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Synthesis, radiolabeling, and baboon SPECT imaging of 2 β -carbomethoxy-3 β -(3'-[¹²³I]iodophenyl)tropane ([¹²³I]YP256) as a serotonin transporter radiotracer.([¹²³I]YP256) a potential serotonin transporter radiotracer)

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Abstract

To develop a potential SPECT probe to evaluate the integrity of the serotonergic system (5-HTT) whose dysfunction is linked to several disease conditions such as Parkinson's, Alzheimer's diseases and depression, we report the synthesis, radiolabeling and *in vivo* baboon imaging of 2 β -carbomethoxy-3 β -(3'-[¹²³I]iodophenyl) tropane (**YP256, 6**). The radiolabeling was performed by iododestannylation using sodium [¹²³I]iodide and peracetic acid. Although the ligand displayed high selectivity for 5-HTT over dopamine transporter (DAT) *in vitro*, SPECT imaging in baboons did not reveal selective 5-HTT accumulation in brain *in vivo*.

Keywords

radiolabeling; serotonin transporter; monkey study; brain imaging; SPECT

1. Introduction

Several disease conditions are strongly linked to the degeneration (Parkinson's disease ^{1–3} and schizophrenia ⁴) or the dysfunction (pathogenesis of depression ⁵) of the serotonergic neurons in the human brain. Cocaine (**1**) exerts multiple physiological effects on the mammalian central nervous system through its ability to bind to the dopamine (DA), serotonin (5-HT) and norepinephrine (NE) transporters in brain, resulting in a loss of their respective function. The development of new SPECT or PET radiolabeled analogs of cocaine binding specifically to 5-HTT has been used to provide potential tools to explore the brain serotonergic system *in vivo* in these diseases. The different structure-activity relationship studies in pursuit of an enhanced selectivity for 5-HTT have yielded several potential candidates, mostly tropane derivatives such as β -CIT (RTI-55, **2**) and its N-desmethyl analog norCIT ⁶, but have shown

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limited results due to their poor selectivity for 5-HTT over DAT. The search for a high-affinity, selective 5-HTT radiotracer has proved elusive (for reviews, see ^{7–10}). The PET tracer [¹¹C] (+)-McN-5652-Z has been used for several years ¹¹, even though its low target to background ratio limits its use to high density regions like the midbrain ^{12,13}. A fluorine-18 labeled analog [¹⁸F]FMe-McN5652 has shown somewhat better properties in pigs and rats ^{14,15}. Reports of diphenyl sulfide-related ligands of the “IDAM” type ¹⁶ led to tracers with higher in vivo selectivity, and two of these, [¹¹C]DASB ¹⁷ and [¹¹C]MADAM ¹⁸, have found their way into human studies. Further refinements have also been reported in animals with fluorinated analogs [¹¹C]AFM and [¹¹C]AFA.

Based on the observations of Carroll ¹⁹ and Davies ^{20,21} that selectivity for 5-HTT was enhanced by unsaturated substituents on the phenyl ring and by *N*-demethylation (trends confirmed by preliminary results with 4'-substituted analogs ^{22,23}), we synthesized a series of 3'-substituted tropane analogs and measured their binding affinity towards the three monoamine transporters. The potency (*K_i*, nM) of the compounds was evaluated by competition against radiolabeled ligands selective for 5-HTT, DAT, and NET in rat forebrain tissue and human cell membranes and binding selectivity was calculated as the inverse ratio of *K_i* values. The results pointed out an unexpected ability for the position 3' to increase the selectivity for 5-HTT over DAT without substantial loss of binding affinity for 5-HTT, especially in the case of the 3' analog of β-CIT **5** (Table 1), which was less potent than the 4'-isomer β-CIT (21-fold in rat and 14-fold in human), but more so at DAT than 5-HTT, so that the selectivity for 5-HTT was greater (Table 2). Similar to previous reports ⁵, selectivity for 5-HTT was increased even further by *N*-demethylation: the nor-compound **6** (or **YP256**) exhibited 16-fold (rat) or 415-fold (human) selectivity towards 5-HTT over DAT. The radiolabeling and *in vivo* study of the ¹²³I labeled form of this compound are reported here.

2. Materials and Methods

General

¹H NMR spectra were obtained using a Bruker AM 500 MHz spectrometer and ¹³C NMR spectra were obtained on Bruker 400 MHz. Mass spectra were obtained with a Micromass Q-ToF spectrometer. Elemental analyses were performed by Atlantic Microlab Inc. (Knoxville, TN), and values within 0.4% of the theoretical values were accepted as valid. Flash chromatography ²⁴ was performed with 40-μm mesh silica gel 60 (J. T. Baker) using eluents as indicated. Starting materials for syntheses were purchased either from Sigma-Aldrich Chemicals (St. Louis, MO) or from Acros Corp. (Pittsburgh, PA). No-carrier-added sodium [¹²³I]iodide in dilute NaOH was obtained from Nordion International, Vancouver, B.C., Canada.

2.1. Chemistry

2.1.1. 8-Methyl-3β-(3'-trimethylsilylphenyl)-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester **4**—To a suspension of powdered Mg (2.78 g, 116 mmol) in anhydrous ether (300 mL) was added a solution of 1-bromo-3-trimethylsilylbenzene (18.3 g, 80 mmol) in anhydrous ether (150 mL) under stirring and Ar (g). After 10 min, a few drops of a solution of 1,2-dibromoethane in ether (10 mL) was added and the mixture was stirred slowly for 20 min. The reaction was cooled to −78°C and ecgonidine methyl ester **3** (5 g, 27.6 mmol) in ether (200 mL) was added drop-wise. The temperature was raised to −20°C and after 1 h at this temperature, the reaction was cooled to −70°C, and trifluoroacetic acid (3 mL) in ether (5 mL) was slowly added. The temperature was allowed to increase to −40°C and H₂O (20 mL) was slowly added. Without delay, the mixture was basified with 15 M NH₄OH. The mixture was extracted with ether and the organic layer was washed with saturated NaCl, dried over anhydrous Na₂SO₄, and the solvent was removed on a rotary evaporator. The residue was

purified by column chromatography (hexane/ether/triethylamine 9:1:0.5) to give 2.97 g (32%) of product as a white powder. ^1H NMR (500.13 MHz, CDCl_3) : δ 7.41 (s, 1H, H-2'), 7.35 (m, 1H, H-4'), 7.30 (m, 2H, H-6', H-5'), 3.59 (m, 1H, H-1), 3.51 (s, 3H, OCH_3), 3.41 (m, 1H, H-5), 3.05 (m, 1H), 2.95 (m, 1H), 2.65 (dt, 1H, $J_1 = 13.0$ Hz, $J_2 = 2.5$ Hz, H-4 β), 2.27 (s, 3H, N- CH_3), 2.23 (m, 1H), 2.15 (m, 1H), 1.72 (m, 3H), 0.01 (s, 9H). ^{13}C NMR (125.7, CDCl_3) : δ 173.3, 143.0, 140.6, 133.3, 131.9, 129.1, 128.4, 66.5, 63.4, 54.0, 52.1, 43.0, 35.2, 35.0, 27.0, 26.2, 0.0.

2.1.2. 3β -(3-Iodophenyl)-8-methyl-8-aza-bicyclo[3.2.1]octane-2 β -carboxylic acid methyl ester **5**—To a solution of **4** (2.9 g, 8.7 mmol) in HOAc (100 mL) was added ICl (1.56 g, 1.1 eq.) under stirring and argon. The mixture was heated at 60°C for 24 h and, after cooling, ice-water was added. The mixture was basified with 15 M NH_4OH , then extracted with CH_2Cl_2 . The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The crude material was purified by column chromatography (hexane/ether/triethylamine 9:1:0.5) of silica gel, to give 2.35 g of product as a colorless oil (70 %).

^1H NMR (399.98 MHz, CDCl_3) : δ 7.44 (s, 1H, H-2'), 7.34 (d, 1H, $J = 7.99$, H-4'), 7.12 (d, 1H, $J = 7.99$, H-6'), 6.87 (t, 1H, $J = 7.99$, H-5'), 3.44 (m, 1H, H-1), 3.40 (s, 3H, OCH_3), 3.22 (m, 1H, H-5), 2.79 (m, 2H, H-2, H-3), 2.40 (m, 2H), 2.09 (s, 3H, N- CH_3), 1.99 (m, 2H), 1.52 (m, 3H).

^{13}C NMR (100.57 MHz, CDCl_3): δ 172.2, 146.1, 136.8, 135.2, 130.1, 127.0, 94.6, 65.6, 62.5, 52.9, 51.6, 42.3, 34.2, 33.9, 26.2, 25.6.

2.1.3 3β -(3'-Iodophenyl)-8-aza-bicyclo[3.2.1]octane-2 β -carboxylic acid methyl ester **6**—To a stirred solution of **5** in anhydrous 1,2-dichloroethane under N_2 (g), was added drop-wise 1-chloroethyl chloroformate (ACE-Cl, 6 eq.), and the mixture was refluxed for 12 h. After cooling, the solvent was removed on a rotary evaporator and MeOH (50 mL) was added. After being refluxed for 8 h, the solution was evaporated to dryness. Water (150 mL) was added, the mixture was basified with NH_4OH and extracted with CH_2Cl_2 . The organic layer was washed with H_2O , saturated NaCl, dried over anhydrous Na_2SO_4 , and evaporated to dryness. The crude material was purified by column chromatography with hexane/ether/ Et_3N (4/6/0.2), to afford 1.40 g (62%) as a colorless oil. ^1H NMR (400.13 MHz, CDCl_3) : δ 7.47 (s, 1H, H-2'), 7.35 (d, 1H, $J = 7.99$, H-4'), 7.15 (d, 1H, $J = 7.99$, H-6'), 6.90 (t, 1H, $J = 7.99$, H-5'), 3.71 (m, 2H, H-1+H-5), 3.39 (s, 3H, OCH_3), 3.20 (m, 1H, H-3), 2.72 (m, 1H, H-2), 2.40 (m, 1H), 2.05 (m, 2H), 1.68 (m, 3H). ^{13}C NMR (100.57 MHz, CDCl_3) : δ 172.8, 146.8, 136.5, 135.7, 131.2, 127.5, 94.5, 56.5, 53.9, 51.2, 52.1, 35.9, 33.6, 29.4, 27.8.

2.1.4. 3β -(3'-Iodophenyl)-8-t-butoxycarbonyl-8-aza-bicyclo[3.2.1]octane-2 β -carboxylic acid methyl ester **7**—To a 0°C cooled and stirred solution of nortropane **6** (280 mg, 0.75 mmol) in anhydrous CH_3CN under N_2 (g) was added, drop-wise, 4-dimethylaminopyridine (138 mg, 1.5 eq.) in CH_3CN (2 mL). The mixture was stirred at 0°C for 10 min. Di-t-butyl dicarbonate (330 mg, 2 eq.) in CH_3CN (2 mL) was then added and the mixture was stirred at room temperature overnight. The solution was then evaporated to dryness. The crude material was purified by column chromatography using Et_2O /hexane/ Et_3N (2/8/0.2), to afford 261 mg (73%) of **7** as an oil. ^1H NMR (400.13, CDCl_3) : δ 7.49 (s, 1H, H-2'), 7.46 (d, $J = 7.6$ Hz, 1H, H-4'), 7.14 (brs, 1H, H-6'), 6.93 (t, $J = 7.6$ Hz, 1H, H-5'), 4.47 (m, 2H, H-1+H-5), 3.40 (s, 3H, OMe), 3.12 (s, 1H), 2.78 (m, 1H), 2.63 (m, 1H), 2.06 (m, 1H), 1.97 (m, 1H), 1.76 (m, 1H), 1.59 (m, 2H), 1.6 (m, 9H). ^{13}C NMR (100.57 MHz, CDCl_3) : δ 172.3, 171.8, 146.7, 146.6, 136.7, 136.5, 135.8, 135.7, 131.4, 13.1, 128.0, 127.8, 94.5, 94.3, 79.56, 55.6, 55.3, 53.9, 52.7, 52.1, 51.9, 51.6, 51.5, 35.5, 35.2, 32.3, 29.7, 28.8, 28.5, 27.7.

2.1.5. 3 β -(3-Trimethylstannylphenyl)-8-t-butoxycarbonyl-8-aza-bicyclo[3.2.1]octane-2 β -carboxylic acid methyl ester **8**—To a solution of **7** (120 mg, 0.25 mmol) and tetrakis(triphenylphosphine) palladium (25 mg, 0.1 eq.) in anhydrous toluene (20 mL) under argon and stirring, was added hexamethylditin (140 mg, 2 eq.). After being refluxed for 24 h, toluene was evaporated. Purification of the crude material by column chromatography (hexane/Et₂O/Et₃N 70:30:0.5) afforded 109 mg (84%) of colorless oil. ¹H NMR (500.13 MHz, CDCl₃): δ 7.05 (m, 4H), 4.3 (m, 2H), 3.24 (s, 3H, OMe), 3.03 (m, 1H), 2.69 (m, 1H), 2.57 (m, 1H), 1.94 (m, 1H), 1.82 (m, 1H), 1.66 (m, 1H), 1.49 (m, 2H), 1.23 (m, 9H, CH₃)₃-C), 0.06 (s, 9H, Sn(CH₃)₃). ¹³C NMR (125.7 MHz, CDCl₃): δ 171.9, 171.8, 153.0, 152.68, 142.3, 141.5, 135.4, 135.2, 135.1, 134.2, 128.4, 128.2, 127.9, 127.7, 79.6, 55.8, 55.5, 53.7, 52.4, 52.3, 51.8, 51.7, 51.4, 36.1, 35.9, 33.1, 30.1, 29.3, 28.6, 27.1, – 9.09.

2.2. In vitro Binding

Assay procedures were based on the following reports: SERT²⁵, DAT²⁶, and NET²⁷, using membrane-containing homogenates of male Sprague-Dawley rat (Charles River Labs) forebrain tissue (cerebral cortex for SERT and NET; corpus striatum for DAT), or membranes from cell lines transfected to express human genes of the same transporter proteins (RBI-Sigma; Peng et al. 2004)²⁸. Test agents typically evaluated at ≥ 6 concentrations in duplicate with membrane homogenates in the presence of a [³H]radioligand, with and without a blank agent, as follows: SERT: [³H]cyanoimipramine (DuPont-NEN; 700 pM; blank: 10 μ M fluoxetine (donated by Eli Lilly); DAT: [³H] $\square$ -CIT (Tocris-Cookson; 300 pM; blank: 10 μ M GBR-12909 (RBI-Sigma); NET: [³H]nisoxetine (DuPont-NEN; 350 pM; blank: 10 μ M desipramine (RBI-Sigma). Tubes were incubated at RT for 120 min (SERT), on ice for 45 min (DAT), or ice for 180 min (NET). Labeled tissue samples were recovered on a Brandel Cell Harvester on glass fiber filter sheets, washed with ice-cold physiological saline, and counted for tritium in a Wallac liquid scintillation spectrophotometer. Concentration-inhibition curves were computer-fit to determine IC₅₀ $\pm$ SEM, and converted to K_i values from the relationship of Cheng & Prusoff: K_i = IC₅₀/(1 + [L/K_d])²⁶ (Kula et al. 1999). Selectivity for the SERT was indicated as the ratio of K_i for the DAT or NET to SERT (preference for the SERT site was indicated by a reciprocal).

2.2. Radiochemistry

To a vial containing 333 μ L (721 MBq; 19.5 mCi) Na[¹²³I]I solution in a 1-mL conical V-vial was added, in the following order: 67 μ L 0.5 M H₃PO₄ (one-fifth the volume of Na[¹²³I]I), a solution of 50 μ g (ca 0.1 μ mol) of 3 β -(3-trimethylstannylphenyl)-8-t-butoxycarbonyl-8-aza-bicyclo[3.2.1]octane-2 β -carboxylic acid methyl ester **8** in 50 μ L MeOH, and, without delay, 50 μ L 6.4% peracetic acid (freshly prepared from 32% CH₃CO₃H). After 10–20 min at room temperature, 50 μ L (0.5 mg) 10 mg/mL aqueous NaHSO₃ solution was added and the vial head space was purged into charcoal with a stream of N₂ (g) for 10 min. After drying under vacuum, 400 μ L MeOH and 2.5 mL 2 M HCl/Et₂O was added to the flask and reacted for 10–15 min. After removing the solvent on a rotary evaporator, 300 μ L mobile phase and 300 μ L saturated NaHCO₃ were added and the mixture was purified by HPLC (MeOH/H₂O/Et₃N: 70/30/0.2), 1 mL/min (retention time of **YP256** = 17.4 min). After removal of solvent on the rotary evaporator, the residue was formulated in 5% ethanol/normal saline containing ascorbic acid and filtered through a 0.22 μ m sterile filter (Gelman, Acrodisc13) into a sterile 10-mL serum vial. Radiochemical and chemical purity were assessed by HPLC in the same system, with sequential gamma and UV detection, comparing to a standard of nonradioactive authentic YP256.

2.3. *In vivo* SPECT Imaging

Baboon SPECT imaging was carried out as previously described²⁹, under institutional animal care protocols complying with Federal regulations. A single female baboon (ovariectomized *Papio anubis*, 20 kg) was fasted for 18–24 h before the study. At 2 h before injection, the animal was immobilized with intramuscular ketamine (10 mg/kg) and glycopyrrolate (0.01 mg/kg), transferred to the SPECT camera, and immediately intubated with an endotracheal tube for administration of 2.5% isoflurane. Body temperature was kept constant using a heated water blanket, at $36.4 \pm 0.3^\circ\text{C}$. Vital signs, including heart rate, respiration rate, oxygen saturation, and body temperature, were monitored every 30 min during the study. An intravenous perfusion line with 0.9% saline was placed and was used for the single bolus injection of [^{123}I]YP256. The baboon's head was immobilized within the gantry with a "bean bag" that hardens upon evacuation (Olympic Medical, Seattle, WA, USA).

2.3.2. SPECT Data Acquisition—SPECT data were acquired with the brain-dedicated multislice CERASPECT camera (Digital Scintigraphics, Waltham, MA, USA) with a resolution in all three axes of approximately 12 mm full width half-maximum measured using an ^{123}I line source and 20-cm water-filled cylindrical phantom. The distribution of radioactivity was assessed after administration of a single injected bolus of 232 MBq (6.28 mCi). Brain images ($128 \times 128 \times 64$ matrix; pixel size = 1.67×1.67 mm, slice, thickness = 1.67 mm, voxel volume = 4.66 mm^3) were acquired at 159 keV, in step-and-shoot mode at 15 min each for a total of 340 min and 20 acquisitions. Images were reconstructed using a ramp and a Butterworth filter (cutoff = 0.65 cm, power factor = 10). To identify brain regions, MRI scans of 1.5-mm contiguous slices were obtained with a 1.5 Tesla GE Signa device (General Electric, Milwaukee, WI, USA). Axial images were acquired using a spoiled GRASS (gradient recall acquisition in the steady state) sequence with TR = 25 ms, TE = 5 ms, NEX = 2, matrix = 256×192 , field of view = 16 cm.

2.3.3. Image Analysis—SPECT emission images were reconstructed and attenuation corrected as described previously²⁹. The images were then reoriented to the approximate canthomeatal line by using spatial 3-D reorientation in MEDx (Sensor Systems, Sterling, VA, USA). The first 10 reoriented SPECT images were summed to create an image for which anatomical boundaries could be clearly delineated for coregistration to the MRI scan. The summed image was coregistered to a MRI image of the baboon brain using surface registration in MEDx. .\). Region of interest (ROI) analyses were performed using ROI maps generated from placement of standardized 2-D ROI templates on the coregistered MRI image using a baboon brain atlas as a reference. The regional radioactive densities (counts/pixel) were determined by forming 3-D volumes between 2-D ROIs placed on three adjacent slices (except for the brainstem, which included six adjacent slices) in MEDx. The final 3-D volumes (cm^3) were cingulate cortex (1.2), , right and left parietal (R 2.9, L 2.9), frontal (R 3.4, L 3.4), temporoinsular (R 2.2, L 2.2), and occipital (R 2.0, L 2.0) cortices, the right and left striatum (R 1.1, L 1.1), which included the caudate, putamen, and globus pallidus, , and cerebellum (R 1.4, L 1.4), and the diencephalon (1.1) and brainstem (1.4).. The regional brain activities are reported as the average value of the right and left hemispheres. Regional activities were decay-corrected to the time of injection.

3. Results

3.1. Chemistry

Compound **4**, prepared in 43% yield by Grignard addition of 3-trimethylsilylphenyl magnesium bromide on ecgonidine methyl ester **3**, upon treatment with a solution of iodine monochloride in acetic acid at 60°C , afforded **5** (60%) by electrophilic substitution. N-Demethylation of **5** with 1-chloroethyl chloroformate in 1,2-dichloroethane at reflux gave the

nortropine analog **6**. N-Protection with di-*t*-butyl dicarbonate in the presence of 4-*N,N*-dimethylaminopyridine produced the N-Boc derivative **7** (73%), which upon Stille transformation with hexamethylditin and tetrakis(triphenylphosphine)-palladium gave the corresponding trimethylstannyl derivative **8** (84%).

3.2. Radiochemistry

[¹²³I]YP256 (**6**; scheme 2) was prepared by iododestannylation of the N-*t*-Boc-protected trimethylstannyl precursor **8** with [¹²³I]sodium iodide and peracetic acid at room temperature ³⁰. Removal of the N-Boc protecting group by treatment with 2M HCl in ether afforded the radiolabeled nortropine [¹²³I]**6**. Purification by reverse phase HPLC with concomitant UV and radioactivity detection, allowed identity confirmation of [¹²³I]**6** by comparison to its corresponding unlabelled analog. [¹²³I]YP256 was obtained with a labeling yield of 84.9% but isolated yield of final product was 55.5%, owing to adsorption of radiotracer to the membrane filters used for final filtration. Radiochemical purity was 99.0 % and specific activity >185,000 GBq/μmol (>5,000 Ci/mmol), based on the limit of detection of the HPLC UV detector.

3.3. SPECT Imaging

For all brain regions, peak activity was reached between 8 and 24 min pi, then, for the following 4 h, decreased rapidly at rates 9–17%/h. During the last 4 to 6 h, the activity levels declined to 1.5–7%/h rates in all brain regions. Overall there was no significant differences in regional [¹²³I]YP256 activity at 4 h (2–3%)

4. Discussion

In the present study, YP256 was labeled with ¹²³I by iododestannylation of the trimethylstannyl precursor and the time course of ¹²³I distribution in brain was assessed by SPECT imaging in a baboon after a single bolus injection. [¹²³I]YP256 entered the brain rapidly and reached peak uptake between 16 and 32 min pi, at a level of 1.2% of the injected dose. *In vitro* studies on human 5-HTT membranes have shown that YP256 is a high affinity ($K_i = 1.23 \pm 0.04$ nM) 5-HT transporter inhibitor that exhibits greater than 400-fold selectivity for 5-HTT compared to DAT ($K_i = 510 \pm 71$ nM). The distribution of [¹²³I]YP256 was highest in thalamus and striatum and lowest in cerebellum. The highest ratio between the low and high density region was 1.5 at 90 min PI. The overall low availability of YP256 to the CNS in nonhuman primates may be due, in part, to the high degree of plasma protein binding (*e.g.*, 68%).

These results suggest that *in vivo*, at tracer doses, *in vivo* tissue uptake [¹²³I]YP256 may be nonspecific or may be binding to targets distinct from the 5HT transporter.

Figure 2. *in vivo* distribution of [¹²³I]YP256 in non human primate brain.

Figure 3. Time-activity curves for [¹²³I]YP256 uptake in nonhuman primate brain after bolus injection.

5. Conclusion

The present study sought to assess [¹²³I]YP256 as a potential tool for *in vivo* imaging of the serotonin transporter system. Even if YP256 is selective for 5-HTT over DAT *in vitro* (415-fold) and [¹²³I]YP256 meets requirements of a potential brain radiotracer (*e.g.*, readily labeled in high yield and purity, high total brain uptake), it did not display the expected selective anatomical distribution *in vivo* in primate brain. Why the *in vivo* selectivity did not match the *in vitro* behavior is not clear. Most likely the explanation lies in the different environment that the radiotracer encounters in its interaction with the target protein (DAT vs 5-HTT). In the *in*

vitro binding assay, the transporter protein or tissue is isolated and diluted with assay buffer, so that the behavior can be attributed primarily to the equilibrium between association and dissociation of the radioligand-protein binding. In the *in vivo* case, on the other hand, the target protein is immobilized on a membrane and the radiotracer is exposed to a sea of competing proteins. This nonspecific binding or weak interactions may raise the background level sufficiently to mask the innate binding specificity, just as it is difficult to accurately measure the difference between two large numbers. Furthermore, *in vitro* binding assays are carried out at or below 25°C, compared to the 37°C environment *in vivo*. Indeed, we observed a temperature dependence on binding of the benzodiazepine receptor radioligand [¹²³I]iomazenil both *in vitro*³¹ and *in vivo*³². Based on the results presented in this report, [¹²³I]YP256 is not suitable as a radiotracer for *in vivo* imaging of 5-HTT. Since we started this project, a large number of radiotracers for PET or SPECT imaging of the serotonin transporter have been reported. All compounds reported for *in vivo* imaging have very high affinity for SERT ([¹¹C]DASB, [¹¹C] McN5652, [¹²³I]mZIEN, [¹²³I]ADAM). However, as reported recently, SERT radioligands bind to different classes of the transporter and only compounds with K_d between 0.03 and 0.3 nM at 37°C are useful for imaging studies.³³

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Figure 1.

Figure 2.
Co-registration of radioactive distribution of ^{123}I with anatomical imaging by MRI in baboon brain after i.v. administration of [^{123}I]YP256.

Figure 3.

Time-activity curve of ^{123}I in baboon brain after single bolus administration of 232 MBq (6.28 mCi) $[^{123}\text{I}]\text{YP256}$. *Legend:* FC, frontal cortex; PC, parietal cortex; CC, cingulate cortex; OC, occipital cortex; TIC, temporoinsular cortex; BG, basal ganglia; Brstm, brain stem; HA, hippocampal-amygdaloid area; CB, cerebellum.

Reagents: (a) 3-SiMe₃-C₆H₅MgBr, Et₂O, b) ICl, AcOH, 60°C; (c) 1) ACE-Cl, C₂H₄Cl₂, Δ 2) MeOH, Δ, c) di-*t*-butyl dicarbonate, DMAP, CH₃CN, RT; e) Me₃SnSnMe₃, Pd(PPh₃)₄, toluene, Δ.

Scheme 1.

Synthesis of 3-(3-Trimethylstannyl-phenyl)-8-aza-bicyclo[3.2.1]octane-2,8-dicarboxylic acid 8-*tert*-butyl ester 2-methyl ester **8**.

Reagents: (f) 1) H₃PO₄, CH₃CO₃H, ¹²³I] NaI 2) 2M HCl, Et₂O.

Scheme 2.

Radiosynthesis of 2β-carbomethoxy-3β-(3'-[¹²³I]iodophenyl) tropane **6**

Affinity of 3'-substituted phenyltropanes for rat (r) and human (h) dopamine, serotonin and norepinephrine transporters ($K_i \pm$ SEM, nM). SERT was assayed with [3 H]cyanoimipramine (700 pM; blank: fluoxetine, 10 μ M) and rat cerebral cortex and hSERT membranes; DAT with [3 H] β -CIT (300 pM; blank: GBR-12909, 10 μ M) and homogenates of rat caudate nucleus and hDAT membranes; and NET with [3 H]nisoxetine (350 pM, blank: desipramine, 10 μ M) with rat cerebral cortex and hNET membranes.

Table 1

Compound	DAT	h DAT	5-HTT	h 5-HTT	NET	h NET
5^a	100 $\pm$ 11	279 $\pm$ 41	9.82 $\pm$ 0.80	9.63 $\pm$ 0.4	> 10,000	1039 $\pm$ 53
6	21.2 $\pm$ 4.6	510 $\pm$ 71	1.35 $\pm$ 0.13	1.23 $\pm$ 0.04	325 $\pm$ 99	118 $\pm$ 3
β-CIT	0.96 $\pm$ 0.15	0.48	0.46 $\pm$ 0.06	0.67	2.80 $\pm$ 0.40	NM

^a tested as the HCl salt.
NM: Not measured.

Table 2

Binding Selectivity Ratios (Larger numbers are more selective)

Compound	5 HTT/DAT	h 5-HTT/h DAT	5-HTT/NET	h 5-HTT/h NET
5	10.2	29.0	>1020	108
6	15.7	415	241	95.9
β-CIT	2.08	1.40	6.08	