Communications to the Editor

Enantioselectivity of Cocaine Recognition Sites: Binding of (1S)- and (1R)-2β-Carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT) to Monoamine Transporters

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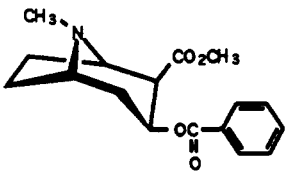
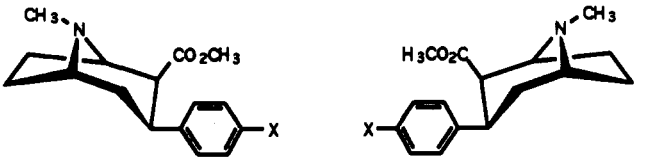
(1R)-Cocaine (1) is one of the most powerful reinforcing drugs known[1] and exhibits a complex set of pharmacological actions, primarily by inhibiting the reuptake of monoamines.[1] The inhibition of dopamine uptake by cocaine leads to a buildup of dopamine in the synaptic cleft and results in significant potentiation of dopaminergic transmission. This potentiation has been implicated in the reinforcing properties of cocaine and perhaps for some of its euphoric effects as well.

Multiple saturable binding sites at the dopamine transporter for natural (1R)-cocaine (1) have been identified in striatal tissue of rodents,[2-6] humans,[7] and nonhuman primates.[8] Affinities of natural (1R)-cocaine (1) at one of these binding sites parallel their potencies for inhibiting dopamine uptake in this brain region.[3,4,7,8] Thus, these binding sites have revealed several characteristic properties of biologically relevant receptors. One of the important properties is the stereoselectivity of (1R)-cocaine (1) for its binding site. [^{3}H]Cocaine (1) stereoselectively binds to dopamine transporter with preference for (1R)-cocaine over its enantiomer (1S)-cocaine or its stereoisomer pseudococaine.[9] All seven stereoisomers of (1R)-cocaine show a 60-fold or greater reduction in potency for inhibiting [^{3}H]CFT binding. The inversion of the configuration (1S)-cocaine vs (1R)-cocaine (1) reduces the binding affinity by 150-fold.[9] Gatley et al.[10] reported that the amount of (1S)-[^{11}C]cocaine that entered the brain was below the detectable limits with PET (positron emission tomography) when it was given to baboon, while (1R)-cocaine (1) localized in striatum with a time course similar to that of the subjective behavioral "high" in humans.[11] They found that the hydrolysis of (1S)-cocaine by baboon plasma butyrylcholinesterase was at least 1000 times faster than that of its enantiomer, (1R)-cocaine (1). Consequently, the lack of brain uptake of the (1S)-isomer could be explained by its rapid stereoselective hydrolysis in plasma.

Some of (1R)-cocaine (1) analogues with an aromatic ring directly connected to the 3β-position of the tropane ring showed enhanced biological activity and, particularly,



1 (1R)-Cocaine



2 X = H CPT
3 X = F CFT
4 X = I (1R)-β-CIT

5a X = I (1S)-β-CIT
b X = Sn(CH$_3$)$_3$
c X = $^{123/125}$I

Figure 1. Cocaine and 3β-phenyl analogues.

increased behavioral stimulation and higher binding affinity at monoamine reuptake sites with lower intravenous toxicity.[12,13] Cocaine congeners such as 2β-carbomethoxy-3β-phenyltropane (CPT, 2), 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (3, CFT, WIN 35,428), and 2β-carbomethoxy-3β-(4-iodophenyl)tropane (designated as β-CIT, 4, in analogy to CFT) all exhibit higher binding affinities to monoamine transporters than cocaine.[2,9,12-16] Among them (1R)-β-CIT (4) is perhaps the most potent ligand for the dopamine and serotonin transporters from baboon brain, with an IC_{50} of 1.6 nM in displacing [^{3}H]-β-CFT (DA) and 3.8 nM in displacing [^{3}H]paroxetine (5-HT).[14,15] The brain binding of [^{123}I]-(1R)-β-CIT (4) by SPECT (single-photon emission computed tomography) would be expected to include specific binding to monoamine reuptake sites as well as nonspecific binding, with the specific binding presumably being the behaviorally significant component. Recently, Laruelle et al.[16] found that the target to background ratios of [^{123}I]-(1R)-β-CIT (4) were quite high, particularly in the striatum (5.82 at 180 min and 7.33 at 300 min). We have prepared the (1S)-enantiomer (**5a**) of β-CIT (4) because of its potential use in homogenate binding studies to characterize nonspecific binding and in SPECT studies to characterize both the kinetics of passage through the blood–brain barrier and the kinetics of nonspecific binding. The affinities of the (1S)- and (1R)-enantiomers of β-CIT for dopamine (DA) and serotonin (5-HT) transporter were assessed by their potencies to displace [^{125}I]-β-CIT binding to tissue homogenates prepared from rat caudate (predominantly DA transporters) and rat cortex (predominantly 5-HT transporters) (Figure 2).[14,17] Because the radiolabeled inactive enantiomer **5c** could be used for *in vivo* brain imaging studies, we used a physiological buffer containing solutes at concentrations comparable to those in cerebral spinal fluid (artificial cerebral spinal fluid, ACSF). In addition, since Kirifides et al.[18] reported that freezing brain tissue altered binding to the DA transporter, we have used freshly prepared tissue homogenates.

Chemistry. The (1S)-enantiomers **5a–b** of (1R)-β-CIT

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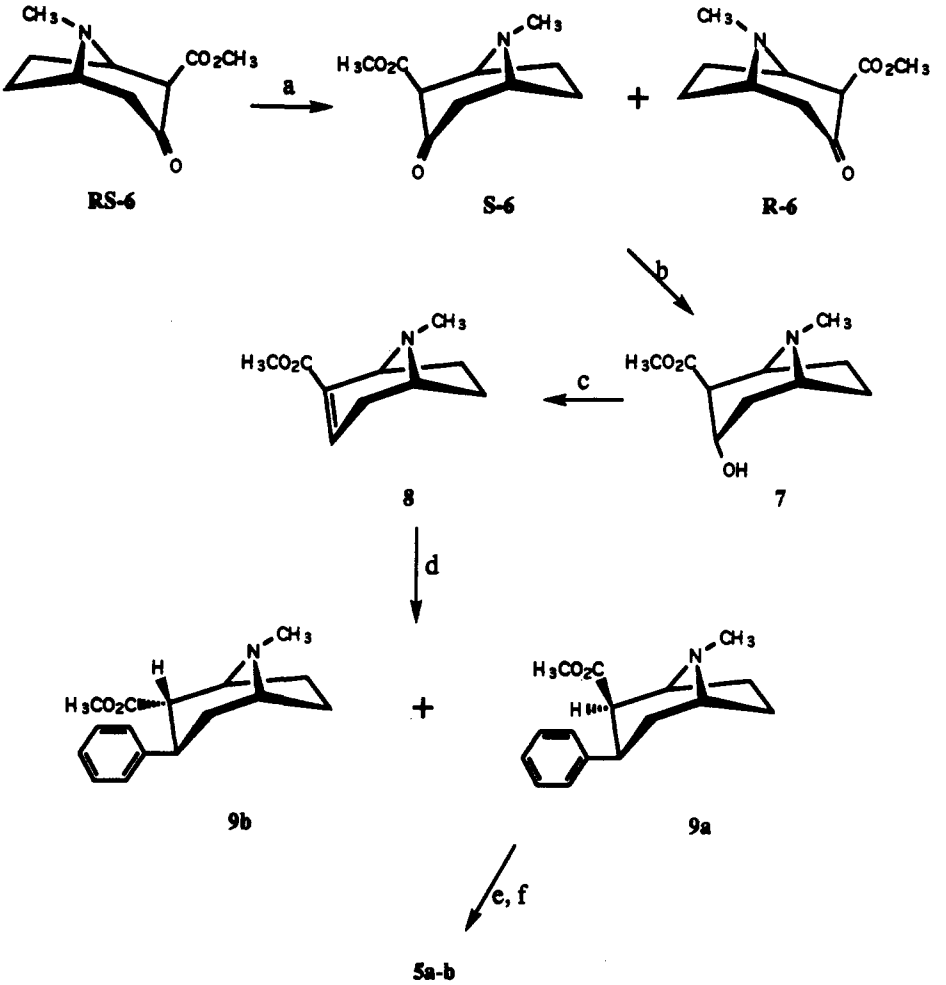
Scheme I[a]



[a] (a) (-)-Tartaric acid. (b) H_2/PtO_2. (c) $POCl_3$. (d) PhMgBr. (e) I_2/HNO_3-H_2SO_4. (f) $[(CH_3)_3Sn]_2/[(C_6H_5)_3]_4Pd$.

(4) were prepared starting from commercially available 3-tropinone (Scheme I). Thus, the sodium hydride catalyzed carbomethoxylation[19] of 3-tropinone with dimethyl carbonate followed by resolution with (-)-tartaric acid gave (1*S*)-2-carbomethoxy-3-tropinone (**6**).[9] The PtO_2 catalytic hydrogenation of 6 as described by Clarke et al.[12] provided (1*S*)-allopseudoecgonine methyl ester (**7**), which on refluxing in phosphorus oxychloride gave the key intermediate (1*S*)-anhydroecgonine methyl ester (**8**).[12] Grignard reaction, iodination, and stannylation were carried out as previously reported for (1*R*)-β-CIT (**4**).[14] The treatment of 8 with 2 equiv of phenylmagnesium bromide at −40 to 45 °C gave a 2:1 mixture of (1*S*)-2β-carbomethoxy-3β-phenyltropane [(1*S*)-β-CPT (**9a**)] and (1*S*)-2α-carbomethoxy-3β-phenyltropane [(1*S*)-α-CPT (**9b**)] in 94% yield. Separation of **9a** from **9b** with flash chromatographic column of silica gel, eluting with methylene chloride/triethylamine (10:1), gave **9a** in 63.6% yield: mp 62–63 °C; $[\alpha]^{26}_D$ +5.6° (*c* = 1, $CHCl_3$) [lit.[12] (1*R*)-enantiomer mp 62–64.5 °C; $[\alpha]^{26}_D$ −5.3°, 1% $CHCl_3$]; HPLC (Phenomenex Bondclone C18, 300 × 3.9 mm; methanol/water/triethylamine 65/35/0.05; 1.5 mL/min; t_R 6.1 min) 100%. Anal. ($C_{16}H_{21}NO_2$) C, H, N. Direct iodination of **9a** with iodine in the presence of HNO_3/H_2SO_4 (1/1) gave (1*S*)-β-CIT (**5a**): mp 110.5–111.5 °C; $[\alpha]^{25}_D$ −2.1° (*c* = 1, $CHCl_3$) [lit.[14] (1*R*)-enantiomer mp 110–111 °C; $[\alpha]^{25}_D$ +2.2° (*c* = 1, $CHCl_3$)]; HPLC (Phenomenex Bondclone C18, 300 × 3.9 mm; methanol/water/triethylamine 65/35/0.05; 1.5 mL/min, t_R 16.5 min) 98%. Anal. ($C_{16}H_{20}NO_2I$) C, H, N. The treatment of **5a** with hexamethylditin in the presence of palladium(0) tetrakis(triphenylphosphine) gave (1*S*)-2β-carbomethoxy-3β-[4-(trimethylstannyl)phenyl]tropane (**5b**): 93.4% yield; mp 116–117 °C; $[\alpha]^{25}_D$ −0.71° (*c* = 1, $CHCl_3$) [lit.[20] (1*R*)-enantiomer mp 113–114 °C; $[\alpha]^{18}_D$ + 1.6° (*c* = 1, $CHCl_3$)]; HPLC (E Merck Aluspher RP select B 250-4; methanol/water/triethylamine 65/35/0.05; UV detection at 254 nm; 1.0 mL/min, t_R 11.5 min) >98%. Anal. ($C_{19}H_{29}NO_2Sn$) C, H, N. [^{125}I]-(1*R*)-β-CIT was prepared by iododestannylation of the corresponding 4-(trimethylstannyl)phenyl derivatives as previously described.[14,16] Radiochemical purity was >95% measured by HPLC. The specific activity, estimated from HPLC with UV detection, was >2000 Ci/mmol and was assumed to be equal to the theoretical value of 2200 Ci/mmol for the binding experiments.

Homogenate Binding. Materials and Methods. Male Sprague–Dawley rats (200–250 g) were euthanized, and the brains were immediately removed and sectioned in a mold. Striatum and cortex anterior to coronal section Bregma ~20 mm (including frontal, parietal, and cingulate cortices) were dissected, weighed, and homogenized at a 1/40 dilution (weight/volume) with a Brinkmann Polytron (setting 6 for 10 s). The tissue homogenates were centrifuged (20 000*g* at 4 °C for 10 min) and resuspended twice prior to final resuspension in ACSF buffer (128 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 10 mM D-glucose,

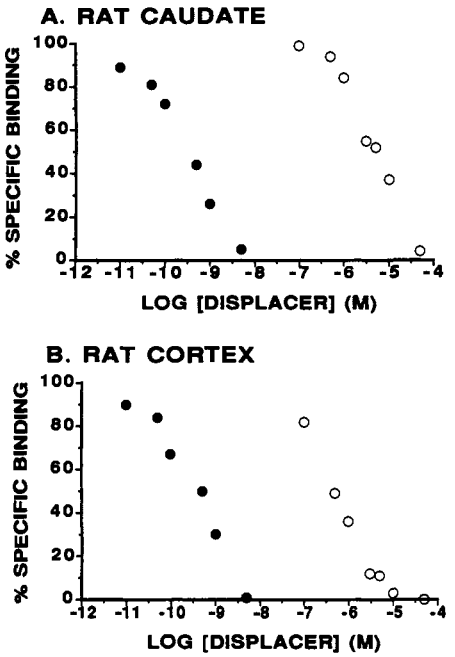


Figure 2. Displacement of [^{125}I]-(1*R*)-β-CIT from cortical membranes prepared from rat caudate (A) and cortex (B) by (1*R*)-β-CIT (●) and (1*S*)-β-CIT (○). Each symbol represents the mean of triplicate incubation, and this representative experiment was performed three times. Total and nonspecific binding values, respectively, of [^{125}I]-(1*R*)-β-CIT in this experiment were as follows: caudate (6770 and 920 dpm) and cortex (1810 and 350 dpm).

2 mM $CaCl_2$, 2 mM $MgSO_4$, and 15 mM $NaHCO_3$, which provided a pH of 7.40 at 22 °C).

The binding assay was initiated by the successive addition of 100 μL of [^{125}I]-β-CIT (final concentration of 10 pM), 100 μL of buffer with displacing drug, and 800 μL of tissue solution. Final tissue dilution (1/4000 for striatum and 1/1600 for cortex expressed as weight/volume) was adjusted so that total binding was between 5 and 10% of total ligand concentration. Tubes were incubated for 90 min at 22 °C. Preliminary experiments showed that equilibrium binding was achieved by 45–60 min and showed no degradation during incubations up to 90 min. The assay was terminated by rapid filtration over GF/B filers on a 48-channel cell harvester (Brandel, Gaithersburg, MD). Filters were rapidly washed three times with 5 mL of ice-cold buffer and counted in a COBRA 5010 γ-counter (Packard, Meriden, CT) with an efficiency of 80%.

Three separate displacement experiments were performed for the (1*S*)- and (1*R*)-enantiomers (**5a** and **4**, respectively) of β-CIT with both caudate and cortex tissue (Figure 2). Triplicate incubation tubes contained each enantiomer at 6–7 concentrations which fairly symmetrically encompassed a 10-fold range both above and below the IC_{50} value (i.e., the concentration required to occupy 50% of the target sites). The IC_{50} value was determined with a logit–log analysis performed with G. A. McPherson's EBDA program (version 2.0, Elsevier BIOSOFT, Cambridge, UK). Nonspecific binding was measured in the presence of 60 μM (1*R*)-cocaine (**1**) and represented 13–21% of total binding.

Results. The IC_{50} values of the (1*R*)-β-CIT (**4**) to displace [^{125}I](1*R*)-β-CIT were 0.42 ± 0.07 nM with striatal membranes and 0.55 ± 0.03 nM with cortical membrane (mean ± SEM, $n = 3$ for each tissue). In contrast, the IC_{50} values of its (1*S*)-enantiomer **5a** were 5700 ± 350 nM and 450 ± 48 nM, respectively. Thus, the (1*R*)-β-CIT (**4**) was more potent than the (1*S*)-β-CIT (**5a**) by a factor of 14 000 for caudate tissue and 800 for cortical tissue.

Discussion. Because rat caudate is enriched in DA transporters and cortex in 5-HT transporters, these tissues have been used in homogenate binding studies with radiolabeled analogues of cocaine to provide a measurement of the relative affinities of displacing agents at these two monoamine transporters.[14,17] Our homogenate binding studies show that the (1*S*)-enantiomer **5a** has 3–4 orders of magnitude lower affinity for these two monoamine transporters than the (1*R*)-enantiomer **4**. Thus, although significant and important effects on activity are obtained by replacement of the benzoyl group at C-3 of tropane ring with an aryl group,[4] the inversion of (1*R*)-configuration results in significant reduction in the binding affinity at monoamine transporters. The present work extends the previous observations that the structural requirement for the binding of cocaine analogues to the monoamine transporters is (*R*)-configuration at C_1 position of the tropane ring.[4,9,13] We have not examined the relative potencies of (1*S*)- and (1*R*)-β-CIT at norepinephrine (NE) transporters, in part because of the lack of a moderately size brain region with relative enrichment in this target site. However, our SPECT imaging studies with [^{123}I]-β-CIT in monkeys suggest that almost none of the brain activity is related to the NE transporter, since the injection of maprotiline, an agent selective for this transporter, caused no measurable displacement of regional brain activities.[16] Our studies suggest that [^{125}I]-labeled (1*S*)-β-CIT will be a useful probe of nonspecific binding for homogenate binding studies and that [^{123}I]-(1*S*)-β-CIT will be a useful tracer for *in vivo* evaluation of both the blood–brain barrier transfer and the kinetics of nonspecific binding in SPECT.

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References

(1) Johanson, C. E.; Fischman, M. W. The pharmacology of cocaine related to its abuse. *Pharmacol. Rev.* **1989**, *41*, 3–52.
(2) Reith, M. E. A.; Sershen, H.; Lajtha, A. Saturable [^{3}H]cocaine binding in central nervous system of mouse. *Life Sci.* **1980**, *27*, 1055–1062.
(3) Kennedy, L. T.; Hanbauer, I. Sodium-sensitive cocaine binding to rat striatum membrane: possible relationship to dopamine uptake sites. *J. Neurochem.* **1983**, *41*, 172–178.
(4) Reith, M. E.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structure requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotype behavior. *Biochem. Pharmacol.* **1986**, *35*, 1123–1129.
(5) Calligaro, D. O.; Elderfrawi, M. E. Central and peripheral cocaine receptors. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 61–67.
(6) Calligaro, D. O.; Elderfrawi, M. E. High affinity stereospecific binding of [^{3}H]cocaine in striatum and its relationship to the dopamine transporter. *Membr. Biochem.* **1988**, *7*, 87–106.
(7) Schoemaker, H.; Pimoule, C.; Arbilla, S.; Scatton, B.; Javoyagid, F.; Langer, S. Z. Sodium dependent [^{3}H]cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinson's disease. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *329*, 227–235.
(8) Madras, B. K.; Fahey, M. A.; Bergman, J.; Canfield, D. R.; Spealman, R. D. Effects of cocaine and related drugs in nonhuman primates. I. [^{3}H]Cocaine binding sites in caudate-putamen. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 131–141.
(9) Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. Synthesis and ligand binding of cocaine isomers at the cocaine receptor. *J. Med. Chem.* **1991**, *34*, 883–886.

(10) Gatley, S. J.; MacGregor, R. R.; Fowler, J. S.; Wolf, A. P.; Dewey, S. L.; Schlyer, D. J. Rapid stereoselective hydrolysis of (+)-cocaine in baboon plasma prevents its uptake in the brain: Implication for behavioral studies. *J. Neurochem.* **1990**, *54*, 720–723.
(11) Cook, C. E.; Jeffcoat, A. R.; Perez-Reyes, M. Pharmacokinetic studies of cocaine and phenylcyclidine in man. In *Pharmacokinetics and pharmacodynamics of psychoactive drugs*; Barentt, G., Chang, M. C., Eds.; Biomedical Publication: Foster City, CA, 1985; pp 48–74.
(12) Clarke, R. L.; Daum, S. J.; Gambino, A. J.; Aceto, M. D.; Pearl, J.; Levitt, M.; Cumiskey, W. R.; Bogado, E. F. Compounds affecting the central nervous system. 4. 3β-Phenyltropane-2-carboxylic esters and analogs. *J. Med. Chem.* **1973**, *16*, 1260–1267.
(13) Milius, R. A.; Saha, J. K.; Madras, B. K.; Neumeyer, J. L. Synthesis and receptor binding of N-substituted tropane derivatives: High affinity ligands for the cocaine receptor. *J. Med. Chem.* **1991**, *34*, 1728.
(14) Neumeyer, J. L.; Wang, S.; Milius, R. A.; Baldwin, R. M.; Zea-Ponce, Y.; Hoffer, P. B.; Symbirska, E.; Al-Tikriti, M.; Charney, D. S.; Malison, R. T.; Laruelle, M.; Innis, R. B. [^{123}I]-2β-carbomethoxy-3β-(4-iodophenyl)tropane: High affinity SPECT radiotracer of monoamine reuptake sites in brain. *J. Med. Chem.* **1991**, *34*, 3144–3146.
(15) Innis, R. B.; Baldwin, R. M.; Sybirska, E.; Zea-Ponce, Y.; Laruelle, M.; Al-Tikriti, M.; Charney, D. S.; Zoghbi, S. S.; Smith, E. O.; Wisnieski, G.; Hoffer, P. B.; Wang, S.; Milius, R. A.; Neumeyer, J. L. Single photon emission computed tomography imaging of monoamine reuptake sites in primate brain with [^{123}I]CIT. *Eur. J. Pharmacol.* **1991**, *200*, 369–370.
(16) Laruelle, M.; Baldwin, R. M.; Malison, R. T.; Zea-Ponce, Y.; Zoghbi, S. S.; Al-Tikriti, M.; Sybirska, E.; Zimmermann, R. C.; Wisnieski, G.; Neumeyer, J. L.; Milius, R. A.; Wang, S.; Smith, E. O.; Roth, R. H.; Charney, D. S.; Hoffer, P. B.; Innis, R. B. SPECT imaging of dopamine and serotonin transporters with [^{123}I]-β-CIT: pharmacological characterization of brain uptake in nonhuman primates. *Synapse* **1993**, *13*, 295–309.
(17) Boja, J. W.; Mitchell, W. M.; Patel, A.; Kopajtic, T. A.; Carroll, F. I.; Lewin, A. H.; Abraham, P.; Kuhar, M. J. High affinity binding of [^{125}I]-RTI-55 to dopamine and serotonin transporters in rat brain. *Synapse* **1992**, *12*, 27–36.
(18) Kirifides, A. L.; Harvey, J. A.; Aloyo, V. J. The low affinity binding site for the cocaine analog Win 35,428 is an artifact of freezing caudate tissue. *Life Sci.* **1992**, *50*, PL139–142.
(19) Findlay, S. P. Concerning 2-carbomethoxy tropinone. *J. Org. Chem.* **1957**, *22*, 1385–1393.
(20) Carroll, F. I.; Adur-Rahman, M.; Abraham, P.; Parham, K.; Lewin, A. H.; Donnals, R. F.; Shaya, E. K.; Scheffel, U.; Wong, D. F.; Boja, J. W.; Kuhar, M. J. [^{123}I]-3β-(4-iodophenyl)tropan-2β-carboxylic acid methyl ester (RTI-55). A unique cocaine receptor ligand for the imaging the dopamine and serotonin transporters in vivo. *Med. Chem. Res.* **1991**, *1*, 289–294.