A Technetium-99m SPECT Imaging Agent Which Targets the Dopamine Transporter in Primate Brain

Peter C. Meltzer,*,† Paul Blundell,† Alun G. Jones,‡ Ashfaq Mahmood,‡ Basem Garada,‡ Robert E. Zimmerman,‡ Alan Davison,§ B. Leonard Holman,‡ and Bertha K. Madras||

Organix Inc., 65 Cummings Park, Woburn, Massachusetts 01801, Department of Psychiatry, Harvard Medical School and New England Regional Primate Center, Southborough, Massachusetts 01772, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, and Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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The dopamine transporter (DAT), located presynaptically on dopamine neurons, provides a marker for certain neurological diseases. In particular, the DAT is depleted in Parkinson's disease, and the extent of depletion correlates with the loss of dopamine. Herein we describe the design, synthesis, and biological evaluation of technepine, the first ^{99m}Tc-labeled SPECT imaging agent which targets the dopamine transporter in striatum. We have demonstrated that the DAT can accommodate a chelating unit attached to the 8-amine function of a tropane skeleton. Further, we have demonstrated for the first time that a molecule can be designed to carry the radionuclide ^{99m}Tc across the blood−brain barrier in sufficient quantity to obtain *in vivo* images of the striatum in monkeys. This advance will undoubtedly lead to the design of new receptor and transporter-mediated ^{99m}Tc agents which can label specific transporter and receptor targets in the central nervous system.

Introduction

The dopamine transporter (DAT), located presynaptically on dopamine neurons, is a principal target of cocaine in the mammalian brain.[1,2] The stimulant and reinforcing properties of cocaine have been ascribed to cocaine's ability to inhibit the DAT.[3−6] The DAT also provides a marker for certain neurological diseases. In particular, Madras and others have shown in primates, as well as in *in vitro* studies of human Parkinsonian patients,[7,8] that the DAT is depleted in Parkinson's disease (PD) and the extent of depletion correlates with the loss of dopamine.

Considerable research has focused on the design of positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging agents which could provide a quantitative measure of the DAT density in living brain.[9−13] The lead compound and a widely used PET imaging agent is WIN35,428.[8,9,14] SPECT imaging agents, however, have greater clinical potential than do PET agents, and in 1991 the synthesis of [^{123}I]RTI55 (βCIT), an analog of WIN35,428, was reported.[15] RTI55 has an affinity (IC_{50}) of 1.08 nM and selectivity for the DAT over the serotonin (5HT) transporter (SERT) of 2.[12]

We recently introduced altropane,[11,16] an *N*-[^{123}I]-iodoallyl analog of WIN35,428, as a novel SPECT imaging agent. This compound binds potently (IC_{50} = 6.9 nM) and selectively (DAT:SERT = 28) to the DAT. It has an *in vivo* striatum to cerebellum ratio of 8 in monkey brains and provides excellent SPECT images in humans. Altropane is currently in human trials.

The most widely accepted radionuclide in nuclear medicine is technetium-99m (^{99m}Tc; $T_{1/2}$ = 6.0 h, 140 K eV γ emission). ^{99m}Tc offers important advantages, particularly the ability to generate the isotope in the laboratory from a commercially available kit and without need of a cyclotron. Consequently, ^{99m}Tc-based compounds are widespread in clinical use[17,18] as, for example, blood perfusion agents[19] and cardiovascular imaging agents.[20] However, while iodine can be attached covalently to the specific ligand, ^{99m}Tc must be bound by means of a chelating agent. Consequently, notwithstanding considerable effort[21−26] to design a ^{99m}Tc-chelated imaging agent which can image *specific receptor or transporter systems*, no such agent had been demonstrated to be successful *in vivo*.[44] Herein we report the design and synthesis of the first transporter-mediated technium-99m-based *in vivo* SPECT imaging agent, [*N*-[2-((3′-*N*′-propyl-3″β-(4-fluorophenyl)tropane-2″β-carboxylic acid methyl ester)(2-mercaptoethyl)amino)acetyl]-2-aminoethanethiolato] technetium-99m(V) oxide, technepine, **1** (Figure 1), which labels the DAT in primates. Technepine was first disclosed in a communication in 1996.[27]

Chemistry

The imaging agent **1** (technepine; O-861T) is composed of three parts: (i) a potent and selective tropane, (ii) a chelating unit to which the radionuclide ^{99m}Tc is bound, and (iii) a tether which connects the chelating entity to the tropane skeleton. The synthetic route developed to obtain the target compound **1** is presented in Scheme 1.

The final compounds reported here are, with the exception of technepine itself, rhenium complexes. Rhenium is an excellent model for the radioactive ^{99m}Tc. It forms square pyramidal complexes very similar to those formed by ^{99m}Tc and behaves similarly to technetium. Its N_2S_2 complexes are equally stable.[28] Furthermore, the lipophilicity and the biological availability of rhenium chelates has proved similar to that of ^{99m}Tc chelates.[28] Consequently, it has been utilized in place

† Organix, Inc.
‡ Brigham and Women's Hospital.
§ Massachusetts Institute of Technology.
|| Harvard Medical School and New England Regional Primate Center.
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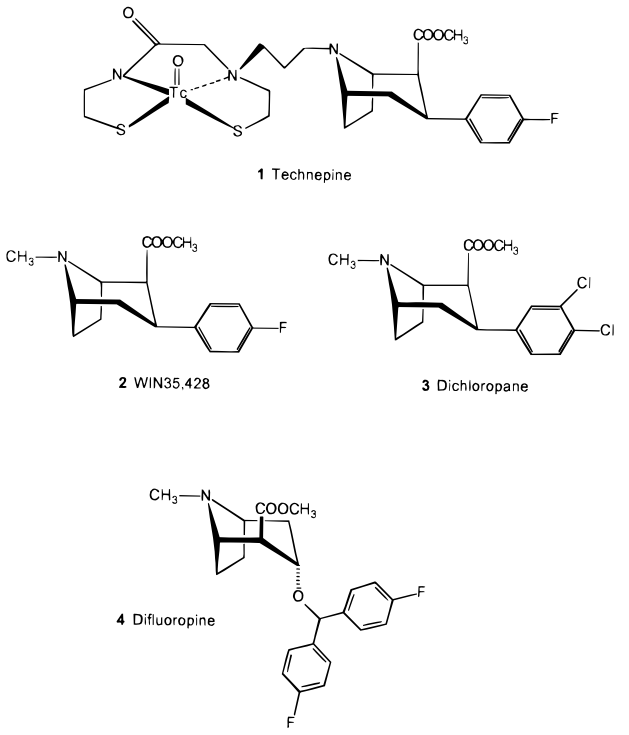
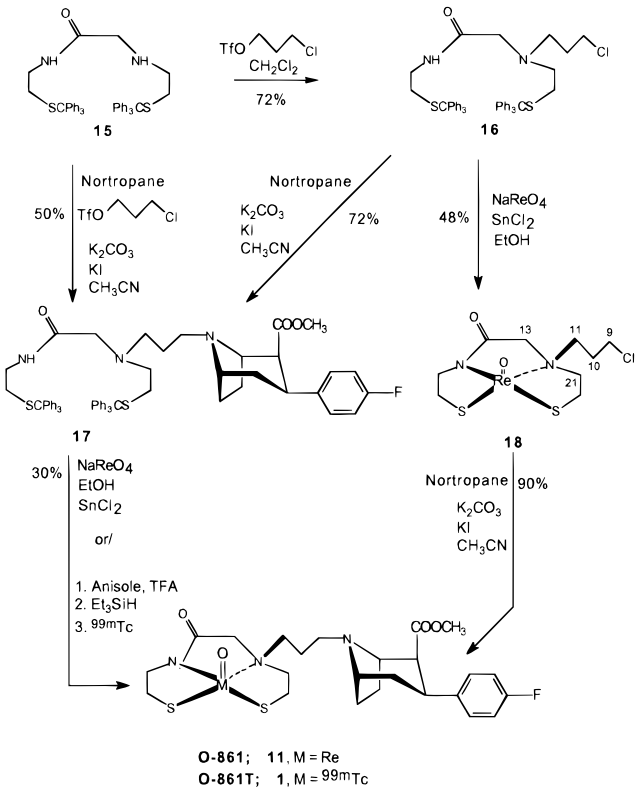


Figure 1. Structure of technepine, **1**, and the three "guiding ligands".

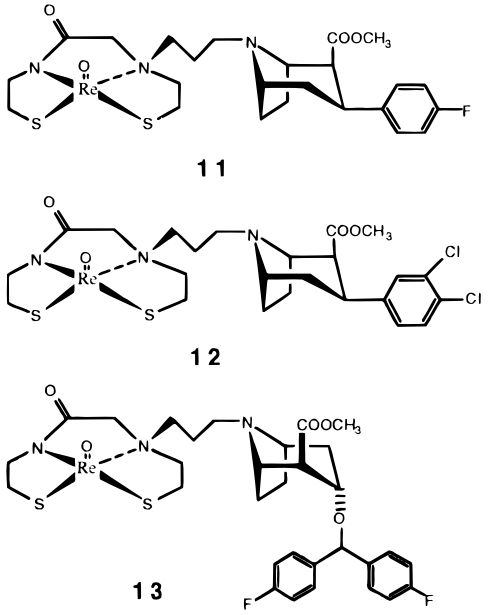
Scheme 1



of ^{99m}Tc in studies which do not require the presence of a radiolabel.[29] A critical advantage is that rhenium can be handled under normal laboratory conditions since it is not radioactive.

Synthesis of the final compounds **11**−**14** (Table 1) reported here was therefore facilitated by the use of cold rhenium. Three pathways were elaborated in order to establish routes for rapid introduction of rhenium and/or technetium-99m (Scheme 1).

Table 1. Inhibition of [^{3}H]-3β-(4-Fluorophenyl)tropane-2β-carboxylic Acid Methyl Ester Binding to the Dopamine Transporter (DAT) and [^{3}H]Citalopram Binding to the Serotonin Transporter (SERT) by Rhenium Analogs **11**−**13** in Cynomolgus Monkey Caudate−Putamen[a]



	IC$_{50}$ (nM)		
compd	dopamine transporter [^{3}H]WIN35,428	serotonin transporter [^{3}H]citalopram	selectivity DAT/SERT
O-861, **11**	5.99 ± 0.81	124 ± 17	21
O-927, **11a**	7.38 ± 1.33	66.9 ± 3.0	9
O-928, **11b**	4.04 ± 0.98	299 ± 23	74
O-863,[b] **12**	37.2 ± 3.4	264 ± 16	7.1
O-864,[b] **13**	616 ± 88	55200 ± 20000	90
O-918, **13a**	482		
O-919, **13b**	343		
O-862,[b] **14** (2α-COOCH$_3$)	2960 ± 157	5020 ± 1880	1.7

[a] Each radioligand was incubated with tissue (4 mg/mL original wet tissue weight) and 7−14 concentrations of a cocaine congener as described in the experimental section. Nonspecific binding of [^{3}H]WIN35,428 was measured with 30 μM (−)-cocaine and of [^{3}H]citalopram with 1 μM fluoxetine. IC$_{50}$ values were computed by the EBDA computer program and are the means (±SD) or SEM of one to four independent experiments, each conducted in triplicate. [b] Madras et al. *Synapse* **1996**, *22*, 239−246.

The N_2S_2 ligand (MAMA′) **15**, as described by O'Neil et al.[30] and DiZio et al.,[29,31] was attached to 1-chloro-3-propyl triflate in methylene chloride to provide **16** in 72% yield. Rhenium could then be introduced directly upon reaction of the bistrityl-protected compound **16** with sodium perrhenate under reductive conditions ($SnCl_2$) in ethanol. A 48% yield of the chelate **18** was obtained after flash column chromatography. The IR stretching frequency for **18** shows a strong band at 952 cm^{-1}. This compares favorably with a stretching frequency of 955 cm^{-1} reported by O'Neil et al.[30] for their *N*-benzyl *syn* analog (*syn* with respect to the Re═O core). In contrast, the *anti N*-benzyl analog shows a strong band at 980 cm^{-1}.[32] No *anti* isomer was evident for **18**.

^{1}H-NMR decoupling experiments confirmed the *syn* relationship of the *N*-alkyl linker of **18**. Thus the triplet at δ 3.64 (J = 6 Hz) assigned to the methylene protons α to the chlorine at C_9 was irradiated and confirmed the position of the C_{10} protons (m, δ 2.1−2.5, 2H) (see Figure 2 for compound numbering). The protons at C_{10}

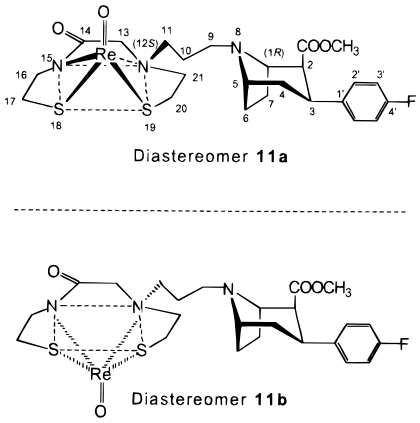


Figure 2. The two diastereoisomers, **11a** and **11b**, of the rhenium analog of technepine, **1**.

were then irradiated, and this provided a singlet for those at C_9 at δ 3.64 and simplified the downfield multiplet at δ 3.7−4.2. (Note: the C_{11} methylene protons of the precursor **16** resonate at δ 2.35.) This is consistent with the findings of Lever et al.[32] who report the N-CH_3 *syn* downfield at δ 3.4 ppm and the N-CH_3 *anti* at δ 1.9, thus implying the C_{11} *syn* relationship with the Re═O core, which correlated well with data reported by O'Neil et al.[30] for an *N*-benzyl analog.

The X-ray structural analysis (see below) of **11a** shows the *N*-alkyl subsitituent with *syn* geometry and thus confirms the *syn* relationship in the intermediate, **18**.

The preformed rhenium chelate **18** could then be attached to an appropriate nortropane. Thus, the nortropanes[12,33] (exemplified in Scheme 1 for WIN35,-428) were then alkylated by this preformed N_2S_2 chelate attached to the propyl halide, to provide the novel rhenium chelates, **11**−**14** (Table 1). In all cases, none of the *anti* isomers could be detected.

Alternately, the chloropropyl chelator **16** could be attached directly to the appropriate nortropane by reaction in acetonitrile in the presence of potassium carbonate and potassium iodide to provide the bistrityl-protected chelator **17** in 72% yield. Introduction of rhenium (or ^{99m}Tc) could then be effected. Thus **17** was treated with sodium perrhenate under reductive conditions ($SnCl_2$) in ethanol. A 30% yield of the chelate **11** was obtained after flash column chromatography.

Compound **11** exists in two diastereomeric forms (Figure 2), **11a** and **11b**. These two diastereomers coelute on TLC in a variety of solvent systems, and therefore chromatographic separation is difficult. However, column chromatography with a ratio of silica gel to compound (ca. 40 g:171 mg) and collection of multiple small fractions allowed pooling of early versus late fractions to provide purified samples of each diastereoisomer. Diastereomers **13a** and **13b** could be similarly isolated. However, in this case TLC could be used to monitor separation.

Structural assignment of the rhenium chelates was again facilitated by NMR analysis. The presence of diastereomeric mixtures was clearly evidenced in the ^{1}H-NMR spectra of these compounds. In this regard, the C_{13} protons are particularly diagnostic for the Re═O complexes. For example, in the ligand **16** these protons appear as a singlet at δ 2.85. In the Re═O chelate **11**, both C_{13} protons appear considerably downfield in the region of δ 4.0−4.8. This downfield shift is due, in part, to the anisotropic effect of the Re═O bond and the quaternary nature of the nitrogen (N_{12}). The diastereotopy of the C_{13} protons is evidenced in that the $C_{13\beta}$ proton manifests a four-line multiplet [**11a**, δ 4.79 (d, J = 16 Hz, 1H H-13β), and **11b**, 4.77 (d, J = 16 Hz, 1H, H-13β]. The corresponding 13α proton also shows a four-line multiplet [**11a**, δ 4.07 (d, J = 16 Hz, 1H, H-13α); **11b**, 4.04 (d, J = 16 Hz, 1H, H-13α) with almost 0.8 ppm upfield shift from the 13β.

The effect of the Re═O bond is also apparent on the 16α,β protons. The 16α is a four-line multiplet for **11a** at δ ∼4.02 and the 16β is a four line multiplet at δ ∼4.57. In the diastereomeric mixture of **11**, the 16β protons overlap, resulting in a six-line multiplet at δ ∼4.55. It is also interesting that the carbomethoxy moieties in the 2β position (**11**, **12**, and **13**) all show different chemical shifts (two singlets) for the two different diastereomers (**11**, δ 3.44, 3.49; **12**, δ 3.50, 3.55; **13**, δ 3.65, 3.70). When the carbomethoxy group is in the 2α configuration, as in **14**, both diastereomers have the same chemical shift, and a singlet (δ 3.52) is seen. This implies that the chelate in **14** is no longer in a position to influence the carbomethoxy group. The X-ray analysis of **11a** confirmed (Figure 4) this interaction as, in this case, the 2-CO_2Me group is forced toward the chelate.

The absolute structure (Figure 4) of diastereoisomer **11a** was established by X-ray crystallography and confirmed that the propyl group attached at N_{12} bears a *syn* relationship to the Re═O bond. Further, the absolute structure of **11a** is as shown in Figure 2. The chirality of the tropane moiety is derived from (1*R*)-cocaine and is therefore fixed. However, the introduction of a chiral rhenium chelate leads to the formation of these diastereoisomers. Therefore, diastereoisomer **11a** is the 1*R*,12*S* isomer [(1*R*,12*S*)-*N*-(2-((3′-*N*′-propyl-3″β-(4-fluorophenyl)tropane-2″β-carboxylic acid methyl ester)(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]rhenium(V) oxide and diastereoisomer **11b** is the 1*R*,12*R* isomer [(1*R*,12*R*)-*N*-(2-((3′-*N*′-propyl-3″β-(4-fluorophenyl)tropane-2″β-carboxylic acid methyl ester)(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]rhenium(V) oxide.

Biology

The final compounds reported here are, with the exception of technepine itself, rhenium complexes. Since, as noted above, rhenium is an excellent model for the radioactive ^{99m}Tc, the *in vitro* biological data reported here were obtained for the rhenium chelates. Since ^{99m}Tc will be introduced in a final preparative step for routine use, a diastereomeric mixture of the imaging agents is likely to be used. Therefore the biological activity of the diastereomeric mixture of **11**, the model for technepine itself, was obtained. In addition, binding constants for each of the diastereoisomers **11a** and **11b** were also measured.

The affinities (IC_{50}) of compound **11**−**14** for the dopamine (DAT) and serotonin (SERT) transporters were determined in competition studies using [^{3}H]-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester ([^{3}H]WIN35,428 or [^{3}H]CFT) to label the dopamine transporter[5] and [^{3}H]citalopram to label the serotonin transporter.[27] Studies were conducted in cynomolgus monkey striatum since these compounds are part of an

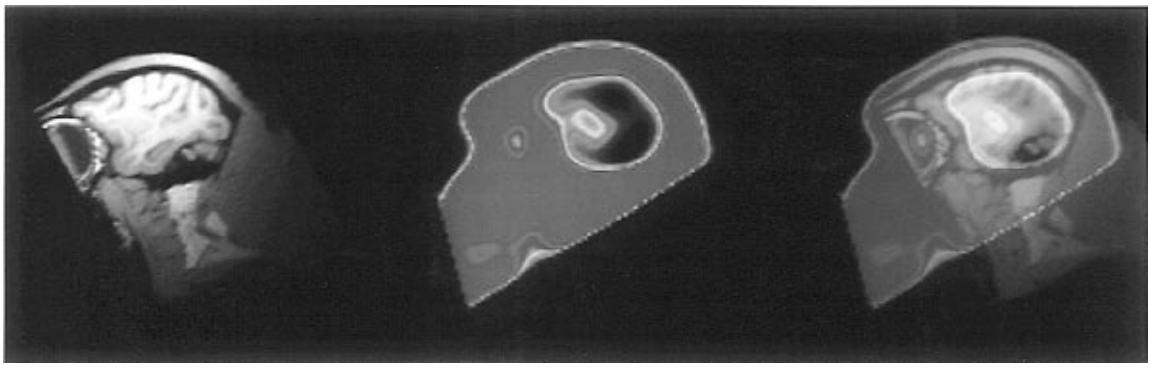


Figure 3. SPECT and MRI image of a rhesus monkey with technepine, **1**, as imaging agent, processed 1−1.5 h after injection of the radioactive probe. Panel 1 shows an MRI image. Panel 2 shows a SPECT image. Panel 3 shows coregistration of the MRI and SPECT images.

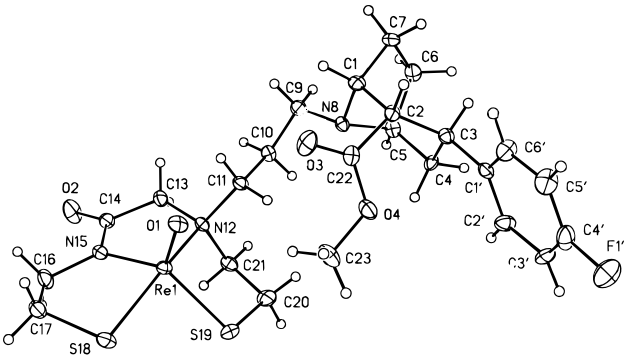


Figure 4. ORTEP diagram of diastereoisomer **11a**.

ongoing investigation of structure−activity relationships at the dopamine transporter in this tissue[5,12,33] and meaningful comparisons with an extensive data base and *in vivo* imaging data can be made. Competition studies were conducted with a fixed concentration of radioligand and a range of concentrations of the test drug. All drugs inhibited [^{3}H]WIN35,428 and [^{3}H]-citalopram binding in a concentration-dependent manner. Binding constants are presented in Table 1.

It is apparent that the WIN35,428 template provides a potent compound (**11**: IC_{50} = 5.99 ± 0.81 nM). Of the two diastereomeric forms of O-861 (**11**), **11b** (O-928) was more potent and conferred the higher level of transporter selectivity on the molecule. In contrast, **11a** (O-927) was of reduced potency and transporter selectivity.

Synthesis of [^{99m}Tc]Technepine

Synthesis of technepine, **11** (O-861T: M = ^{99m}Tc), as a 1:1 diastereomeric mixture, was achieved by deprotection of the bistritylated compound **17** with trifluoroacetic acid under cation trapping conditions (triethylsilane). The bisthiol obtained was treated with technetium-99m glucoheptonate. The product was purified by HPLC to provide 6 mCi of the ligand **1** for *in vivo* studies.

SPECT Imaging Data

SPECT images in rhesus monkeys were obtained over 3 h. The striatum was localized by coregistration of the SPECT images with Magnetic Resonance Imaging (MRI). Radioligand accumulated within the striatum within minutes and was detectable in this region for a period of 3 h. Figure 3 shows a lateral view of a SPECT image taken 60−95 min after administration of 7.5 mCi of **1**. The first panel shows an MRI image of the brain, the second shows accumulation of **1** in the striatum, and the third shows a coregistration of MRI and SPECT images. Coregistration of the SPECT images obtained in the coronal plain with coronal images generated by MRI confirmed the location of the radiolabeled probe within the striatum at several anterior-to-posterior planes. The selectivity of technepine was confirmed by a comparison of striatum versus cerebellum radioactive labeling and found to be 2:1 in the female monkey and 3:1 in the male monkey.

Discussion

The focus of this work is the design and synthesis of a ^{99m}Tc-labeled ligand which targets the DAT in primate brain. In order to achieve this, four rhenium-labeled model ligands were prepared, and their binding potencies and selectivities to neurotransmitter uptake systems (DAT, SERT, NET) were measured. The most potent and selective of these models was then labeled with the SPECT radionuclide, ^{99m}Tc, to provide technepine (O-861T; **1**), and successful images in primate brain were obtained. Herein we discuss the design of technepine as well as the biology of the rhenium complexes, **11**−**14**. Technepine is the first ^{99m}Tc-labeled chemical entity to enable an *in vivo* SPECT image in primate brain. Although others[26] have reported the development of a ^{99m}Tc agent that binds with high affinity to the dopamine transporter, SPECT images have not yet been published for this compound. These authors[26] later reported that their compound failed to accumulate in sufficient quantity to obtain an *in vivo* image.[34]

Design of Technepine. The stereoselectivity of transporter systems, and in particular the DAT, imposes specific constraints on the design of transporter-targeted imaging agents. Not least among these is the steric impediment offered by a bulky ^{99m}Tc chelating unit attached to a ligand selected to guide the imaging agent to its biological target. It may be anticipated that, in the absence of careful design, the sterically sensitive uptake mechanism may preclude potent binding and selectivity for the DAT. The design of technepine hinged upon the careful selection of a "guiding" tropane moiety to which a neutral ^{99m}Tc chelate could be attached by a methylene chain linking group. The ligand is therefore composed of three parts: (i) a tropane skeleton specifically selected to be both potent and selective for the DAT (the "guiding ligand"), (ii) a

chelating unit to which the radionuclide ^{99m}Tc (or Re) is bound (the "chelator"), and (iii) a "tether" which connects the chelating entity to the tropane skeleton.

(A) Guiding Ligand. Selection of a potent guiding ligand was aided by structure−activity relationships (SAR)[12,33] of the tropane family of compounds. Specifically, SAR of the tropanes indicated that three templates might be appropriate (see Figure 1). First, the 3,4-dichloro analog **3** (dichloropane: IC_{50} = 0.9 nM)[12] of WIN35,428, **2**, might provide a ligand of high potency, albeit low selectivity. Second, the biological data obtained for difluoropine, **4** (IC_{50} = 10.9 nM; DAT:SERT = 325),[33] indicated that this compound might provide a potent and selective guiding ligand for use in a ^{99m}Tc-chelated SPECT agent. Third, WIN35,428, the prototypical tropane for DAT inhibition (IC_{50} = 11.0 nM; DAT:SERT = 15), had already been demonstrated to provide an exceptional template from which to construct SPECT imaging agents.[11,16] Upon assessment of all three ligands, the optimum, designated technepine, was based upon the WIN35,428 template.

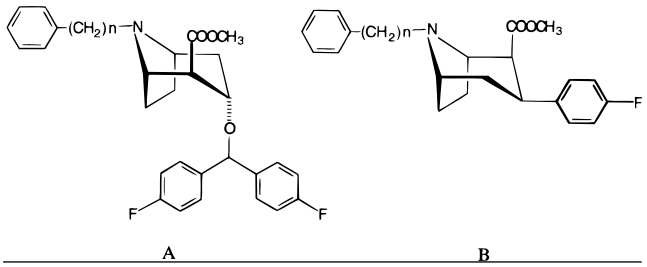
(B) Point of Attachment. Upon examination of the parent compounds, **2**−**4**, it is apparent that there are two obvious potential points of attachment which are chemically readily accessible. First, the 2-carbomethoxy moiety which has been demonstrated to tolerate wide molecular modification[35] and, second, the 8-nitrogen which had not yet been explored as to transporter tolerance for the substantial bulk envisioned for a technetium chelate. In order to avoid the potential of a labile label placed directly at the 2-carbomethoxy (hydrolyzable ester) position, we elected, first, to focus attention on the 8-amino moiety.

A model study of both WIN35,428 and difluoropine[36] was conducted to explore the available transporter space in the region of the 8-amine. Six *N*-arylalkyl analogs, **5**−**10**, were prepared (Table 2). Potency at the dopamine transporter was measured in the striatum of cynomolgus monkey brain using [^{3}H]WIN35,428 to label the sites. Affinity for the serotonin transporter was measured in the same tissue using [^{3}H]citalopram to label those sites. In both the WIN35,428 and the difluoropine family, it was evident that highest affinity for the DAT was obtained with a propyl linker (IC_{50} = 22.0 nM for **6**, and IC_{50} = 1.4 nM **9**). However transporter selectivity was low in the difluoropine analog (**6**), but favorable for the WIN35,428 analog (**9**, DAT:SERT = 95). From these model studies we inferred that reasonable binding potency could be expected for a suitably selected ^{99m}Tc chelate if it were bound to the tropane by a propyl tether. We reasoned that the metal chelate, which is larger than the phenyl group of the model, would then reside in the transporter space occupied by the phenyl group of a pentane-linked model, and thus effect binding minimally.

(C) Chelator. Substantial work has been carried out on the design and synthesis of ^{99m}Tc brain perfusion imaging agents. These agents need to cross the blood−brain barrier, and it has been established[17−19,24,28,30,31,37−42] that to do so the ligand should be neutral and should have sufficient lipid solubility.

The selection of the ^{99m}Tc chelate utilized here (MAMA′) was based upon the work of DiZio et al.[29,31] They demonstrated that, for a lipophilic steroid, selection of a highly lipophilic chelator detracted from its ability to cross the blood−brain barrier and bind selectively to its biological target. In contrast, it was demonstrated that use of a simple N_2S_2 chelating unit[38,39] provided a reasonable match of lipophilicity for the steroid. These tetradentate N_2S_2 ligands give rise to some interesting characteristics such as *syn* and *anti* geometry, and in the case of a chiral ligand such as a tropane, the existence of diastereomers as discussed earlier. We reasoned that the alternate symmetrical tridentate (and therefore diastereomer-free) SNS ligands[41] require that the tropane be linked to the chelate *via* the remaining electron donor. This was anticipated to lead to a potential lack of *in vivo* stability of the chelated ligand, since the exogenous sulfur could readily be replaced by an endogenous sulfur (e.g. cysteine). We therefore selected the N_2S_2 ligand, MAMA′,[30] for use in our studies. Indeed, since completion of this work, it has been reported that an SNS ligand has failed to provide an approach for primate imaging since the amount of label in brain proved too low (0.1% dose/organ at 2 min post iv injection).[34]

Table 2. Inhibition of [^{3}H]-3β-(4-Fluorophenyl)tropane-2β-carboxylic Acid Methyl Ester Binding to the Dopamine Transporter (DAT) and [^{3}H]Citalopram Binding to the Serotonin Transporter (SERT) by Arylalkyl Analogs **5**−**10** in Cynomolgus Monkey Caudate−Putamen[a]



A B

			IC_{50} (nM)		
compd	series	n	dopamine transporter [^{3}H]WIN35,428	serotonin transporter [^{3}H]citalopram	selectivity DAT/SERT
O-755, **5**	A[b]	1	223 ± 53	4,970 ± 700	22
O-747, **6**	A[b]	3	22.0 ± 11.9	19.7 ± 0.9	0.9
O-764, **7**	A[b]	5	99.0 ± 28	550 ± 63	6
O-934, **8**	B	1	58.9 ± 1.65	1073 (n = 1)	18
O-932, **9**	B	3	1.4 ± 0.20	133 ± 7	95
O-933, **10**	B	5	3.4 ± 0.83	49.9 ± 10.2	15

[a] Each radioligand was incubated with tissue (4 mg/mL original wet tissue weight) and 7−14 concentrations of a cocaine congener as described in the experimental section. Nonspecific binding of [^{3}H]WIN 35,428 was measured with 30 μM (−)-cocaine and of [^{3}H]citalopram with 1 μM fluoxetine. IC_{50} values were computed by the EBDA computer program and are the means (±SD) of two to four independent experiments, each conducted in triplicate. [b] Meltzer, P. C.; Liang, A. Y.; Madras, B. K. *J. Med. Chem.* **1996**, *39*, 371−379.

The affinities (IC_{50}) of compounds **11**−**14** for the dopamine (DAT) and serotonin (SERT) transporters are presented in Table 1. The most potent compound is the 12*R* diastereomer, **11b**, which has an IC_{50} of 4.04 nM and a selectivity of 74 (DAT:SERT). Of interest is the fact that the two diastereomers **11a** and **11b**, while similar in DAT potency (**11a**, IC_{50} = 7.38 nM; **11b**, IC_{50} = 4.04 nM), differ markedly in selectivity for the DAT (9 vs 74). Most important, the mixture **11** is sufficiently potent and selective (DAT:SERT = 21) to provide a promising SPECT ligand.

The 3,4-dichloro analog **12** is of intermediate potency (DAT: IC_{50} = 37.2 ± 3.4 nM) and of moderate selectivity

(DAT:SERT = 7). In comparison, SAR of the classical tropane series of compounds[5,12] has shown that the 3,4-dichloro analog of WIN35,428 is among the most potent of DAT inhibitors (IC_{50} = 0.9 ± 0.02 nM). Although highly selective for the DAT over the SERT, the least potent compound proved to be the difluoropine analogs **13** (IC_{50} = 616 ± 88 nM; selectivity, DAT:SERT = 90).

It is noteworthy that, as for the tropane series in general, biological selectivity is maintained for the 2β-$COOCH_3$ (**11**) vs the 2α-$COOCH_3$ (**14**; DAT: IC_{50} = 2960 nM) compound.

Finally, norepinephrine binding of **11** evidenced considerable selectivity for the DAT over the NET (**11**: DAT:NET = 6700). On the basis of these data, compound **11** was selected for introduction of ^{99m}Tc and subsequent SPECT imaging studies.

The rank order of potency at the DAT for each of the diastereomeric pairs is **11** > **12** > **13** > **14**. This is in contrast to the rank order for the parent compounds: **3** (IC_{50} = 0.9 nM) > **4** (IC_{50} = 10.9 nM) ≈ **2** (IC_{50} = 11.0 nM). This difference in rank order indicates that the bulky N-substituent causes the C_3 aromatic ring to interact differently at the transporter binding site. This difference is likely a π-stacking or steric bulk (protrusion) effect rather than an electronic one. Indeed, from these data in conjunction with those obtained from a family of oxytropanes (compounds in which oxygen has been substituted for nitrogen[43] where the differences encountered on modification of the aromatic substituents are considerably more marked) it is increasingly evident that the aromatic system can play a dominant role in transporter−ligand interactions.

Conclusions

Herein we describe the design, synthesis, and biological evaluation of the first ^{99m}Tc-labeled SPECT imaging agent, technepine, **1**, for the dopamine transporter in striatum. We have demonstrated that there is sufficient space in the transporter to accommodate a bulky chelating unit in the region of the 8-amine function of the tropane skeleton. Further, we have demonstrated for the first time that a molecule can be designed to carry the radionuclide ^{99m}Tc across the blood−brain barrier in sufficient quantity to obtain *in vivo* images of the striatum in monkeys. This critical advance will undoubtedly lead to the design of new receptor and transporter-mediated ^{99m}Tc agents which can label specific transporter and receptor targets in the central nervous system.[44]

Experimental Section

NMR spectra were recorded on either a Bruker 100, a Varian XL 400, or a Bruker 300 NMR spectrometer. TMS was used as internal standard. Melting points are uncorrected and were measured on a Gallenkamp melting point apparatus. Thin layer chromatography (TLC) was carried out on Baker Si 250F plates. Visualization was accomplished with either iodine vapor, UV exposure, or treatment with phosphomolybdic acid (PMA). Preparative TLC was carried out on Analtech uniplates silica gel GF 2000 μm. Flash chromatography was carried out on Baker silica gel 40 μM. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. A Beckman 1801 scintillation counter was used for scintillation spectrometry; 0.1% bovine serum albumin and (−)-cocaine were purchased from Sigma Chemicals. All reactions were carried out under an inert atmosphere (N_2).

[^{3}H]WIN35,428 ([^{3}H]CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-[^{3}H]methyltropane, 79.4−87.0 Ci/mmol) and [^{3}H]citalopram (86.8 Ci/mmol) were purchased from DuPont-New England Nuclear (Boston, MA). (*R*)-(−)-Cocaine hydrochloride for the pharmacological studies was donated by the National Institute on Drug Abuse (NIDA). Fluoxetine was donated by E. Lilly & Co.

***N*-[[[2-[(Triphenylmethyl)thio]ethyl]amino]carbonyl]-methyl]-*N*-(3′-chloropropyl)-*S*-(triphenylmethyl)-2-aminoethanethiol (16).** The amine *N*-[[[2-[(triphenylmethyl)-thio]ethyl]amino]carbonyl]methyl]-*S*-(triphenylmethyl)-2-aminoethanethiol, **15**[30] (10.86 g, 16 mmol), was dissolved in dry methylene chloride (10 mL), and to this solution was added the 3-chloropropyl triflate (1.81 g, 8 mmol, prepared from 3-chloropropanol). The resulting solution was stirred at room temperature for 2 h, at which point a further 90 mL of methylene chloride was added. The solution was filtered to remove excess amine triflate salt, and the filtrate was chromatographed (SiO_2, ethyl acetate/hexanes, 1:1). In this manner 4.36 g (72% based on the triflate) of the desired product, **16**, was isolated as a white foam: mp 55−56 °C; R_f 0.5 (EtOAc/hexanes, 1:1); IR (KBr disk) 1640 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.6−1.9 (m, 2H), 2.2−2.55 (m, 8H), 2.85 (s, 2H), 3.02 (q, 2H, *J* = 6 Hz), 3.45 (t, 2H, *J* = 6 Hz), 7.1−7.5 (m, 30H); HRCIMS calculated for $C_{47}H_{48}ClN_2S_2O$ $[MH]^+$ 755.2896, found 755.2899.

[*N*-[2-[(3′-Chloropropyl)(2-mercaptoethyl)amino]acetyl]-2-aminoethanethiolato]rhenium(V) Oxide (18). *N*-[[[[2-(Triphenylmethyl)thio]ethyl]amino]carbonyl]methyl]-*N*-(3′-chloropropyl)-*S*-(triphenylmethyl)-2-aminoethanethiol, **16** (2.5 g, 3.3 mmol), was dissolved in boiling ethanol (50 mL). To this was added a solution of tin(II) chloride (690 mg, in 6.25 mL of 0.05 M HCl), followed immediately by a solution of sodium perrhenate (1 g in 6.25 mL of 0.05 M HCl). Reflux was continued overnight, after which boiling acetonitrile (200 mL) was added and the resulting solution filtered through a pad of Celite. The cake was further washed with boiling acetonitrile (2 × 200 mL). To the filtrate was added silica gel (30 g), and the solvent was evaporated. The solid was then layered onto a silica gel column and eluted with ethyl acetate. A racemate, **18**, was isolated in 48% yield (740 mg): mp 218.4−218.8° C; R_f 0.32 (EtOAc); IR (KBr disk) 1640, 952 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.67 (dt, 1H, *J* = 12, 4.6 Hz), 2.1−2.5 (m, 2H), 2.90 (dd, 1H, *J* = 13, 4.6 Hz), 3.1−3.5 (m, 4H), 3.64 (t, 2H, *J* = 6 Hz), 3.7−4.2 (m, 3H), 4.09 (d, 1H, *J* = 16 Hz), 4.5−4.7 (m, 1H), 4.68 (d, 1H, *J* = 16 Hz); HRCIMS calculated for $C_9H_{17}ClN_2S_2O_2Re$ $[MH]^+$ 470.9950 found 470.9971. Anal. ($C_9H_{17}ClN_2S_2O_2Re$·0.2 $CH_3CO_2C_3H_5$) C, H, N.

[*N*-[2-[(3′-*N*′-Propyl-3″β-(4-fluorophenyl)tropane-2″β-carboxylic acid methyl ester)(2-mercaptoethyl)amino]-acetyl]-2-aminoethanethiolato]rhenium(V) oxide (11). Route A. To a solution of nor-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester[12,33] (253 mg, 0.96 mmol) in dry acetonitrile (10 mL) was added in succession [*N*-[2-[(3′-chloropropyl)(2-mercaptoethyl)amino]acetyl]-2-aminoethanethiolato]rhenium(V) oxide, **18** (451 mg, 0.96 mmol), potassium iodide (159 mg, 0.96 mmol), and potassium carbonate (1.3 g, 9.6 mmol). The resulting slurry was then brought to reflux overnight. Upon completion of reaction (as shown by TLC) the solution was allowed to cool to room temperature, silica gel (10 g) was added, and the solvent was evaporated. The resulting solid was layered onto a silica gel column and eluted with 0.5% ammonium hydroxide in ethyl acetate. In this manner the title compound, **11**, was obtained as a mixture of diastereomers in 90% yield (608 mg); mp 101.9 °C; R_f 0.55 (94% EtOAc/5% MeOH/1% NH_4OH); IR (KBr disk) 1720, 1666, 957 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14−7.24 (m, 2H), 6.92−6.96 (m, 2H), 4.79, 4.77 (2d, 1H, *J* = 16 Hz), 4.52−4.59 (m, 1H), 3.95−4.11 (m, 3H), 3.55−3.75 (m, 2H), 3.35−3.55 (m, 2H), 3.44, 3.49 (2s, 3H), 3.10−3.30 (m, 3H), 2.95−3.05 (m, 1H), 2.80−2.90 (m, 2H), 2.35−2.60 (m, 2H), 2.15−2.30 (m, 1H), 1.95−2.10 (m, 2H), 1.05−1.95 (m, 6H); HRCIMS calcd for $C_{24}H_{34}FN_3S_2O_4Re$ $[MH]^+$ 698.1505, found 698.1557. Compound **11** was converted to the hydrochloride for elemental analysis. Anal. ($C_{24}H_{33}FN_3O_4S_2Re$·HCl·2H_2O) C, H, N.

Route B. *N*-[2-[(3′-*N*′-Propyl-3″β-(4-fluorophenyl)tropane-2″β-carboxylic acid methyl ester)[2-[(triphenylmethyl)thio]-

ethyl]amino]acetyl]-*S*-(triphenylmethyl)-2-aminoethanethiol, **17** (98 mg, 0.1 mmol), was dissolved in boiling ethanol (1.5 mL). To this was added a solution of tin(II) chloride (21 mg in 200 mL of 0.05 M HCl), followed immediately by a solution of sodium perrhenate (30 mg in 200 mL of 0.05 M HCl). Boiling was continued overnight, after which boiling acetonitrile (10 mL) was added and the resulting solution filtered through a pad of Celite. The cake was further washed with boiling acetonitrile (2 × 20 mL). To the filtrate was added silica gel (1 g), and the solvent was removed on a rotary evaporator. The resultant solid was layered onto a silica gel column and eluted with ethyl acetate. Compound **11** was obtained as a mixture of diastereomers in 30% yield (21 mg) and was identical (TLC, NMR) to that prepared by route A above.

Separation of Diastereiosomers 11a and 11b. A mixture of **11a** and **11b** (171 mg) was chromatographed (40 g of SiO_2 prewashed with Et_3N (4 mL in 100 mL EtOAc); eluent EtOAc with 0.5% NH_4OH). Compound **11a** eluted first (31.5 mg) followed by a mixture of **11a** and **11b** (112.7 mg) and finally by **11b** (26.8 mg) [TLC: R_f for both **11a** and **11b** is 0.55 (94% ethyl acetate, 5% MeOH, 1% NH_4OH)].

11a: mp 261−262 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50−1.92 (m, 6H), 1.94−2.10 (m, 2H), 2.16−2.24 (m, 1H), 2.38−2.46 (m, 1H), 2.50−2.60 (m, 1H), 2.80−2.90 (m, 2H), 2.94−3.02 (m, 1H), 3.10−3.28 (m, 3H), 3.36−3.48 (m, 2H), 3.44 (s, 3H, 2′β-CO_2CH_3), 3.54−3.60 (m, 1H), 3.64−3.74 (m,1H), 3.92−4.08 (m, 2H), 4.07 (d, 1H, J = 16 Hz, *H-6*α), 4.54−4.60 (m, 1H, *H-4*β), 4.79 (d, 1H, J = 16 Hz, *H-6*β), 6.90−7.00 (m, 2H), 7.10−7.30 (m, 2H).

11b: ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.50−3.80 (m, 21H), 3.49 (s, 3H, 2′β-CO_2CH_3), 3.85−4.30 (m, 2H), 4.04 (d, 1H, J = 16 Hz, *H-6*α), 4.40−4.70 (m, 1H, *H-4*β), 4.77 (d, 1H, J = 16 Hz, *H-6*β), 6.80−7.40 (m, 4H).

[*N*-[2-[(3′-*N*′-propyl-3″β-(3,4-dichlorophenyl)tropane-2″β-carboxylic acid methyl ester)(2-mercaptoethyl)amino]acetyl]-2-aminoethanethiolato]rhenium(V) Oxide (12). This compound was prepared as described above for route A and purified by chromatography: 59% yield; mp 108 °C dec; R_f 0.18 (EtOAc); IR (KBr disk) 1724, 1653, 957 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.5−3.9 (m, 22H), 3.55, 3.50 (2s, 3H), 3.9−4.2 (m, 2H), 4.5−4.7 (m, 1H), 4.80 (d, 1H, J = 16.4 Hz), 7.0−7.4 (m, 3H); HRCIMS calcd for $C_{24}H_{33}Cl_2N_3S_2O_4Re$ $[MH]^+$ 748.0819, found 748.0856. Anal. ($C_{24}H_{33}Cl_2N_3O_4S_2Re$) C, H, N.

[*N*-[2-[(3′-*N*′-Propyl-3″α-(bis(4-fluorophenyl)methoxy)-tropane-2″β-carboxylic acid methyl ester)(2-mercaptoethyl)amino]acetyl]-2-aminoethanethiolato]rhenium-(V) Oxide (13). This compound was prepared as described above for route A and purified by column chromatography: 68% yield; mp 102 °C dec; R_f 0.47 (EtOAc); IR (KBr disk) 1709, 1640, 957 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.4−2.4 (m, 9H), 2.6−4.3 (m, 15H), 3.70, 3.65 (2s, 3H), 4.4−4.7 (m, 1H), 4.85, 4.69 (2d, 1H, J = 16 Hz), 5.35 (s, 1H), 6.8−7.4 (m, 8H); HRCIMS calcd for $C_{31}H_{39}F_2N_3S_2O_5Re$ $[MH]^+$ 822.1829, found 822.1818. Anal. ($C_{31}H_{38}F_2N_3O_5S_2Re\cdot 2H_2O$) C, H, N.

Separation of Diastereoisomers 13a and 13b. The diastereomeric mixture of **13a** and **13b** was separated by silica gel column chromatography (eluent 2% NH_4OH/EtOAc). Compound **13a** eluted first (R_f 0.51 (EtOAc)) and **13b** second (R_f 0.43 (EtOAc)).

13a: ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.4−2.4 (m, 9H), 2.6−4.3 (m, 15H), 3.70 (s, 3H), 4.4−4.7 (m, 1H), 4.85 (d, 1H, J = 16 Hz), 5.35 (s, 1H), 6.8−7.4 (m, 8H).

13b: ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.4−2.4 (m, 9H), 2.6−4.3 (m, 15H), 3.65 (s, 3H), 4.4−4.7 (m, 1H), 4.69 (d, 1H, J = 16 Hz), 5.35 (s, 1H), 6.8−7.4 (m, 8H).

[*N*-[2-[(3′-*N*′-Propyl-3″β-(4-fluorophenyl)tropane-2″α-carboxylic acid methyl ester)(2-mercaptoethyl)amino]-acetyl]-2-aminoethanethiolato]rhenium(V) Oxide (14). This compound was prepared as described above for route A and purified by column chromatography: 70% yield; mp 98.6−99.6 °C; R_f 0.29 (93% EtOAc/5% MeOH/2% NH_4OH); IR (KBr disk) 1716, 1646, 957 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.5−2.1 (m, 7H), 2.57 (t, 2H, J = 7 Hz), 2.8−4.0 (m, 13H), 3.52 (s, 3H), 4.0−4.3 (m, 2H), 4.5−4.7 (m, 1H), 4.75, 4.74 (2d, 1H, J = 16 Hz), 6.85−7.30 (m, 4H); HRCIMS calcd for $C_{24}H_{34}N_3S_2O_4Re$ $[MH - F]^+$ 679.1521, found 679.1569. Anal. ($C_{24}H_{33}FN_3O_4S_2Re\cdot 1.5CHCl_3$) C, H, N.

2β-Carbomethoxy-3β-(4-fluorophenyl)-8-[3-[*N*-[2-[[[[2-[(triphenylmethyl)thio]ethyl]amino]carbonyl]methyl]-*N*-[*S*-(triphenylmethyl)thio]ethyl]amino]propyl]nortropane (17). Route A. To a solution of nor-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester[12] (52.6 mg, 0.2 mmol) in dry acetonitrile (10 mL) was added in succession *N*-[[[[2-[(triphenylmethyl)thio]ethyl]amino]carbonyl]methyl]-*N*′-(3′-chloropropyl)-*S*-(triphenylmethyl)-2-aminoethanethiol, **16** (151 mg, 0.2 mmol), potassium iodide (33 mg, 0.2 mmol), and potassium carbonate (280 mg, 2.0 mmol). The resulting slurry was then boiled overnight. Once the reaction was complete, the solution was allowed to cool to room temperature, and then 2 g of silica gel was added and the solvent evaporated. The resulting solid was layered onto a silica gel column and eluted with 0.5% ammonium hydroxide in a 1:1 solution of ethyl acetate and hexanes. In this manner compound **17** was recovered as a foam in 72% yield (141 mg). This was converted to the dihydrochloride: mp 166−168 °C; R_f 0.42 (EtOAc/hexanes (1:1) + 0.5% NH_4OH); IR (KBr disk)1666 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.8−3.8 (m, 24H), 3.3 (s, 3H), 3.9−4.0 (m, 1H), 4.2−4.3 (m, 1H), 4.4−4.5 (m, 1H), 6.9−7.4 (m, 34H), 8.7−8.9 (m, 1H), 9.3−9.4 (m, 1H). Anal. ($C_{62}H_{64}N_3O_3S_2F\cdot 2HCl\cdot 2H_2O$) C, H, N.

Route B. Nor-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester[12] (52.6 mg, 0.2 mmol) was dissolved in dry methylene chloride (1 mL). To this solution was added the 3-chloropropyl triflate (22.6 mg, 0.1 mmol, prepared from 3-chloropropanol). The resulting solution was stirred at room temperature for 2 h at which point a further 10 mL of methylene chloride was added. The solution was filtered to remove excess amine triflate salt, and the filtrate was evaporated and immediately dissolved in dry acetonitrile (10 mL). To this solution was added, in succession, *N*-[[[[2-(triphenylmethyl)thio]ethyl]amino]carbonyl]methyl]*N*-(3′-chloropropyl)-*S*-(triphenylmethyl)-2-aminoethanethiol, **16** (136 mg, 0.2 mmol), potassium iodide (33 mg, 0.2 mmol), and potassium carbonate (280 mg, 2.0 mmol). The resulting slurry was then maintained at reflux overnight. Upon completion of reaction (by TLC), the solution was allowed to cool to room temperature, 2 g of silica gel was added, and the solvent was evaporated. The resulting solid was layered onto a silica gel column and eluted with 0.5% ammonium hydroxide in ethyl acetate/hexanes (1:1). In this manner compound **17** was obtained as a foam (98 mg; 50%) and was identical (TLC, NMR) to that prepared by route A.

General Procedure for Preparation of Arylalkyltropanes. The arylalkyl halide was brought to reflux overnight with nor-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester[12] in acetonitrile in the presence of potassium iodide (1 equiv) and potassium carbonate (10 equiv). The solvent was evaporated and the residue purified by chromatography. The following compounds were obtained by this standard procedure.

(1*R*)-*N*-(3′-Benzyl)-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester (8): yield 93%; mp 132−133 °C; R_f 0.56 (20% EtOAc/hexanes + 0.5% NH_4OH); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.6−2.4 (m, 6H), 2.7−3.2 (m, 4H), 3.4 (s, 3H), 3.4−3.7 (m, 2H), 6.8−7.5 (m, 9H). Anal. ($C_{22}H_{24}FNO_2\cdot 0.5H_2O$) C, H, N.

(1*R*)-*N*-(3′-Phenylpropyl)-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester (9): yield 99%; oil; R_f 0. 42 (20% EtOAc/hexanes + 0.5% NH_4OH); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.5−2.2 (m, 7H), 2.3 (t, 2H, J = 7 Hz), 2.4−3.2 (m, 5H), 3.45 (br s, 1H), 3.53 (s, 3H), 3.75 (br s, 1H), 6.8−7.4 (m, 9H); analyzed as the hydrochloride. Anal. ($C_{24}H_{28}FNO_2\cdot 1.5HCl\cdot 1H_2O$) C, H, N.

(1*R*)-*N*-(3′-Phenylpentyl)-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester (10): yield 66%; oil; R_f 0. 31 (20% EtOAc/hexanes + 0.5% NH_4OH); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.2−2.3 (m, 13H), 2.3−3.2 (m, 5H), 3.4 (br s, 1H), 3.5 (s, 3H), 3.7 (br s, 1H), 6.8−7.4 (m, 9H); analyzed as the hydrochloride. Anal. ($C_{26}H_{32}FNO_2\cdot 2HCl\cdot 1.5H_2O$) C, H, N.

[*N*-[2-[(3′-*N*′-propyl-3″*β*-(4-fluorophenyl)tropane-2″*β*-carboxylic acid methyl ester)(2-mercaptoethyl)amino]-acetyl)-2-aminoethanethiolato]technetium-99m(V) Oxide (1). 2*β*-Carbomethoxy-3*β*-(4-fluorophenyl)-8-[3-[*N*-[2-[[[[2-[(triphenylmethyl)thio]ethyl]amino]carbonyl]methyl]-*N*-[*S*-(triphenylmethyl)thio]ethyl]amino]propyl]nortropane, **17** (1.0 mg), was dissolved in 0.2 mL of anhydrous anisole and cooled in an ice bath to 5 °C. Anhydrous trifluoroacetic acid (10 mL) was added to obtain a bright yellow solution which was stirred at 5 °C for 5 min. The reaction mixture was then titrated with triethylsilane until the disappearance of the yellow color. The solution was evaporated to dryness by rotary evaporation at room temperature and dried under high vacuum drying for 1 h. The dry solid was reconstituted in 0.5 mL of distilled water. A portion of of this aqueous solution (100 μL) was then added to 300 μL of a 58 mCi ^{99m}Tc−glucoheptonate solution (Glucoscan kits from Du Pont, Billerica, MA) and incubated for 45 min at 40−45 °C. HPLC separation on a C_8 reverse phase column equipped with a C_{18} guard column, eluted with 0.1 M ammonium acetate and acetonitrile under a linear gradient, provided the major ^{99m}Tc-labeled product having a retention time of 21.5 min. The major peak was collected in a round bottom flask and evaporated on a high-vacuum rotary evaporator at room temperature. The product was subsequently reconstituted in 1 mL of sterile saline for injection.

Biology. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and of the "Guide for Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication No. (NIH)85-23, revised 1985.

Tissue Sources and Preparation. Brain tissue from adult male and female cynomolgus monkeys (*Macaca fascicularis*) was stored at −85 °C in the primate brain bank at the New England Regional Primate Research Center. The caudate−putamen was dissected from coronal slices and yielded 1.4 ± 0.4 g tissue. Membranes were prepared as described previously.[5,45] Briefly, the caudate−putamen was homogenized in 10 volumes (w/v) of ice-cold Tris·HCl buffer (50 mM, pH 7.4 at 4 °C) and centrifuged at 3800*g* for 20 min in the cold. The resulting pellet was suspended in 40 volumes of buffer, and the entire was procedure was repeated twice. The membrane suspension (25 mg original wet weight of tissue/mL) was diluted to 12 mL/ml for [^{3}H]WIN 35,428 or [^{3}H]-citalopram assay in buffer just before assay and was dispersed with a Brinkmann Polytron homogenizer (setting 5) for 15 s. All experiments were conducted in triplicate, and each experiment was repeated in each of two or three preparations from individual brains.

Dopamine Transporter Assay. The dopamine transporter was labeled with [^{3}H]WIN35,428 ([^{3}H]CFT, 2*β*-carbomethoxy-3*β*-(4-fluorophenyl)-*N*-[^{3}H]methyltropane, 81−84 Ci/mmol, DuPont-NEN).[45] The affinity of [^{3}H]WIN35,428 for the dopamine transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]WIN35,-428 and a range of concentration of unlabeled WIN35,428. The assay tubes received, in Tris·HCl buffer (50 mM, pH 7.4 at 0−4 °C; NaCl 100 mM), the following constituents at a final assay concentration: WIN35,428, 0.2 mL (1 pM to 100 or 300 nM), [^{3}H]WIN35,428 (0.3 nM); membrane preparation 0.2 mL (4 mg original wet weight of tissue/ml). The 2 h incubation (0−4 °C) was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters presoaked in 0.1% bovine serum albumin (Sigma Chemical Co.). The filters were washed twice with 5 mL of Tris·HCl buffer (50 mM) and incubated overnight at 0−4 °C in scintillation fluor (Beckman Ready-Value, 5 mL), and radioactivity was measured by liquid scintillation spectrometry (Beckman 1801). Cpm were converted to dpm following determination of counting efficiency (>45%) of each vial by external standardization. Total binding was defined as [^{3}H]WIN35,428 bound in the presence of ineffective concentrations of unlabeled WIN35,428 (1 or 10 pM). Nonspecific binding was defined as [^{3}H]WIN35,428 bound in the presence of an excess (30 μM) of (−)-cocaine. Specific binding was the difference between the two values. Competition experiments to determine the affinities of other drugs at [^{3}H]WIN35,428 binding sites were conducted using procedures similar to those outlined above. Stock solutions of water-soluble drugs were dissolved in water or buffer, and stock solutions of other drugs were made in a range of ethanol/HCl solutions. Several of the drugs were sonicated to promote solubility. The stock solutions were diluted serially in the assay buffer and added (0.2 mL) to the assay medium as described above.

Serotonin Transporter Assay. The serotonin transporter was assayed in caudate−putamen membranes using conditions similar to those for the dopamine transporter. The affinity of [^{3}H]citalopram (spec. act.: 82 Ci/mmol, DuPont-NEN) for the serotonin transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]citalopram and a range of concentrations of unlabeled citalopram. The assay tubes received, in Tris·HCl buffer (50 mM, pH 7.4 at 0−4 °C; NaCl 100 mM), the following constituents at a final assay concentration: citalopram, 0.2 mL (1 pM to 100 or 300 nM), [^{3}H]citalopram (1 nM); membrane preparation 0.2 mL (4 mg original wet weight of tissue/mL). The 2 h incubation (0−4 °C) was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters presoaked in 0.1% polyethylenimine. The filters were washed twice with 5 mL of Tris·HCl buffer (50 mM) and incubated overnight at 0−4 °C in scintillation fluor (Beckman Ready-Value, 5 mL), and radioactivity was measured by liquid scintillation spectrometry (Beckman 1801). Cpm were converted to dpm following determination of counting efficiency (>45%) of each vial by external standardization. Total binding was defined as [^{3}H]citalopram bound in the presence of ineffective concentrations of unlabeled citalopram (1 or 10 pM). Nonspecific binding was defined as [^{3}H]citalopram bound in the presence of an excess (10 μM) of fluoxetine. Specific binding was the difference between the two values. Competition experiments to determine the affinities of other drugs at [^{3}H]citalopram binding sites were conducted using procedures similar to those outlined above.

Data Analysis. Data were analyzed by the EBDA computer software programs (Elsevier-Biosoft, U.K.). Final estimates of IC_{50} values were computed by the EBDA program. Baseline values for the individual drugs were established from the competition curves, and these generally were similar to baseline values established by 30 μM (−)-cocaine or 1 μM fluoxetine.

Single-Crystal X-ray Analysis of Diasterisomer 11a. Orthorhombic crystals of the purified **11a** were obtained by slow growth at the interface of a hexane/anhydrous ethanol suspension maintained at room temperature. A representative crystal was selected, and a 1.451 78 Å data set was collected at room temperature. Pertinent crystal, data collection, and refinement parameters: crystal size, 0.40 × 0.38 × 0.14 mm; cell dimensions, a = 7.4147(5) Å, b = 15.9123(11) Å, c = 22.2645(14) Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$; formula, $C_{24}H_{33}FN_3O_4ReS_2$; formula weight = 696.85; volume = 2626.9 (3) Å^3; calculated density = 1.762 g cm^{-3}; space group = $P2_12_12_1$; number of reflections = 2723 of which 2512 were considered independent (R_{int} = 0.0232). Refinement method was full-matrix least-squares on F^2. The final R indices were [$I > 2\sigma(I)$] R1 = 0.0322, wR2 = 0.0836.

Coordinates, anisotropic temperature factors, distances, and angles are available as Supporting Information.

Single-Photon Emission Computed Tomography. SPECT and MRI imaging was performed on male and female rhesus monkeys. The SPECT imaging technique was similar to that used for human studies with HMPAO and ECD.[46] The monkey was placed within the gantry of the dedicated brain imaging instrument (Ceraspect). Sixty-four slices of 128 × 128 pixels (voxel size 1.67 mm on a side) were obtained. Reconstruction was by conventional filtered backprojection with a Butterworth filter with power 10 and cutoff of 0.95 cm.

MRI images were obtained using standard sequences for T1, T2, and proton density images. Image registration was performed using the techniques described by Holman et al.[47]

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Supporting Information Available: ORTEP drawing of **11a**, crystal data and refinement parameters, coordinates, anisotropic temperature factors, distances, and angles (8 pages). Ordering information is given on any current masthead page.

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