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Synthesis, radiosynthesis and *in vivo* evaluation of [^{123}I]-4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-1-(4-iodobenzyl)piperidine as a selective tracer for imaging the dopamine transporter

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Dopamine transporter (DAT) neuroimaging is a useful tool in Parkinson's disease diagnosis, staging and follow-up providing information on the integrity of the dopaminergic neurotransmitter system *in vivo*. 4-(2-(Bis(4-fluorophenyl)-methoxy)ethyl)-1-(4-iodobenzyl)piperidine (7) has nanomolar affinity for DAT and better selectivity over the other monoamine transporters compared with the existing SPECT radioligands for DAT. The aim of this study was to synthesize and evaluate [^{123}I]-7 as an *in vivo* tracer for DAT.

The tributylstannyl precursor was synthesized with an overall yield of 25%. [^{123}I]-7 was synthesized by electrophilic destannylation with a yield of 40 ± 10%. Radiochemical purity appeared to be > 98%, whereas specific activity was at least 667 GBq/μmol. Biodistribution studies in mice showed brain uptake of 0.96 ± 0.53%ID/g at 30 s post injection (p.i.) and 0.26 ± 0.02%ID/g at 3 h p.i. High blood activity was observed at all time points. Pretreatment with Cyclosporin A raised brain uptake indicating that [^{123}I]-7 is transported by P-glycoprotein (P-gp) pumps. In rats, regional brain distribution of [^{123}I]-7 was not in agreement with DAT distribution. These results indicate that [^{123}I]-7 is not suitable for mapping DAT *in vivo* but could be a useful tracer for the P-gp transporter.

Keywords: dopamine transporter; SPECT; [^{123}I]-4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-1-(4-iodobenzyl)piperidine; brain

Introduction

Dopamine is a monoamine neurotransmitter with a crucial role in the central nervous system. Dopamine is associated with locomotor control and the brain reward system. Once released in the synapse, dopamine is able to stimulate five different dopamine receptors. These receptors are all G-protein coupled and divided into the D1- and D2-like receptors. D1 and D5 belong to the D1-subfamily, whereas D2, D3 and D4 are members of the D2-like receptors.[1] The dopaminergic neurotransmission is terminated in two ways: by a plasma membrane protein called the dopamine transporter (DAT) or by enzymatic degradation. Of those, reuptake by DAT is the most important. The protein reaccumulates dopamine into the presynaptic neuron thereby regulating the synaptic concentration of dopamine, available for binding to the receptors. DAT consists of 12 transmembrane domains with intracellular localization of the carboxy- and aminoterminals. Uptake of the substrate requires co-transport of two Na^+ and one Cl^- ion.[2–4] In the brain, DAT is predominantly localized in the striatum and olfactory tubercle. The cerebellum seems to receive negligible dopaminergic innervation.[5–7] Outside the central nervous system, localization of DAT is found in the gastrointestinal tract, stomach, pancreas[8] and kidney.[9]

Cocaine is one of the most powerful positively reinforcing psychoactive drugs. The neurochemical mechanism responsible for cocaine's reinforcing properties has been hypothesized to involve the binding of cocaine to DAT. There is still a large need for agents that find use in the treatment of cocaine abuse.[10–13] DAT radioligands could help to evaluate possible therapeutic agents. DAT is also involved in the pathophysiology of Parkinson's disease (PD)[14,15] and other neurological disorders.[16] Ciliax *et al.* demonstrated that DAT is exclusively present in dopamine-synthesizing neurons and is therefore a specific marker for the integrity and number of these neurons.[17] Since PD is characterized by the degeneration of dopaminergic neurons, neuroimaging with DAT radioligands could allow differential diagnosis with nondegenerative movement disorders. Not only diagnosis but also follow-up of the treatment and

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investigation of new therapies could be possible using suitable DAT radioligands.[18–20]

Because of the crucial role of DAT in the etiology of addiction to cocaine and PD, several DAT radioligands have been developed as *in vivo* markers of the dopaminergic system.[21,22] Several SPECT radiotracers for DAT have already been designed, but they all share the same problem of being not selective enough toward the serotonin transporter (SERT). The commercially available [^{123}I]-*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane ([^{123}I]-FP-CIT) (DaTSCAN, GE Healthcare, Little Chalfont, UK) has a K_i value of 3.5 nM for DAT, 0.11 nM for SERT and 63 nM for the norepinephrine transporter (NET), indicating lack of selectivity for DAT.[23–25] Other drawbacks of [^{123}I]-FP-CIT are lack of good *in vivo* kinetics and the formation of four labelled metabolites in plasma.[13,26] Another investigated DAT radioligand, [^{123}I]-(E)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-methylphenyl)nortropane ([^{123}I]-PE2I), has K_i values of 17, 500 and over 1000 nM for DAT, SERT and NET, respectively; therefore, a SERT/DAT selectivity of 29 was calculated. Another disadvantage of [^{123}I]-PE2I is the *in vivo* deiodination of the iodo vinyl group.[27] These shortcomings indicate that there is still a large need for selective radioligands for DAT.

The compound investigated in this study, 4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-1-(4-iodobenzyl)piperidine (**7**), has been synthesized and tested *in vitro* by Boos *et al.*[28] They reported K_i values of 1.9 nM for DAT, 205 nM for SERT and 4110 nM for NET; therefore, the SERT/DAT selectivity is 108. Compared with [^{123}I]-FP-CIT and [^{123}I]-PE2I, the *in vitro* properties of **7** are promising. The present study reports the synthesis, radiolabelling and *in vivo* evaluation of [^{123}I]-**7**.

Results and discussion

Synthesis and radiosynthesis

The synthesis of compounds **2**–**7** is depicted in Figure 1. The starting material **1** was prepared as previously reported by Boos *et al.*[28] The most direct route to the tributylstannyl precursor is generally a palladium-catalyzed stannylation using hexabutyldistannane and tetrakis(triphenylphosphine)palladium where a bromine atom is replaced by a tributylstannyl group. Several attempts to synthesize **5** by this route were unsuccessful, requiring another route. It was found that the triflate analogue was a suitable substrate for the Stille coupling. In this way, the tributylstannyl precursor was synthesized starting from **1** through the triflate intermediate in an overall yield of 25%. Nucleophilic substitution of **1** yielded the cold iodinated product **7** in a yield of 50% (Figure 2). Radiolabelling was conducted by an electrophilic iododestannylation of the tributylstannyl precursor (Figure 3). [^{123}I]-**7** was obtained in a radiochemical yield of 40 ± 10%.

Radioanalytical data

The radiolabelled compound [^{123}I]-**7** was co-injected with the cold compound. Similar retention times were observed for [^{123}I]-**7** and **7**, confirming the identity of the synthesized product. The average radiochemical purity of [^{123}I]-**7** was found to be greater than 98%. After 24 h, the radiochemical purity of [^{123}I]-**7** remained higher than 95 % both in phosphate buffered saline (PBS) as well as in the injectable solution.

Since no UV signal was observed for the amount of [^{123}I]-**7** synthesized, the detection limit was determined by a calibration curve and used for calculation of the specific activity. Using the described radioanalytical method, the detection limit for [^{123}I]-**7** was 1.5×10^{-6} M. Specific activity appeared to be at least 667 GBq/μmol.

In vitro lipophilicity

An ideal log *P* value for brain radiotracers is between 1.5 and 3.5. The determined octanol/PBS partition coefficient was found to be 1.42 ± 0.12%, which is suitable for brain penetration.

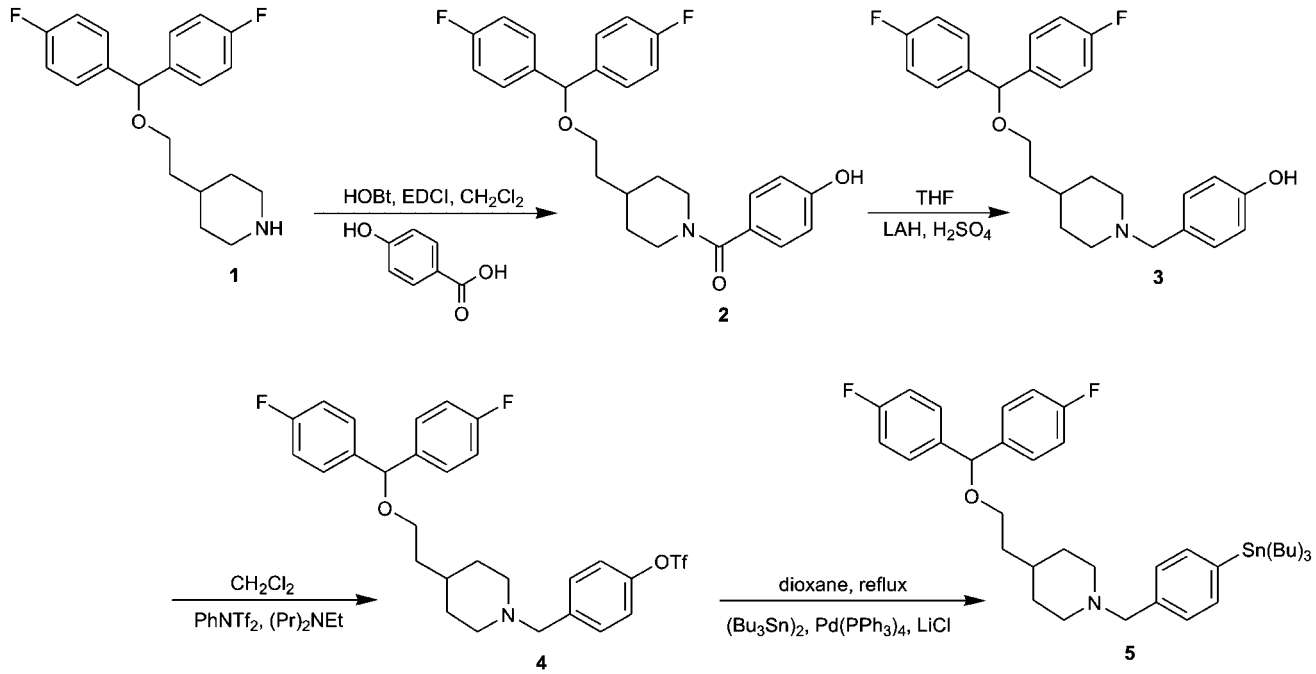

Figure 1. Synthesis of the tributylstannylprecursor **5**.

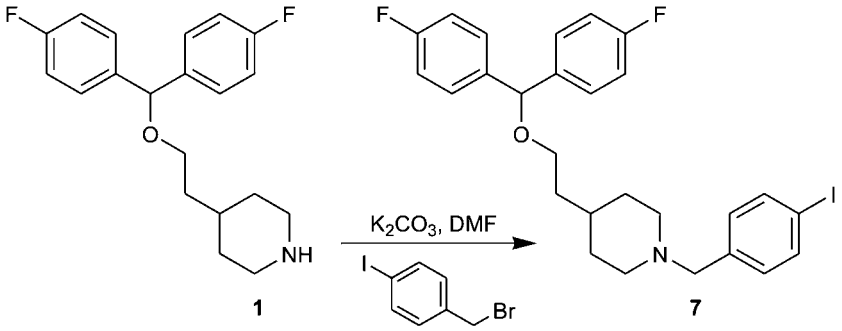

Figure 2. Synthesis of 4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-1-(4-iodobenzyl)piperidine (**7**).

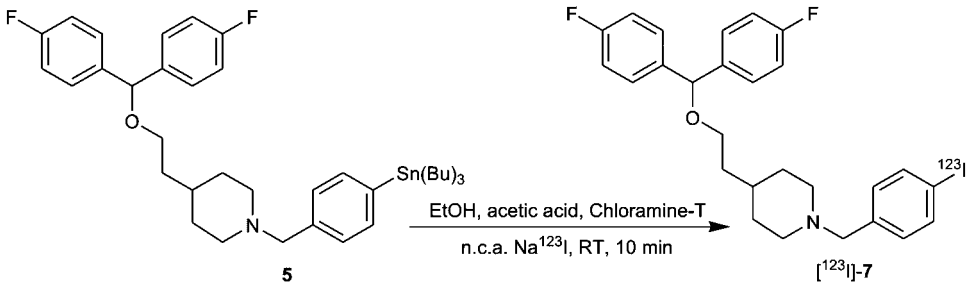

Figure 3. Radiosynthesis of [^{123}I]-4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-1-(4-iodobenzyl)piperidine ([^{123}I]-**7**).

In vivo evaluation of [^{123}I]-7

Results of the biodistribution study are given in Table 1. Uptake of the tracer in mouse brain was demonstrated with a maximum value of 0.96 ± 0.53%ID/g in brain at 30 s post injection (p.i.). The tracer was rapidly cleared out of the blood. At all time points, blood activity remained higher than brain activity. Other organs with high tracer uptake were heart (11.14 ± 3.79%ID/g at 30 s p.i.) and lungs (43.27 ± 15.46%ID/g at 30 s p.i.). High uptake was seen in liver (11.43 ± 4.53%ID/g at 20 min p.i.), kidneys (4.91 ± 2.14%ID/g at 30 s p.i.) and urine (data not shown), and uptake in the intestines was low, suggesting mainly urinary and no biliary clearance of [^{123}I]-**7**.

The uptake of radioactivity in various rat brain regions as a function of time following intravenous administration of [^{123}I]-**7** is given in Table 2. The regional distribution of [^{123}I]-**7** in the rat brain was almost homogeneous, which is not consistent with the current knowledge of DAT localization.[5–7] The uptake in the striatum was not higher compared with the uptake in the other brain regions. No difference in radioactivity uptake was observed between the striatum (0.053 ± 0.038%ID/g at 3 h p.i.) and the reference region (cerebellum (0.029 ± 0.025%ID/g at 3 h p.i.)) suggesting that no specific binding of [^{123}I]-**7** to DAT is accomplished.

P-glycoprotein (P-gp) is an efflux pump for a wide range of xenobiotics at the blood–brain barrier. Hence, P-gp can be a severe obstacle to the penetration of drugs into the brain. The efflux action of P-gp pumps can be reduced by the so-called modulators of which Cyclosporin A (Cs A) is an example. Pretreatment of mice with Cs A indicated a significantly higher brain uptake compared with the control group ($p = 0.02$ at both time points). Blood values were not affected by Cs A pretreatment ($p = 0.09$ at 2 min p.i.; $p = 0.45$ at 3 h p.i.). Cs A treatment increased both the brain uptake (4.1–5.4-fold) and subsequently the brain-to-blood ratio in a 2.3–4.5 way, indicating the contribution of P-gp transporters to the low brain uptake of [^{123}I]-**7** (Table 3).

Experimental

General

The starting material, 4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-piperidine (**1**), was described and synthesized by Boos *et al.*[28] All melting points (m.p.) were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Varian XL-300 instrument with DMSO-d_6 as the solvent; δ values were recorded in ppm (TMS as internal standard) and *J* (Hz) assignments of ^{1}H resonance coupling were done. Electron ionization mass spectra (EIMS) were obtained using a VG-Micro Mass 7070F mass spectrometer. Thin-layer chromatography (TLC) was performed on 250 mm Analtech GHLF silica gel plates. Visualization was accomplished under UV. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA).

All solvents and chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). No carrier added (n.c.a.) [^{123}I]-sodium iodide (in 0.05 M NaOH) was purchased from GE Healthcare (Cygne, The Netherlands). Cs A (Sandimmun) was purchased from Novartis Pharma (Vilvoorde, Belgium).

The HPLC system used consists of a Waters 515 HPLC pump, a Waters 2487 UV detector (Waters, Milford, USA) and a Ludlum model 2200 scalar ratemeter equipped with a Geiger Müller tube (Ludlum Measurements Inc., Sweetwater, USA). Chromatograms were recorded at a wavelength of 254 nm. Absorption unit full scale was set to 0.0001. The columns, mobile phases and flow rates used are indicated in the text below.

All radioactivity was counted with an automated gamma-ray spectrometer equipped with five 1 × 1 in NaI(Tl) crystals (Cobra Autogamma, Packard Canberra).

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Table 1. Tissue uptake of radioactivity in mice at various time points following i.v. administration [^{123}I]-4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-1-(4-iodobenzyl)piperidine ([^{123}I]-**7**)[a]

	Time (min)										
	0.5	1	2	3	5	10	20	40	60	120	180
Blood	11.14 ± 2.28	8.95 ± 3.97	3.02 ± 0.96	1.73 ± 0.65	1.01 ± 0.35	0.99 ± 0.45	0.67 ± 0.24	0.50 ± 0.11	0.30 ± 0.11	0.31 ± 0.01	0.37 ± 0.02
Brain	0.96 ± 0.53	0.41 ± 0.11	0.26 ± 0.08	0.21 ± 0.07	0.14 ± 0.02	0.23 ± 0.07	0.22 ± 0.07	0.21 ± 0.04	0.13 ± 0.04	0.20 ± 0.06	0.26 ± 0.02
Heart	11.14 ± 3.79	5.48 ± 1.29	4.91 ± 0.59	4.54 ± 2.12	3.20 ± 1.22	5.01 ± 1.36	4.24 ± 1.01	2.35 ± 0.72	1.15 ± 0.36	0.96 ± 0.23	1.06 ± 0.14
Lungs	43.27 ± 15.46	20.03 ± 1.3	14.32 ± 1.54	17.51 ± 8.73	11.80 ± 3.42	13.37 ± 6.75	9.12 ± 7.17	5.70 ± 1.44	3.87 ± 1.35	3.66 ± 1.13	3.73 ± 0.51
Stomach	1.33 ± 0.55	0.81 ± 0.17	0.83 ± 0.25	1.11 ± 0.47	0.50 ± 0.12	0.94 ± 0.29	0.85 ± 0.02	1.65 ± 1.15	0.61 ± 0.28	0.67 ± 0.27	1.06 ± 0.08
Spleen	3.23 ± 1.45	2.42 ± 0.56	3.85 ± 1.78	6.34 ± 3.87	4.23 ± 1.43	6.61 ± 0.69	5.99 ± 1.36	3.84 ± 1.66	1.85 ± 0.82	2.08 ± 0.5	2.46 ± 0.84
Liver	4.91 ± 2.97	3.53 ± 0.86	5.04 ± 1.06	5.17 ± 2.04	3.99 ± 1.17	9.03 ± 1.8	11.43 ± 4.53	10.09 ± 2.76	8.10 ± 4.27	10.30 ± 3.02	14.20 ± 4.35
Kidneys	4.91 ± 2.14	3.67 ± 0.85	3.61 ± 1.05	3.97 ± 1.91	2.76 ± 0.87	4.65 ± 0.3	4.46 ± 1.04	3.55 ± 1.11	2.53 ± 1.35	2.88 ± 0.79	3.00 ± 0.86
Small intestine	0.68 ± 0.28	0.49 ± 0.06	0.53 ± 0.2	0.65 ± 0.21	0.35 ± 0.06	0.74 ± 0.12	0.71 ± 0.21	0.69 ± 0.03	0.48 ± 0.24	0.80 ± 0.15	1.20 ± 0.13
Large intestine	0.37 ± 0.16	0.25 ± 0.03	0.24 ± 0.07	0.29 ± 0.12	0.16 ± 0.05	0.38 ± 0.11	0.28 ± 0.11	0.31 ± 0.06	0.20 ± 0.09	0.36 ± 0.11	0.63 ± 0.22
Bladder	1.25 ± 0.65	0.73 ± 0.08	0.64 ± 0.1	0.68 ± 0.28	0.63 ± 0.28	0.82 ± 0.15	0.85 ± 0.04	1.01 ± 0.32	0.95 ± 0.34	1.34 ± 0.73	1.64 ± 0.54
Pancreas	1.54 ± 055	1.05 ± 0.25	1.08 ± 0.21	1.45 ± 0.96	0.1 ± 0.98	1.54 ± 0.18	1.5 ± 0.33	1.19 ± 0.73	1.05 ± 0.42	1.68 ± 0.28	2.4 ± 0.34
Muscle	0.91 ± 0.28	0.57 ± 0.13	0.75 ± 0.09	0.84 ± 0.15	0.66 ± 0.19	1.30 ± 0.58	0.88 ± 0.07	0.88 ± 0.21	0.61 ± 0.26	0.65 ± 0.17	0.75 ± 0.03
Fat	0.93 ± 0.15	0.85 ± 0.62	0.85 ± 0.34	4.27 ± 5.57	0.72 ± 0.43	0.81 ± 0.38	0.76 ± 0.27	1.12 ± 0.77	1.10 ± 0.56	1.25 ± 0.11	2.03 ± 0.34

[a]Animals were injected intravenously with 185 kBq of [^{123}I]-**7** and sacrificed at designated times. Units are expressed as % injected dose/g of tissue ($n = 3$) ± SD corrected for background radiation and averaged.

Table 2. Tissue uptake of radioactivity in different brain regions in rats at various time points following i.v. administration of [^{123}I]-**7**[a]

	Time (min)				
	10	30	60	180	320
Blood	0.086 ± 0.060	0.089 ± 0.097	0.123 ± 0.084	0.116 ± 0.049	0.087 ± 0.094
Striatum	0.022 ± 0.016	0.013 ± 0.012	0.036 ± 0.031	0.053 ± 0.038	0.016 ± 0.012
Cerebellum	0.013 ± 0.007	0.024 ± 0.019	0.042 ± 0.051	0.029 ± 0.025	0.026 ± 0.012
Frontal cortex	0.013 ± 0.008	0.009 ± 0.011	0.036 ± 0.041	0.027 ± 0.035	0.015 ± 0.011
Occipital cortex	0.018 ± 0.009	0.027 ± 0.033	0.043 ± 0.063	0.015 ± 0.007	0.018 ± 0.016
Temporal cortex	0.015 ± 0.008	0.010 ± 0.013	0.017 ± 0.013	0.019 ± 0.012	0.021 ± 0.025
Parietal cortex	0.015 ± 0.010	0.019 ± 0.024	0.037 ± 0.039	0.026 ± 0.028	0.010 ± 0.007
Hippocampus	0.015 ± 0.008	0.077 ± 0.065	0.007 ± 0.008	0.049 ± 0.058	0.027 ± 0.018
Hypothalamus	0.014 ± 0.006	0.023 ± 0.026	0.030 ± 0.020	0.027 ± 0.013	0.010 ± 0.011
Thalamus	0.003 ± 0.003	0.015 ± 0.013	0.016 ± 0.021	0.026 ± 0.006	0.013 ± 0.012
Pons and medulla	0.022 ± 0.011	0.023 ± 0.019	0.064 ± 0.050	0.073 ± 0.055	0.038 ± 0.032
Total brain	0.017 ± 0.010	0.020 ± 0.010	0.042 ± 0.035	0.052 ± 0.036	0.032 ± 0.026

[a]Animals were injected intravenously with 7.4 MBq of [^{123}I]-**7** and sacrificed at designated times. Units are expressed as % injected dose/g of tissue ($n = 3$) ± SD corrected for background radiation and averaged.

Table 3. Biodistribution of [^{123}I]-**7**[a] with saline (control) or Cyclosporin A (test) in mice

	Control group		Test group	
	2 min	3 h	2 min	3 h
Blood	1.35 ± 0.42	0.54 ± 0.37	2.7 ± 1.15	0.50 ± 0.24
Brain	0.11 ± 0.04	0.13 ± 0.08	0.45 ± 0.14*	0.70 ± 0.25*
Brain/Blood	0.08 ± 0.01	0.33 ± 0.18	0.18 ± 0.06	1.48 ± 0.27*

[a]Animals were injected intravenously with 185 kBq of [^{123}I]-**7** and sacrificed at designated times. Units are expressed as % injected dose/g of tissue ($n = 3$) ± SD corrected for background radiation and averaged.
*$p < 0.05$ (Student's *t*-test, compared with control).

All animal studies were conducted following the principles of laboratory animal care and the Belgian Law on the protection of animals. The performed experiments are approved by the local Ethical Committee of Ghent University (ECD 07/06). All animals were purchased from Bioservices.

Synthesis of precursor and reference compound

*(4-(2-(Bis(4-fluorophenyl)methoxy)ethyl)piperidin-1-yl)(4-hydroxyphenyl)methanone (**2**)*

A solution of 4-hydroxybenzoic acid (2.0 g, 14.5 mmol) and *N*-hydroxybenzotriazole (1.9 g, 13.3 mmol) in dichloromethane (200 mL) was stirred for 15 min, prior to the addition of 4-(2-(bis(4-fluorophenyl)methoxy)ethyl)piperidine (**1**) (4.0 g, 12.1 mmol) and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (5.1 g, 26.6 mmol). The solution was stirred for 96 h after which the solvent was removed under reduced pressure. The semi-solid was dissolved in ethyl acetate (250 mL) and the organic layer was washed with 1 M hydrochloric acid (250 mL), 10% potassium carbonate (250 mL) and brine (250 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. TLC analysis (9:1 (v:v) – $CHCl_3$:MeOH) identified a small amount of unreacted starting material not removed in the acid washing. Flash chromatography provided two compounds that co-migrated in the 9:1 (v:v) – $CHCl_3$:MeOH solvent system. Both compounds were dried under high vacuum and underwent alane reduction without further purification.

*4-((4-(2-(Bis(4-fluorophenyl)methoxy)ethyl)piperidin-1-yl)methyl)-phenol (**3**)*

To a flame dried flask, lithiumaluminumhydride (1.3 g, 33 mmol) was added and cooled to 0°C under argon. Tetrahydrofuran (10 mL) was added drop-wise while stirring. Upon the completion of the addition of tetrahydrofuran, sulfuric acid (1.1 g, 17 mmol) in tetrahydrofuran (10 mL) was added drop-wise. The solution was stirred at 0°C for 45 min. To the ice-cold solution was added **2** (3.0 g, 6.6 mmol) in tetrahydrofuran (10 mL). The solution was stirred for an additional 15 min at 0°C, then allowed to warm to room temperature (RT) and stirred for 2 h. Ten percent sodium hydroxide (200 mL) and ethyl acetate (75 mL) was added to the solution. The layers were separated and the aqueous layer was further extracted with ethyl acetate (2 × 75 mL). The organic extracts were combined and washed with H_2O (200 mL) and brine (200 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to yield **3** as a solid. TLC analysis identified a small quantity of the starting material. The solid was dissolved in ether and 1.1 equivalent of oxalic acid was added. A total of 2.6 g (5.9 mmol) of **3** as the oxalate salt was obtained. The yield of the reaction was 89%.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.7–7.4 (m, 12H, ar); 5.5 (s, 1H, CH-O); 3.8 (bs, 2H, CH_2NH); 3.0–3.4 (m, 4H); 2.5-2.9 (m, 3H); 1.3–1.7 (m, 6H). HRMS (EI) calcd. for $C_{27}H_{30}F_2NO_2$ *m/z*, 438.2245; found 438.2243. Anal. calcd. for $C_{27}H_{29}F_2NO_2{\cdot}0.5\ C_2H_2O_4$: C, 69.69; H, 6.27; N, 2.90. Found: C, 69.17; H, 6.21; N, 2.88. M.p. 198–199.5°C. $R_f = 0.53$ (9:1 – $CHCl_3$:MeOH).

*4-((4-(2-(Bis(4-fluorophenyl)methoxy)ethyl)piperidin-1-yl)methyl)-phenyltrifluoromethane-sulfonate (**4**)*

N-Phenyltrifluoromethanesulfonimide (1.4 g, 3.8 mmol), **3** (1.3 g, 3.0 mmol) and *N*,*N*-diisopropylethylamine (1.0 mL, 6.0 mmol) were combined in dichloromethane (20 mL) and stirred under argon overnight. To the solution was added H_2O (50 mL) and dichloromethane (50 mL). The organic layer was separated and subsequently washed with a 10% sodium carbonate solution, H_2O (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. After chromatography on silica gel using 9:1 (v:v) – $CHCl_3$:MeOH as the solvent system, **4** was obtained as a clear oil 1.5 g (2.6 mmol). The yield of the reaction was 87%.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.0–7.4 (m, 12H, ar); 5.5 (s, 1H, CH-O); 4.0 (s, 2H, CH_2NH); 3.2–3.4 (m, 4H); 3.1(m, 2H); 1.2-1.7 (m, 7H). HRMS (EI) calcd. for $C_{28}H_{29}F_2NO_4S$ *m/z*, 570.1737; found 570.1738. Anal. calcd. for $C_{28}H_{28}F_2NO_4S{\cdot}2\ H_2O$: C, 55.53; H, 5.32; N, 2.31. Found: C, 55.71; H, 5.27; N, 2.31.

*4-(2-(Bis(4-fluorophenyl)methoxy)ethyl)-1-(4-(tributylstannyl)benzyl)-piperidine (**5**)*

A total of 1.1 g (1.9 mmol) of **4** was dissolved in anhydrous dioxane (15 mL) and to the solution was added bis(tributyltin) (1.5 g, 2.5 mmol). Under argon, lithium chloride (0.2 g, 5.7 mmol), tetrakis(triphenylphosphine)palladium (0.7 g, 0.06 mmol) and a catalytic amount of 2,6-di(*tert*)-butyl-4-methylphenol were added. The solution was heated to reflux and stirred overnight. The reaction was quenched upon the addition of ethyl acetate (50 mL) and a 10% aqueous ammonium hydroxide solution (50 mL). The organic layer was filtered through a pad of celite. The filtrate was washed with H_2O (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4. Chromatography with 7:3 (v:v) – hexane:ethyl acetate as the solvent system provided 0.46 g (0.6 mmol) of **5** as a chromatographically pure, clear oil. The reaction yield was 68%.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.1–7.4 (m, 12H, ar); 5.5 (s, 1H, CH-O); 3.3–3.4 (m, 8H); 0.8–1.5(m, 30H). HRMS (EI) calcd. for $C_{39}H_{56}F_2NOSn$ *m/z*, 712.3352; found 710.3344. Anal. calcd. for $C_{39}H_{55}F_2NOSn{\cdot}0.5\ H_2O$: C, 65.09; H, 7.84; N, 1.95. Found: C, 64.83; H, 7.81; N, 1.95.

*4-(2-(Bis(4-fluorophenyl)methoxy)ethyl)-1-(4-iodobenzyl)piperidine (**7**)*

To a solution of **1** (0.5 g, 1.4 mmol) and potassium carbonate (0.6 g, 4.2 mmol) in dimethylformamide (15 mL) was added a catalytic amount of sodium iodide and 4-iodobenzyl bromide (0.5 g, 1.5 mmol). The solution was stirred overnight. TLC analysis identified no remaining starting material and the solution was poured into H_2O (200 mL) and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with H_2O (2 × 200 mL) and brine (200 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The resulting oil was dissolved in ethanol (50 mL) and 1.1 equivalent of oxalic acid in ethanol was added. Upon cooling, crystals were formed and an additional recrystallization provided 0.4 g (0.7 mmol) of a **7** oxalate as a white solid. The reaction proceeded with a yield of 50%.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.1–7.8 (m, 12H, ar); 5.5 (s, 1H, CH-O); 4.0 (bs, 2H, CH_2NH); 3.1–3.5 (m, 4H); 2.5-2.7 (m, 3H); 1.2–1.8 (m, 6H). HRMS (ESI) calcd. for $C_{27}H_{29}IF_2NO$ *m/z*, 548.1262; found 548.1264. Anal. calcd. for $C_{27}H_{28}IF_2NO{\cdot}C_2H_2O_4$: C, 54.64; H, 4.74; N, 2.20. Found: C, 54.37; H, 4.95; N, 2.18. M.p. 157–158°C.

Radiosynthesis

[^{123}I]-**7** was prepared by electrophilic radioiododestannylation of **5**. Briefly, ethanol (50 μL), glacial acetic acid (8 μL), chloramine-T (300 μg in 15 μL deionized H_2O) and n.c.a. [^{123}I]-sodium iodide (37 MBq in 10 μL of 0.05 M NaOH) were added to a reaction vial containing **5** (200 μg, 0.281 mmol). The mixture was allowed to react for 10 min at ambient temperature. The reaction was quenched by adding sodium metabisulfite (300 μg in 15 μL deionized H_2O). Purification was performed by HPLC on an Apollo C18 column (250 mm × 4.6 mm, 5 μm) using 90:10:0.1 (v:v) – MeOH:H_2O:NH_4OH as the solvent system at a flow rate of 1 mL/min. [^{123}I]-**7** eluted with a retention time of 24–25 min. The collected fraction was diluted with sterile water and concentrated on an activated C18 Sep-pak cartridge (Alltech Maxi-Clean Prevail C18). [^{123}I]-**7** was eluted from the Sep-pak with 1 mL ethanol. Finally, the ethanol was diluted with isotonic saline to obtain an injectable solution (<10% (v:v) EtOH).

Quality control and stability

The radioanalytical data were obtained by injection of 100 μL test solution on a reversed-phase analytical HPLC (Alltima C18, 250 mm × 4.6 mm, 5 μm) using 91:9:0.1 (v:v) – MeOH:H_2O:NH_4OH as the mobile phase at a 1 mL/min flow rate.

Radiochemical purity and identity was determined by co-injection of an aliquot of [^{123}I]-**7** (after Sep-pak purification) with authentic cold reference product (**7**).

To test the *in vitro* stability, [^{123}I]-**7** was maintained in 0.01 M PBS pH 7.4 or in the injection solution at RT. After 24 h, [^{123}I]-**7** was analyzed by HPLC.

Lipophilicity determination

The log *P* value gives an indication whether the compound could pass the blood–brain barrier and if the nonspecific binding will be low. The partition coefficient was measured according to the shake flask method.[29,30] Approximately 185–370 kBq of [^{123}I]-**7** was added to a test tube containing 3 mL PBS and 3 mL *n*-octanol. The mixture was shaken vigorously by hand, vortexed for 3 min and centrifuged for 3 min at 3000*g*. To remove hydrophilic impurities, the phosphate buffer was discarded and replaced by fresh one. Again the mixture was shaken vigorously by hand, vortexed for 3 min and centrifuged for 3 min at 3000*g*. An aliquot of 0.5 mL of both *n*-octanol phase and buffer phase was taken and counted separately for radioactivity in an automated gamma counter. The aqueous phase was discarded and 2.5 mL of fresh buffer was added. This process was repeated twice. The partition coefficient was determined by calculating the ratio of radioactivity in *n*-octanol to buffer: (counts in *n*-octanol)/(counts in PBS buffer). The measurements were done in triplicate.

Biodistribution studies in mice

Male NMRI mice of 4–6 weeks old, weighing 22−30 g, were injected in the tail vein with 200 μL of 8:92 (v:v) – ethanol:saline solution containing approximately 185 kBq of [^{123}I]-**7**. At various time points p.i., mice (three animals per time point) were sacrificed under isoflurane anesthesia and dissected. Blood, urine and organs were removed, weighed and counted for radioactivity in the gamma counter. All organs were rinsed with water prior to weighing and counting. For calculation of the injected dose, five aliquots of the injection solution were weighed and counted for activity. Results are decay corrected and expressed as a percentage of the injected dose per gram of tissue (%ID/g) with standard deviation (SD).

Blood–brain barrier transporter inhibition study

To investigate whether the low brain uptake of [^{123}I]-**7** is due to P-gp interference, a biodistribution study with pretreatment of the mice with Cs A was performed. A 50 mg/kg dose and a 30 min time period between Cs A and tracer injection have been reported to inhibit the efflux action of P-gp pumps in rodent brain.[31] Male NMRI mice with a body mass of 20–25 g ($n = 3$) were intravenously injected with either 50 mg/kg Cs A (test group) or the same volume of physiological saline (control group). After 30 min, [^{123}I]-**7** (185 kBq, 150 μL) was injected in the tail vein. The mice were sacrificed and dissected at 2 min and 3 h p.i. of [^{123}I]-**7**. Blood and organs were weighed and counted for radioactivity. Results are expressed as %ID/g ± SD. Statistical analyses were performed using the one-side unpaired Student's *t*-test. A p value < 0.05 was interpreted as statistically significant.

Cerebral biodistribution studies in rats

Male rats (250–300 g, Sprague–Dawley) were injected in the tail vein with 7.4 MBq of [^{123}I]-**7** dissolved in 300 μL 8:92 (v:v) – ethanol:water. At 10, 30 min, 1, 3 and 6 h p.i., animals ($n = 3$) were sacrificed by intravenous injection of T61. Blood was taken and the brain was removed and dissected. Blood samples and the different brain parts were weighed and counted for radioactivity with the gamma counter. Radioactivity is decay corrected and expressed as %ID/g ± SD.

Conclusion

It has been demonstrated that DAT neuroimaging is useful in PD diagnosis, providing information on the integrity of the dopaminergic neurotransmitter system *in vivo*.[18–20] Because of the crucial role of DAT in the etiology of PD as well as other neurological disorders and addiction, several DAT ligands have been developed for *in vivo* SPECT imaging.[21,22] These ligands, for example, [^{123}I]-FP-CIT, are not selective toward SERT.[23–25] The investigated SPECT tracer, [^{123}I]-4-(2-(bis(4-fluorophenyl)methoxy)-ethyl)-1-(4-iodobenzyl)piperidine, has good *in vitro* selectivity over the other monoamine transporters (108 for SERT/DAT and 2163 for NET/DAT).[28] We therefore evaluated the potential of [^{123}I]-**7** as a suitable tracer for DAT *in vivo*.

The preparation and purification of the precursor molecule, as well as the reference compound, were accomplished without any significant problems. The radiosynthesis of [^{123}I]-**7** was achieved by a one-pot synthetic procedure in a 40 ± 10% yield and with good specific activity. After purification, the radiochemical purity appeared to be higher than 98%. The authenticity of the tracer was determined by co-elution with the nonradioactive reference compound. *In vitro*, [^{123}I]-**7** remained stable for at least 24 h.

Upon intravenous administration, low levels of activity were observed in mouse brain indicating low blood–brain penetration of [^{123}I]-**7**. At none of the selected time points, radioactivity concentration of brain exceeded blood activity. A possible reason for the low brain uptake of [^{123}I]-**7** is the contribution of P-gp pumps or other multidrug resistance protein transporters in the brain distribution of [^{123}I]-**7**. We examined the effect of pretreatment with Cs A, a modulator of the P-gp pumps, on the brain uptake of [^{123}I]-**7**. A significant increase in the brain uptake (4.1–5.4-fold) of [^{123}I]-**7** was observed compared with the control group indicating that [^{123}I]-**7** is transported by the P-gp pumps out of the brain. These findings might be a possible explanation for the low brain accumulation of [^{123}I]-**7**. In rat brain, the regional distribution of DAT was not reflected in the radioactivity distribution. Although **7** is selective for DAT *in vitro*, it did not display the expected selective distribution *in vivo* in rat brain. Based on these results, one can conclude that [^{123}I]-**7** is not suitable as a radioligand for *in vivo* SPECT visualization of DAT but could be a useful tracer for the P-gp transporter. Further research to validate the usefulness of [^{123}I]-**7** as a SPECT tracer for P-gp pumps *in vivo* is ongoing.

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