8-{[(1*S*,2*S*)-2-(fluoromethyl)cyclopropyl]methyl}-3-phenyl-8-azabicyclo[3.2.1]octane-2-carboxylate (**1**) (Figure 1) has been designed through the use of a conformational restriction-based approach.[3,5] Compound **1** provides a moderate inhibition potency of human DAT (hDAT; IC_{50} = 11 nM) which is 29-fold higher than that of (−)-cocaine (**2**). Its inhibition selectivity over the human serotonin transporter (SERT; IC_{50} = 1.4 μM) is 67-fold improved, whereas its inhibition selectivity over the human noradrenalin transporter (NET; IC_{50} = 175 nM) is 28-fold improved relative to **2**.[3]

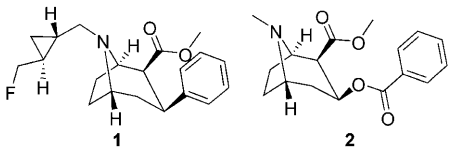
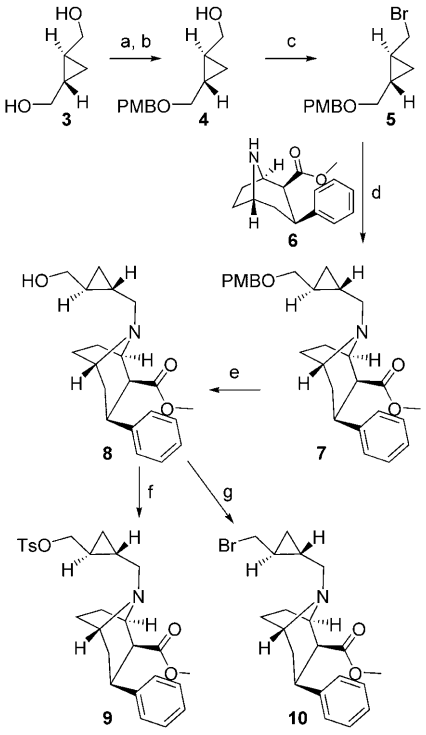


Figure 1. Molecular structures of PR17.MZ (**1**) and (−)-cocaine (**2**).

A labeling precursor for direct nucleophilic radiofluorination was synthesized from (1*S*,2*S*)-cyclopropane-1,2-diyldimethanol (**3**) in five consecutive steps as shown in Scheme 1.[6,7] Desymmetrization of **3** with sodium hydride and *p*-methoxybenzyl chloride in *N*,*N*-dimethylformamide gave compound **4** in 92%



Scheme 1. Synthetic route to labeling precursors **9** and **10**: a) NaH, DMF, 0 °C→RT, 30 min; b) PMBCl, TBAI (10 mol%), RT, 2 h, 92% over two steps; c) CBr_4, PPh_3, CH_2Cl_2, RT, 90 min, 94%; d) **6**, DIPEA, MeCN, 75 °C, 14 h (overnight), 89%; e) CAN, 20 min, then MeCN/H_2O (9:1), RT, 20 min, 95%; f) Ts_2O, $Y(OTf)_3$, CH_2Cl_2, 0 °C→RT, 14 h (overnight), 88%; g) CBr_4, PPh_3, CH_2Cl_2, RT, 90 min, 95%.

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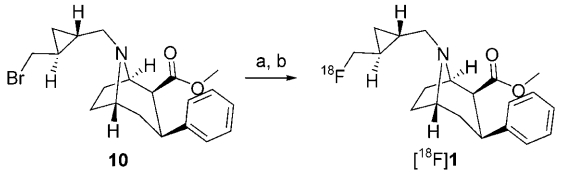
yield. Appel bromination proceeded in 94% yield to obtain electrophile **5**. (1*R*,2*S*,3*S*,5*S*)-Methyl-3-phenyl-8-azabicyclo-[3.2.1]octane-2-carboxylate (**6**) was alkylated using **5** in acetonitrile with *N*,*N*-diisopropylethylamine as base in 89% yield, followed by oxidative cleavage of the *p*-methoxybenzyl protecting group with ceric ammonium nitrate (CAN) in acetonitrile/water in 95% yield. Esterification with *p*-toluenesulfonic acid anhydride afforded labeling precursor **9** in 88% yield and 64% overall yield. Unfortunately, compound **9** was found to degrade at 4 °C, and multiple radioactive products were formed when it was reacted with $^{18}F^-$ after a few weeks of storage. To overcome this issue, bromide labeling to give precursor **10** was investigated. Compound **10** was prepared in 95% yield via Appel bromination of alcohol **8**.

Compound **10** still degraded, albeit slowly, at 4 °C; nevertheless, it was sufficiently stable for systematic labeling studies. It was used as a labeling precursor for all subsequent animal experiments. Direct nucleophilic radiofluorination of precursor **10** was conducted with the potassium Kryptofix® K222 cryptand $^{18}F^-$ complex ($[K^+ \subset K222]^{18}F^-$) in acetonitrile at 90 °C for 3 min to obtain [^{18}F]**1** in up to 45% radiochemical yield (see Scheme 2 and Figure 2). Alternatively, yields up to 86% were obtained after heating in a focused microwave reactor (250 W, 180 °C) for 50 s using a sealed reaction vessel, as shown in Figure 2. The formulated radiotracer was obtained in a radiochemical purity of >98% and a specific activity of up to 180 MBq nmol^{-1} after HPLC purification, solid-phase extraction, and formulation in phosphate-buffered saline.

Specificity for rat DAT was determined in vitro by autoradiography of 14-μm-thick cryosections from Wistar rat brains.



Scheme 2. Synthesis of [^{18}F]**1** via direct nucleophilic radiofluorination of **10**: a) $^{18}F^-$, K_2CO_3, K222, MeCN, 90 °C, 3 min, 45%; b) $^{18}F^-$, K_2CO_3, K222, MeCN, MW (250 W), 50 s, 81%.

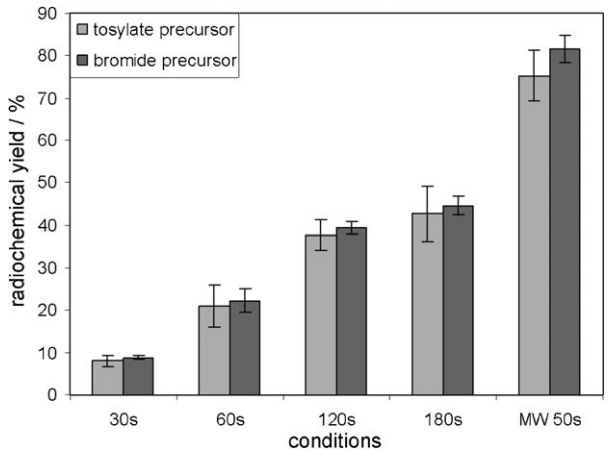


Figure 2. Radiochemical yields of [^{18}F]**1** as a function of indicated reaction times; MW: microwave irradiation at 250 W. Error bars represent ±1 SD (n = 4–7).

Compound [^{18}F]**1** was applied to coronal brain sections at a concentration of 11 nM. The sections were incubated for 1 h and washed with buffer twice. Some sections were pre-incubated with the selective DAT inhibitor WIN35,428 (β-CFT) to saturate the available DAT binding sites prior to application of the radioligand. Detection was performed with a Fuji® BAS-TR 2025 phosphoimaging plate, and data were processed with Fuji® Multigauge v. 3.0 and Gimp 2 (http://www.gimp.org/) software.[5] The results illustrate the specific accumulation of [^{18}F]**1** in the striatum and low uptake into the cerebellum (Figure 3a and b). In contrast, no striatal uptake of the radiotracer was detected in sections pre-incubated with 1 μM WIN35,428 (Figure 3c). These are the expected binding characteristics of a selective DAT ligand in the rat brain.[8]

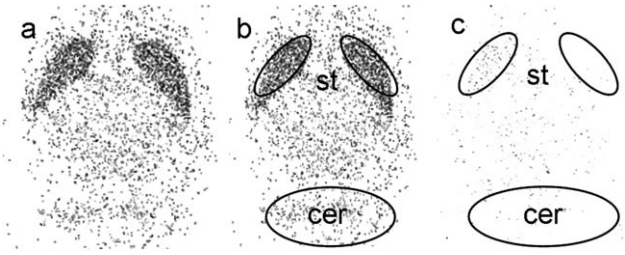


Figure 3. In vitro autoradiography of [^{18}F]**1** on coronal rat brain sections (thickness: 14 μm). a) Binding pattern of the tracer; b) brain section with ROI in left and right striatum (st) and cerebellum (cer); c) brain section pre-incubated with 1 μM WIN35,428 (β-CFT) in Tris·HCl buffer for 30 min with ROI in left and right striatum (st) and cerebellum (cer).

In a preliminary in vitro metabolism study using heparinized Wistar rat blood, [^{18}F]**1** was slowly metabolized over the course of 2 h. More than 95% of intact tracer was observed after 5 min, whereas the amount of intact tracer was decreased to 83.6±1.2% after 60 min and 77.6±1.6% after 120 min (Figure 4).

A dynamic μPET study was conducted with three male Wistar rats (280±20 g). Anesthesia was performed by i.p. injection of ketamine/xylazine. Animals were scanned for 90 min in a Siemens/CTI Focus 120 small-animal PET scanner. The list-mode data were reconstructed by two-dimensional ordered subset expectation maximization (OSEM 2D) algorithms, and three-dimensional image data were generated. Analyze 8.1 (http://www.mayo.edu/bir/Software/Analyze/Analyze.html) and

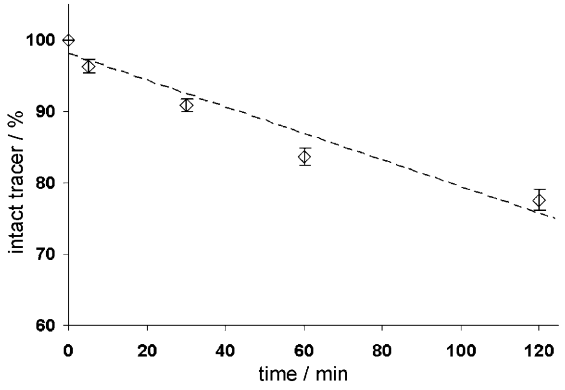


Figure 4. In vitro metabolism of [^{18}F]**1** in Sprague Dawley rat blood over 120 min. Error bars represent ±1 SD (n = 3).

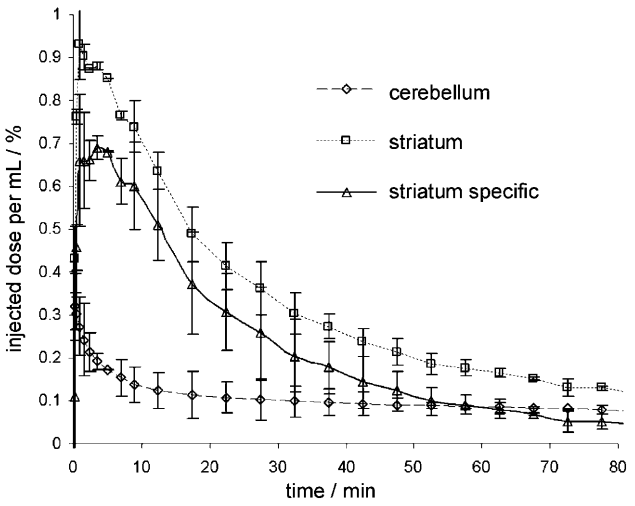


Figure 5. Averaged [^{18}F]**1** TACs for striatum (□) and cerebellum (◇), and proportion of specific binding in the striatal ROI (△). Error bars represent ±1 SD (n = 3).

pmod (http://www.pmod.com) software were used for data evaluation. Time–activity curves (TAC) for a region of interest (ROI) in the DAT-rich striatum as well as for a ROI in the reference region (cerebellum) are shown in Figure 5. The proportion of specific binding in the striatum, obtained by subtraction of the cerebellum TAC from the striatal TAC, is also shown.

[^{18}F]**1** shows very low nonspecific binding and rapid washout from the cerebellum. The initial uptake of 0.32 ± 0.08 % of the injected dose per milliliter of tissue (% ID mL^{-1}) after 10 s was decreased to only 0.12 ± 0.06 % within 20 min. As expected for a DAT ligand, the radioactivity was retained in the striatum.[8]

Peak uptake of 0.92 ± 0.06 % ID mL^{-1} was reached at 70 s post-injection (p.i.), followed by washout to 0.49 ± 0.06 % ID mL^{-1} after 20 min and 0.23 ± 0.03 % ID mL^{-1} after 45 min. Interestingly, [^{18}F]**1** shows a significantly more rapid washout than most DAT tracers investigated so far.[5,9] However, displacement of the residual radioactivity in the striatum to background levels was achieved after 90 min using an i.v. injection of 1.5 mg kg^{-1} β-CFT.

The tracer was found to show an overall pharmacokinetic behavior similar to that of (−)-*N*-[^{11}C]cocaine ([^{11}C]**2**), an extraordinarily valuable tracer for PET studies of presynaptic dopaminergic pathologies.[4,2a] The equilibrium state between the striatal ROI and the reference region becomes linear as soon as 20 min p.i., and a distribution volume ratio (DVR) of 3 was obtained from graphical analysis (Logan plot).[10] The improved pharmacokinetic profile and the early equilibrium result in considerably shorter scanning times for PET studies. In this regard, the value of [^{18}F]**1** is further augmented by the high in vitro selectivity and advantageous kinetic profile.

In conclusion, rapid cocaine-like pharmacokinetics, the use of an ^{18}F label, paired with high in vitro selectivity strongly indicates that the novel DAT tracer [^{18}F]**1** has excellent potential as a highly available DAT tracer for clinical and preclinical research and routine diagnosis. Further studies toward a cGMP-compliant fully automated production of [^{18}F]**1** are in progress.

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