J. Med. Chem. **1998,** *41,* 428−436

Specificity of Diastereomers of [^{99m}Tc]TRODAT-1 as Dopamine Transporter Imaging Agents

Sanath K. Meegalla,[†] Karl Plössl,[†] Mei-Ping Kung,[†] D. Andrew Stevenson,[†] Mu Mu,[†] Steven Kushner,[†] Louise M. Liable-Sands,[§] Arnold L. Rheingold,[§] and Hank F. Kung*[,†,‡]

Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and Department of Chemistry, University of Delaware, Newark, Delaware 19716

Received November 3, 1997

Recently, we reported the first human study of [^{99m}Tc]TRODAT-1, technetium, 2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]amino]-ethanethiolato(3−)-oxo-[1*R*-(*exo-exo*)]-, as an imaging agent of central nervous system (CNS) dopamine transporters. Due to the existence of several chiral centers on this molecule, upon the formation of [^{99m}Tc]TRODAT-1 complex (**2**) several diastereomers could be created. Two major diastereomers of [^{99m}Tc]TRODAT-1 (**2**), designated as peak A (**2A**) and peak B (**2B**), were separated by HPLC. Biodistribution of the purified diastereomers **2A**,**B** was evaluated in rats. It appears that **2A** displayed a higher lipophilicity than **2B** (PC = 305 and 229, respectively), and a similar trend was observed for the initial brain uptake at 2 min postinjection (0.50% and 0.28% dose/organ for **2A**,**B**, respectively). At 60 min post-iv-injection, the specific uptakes, as measured by [striatum − cerebellum]/cerebellum ([ST−CB]/CB) ratio, were 1.72 and 2.79 for **2A**,**B**, respectively. The higher [ST−CB]/CB ratio observed for **2B** was corroborated by the results of an in vitro binding assay. Higher binding affinity for dopamine transporters was observed for **3B** (K_i = 13.87 and 8.42 nM for the analogous rhenium complexes **3A**,**B**, respectively). The structure of the [^{99m}Tc]TRODAT-1 complexes was deduced using nonradioactive rhenium as a surrogate for radioactive technetium complex. Reacting free TRODAT-1 ligand with [Bu_4N][$ReOCl_4$] yielded two major complexes: Re-TRODAT-1A (**3A**) and Re-TRODAT-1B (**3B**) (corresponding with peaks A and B of [^{99m}Tc]TRODAT-1, respectively), whose structures were determined by X-ray analysis. The X-ray structures show that both complexes have a pseudo-square-pyramidal structure of $[Re^{v}O]^{3+}N_2S_2$ core with oxygen occupying the apical position and the *N*-alkyl substitution in syn-configuration to the oxo−rhenium bond. In conclusion, TRODAT-1 formed at least two diastereomers after complexing with a metal(V)−oxo (M = ^{99m}Tc, Re) center core. The two isomers display different binding affinities toward dopamine transporters and distinct properties of localization in the striatum area of the brain where the transporters are located.

Introduction

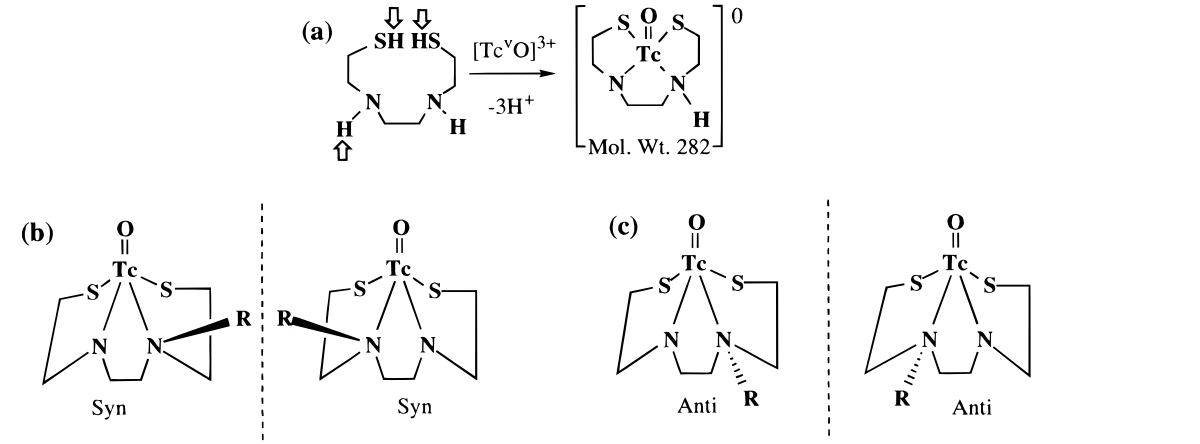
The most commonly used radionuclide in diagnostic nuclear medicine is technetium-99m ($t_{1/2}$ = 6 h, 140 keV).[1−3] Its popularity is mainly due to several unique characteristics: the radionuclide can be readily produced by a ^{99}Mo/^{99m}Tc generator, the medium γ-ray energy emitted by Tc-99m (140 keV) is suitable for gamma camera detection, and the physical half-life is compatible with the biological localization and residence time required for in vivo imaging. In the past few years, our laboratory has been engaged in developing Tc-99m-labeled receptor-specific imaging agents using a $[Tc^{v}O]^{3+}N_2S_2$ core as the complexing moiety. It is well-established that when [^{99m}Tc]pertechnetate (TcO_4^-) is reduced in the presence of a reducing agent, such as stannous chloride, and a soft chelating ligand, such as N_2S_2 or NS_3, a neutral and lipophilic $[Tc^{v}O]^{3+}N_2S_2$ or $[Tc^{v}O]^{3+}NS_3$ complex with a pyramidal structure is formed (Scheme 1a).[2,4−7] When an *N*-alkyl substitution group is added, syn- and anti-isomers may be formed (Scheme 1b,c).[8] Additionally, enantiomers of these syn- and anti-complexes are formed (Scheme 1).[2,9]

Several recent reports demonstrate that it is possible to incorporate a $[Tc^{v}O]^{3+}N_2S_2$ or $[Tc^{v}O]^{3+}NS_3$ core into potential receptor-selective imaging agents for muscarinic receptors,[10,11] vesamicol sites,[12] serotonin receptors,[13−15] dopamine D1[16] and D2 receptors,[14,17,18] and steroid hormone receptors.[19−27] Use of boronic acid adducts of technetium dioxime (BATO type of complexes) as muscarinic receptor imaging agents has been reported.[28] However, these Tc-99m imaging agents have demonstrated limited success in in vivo studies.[27] There are various factors that may contribute to the failure of Tc-99m receptor imaging agents. It appears that designing a high-binding-affinity ligand containing a Tc-99m core can be accomplished; however, achieving the goal of designing a Tc-99m ligand with a high uptake and low nonspecific binding in vivo is much more elusive. Factors that control the biodistribution and nonspecific binding are more difficult to address, since

* Address for correspondence: Department of Radiology, University of Pennsylvania, 3700 Market St., Room 305, Philadelphia, PA 19104. Tel: (215) 662-3096. Fax: (215) 349-5035. E-mail: kunghf@sunmac.spect.upenn.edu.
[†] Department of Radiology, University of Pennsylvania.
[‡] Department of Pharmacology, University of Pennsylvania.
[§] University of Delaware.

S0022-2623(97)00742-5 CCC: $15.00
Published on Web 01/24/1998

Scheme 1. Formation of a Neutral $[Tc^{v}O]^{3+}N_2S_2$ Complex Based on N_2S_2 (BAT) Ligands and Possible Isomers from Mono-N-alkylated N_2S_2 Ligand



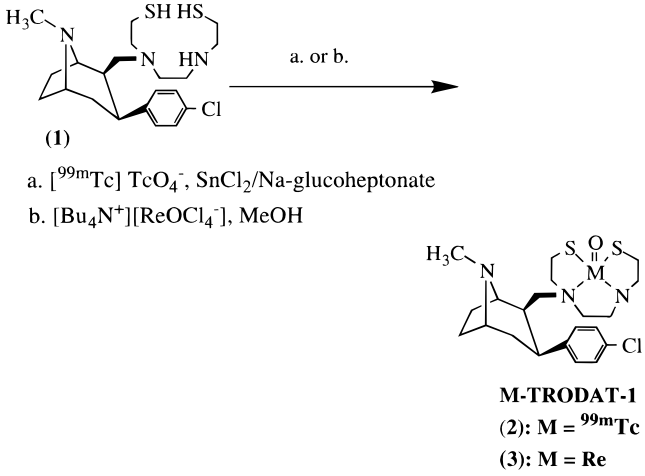
little is known about the factors that regulate in vivo behavior of Tc-99m complexes.

A large number of dopamine transporter imaging agents based on cocaine or its closely related congeners, tropane derivatives, have been reported as useful positron emission tomography (PET) and single-photon emission-computed tomography (SPECT) imaging agents.[29−31] The regional brain distribution of dopamine transporters is largely concentrated in the basal ganglia, where dopamine neurons are located. Several useful agents, including [^{11}C]-*N*-methyl-labeled cocaine,[32,33] [^{3}H]CFT (WIN35,428),[34,35] [^{11}C]methylphenidate,[36] [^{123}I]-β-CIT,[37−41] [^{123}I]IPT,[42−45] [^{123}I]CIT-FP,[41,46,47] and [^{123}I]altropane,[48] display high binding affinity and selectivity to dopamine transporters. These agents, for PET or SPECT imaging, display excellent specific uptake in the striatum (basal ganglia) and are useful in evaluating changes in dopamine transporters in vivo and in vitro, especially for patients with Parkinson's disease (PD), which is characterized by a selective loss of dopamine neurons in the basal ganglia and substantia nigra.

In developing Tc-99m-labeled dopamine transporter imaging agents, several Tc-99m-labeled tropanes have been reported.[49−54] We have prepared tropane derivatives containing a 2β-substitution of bis(aminoethanethiol) (BAT) N_2S_2 as the ligand for forming a $[Tc^{v}O]$ complex.[55,56] One of the compounds, [^{99m}Tc]TRODAT-1, displayed initial uptake in rat brain (0.4% at 2 min post-iv-injection), and the striatal/cerebellar (ST/CB) ratio reached 2.8 at 60 min after an iv injection. After an iv injection of 9 mCi of [^{99m}Tc]TRODAT-1, in vivo images of baboon brain using SPECT exhibited excellent localization in striatum (basal ganglia), where dopamine neurons are known to be concentrated.[57] This series of compounds may provide a convenient source of short-lived imaging agents for routine diagnosis of central nervous system (CNS) abnormality.

In our preliminary publication of [^{99m}Tc]TRODAT-1[58] we reported that upon the formation of the metal−oxo complexes, both the Tc-99m complex and the corresponding Re complex displayed several peaks on HPLC (using a chiral column, Chiracel-AD). To investigate this further, we report herein the preparation and characterization of the major isomers of these complexes by NMR, X-ray crystallography, and in vitro and in vivo biological evaluations.

Scheme 2. Preparation of [^{99m}Tc]TRODAT-1 (**2**) and Re-TRODAT-1 (**3**)



Chemistry

The syntheses of the "free thiol", [Re]TRODAT-1 and [^{99m}Tc]TRODAT-1 (**1**) have been previously reported.[58] Two major diastereomers of [Re]TRODAT-1 were isolated from the crude reaction mixture by preparative thin-layer chromatography on silica gel and were recrystallized from CH_2Cl_2/MeOH (Scheme 2). One of the isomers, Re-TRODAT-1A (**3A**), crystallized as large purple prisms (0.40 × 0.30 × 0.20 mm), while the other isomer, Re-TRODAT-1B (**3B**), crystallized as pale-purple rods (0.3 × 0.03 × 0.02 mm); both were in the space group $P2_12_12_1$. Compound **3A** showed a Re−oxo IR absorption at 944 cm^{-1}, while compound **3B** showed an absorption at 933 cm^{-1}, suggesting a syn-configuration in both isomers.

The ^{1}H NMR signal assignment of these two isomers was somewhat difficult because of the complexity of proton NMR spectra, mainly due to the diastereotopic nature of all the methylene protons, broadness of signals, and shielding effect of the Re−oxo group. However, with the help of DEPT, NOESY, and TOCSY (with a parameter set to emulate COSY) experiments, we were able to make reasonable assignments to all the ^{1}H NMR signals of each isomer, although some of the coupling constants could not be obtained (Figure 1).

The DEPT spectrum of the low-polar isomer showed four methyne C signals at 36.4, 42.8, 61.6, and 67.8 ppm.

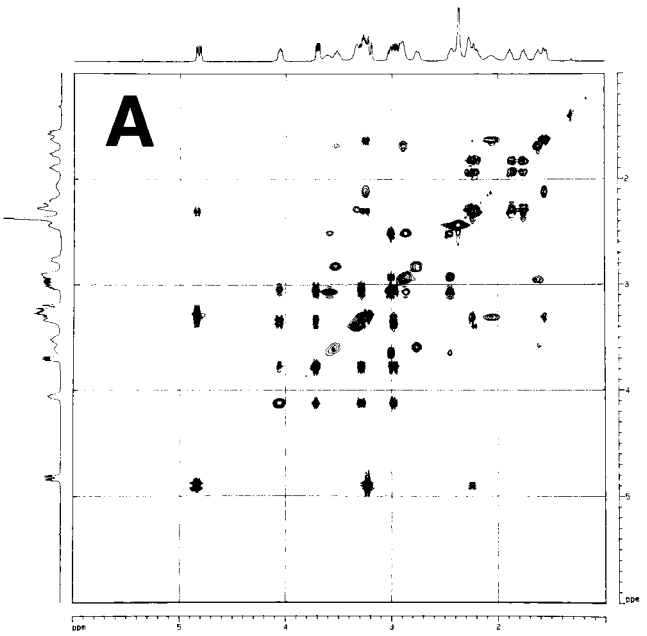


Figure 1. TOCSY spectrum of Re-TRODAT-1A (**3A**).

Signals at 132.7 and 140.7 ppm were assigned to the two quaternary aromatic carbons, out of which the signal at 140.7 ppm was assigned to the carbon bearing the chlorine atom. The only methyl carbon signal appeared at 42.4 ppm. The other four aromatic carbons showed two ^{13}C signals at 128.9 and 129.9 ppm. The signal at 128.9 ppm was assigned to the ortho carbon to the chlorine. The 10 methylene carbons appeared at 25.2, 26.2, 33.7, 40.2, 48.7, 58.6, 60.0, 62.2, 64.9, and 70.7 ppm. The high-field carbon signals at 25.2, 26.2, and 33.7 ppm were clearly due to the methylene carbons of the tropane ring, where the signal at 33.7 ppm was being generated by the C-14 methylene moiety.

From the NOESY spectrum of this compound the signals at 4.7 and 3.2, 4.0 and 3.3, 3.7 and 2.9, 3.55 and 2.95, 3.5 and 2.6, 2.9 and 2.4, 2.9 and 1.6, 2.2 and 1.9, 2.15 and 1.7, 2.05 and 1.5 ppm were identified as geminally coupled pairs of methylene protons. Three methyne protons appeared around 3.5 ppm and another at 2.27 ppm; they were obscured by other signals in their respective areas. Since the H-8a appears to be in the deshielding region of the aromatic ring, one of the methyne proton signals at ~3.3 ppm must be due to this proton. Thus the methyne carbon signal at 36.4 ppm should be the C-8 carbon. On the basis of the chemical shift values of the carbon signals at 61.5 and 67.8 ppm, they were assigned to the C-9 and C-10 methyne carbons of the tropane ring system, which were directly attached to the bridge head nitrogen, thus leaving the signal at 42.8 ppm for the C-15 methyne carbon, carrying the aromatic ring.

Generally the N-substituent of the syn-isomer of the Re−oxo complex of bisaminodithiols would be in the deshielding cone of the Re═O bond. When the substituent is a methylene group with restricted rotation, one of the methylene protons is more deshielded than the other and hence would appear at low field. In the case of Re-TRODAT-1A (**3A**), the doublet of doublet at 4.7 ppm ($J_1 = 15$ Hz, $J_2 = 5$ Hz) was assigned to one of the protons in the methylene bridge. On the basis of the *J*-coupled 1H−1H correlation, the methyne signal

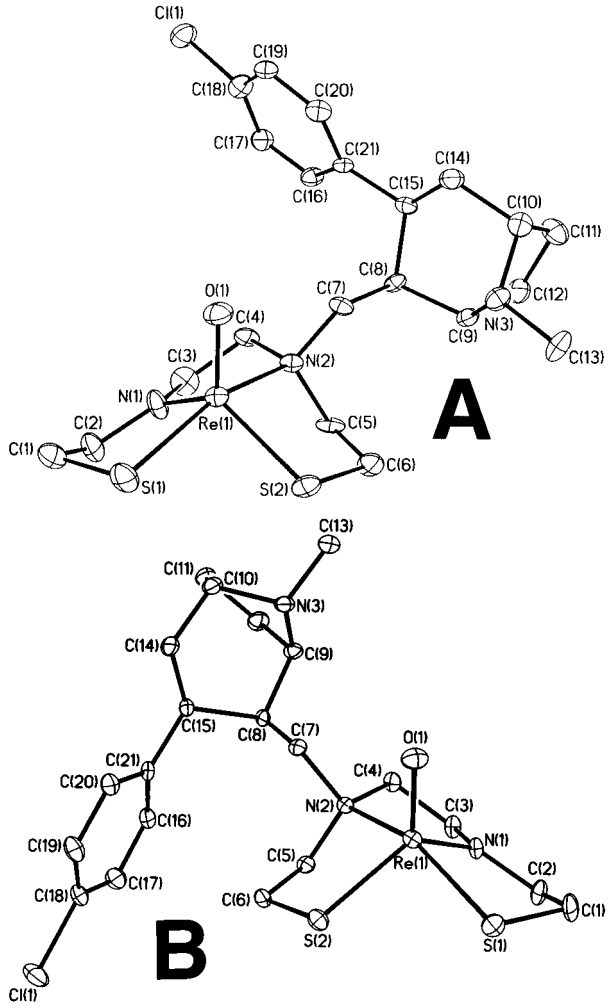


Figure 2. X-ray structures of Re-TRODAT-1A (**3A**) and Re-TRODAT-1B (**3B**).

at 2.27 ppm was assigned to the H-8b of the tropane ring, which was attached to the methylene bridge.

The syn-protons of NCH_2 of the N_2S_2 core should be more deshielded than $S-CH_2$. Among those $N-CH_2$ groups one would expect the syn-protons of H-5 and H-4 methylenes to be more deshielded than the other two because of the quaternary nature of the N carrying the methylene bridge. If that is the case, one should see the vicinal coupling of one of these protons to at least one of somewhat upfield $S-CH_2$ protons. Since no such coupling in the TOCSY spectrum was observed, the signals at 4.1 and 3.6 ppm must be due to those syn-protons of H-4 and H-3 methylenes, respectively. The broad multiplet at 3.55 ppm was assigned to the syn-protons of H-5 and H-2 methylenes.

A slight upfield shift of H-5 methylene protons could be explained as an effect of ring anisotropy of the adjacent aromatic substituent, which was also reflected in the chemical shift of the H-6 methylene group. It should be noted, however, that the X-ray crystal structure of this compound showed that H-4 methylene is in close juxtaposition to the *p*-chlorophenyl moiety, and thus the rotation of the N_2S_2 moiety along the C-7−N bond would leave both H-6 methylene and H-4 in the deshielding area of the aromatic substituent. Syn-H-2, anti-H-1, syn-H-5, and anti-H-6 showed no *J*-coupled correlation in the COSY spectrum. This observation

Table 1. Crystal Data and Structure Refinement for Re-TRODAT-1A (**3A**) and Re-TRODAT-1B (**3B**)

	3A	**3B**
empirical formula	$C_{21}H_{31}ClN_3OReS_2$	$C_{21}H_{31}ClN_3OReS_2$
formula weight	627.26	627.26
temperature (K)	246(2)	218(2)
wavelength (Å)	0.71073	0.71073
crystal system	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$
unit cell dimensions (Å)	$a = 11.479(4)$	$a = 6.7989(3)$
	$b = 12.641(4)$	$b = 10.0580(5)$
	$c = 15.907(5)$	$c = 34.0177(15)$
	$\alpha = \beta = \gamma = 90°$	$\alpha = \beta = \gamma = 90°$
volume (Å^3), Z	2308.2(12), 4	2326.2(3), 4
density (calcd, g/cm^3)	1.805	1.791
absorption coefficient (mm^{-1})	5.579	5.536
$F(000)$	1240	1240
crystal size (mm)	0.40 × 0.30 × 0.20	0.30 × 0.03 × 0.02
crystal color	purple prism	pale-grape rod
range for data collection	2.06−30.00°	1.20−28.33°
reflections collected	4834	13943
independent reflections	4470 ($R_{int} = 0.4125$)	5303 ($R_{int} = 0.0349$)
max, min transmission	0.306, 0.205	0.444, 0.314
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2
data/restraints/parameters	4470/0/263	5301/0/262
goodness-of-fit on F^2	0.863	0.949
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0381$, w$R_2 = 0.0800$	$R_1 = 0.0265$, w$R_2 = 0.0665$
R indices (all data)	$R_1 = 0.0810$, w$R_2 = 0.0954$	$R_1 = 0.0292$, w$R_2 = 0.0772$
extinction coefficient	0.0021(3)	NA
largest diff peak, hole (eÅ^{-3})	0.987, −2.699	1.922, −2.760

was well-supported by the dihedral angles (77.5° and 65.3°, respectively) of these two proton sets obtained from X-ray study. The H-14 methylene protons in the parent tropane derivative are known to appear at 2.54 (axial H) and 2.2 (equatorial H) ppm as multiplets. These protons must show *J*-coupling to adjacent C−H-10, which appeared around 3.3 ppm. From the homonuclear correlation, the only signals that showed this coupling were at 1.5 and 2.1 ppm and were assigned to H-14 methylene protons, the latter being the axial proton. This assignment was in clear agreement with the ^{13}C NMR and NOESY data, as well as with the assignment of the resonance at 33.7 ppm as the signal for the methylene C-14 carbon. Since the H-15 appeared at ∼3.3 ppm, the vicinal coupling of H-15 and H-14 protons could not be firmly established. The proton signal assignment of the other diastereomer, Re-TRODAT-1B (**3B**), was also carried out in the same manner. Although the poor solubility of this compound in $CDCl_3$ did not permit the acquisition of a NOESY spectrum, the data obtained for the **3A** could be utilized in some signal assignments.

X-ray structure analyses (Figure 2) of the two isomers confirmed the syn assignments of the nitrogen substituent relative to the rhenium−oxo bond: the bridging methylene substituent, which is attached to the tropyl moiety at the N(2) nitrogen of both complexes, was in a syn-configuration to the metal−oxo center core. The oxo−rhenium cores of both isomers were complexed by the N_2S_2 in a pseudo-square-pyramidal fashion with the oxygen occupying the apical positions. The rhenium atom was located at a distance of 0.384 and 0.365 Å for **3A**,**B**, respectively, above the plane described by the atoms N(1)N(2)S(1) and S(2). The deviation of the plane was 0.267 and 0.221 Å for **3A**,**B**, respectively. Alternatively, the structures could also be described as trigonal bipyramidal, with N(1)S(2)O(1) defining the plane and N(2) and S(1) sitting in the axial positions. The metal atom sat in the defined plane with deviations of 0.044 and 0.040 Å for **3A**,**B**, respectively. Although few X-ray structures with a Tc/Re−N_2S_2 core are known (most are with the tetramethyl-N_2S_2 ligand), the bond distances of both isomers compared favorably with published data and fall within the expected range: 1.703(6) Å for Re═O in **3A** and 1.703(3) Å for **3B** (lit. range, 1.695 and 1.723 Å),[59] 1.920(7) and 2.176(7) Å for Re−N in **3A** and 1.948(4) and 2.200(4) Å in **3B**, the longer bond being the Re−N to the quaternary nitrogen in both cases, indicating the different Re−N bonding modes (Re−amides vs Re−tertiary amines).[60,61] The Re−S bond lengths (Re−S(1) and Re−(S2)), each 2.282(2),(3) Å in **3A** and 2.2942(12) and 2.3003(11) Å in **3B**, were in the expected range for comparable Re−S bond lengths.[23,54]

The diastereotopy of these isomers was introduced with the creation of quaternary nitrogen chiral center of the bisaminodithiol skeleton. As revealed by the X-ray structure determination, the low-polar isomer **3A** had the *R* absolute configuration while the other isomer, **3B**, was in *S* configuration at the quaternary nitrogen center. The absence of an anti-isomer in complex formation could probably be attributed to the steric factors imposed by the large tropyl methyl substituent at the quaternary nitrogen center. The C(8) and C(15) substituents on the tropyl fragment were in the exo configuration as expected, indicating that the stereochemistry of the tropyl moiety was not affected through all chemical manipulations; the methyl group at nitrogen N(3) is directed toward the C_2 methylene bridge.

The [^{99m}Tc]TRODAT-1 complex was prepared by ligand-exchange reaction between free ligand **1** and Tc-glucoheptonate at acidic pH. The diastereomers of [^{99m}Tc]TRODAT-1 were separated on a chiral HPLC column and evaluated in rats. Since the HPLC profiles of Re isomers **3A**,**B** closely resemble those of [^{99m}Tc]-TRODAT-1 peaks A and B, respectively, the structure of each [^{99m}Tc]TRODAT-1 isomer was presumed to be

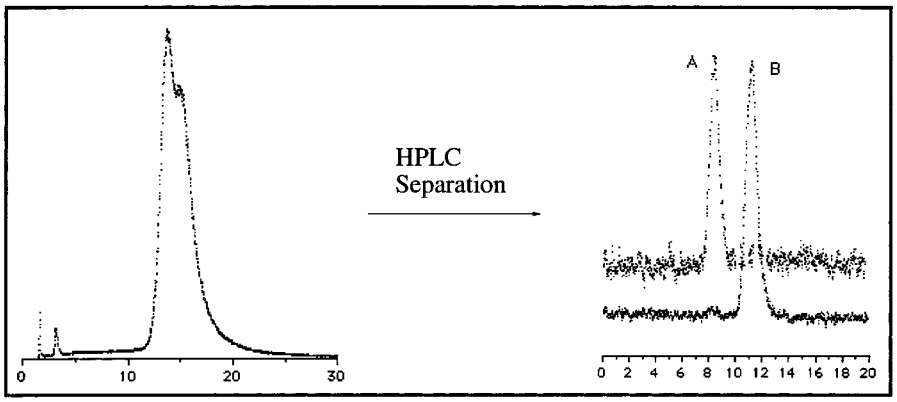


Figure 3. HPLC profile (γ-tracing) obtained by injecting the initial preparation of [^{99m}Tc]TRODAT-1 (diastereomeric mixture) onto a reversed-phase column (PRP-1) (left) eluted with acetonitrile/DMGA buffer (pH 7) in a ratio of 80:20 and a flow rate of 1 mL/min. Afterward the diastereomers were separated by a Chiralpak AD (chiral) column (right) with hexane/EtOH in a ratio of 3:1 and a flow rate of 1 mL/min.

Table 2. Selected Bond Lengths (Å) and Angles (deg) for Re-TRODAT-1A (**3A**) and Re-TRODAT-1B (**3B**)

Re-TRODAT-1A (**3A**)		Re-TRODAT-1B (**3B**)	
Re(1)−O(1)	1.703(6)	Re(1)−O(1)	1.703(3)
Re(1)−N(1)	1.920(7)	Re(1)−N(1)	1.948(4)
Re(1)−N(2)	2.176(7)	Re(1)−N(2)	2.200(4)
Re(1)−S(2)	2.282(2)	Re(1)−S(1)	2.2942(12)
Re(1)−S(1)	2.282(3)	Re(1)−S(2)	2.3003(11)
O(1)−Re(1)−N(1)	120.2(4)	O(1)−Re(1)−N(1)	117.8(2)
O(1)−Re(1)−N(2)	97.3(3)	O(1)−Re(1)−N(2)	98.8(2)
N(1)−Re(1)−N(2)	80.2(3)	N(1)−Re(1)−N(2)	80.1(2)
O(1)−Re(1)−S(2)	117.9(2)	O(1)−Re(1)−S(1)	109.16(13)
N(1)−Re(1)−S(2)	121.1(3)	N(1)−Re(1)−S(1)	82.51(12)
N(2)−Re(1)−S(2)	83.8(2)	N(2)−Re(1)−S(1)	151.62(10)
O(1)−Re(1)−S(1)	107.8(2)	O(1)−Re(1)−S(2)	117.69(13)
N(1)−Re(1)−S(1)	83.0(2)	N(1)−Re(1)−S(2)	123.87(12)
N(2)−Re(1)−S(1)	154.4(2)	N(2)−Re(1)−S(2)	83.83(10)
S(2)−Re(1)−S(1)	88.63(10)	S(1)−Re(1)−S(2)	87.44(4)

Table 3. Brain Uptake of Diastereomers, Peak A (**2A**) or Peak B (**2B**), of [^{99m}Tc]TRODAT-1 in Male Rats

			brain uptake in rats (% dose/organ)		ratio ([ST−CB/CB])[b]	in vitro binding K_i (nM)[c]
Tc complexes	MW	PC[a]	2 min	60 min	60 min	ReOL
diastereomers	539.1	227	0.43	0.12	1.66	14.1 ± 2.08
peak A (**2A**)	539.1	305	0.50	0.21	1.72	13.87 ± 1.73
peak B (**2B**)	539.1	229	0.28	0.12	2.79	8.42 ± 0.67

[a] PC, partition coefficient (*n*-octanol/phosphate buffer, pH 7.4); all compounds were stable in saline for >4 h. [b] [ST−CB]/CB = (percentage dose/g of striatum) minus (percentage dose/g of cerebellum)/(percentage dose/g of cerebellum). [c] In vitro binding assays for ReO complexes were performed in rat striatal homogenates with [^{125}I]IPT as the ligand (K_d = 0.3 nM for dopamine transporters).[65] The ratio of these two complexes appeared to be consistently reproduced (ratio [^{99m}Tc]TRODAT-1A/[^{99m}Tc]TRODAT-1B = 0.75 ± 0.09, n = 25).

similar to the X-ray structure of the corresponding Re isomer (see Tables 1 and 2).

Biodistributions performed on the separated isomers (Figure 3) showed that they have distinctively different properties (Table 3). The more lipophilic isomer, [^{99m}Tc]-TRODAT-1A (**2A**, PC 305), had higher initial brain uptake in rats than [^{99m}Tc]TRODAT-1B (**2B**, PC 229) (0.5% vs 0.28% dose/organ, respectively). After 60 min, the ratios of [ST−CB]/CB were 1.72 and 2.79 for [^{99m}Tc]-TRODAT-1 (**2A**,**B**, respectively). The higher [ST−CB]/CB ratio of isomer B also correlated with the higher binding affinity for dopamine transporters of the corresponding rhenium isomer, as determined in in vitro binding studies (K_i = 13.87 and 8.42 nM for **3A**,**B**, respectively).

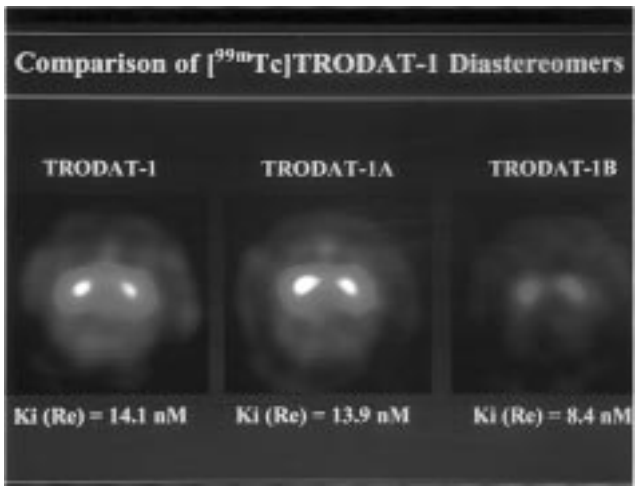


Figure 4. Transaxial SPECT images (2.0 mm thick) of baboon brain at 120−150 min post-iv-injection of 3−10 mCi of [^{99m}Tc]TRODAT-1 (**2**): diastereomeric mixture of **2A**,**B** (left), pure **2A** (middle), and pure **2B** (right). A high accumulation of [^{99m}Tc]TRODAT-1 was observed in caudate and putamen, areas of the brain where dopamine transporters are concentrated.

To further evaluate the potential of [^{99m}Tc]TRODAT-1 as a dopamine transporter imaging agent, a SPECT imaging study was carried out in a female baboon using the diastereomeric [^{99m}Tc]TRODAT-1, [^{99m}Tc]TRODAT-1A (**2A**), and [^{99m}Tc]TRODAT-1B (**2B**) (Figure 4). The SPECT image of **2A** displayed the highest uptake and contrast in caudate and putamen areas, where dopamine transporters are known to be located. It appears that the diastereomeric mixture provided higher quality images which were comparable to those obtained with the pure **2A**. Even though **2B** gave higher [ST−CB]/CB ratios in rats, SPECT images in baboon did not exhibit a better image. Most likely this is due to the lower brain uptake of **2B**, which led to a lower count rate in the brain. These results suggest that higher brain uptake may be more important than a higher target-to-nontarget ratio.

In conclusion, receptor binding assays, biodistribution studies, and imaging studies of the diastereomers of [^{99m}Tc]TRODAT-1 showed the importance of the stereo-

chemistry of these diastereomers in affecting in vivo localization of dopamine transporters. For developing receptor or site-specific imaging agents, it is important to consider the potential mixture of diastereomers as a confounding factor that may influence image interpretation.

Materials and Methods

General. Reagents used in the syntheses were purchased from Aldrich (Milwaukee, WI) or Fluka (Ronkonkoma, NY) and were used without further purification unless otherwise indicated. Anhydrous Na_2SO_4 was used as a drying agent. Reaction yields are reported without attempts at optimization. Thin-layer chromatography was performed on EM Science (Gibbstown, NJ) precoated (0.2 mm) silica gel 60 plates, and the spots were detected with iodine vapor and/or UV light. Silica gel 60 (70−230 mesh), obtained from EM Science, was used for column chromatography. ^{1}H NMR spectra and 2D NMR data were obtained on Bruker spectrometers (Bruker AC 300 and AMX 500, respectively). All samples prepared for NMR analysis were dissolved in $CDCl_3$, purchased from Aldrich. Chemical shifts are reported as δ values with chloroform or TMS as the internal reference. Coupling constants are reported in Hz. The multiplicity is defined by s (singlet), d (doublet), t (triplet), brs (broad signal), dt (doublet of triplet), and m (multiplet). IR spectra were recorded with a Mattson Polaris FT-IR spectrometer and are reported in cm^{-1}. Melting points were determined on a Meltemp apparatus (Cambridge, MA) and are uncorrected. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA). High-resolution mass spectrometry was performed by the Nebraska Center for Mass Spectroscopy, University of Nebraska (Lincoln, NE). $[Bu_4N][ReOCl_4]$ was prepared according to the literature method.[62] The diastereomeric mixture of Re-TRODAT-1 (**3**) was prepared as previously described.[58] Preparative thin-layer chromatography (silica gel) of the mixture of the Re complexes with NH_4OH/MeOH/EtOAc (0.3:0.7:9) separated two compounds which were recrystallized from CH_2Cl_2/MeOH using slow evaporation technique.

Rhenium(V), 2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]amino]ethanethiolato(3−)-oxo-[1*R*-(*exo-exo*)]-; 2*β*-{Oxo[*N*(*R*),*N*′-bis(2-mercaptoethyl)ethylenediaminato]rhenium(V)methyl}-3*β*-(4-chlorophenyl)tropane (Re-TRODAT-1A, 3A):** isolated yield 18%; mp >200 °C; IR (KBr) 944 cm^{-1}; ^{1}H NMR ($CDCl_3$) (500 MHz) δ (ppm) 1.55 (1H, m, H_{14eq}), 1.65 (1H, m, H_{6a}), 1.75 (1H, m, H_{11eq}), 1.9 (1H, m, H_{12eq}), 2.0 (1H, m, H_{14eq}), 2.1−2.5 (7H, m, H_{12ax}, H_{11ax}, H_{8eq}, H_{1a}, N−CH_3), 2.77 (1H, m, H_{2a}), 2.9−3.1 (4H, m, H_{1b}, H_{3a}, H_{5a}, H_{6b}), 3.15−3.35 (5H, m, H_{4a}, H_{7a}, H_9, H_{10}, H_{15a}), 3.5−3.55 (m, 2H, H_{2b}, H_{5b}), 3.7 (dd, 1H, J_1 = 11.1 Hz, J_2 = 5.2 Hz, H_{3b}), 4.05 (dd, 1H, J_1 = 15.2 Hz, J_2 = 6.2 Hz, H_{4b}), 4.8 (dd, 1H, J_1 = 15.2 Hz, J_2 = 6.2 Hz, H_{7b}), 7.2 and 7.36 (d, 2H each, J = 8.4 Hz, aromatic H); ^{13}C NMR ($CDCl_3$) (125 MHz) δ 25.2 (*C*H_2), 26.26 (*C*H_2), 33.72 (*C*H_2), 36.37 (*C*H), 40.19 (*C*H_2), 42.45 (*C*H_3), 42.85 (*C*H), 48.72 (*C*H_2), 58.65 (*C*H_2), 60.03 (*C*H_2), 61.65 (*C*H), 62.20 (*C*H_2), 64.95 (*C*H_2), 67.77 (*C*H), 70.77 (*C*H_2), 128.9 (*C*H), 129.9 (*C*H), 132.7 (*C*), 140.77 (*C*); HRMS (CI) *m*/*z* 627.1154, calcd for $C_{21}H_{31}ON_3S_2ClRe$, $M^+ + 1$, 628.1249.

Rhenium(V), 2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]amino]ethanethiolato(3−)-oxo-[1*R*-(*exo-exo*)]-; 2*β*-{Oxo[*N*(*S*),*N*′-bis(2-mercaptoethyl)ethylenediaminato]rhenium(V)methyl}-3*β*-(4-chlorophenyl)tropane (Re-TRODAT-1B, 3B):** isolated yield 13%; mp >200 °C; IR (KBr) 933 cm^{-1}; ^{1}H NMR ($CDCl_3$) (500 MHz) δ (ppm) 1.5 (2H, m), 1.68 (1H, m), 1.78 (1H, m), 1.9 (1H, m), 2.03 (1H, m), 2.1−2.5 (7H, m), 2.8 (1H, m), 2.9−3.4 (m, 8H), 3.7 (m, 2H), 4.0 (m, 1H), 4.1 (dd, 1H, J_1 = 10.2 Hz, J_2 = 6.1 Hz, H_{5b}), 4.8 (dd, 1H, J_1 = 13.2 Hz, J_2 = 6.5 Hz, H_{7b}), 7.2 and 7.41 (d, 2H each, J = 8.4 Hz, aromatic H); ^{13}C NMR ($CDCl_3$) (125 MHz) δ 25.01, 26.17, 33.67, 35.95, 39.83, 42.08, 42.83, 49.14, 60.23, 61.88, 65.21, 67.93, 70.94, 128.9, 129.06, 130.03, 140.54; HRMS (CI) *m*/*z* 627.1154, calcd for $C_{21}H_{31}ON_3S_2ClRe$, $M^+ + 1$, 628.1249.

Radiolabeling with Tc-99m. The ligand (TRODAT-1; 0.2−0.4 μmol) was dissolved in 100 μL of EtOH and 100 μL of HCl (1 N). HCl (500 μL, 1 N), 1 mL of Sn-glucoheptonate solution (containing 136 μg of $SnCl_2$ and 200 μg of Na-glucoheptonate, pH 6.67), and 50 μL of EDTA solution (0.1 N) were successively added. [^{99m}Tc]Pertechnetate (100−200 μL; ranging from 1 to 20 mCi) in saline solution was then added. The reaction mixture was heated for 30 min at 100 °C (or heated at 121 °C in an autoclave for 30 min), cooled to room temperature, and neutralized with a saturated $NaHCO_3$ solution. After the complex was extracted from the aqueous reaction medium with ethyl acetate (1 × 3, 2 × 1.5 mL) and passed through a small column of Na_2SO_4, the ethyl acetate extracts were condensed under a flow of N_2. The residue was dissolved in 200 μL of EtOH and purified by HPLC (PRP-1 column, 250 × 4.1 mm, CH_3CN/3,3-dimethylglutarate buffer, 5 mM, pH 7, volume ratio 8:2, flow rate 1 mL/min; radiochemical yield 88%, radiochemical purity >97%). The diastereomers were then separated by a Chiralpak AD column eluted with hexane/EtOH in a ratio of 3:1 and a flow rate of 1 mL/min. The retention times for the [^{99m}Tc]TRODAT-1 diastereomers were 8.2 and 11.2 min for peaks A and B, respectively. The complexes displayed in vitro stability for up to 24 h after preparation.

Partition Coefficients. Partition coefficients were measured by mixing each Tc-99m compound with 3 g each of 1-octanol and buffer (pH 7.4, 0.1 M phosphate) in a test tube. The test tube was vortexed for 3 min at room temperature and then centrifuged for 5 min. Two weighed samples (0.5 g each) from the 1-octanol and buffer layers were counted in a well counter. The partition coefficient was determined by calculating the ratio of cpm/g of 1-octanol to that of buffer. Samples from the 1-octanol layer were repartitioned until consistent partition coefficient values were obtained. The measurement was repeated three times.

Biodistribution in Rats. Male Sprague−Dawley rats (225−300 g) were allowed free access to food and water and were used for in vivo biodistribution studies.[63,64] While under ether anesthesia, 0.2 mL of a saline solution containing either the mixture of diastereomers **2A**,**B**, **2A**, or **2B** (50−100 μCi) was injected directly into the femoral vein of rats. The rats were sacrificed by cardiac excision at various time points postinjection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic gamma counter (Packard 5000). The percentage dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Total activities of blood and muscle were calculated under the assumption that they were 7% and 40% of the total body weight, respectively.

Regional brain distribution in rats was obtained after an injection of diastereomers, peak A (**2A**) or peak B (**2B**) of [^{99m}Tc]TRODAT-1. Samples from different brain regions (cortex, striatum, hippocampus, and cerebellum) were dissected, weighed, and counted, and the percentage dose per gram of sample was calculated by comparing the sample counts with the count of the diluted initial dose. The uptake ratio of each region was obtained by dividing the percentage dose per gram of that region by that of the cerebellum. The rats were dissected, and brain tissue samples were counted as described above. The specific uptake of the compound was expressed as the ratio of [ST−CB]/CB ((percentage dose/g of striatum) minus (percentage dose/g of cerebellum) divided by (percentage dose/g of cerebellum)).

SPECT Imaging in a Baboon. A baboon (~15 kg) was the subject of a SPECT imaging study using the diastereomers, peak A (**2A**) or peak B (**2B**), of [^{99m}Tc]TRODAT-1. Prior to imaging, the animal was fasted, immobilized with ketamine (10−20 mg/kg, im) and xylazine (2−3 mg/kg, im), intubated, and maintained on a 1.5−2.0% isofluorane/98.5% oxygen mixture (flow rate of 200−500 cm^3/min). The animal was injected with glycopyrrolate (10 μg/kg, sc), an anticholinergic

drug that does not cross the blood−brain barrier, to decrease digestive and respiratory secretions. Body temperature was maintained using a hot water circulating heating pad and was monitored with a rectal thermometer. The animal's head was immobilized using a vacuum-packed bean-bag device that hardens upon evacuation when molded around the head. No-carrier-added [^{99m}Tc]TRODAT-1 (mixture of **2A**,**B**, pure **2A**, or pure **2B**; 3−6 mCi) was administered as an iv bolus in the saphenous vein of the baboon. Immediately after injection, sequential 5 min/frame dynamic SPECT scans were acquired on a triple-headed Picker Prism 3000 camera (fwhm: 7 mm) equipped with fan beam collimators for 2 h. The acquisition parameters were a 20% energy window at 140 keV, 120 projection angles over 360°, a 128 × 128 matrix, and a zoom factor of 1.78 in a slice thickness of 2 mm cubic voxels. The projection data were reconstructed with a count-dependent 3-D Wiener filter. Chang's first-order correction method was used to compensate for attenuation. The image was reformatted to display transaxial sections for each study.

Crystallographic Structural Determination. Crystal, data collection, and refinement parameters are given in Table 1. Suitable crystals were selected and mounted on the top of a glass fiber with epoxy cement. The systematic absences in the diffraction data are uniquely consistent with the orthorhombic space group, $P2_12_12_1$. Empirical corrections for absorptions were applied to the data. The structures were solved using direct methods, completed by subsequent difference Fourier syntheses, and refined by full-matrix least-squares procedures. An empirical absorption correction was applied. The absolute configurations of the structures have been determined (Flack parameter = −0.002(13) for **3A** and 0.012(7) for **3B**). All non-hydrogen atoms were refined with anisotropic displacement coefficients, and hydrogen atoms were treated as idealized contributions. Two peaks on the final difference map of **3B** (1.25−1.92 *e*/Å^3) remained but were in chemically unreasonable positions (0.91−1.02 Å from Re) and were considered noise. All software and sources of the scattering factors are contained in the SHELXTL (5.03) program library (G. Sheldrick, Siemens XRD, Madison, WI).

Acknowledgment. The authors thank Dr. George Furst for assistance with the NMR (TOCSY, etc.) experiments of the Re compounds and Ms. Susan West for editorial assistance. This work was supported by grants awarded by the National Institutes of Health (to H.F.K., DOE-ER61657, NS24538, and NS18509). The University of Delaware acknowledges the National Science Foundation for their support of the purchase of the CCD-based diffractometer (CHE-9628768).

References

(1) Jurisson, S. S.; Berning, D.; Jia, W.; Ma, D.-S. Coordination compounds in nuclear medicine. *Chem. Rev. (Washington, D.C.)* **1993**, *93*, 1137−1156.
(2) Steigman, J.; Eckelman, W. C. *The Chemistry of Technetium in Medicine*; National Academy: Washington, DC, 1992.
(3) Tisato, F.; Refosco, F.; Bandoli, G. Structural survey of technetium complexes. *Coord. Chem. Rev.* **1994**, *135/136*, 325−397.
(4) Davison, A.; Jones, A. G.; Orvig, C.; Sohn, M. A new class of oxotechnetium(+5) chelate complexes containing a $TcON_2S_2$ core. *Inorg. Chem.* **1981**, *20*, 1629−1632.
(5) Mahmood, A.; Halpin, W. A.; Baidoo, K. E.; Sweigart, D. A.; Lever, S. Z. Structure of a neutral N-alkylated diaminedithiol (DADT) $^{99}Tc^{V}$ complex: syn[^{99}TcO(NET-TMDADT)]. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1991**, *47*, 254−257.
(6) Lever, S. Z.; Burns, H. D.; Kervitsky, T. M.; Goldfarb, H. W.; Woo, D. V.; Wong, D. F.; Epps, L. A.; Kramer, A. V.; Wagner, H. N., Jr. Design, preparation and biodistribution of a technetium-99m triaminedithiol complex to access regional cerebral blood flow. *J. Nucl. Med.* **1985**, *26*, 1287−1294.
(7) Francesconi, L. C.; Graczyk, G.; Wehrli, S.; Shaikh, S. N.; McClinton, D.; Liu, S.; Zubieta, J.; Kung, H. F. Synthesis and characterization of neutral $M^{V}O$ (M = Tc, Re) amine-thiol complexes containing a pendent phenylpiperidine group. *Inorg. Chem.* **1993**, *32*, 3114−3124.
(8) Lever, S. Z.; Baidoo, K. E.; Mahmood, A. Structure proof of syn/anti isomerism in N-alkylated diaminedithiol (DADT) complexes of technetium. *Inorg. Chim. Acta* **1990**, *176*, 183−184.
(9) Wolff, J. A. Synthesis and study of technetium(V) complexes of tetradentate nitrogen-,sulfur-donor ligands. Thesis, Massachusetts Institute of Technology, 1992.
(10) Eckelman, W. C. Designing a molecular probe for muscarinic acetylcholine-receptor (mAChR) imaging. *J. Nucl. Med.* **1995**, *36*, S5−S7.
(11) Lever, S. Z.; Baidoo, K. E.; Mahmood, A.; Matsumura, K.; Scheffel, U. A.; Wagner, H. N., Jr. Novel technetium ligands with affinity for the muscarinic cholinergic receptor. *Nucl. Med. Biol.* **1994**, *21*, 157−164.
(12) del Rosario, R. B.; Jung, Y.-W.; Baidoo, K. E.; Lever, S. Z.; Wieland, D. M. Synthesis and in vivo evaluation of a [$^{99m/99}$Tc]-DADT-benzovesamicol: a potential marker for cholinergic neurons. *Nucl. Med. Biol.* **1994**, *21*, 197−203.
(13) Johannsen, B.; Scheunemann, M.; Spies, H.; Brust, P.; Wober, J.; Syhre, R.; Pietzsch, H.-J. Technetium(V) and rhenium(V) complexes for 5-HT_{2A} serotonin receptor binding: structure-affinity considerations. *Nucl. Med. Biol.* **1996**, *23*, 429−438.
(14) Samnick, S.; Brandau, W.; Sciuk, J.; Steinsträsser, A.; Schober, O. Synthesis, characterization and biodistribution of neutral and lipid-soluble [^{99m}Tc]bisaminoethanethiol spiperone derivatives: possible ligands for receptor imaging with SPECT. *Nucl. Med. Biol.* **1995**, *22*, 573−583.
(15) Baidoo, K. E.; Zhan, Y.; Lin, K.-S.; Finley, P.; Lever, S. Z.; Scheffel, U. Design and synthesis of new neutral technetium diaminedithiolate complexes that bind with high affinity to serotonin (5-HT) receptors. *J. Nucl. Med.* **1997**, *38*, 100P.
(16) Chumpradit, S.; Kung, M.-P.; Mu, M.; Hou, C.; Kung, H. F. Synthesis and characterization of a [^{99m}Tc]SCH23390 derivative as a selective dopamine D_1 receptor imaging agent. *Proceedings of the XIIth International Symposium on Radiopharmacol Chemistry*, Uppsala, Sweden, 1997; Abstract VI-14, pp 437−438.
(17) Lever, S. Z.; Kao, C.-H. K.; Scheffel, U.; Baidoo, K. E. Technetium labeled analogues of D_2 receptor binding benzamides [abstract]. *J. Nucl. Med.* **1997**, *38*, 99P.
(18) Eisenhut, M.; Brandau, W.; Missfeldt, M. Synthesis and in vivo testing of a bromobutyl substituted 1,2-dithia-5,9-diazacycloundecane: a vesatile precursor for new 99m-Tc-bis(aminoethanethiol) complexes. *Nucl. Med. Biol.* **1989**, *16*, 805−811.
(19) Chi, D. Y.; O'Neil, J. P.; Anderson, C. J.; Welch, M. J.; Katzenellenbogen, J. A. Homodimeric and heterodimeric bis(aminothiol) oxometal complexes with rhenium(V) and technetium(V). Control of heterodimeric complex formation and an approach to metal complexes that mimic steroid hormones. *J. Med. Chem.* **1994**, *37*, 928−937.
(20) Chi, D. Y.; Katzenellenbogen, J. A. Selective formation of heterodimeric bis-bidentate aminothiol-oxometal complexes of rhenium(V). *J. Am. Chem. Soc.* **1993**, *115*, 7045−7046.
(21) DiZio, J. P.; Fiaschi, R.; Davison, A.; Jones, A. G.; Katzenellenbogen, J. A. Progestin−rhenium complexes: metal-labeled steroids with high receptor binding affinity, potential receptor-directed agents for diagnostic imaging or therapy. *Bioconjugate Chem.* **1991**, *2*, 353−366.
(22) DiZio, J. P.; Anderson, C. J.; Davison, A.; Ehrhardt, G. J.; Carlson, K. E.; Welch, M. J.; Katzenellenbogen, J. A. Technetium- and rhenium-labeled progestins: synthesis, receptor binding and in vivo distribution of an 11-β-substituted progestin labeled with technetium-99 and rhenium-186. *J. Nucl. Med.* **1992**, *33*, 558−569.
(23) O'Neil, J. P.; Wilson, S. R.; Katzenellenbogen, J. A. Preparation and structural characterization of monoamine-monoamide bis(thiol) oxo complexes of technetium(V) and rhenium(V). *Inorg. Chem.* **1994**, *33*, 319−323.
(24) O'Neil, J. P.; Carlson, K. E.; Anderson, C. J.; Welch, M. J.; Katzenellenbogen, J. A. Progestin radiopharmaceuticals labeled with technetium and rhenium: synthesis, binding affinity, and in vivo distribution of a new progestin N_2S_2-metal conjugate. *Bioconjugate Chem.* **1994**, *5*, 182−193.
(25) Hom, R. K.; Chi, D. Y.; Katzenellenbogen, J. A. Heterodimeric bis(amino thiol) complexes of oxorhenium(V) that mimic the structure of steroid hormones. Synthesis and stereochemical issues. *J. Org. Chem.* **1996**, *61*, 2624−2631.
(26) Sugano, Y.; Katzenellenbogen, J. A. Synthesis of tetradentate bisamino-bisthiol complexes of oxorhenium(V) as structural mimics of steroids. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 361−366.

(27) Hom, R. K.; Katzenellenbogen, J. A. Technetium-99m-labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results [review]. *Nucl. Med. Biol.* **1997**, *24*, 485−498.
(28) Jurisson, S. S.; Pirro, J.; Di Rocco, R. J.; Rosenspire, K. C.; Jagoda, E.; Nanjappan, P.; Eckelman, W. C.; Nowotnik, D. P.; Nunn, A. D. Boronic acid adducts of technetium dioxime (BATO) complexes derived from quinuclidine benzilate (QNB) boronic acid stereoisomers: syntheses and studies of their binding to the muscarinic acetylcholine receptor. *Nucl. Med. Biol.* **1995**, *22*, 269−281.
(29) Carroll, F. I.; Scheffel, U.; Dannals, R. F.; Boja, J. W.; Kuhar, M. J. Development of imaging agents for the dopamine transporter. *Med. Res. Rev.* **1995**, *15*, 419−444.
(30) Carroll, F. I.; Mascarella, S. W.; Kuzemko, M. A.; Gao, Y.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Synthesis, ligand binding, and QSAR (CoMFA and classical) study of 3β-(3′-substituted phenyl)-, 3β-(4′-substituted phenyl)-, and 3β-(3′,4′-disubstituted phenyl)tropane-2β-carboxylic acid methyl esters. *J. Med. Chem.* **1994**, *37*, 2865−2873.
(31) Carroll, F. I.; Kotian, P.; Dehghani, A.; Gray, J. L.; Kuzemko, M. A.; Parham, K. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. Cocaine and 3β-(4′-substituted phenyl)tropane-2β-carboxylic acid ester and amide analogues. New high-affinity and selective compounds for the dopamine transporter. *J. Med. Chem.* **1995**, *38*, 379−388.
(32) Yu, D.-W.; Gatley, S. J.; Wolf, A. P.; MacGregor, R. R.; Dewey, S. L.; Fowler, J. S.; Schlyer, D. J. Synthesis of carbon-11-labeled iodinated cocaine derivatives and their distribution in baboon brain measured using positron emission tomography. *J. Med. Chem.* **1992**, *35*, 2178−2183.
(33) Fowler, J. S.; Volkow, N. D.; MacGregor, R. R.; Logan, J.; Dewey, S. L.; Gatley, S. J.; Wolf, A. P. Comparative PET studies of the kinetics and distribution of cocaine and cocaethylene in baboon brain. *Synapse* **1992**, *12*, 220−227.
(34) Frost, J. J.; Rosier, A. J.; Reich, S. G.; Smith, J. S.; Ehlers, M. D.; Snyder, S. H.; Ravert, H. T.; Dannals, R. F. Positron emission tomographic imaging of the dopamine transporter with [^{11}C]-WIN 35,428 reveals marked declines in mild Parkinson's disease [see comments]. *Ann. Neurol.* **1993**, *34*, 423−431.
(35) Wong, D. F.; Yung, B.; Dannals, R. F.; Shaya, E. K.; Ravert, H. T.; Chen, C. A.; Chan, B.; Folio, T.; Scheffel, U.; Ricaurte, G. A.; Neumeyer, J. L.; Wagner, H. N., Jr.; Kuhar, M. J. In vivo imaging of baboon and human dopamine transporters by positron emission tomography using [^{11}C]WIN35,428. *Synapse* **1993**, *15*, 130−142.
(36) Ding, Y. S.; Fowler, J. S.; Volkow, N. D.; Logan, J.; Gatley, S. J.; Sugano, Y. Carbon-11-*d*-threo-methylphenidate binding to dopamine transporter in baboon brain. *J. Nucl. Med.* **1995**, *36*, 2298−2305.
(37) Innis, R. B.; Seibyl, J. P.; Scanley, B. E.; Laruelle, M.; Abi-Dargham, A.; Wallace, E.; Baldwin, R. M.; Zea-Ponce, Y.; Zoghbi, S.; Wang, S.; Gao, Y.; Neumeyer, J. L.; Charney, D. S.; Hoffer, P. B.; Marek, K. L. Single photon emission computed tomographic imaging demonstrates loss of striatal dopamine transporters in Parkinson's disease. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11965−11969.
(38) Innis, R. B. Single photon emission tomography imaging of dopamine terminal innervation: a potential clinical tool in Parkinson's disease [editorial, review]. *Eur. J. Nucl. Med.* **1994**, *21*, 1−5.
(39) Seibyl, J. P.; Laruelle, M. A.; Van Dyck, C. H.; Wallace, E.; Baldwin, R. M.; Zoghbi, S. S.; Zea-Ponce, Y.; Neumeyer, J. L.; Charney, D. S.; Hoffer, P. B.; Innis, R. B. Reproducibility of iodine-123-β-CIT SPECT brain measurement of dopamine transporters. *J. Nucl. Med.* **1996**, *37*, 222−228.
(40) Kuikka, J. T.; Akerman, K.; Bergstrom, K. A.; Karhu, J.; Hiltunen, J.; Haukka, J.; Heikkinen, J.; Tiihonen, J.; Wang, S.; Neumeyer, J. L. Iodine-123-labeled N-(2-fluoroethyl)-2β-carbomethoxy-3β-(4-iodophenyl)nortropane for dopamine transporter imaging in the living human brain. *Eur. J. Nucl. Med.* **1995**, *22*, 682−686.
(41) Neumeyer, J. L.; Wang, S.; Gao, Y.; Milius, R. A.; Kula, N. S.; Campbell, A.; Baldessarini, R. J.; Zea-Ponce, Y.; Baldwin, R. M.; Innis, R. B. N-ω-Fluoroalkyl analogues of (1*R*)-2β-carbomethoxy-3β-(4-iodophenyl)-tropane (β-CIT): radiotracers for positron emission tomography and single photon emission computed tomography imaging of dopamine transporters. *J. Med. Chem.* **1994**, *37*, 1558−1561.
(42) Goodman, M. M.; Kung, M.-P.; Kabalka, G. W.; Kung, H. F.; Switzer, R. Synthesis and characterization of radioiodinated N-(3-iodopropen-1-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropanes: potential dopamine reuptake site imaging agents. *J. Med. Chem.* **1994**, *37*, 1535−1542.
(43) Malison, R. T.; Vessotskie, J. M.; Kung, M.-P.; McElgin, W.; Romaniello, G.; Kim, H.-J.; Goodman, M. M.; Kung, H. F. Striatal dopamine transporter imaging in nonhuman primates with iodine-123-IPT SPECT. *J. Nucl. Med.* **1995**, *36*, 2290−2297.
(44) Mozley, P. D.; Stubbs, J. B.; Kim, H.-J.; McElgin, W.; Kung, M.-P.; Meegalla, S.; Kung, H. F. Dosimetry of an iodine-123-labeled tropane to image dopamine transporters. *J. Nucl. Med.* **1996**, *37*, 151−159.
(45) Tatsch, K.; Schwarz, J.; Mozley, P. D.; Linke, R.; Pogarell, O.; Oertel, W. H.; Fieber, R. S.; Hahn, K.; Kung, H. F. Relationship between clinical features of Parkinson's disease and presynaptic dopamine transporter binding assessed with [^{123}I]IPT and single-photon emission tomography. *Eur. J. Nucl. Med.* **1997**, *24*, 415−421.
(46) Abi-Dargham, A.; Gandelman, M. S.; DeErausquin, G. A.; Zea-Ponce, Y.; Zoghbi, S. S.; Baldwin, R. M.; Laruelle, M.; Charney, D. S.; Hoffer, P. B.; Neumeyer, J. L.; Innis, R. B. SPECT imaging of dopamine transporters in human brain with iodine-123-fluoroalkyl analogues of β-CIT. *J. Nucl. Med.* **1996**, *37*, 1129−1133.
(47) Neumeyer, J. L.; Tamagnan, G.; Wang, S.; Gao, Y.; Milius, R. A.; Kula, N. S.; Baldessarini, R. J. N-substituted analogues of 2β-carbomethoxy-3β-(4′-iodophenyl)tropane (β-CIT) with selective affinity to dopamine or serotonin transporters in rat forebrain. *J. Med. Chem.* **1996**, *39*, 543−548.
(48) Fischman, A. J.; Babich, J. W.; Elmaleh, D. R.; Barrow, S. A.; Meltzer, P.; Hanson, R. N.; Madras, B. K. SPECT imaging of dopamine transporter sites in normal and MPTP-treated rhesus monkeys. *J. Nucl. Med.* **1997**, *38*, 144−150.
(49) Zoghbi, S. S.; Tamagnan, G.; Baldwin, R. M.; Gao, Y.; Neumeyer, J. L.; Baldessarini, R.; Charney, D. S.; Seibyl, J. P.; Innis, R. B. Synthesis of a dopamine transporter binding cyclopentadiene phenyltropane conjugate complexed with Re and Tc-99m. *J. Nucl. Med.* **1997**, *38*, 100P.
(50) Madras, B. K.; Jones, A. G.; Mahmood, A.; Zimmerman, R. E.; Garada, B.; Holman, B. L.; Davison, A.; Blundell, P.; Meltzer, P. C. Technepine: a high affinity technetium-99m probe to label the dopamine transporter in brain by SPECT imaging. *Synapse* **1996**, *22*, 239−246.
(51) Tamagnan, G.; Gao, Y. G.; Baldwin, R. M.; Zoghbi, S. S.; Neumeyer, J. L. Synthesis of beta-CIT-BAT, a potential Tc-99m imaging ligand for dopamine transporter. *Tetrahedron Lett.* **1996**, *37*, 4353−4356.
(52) Aronson, B.; Enmon, J. L.; Izenwasser, S.; Katz, J. L.; Kelkar, S. V.; Luo, L.; Nolan, S. P.; Trudell, M. L. Synthesis and ligand binding of η^6-(2β-carbomethoxy-3β-phenyltropane) transition metal complexes. *J. Med. Chem.* **1996**, *39*, 1560−1563.
(53) Meltzer, P. C.; Blundell, P.; Jones, A. G.; Mahmood, A.; Garada, B.; Zimmerman, R. E.; Davison, A.; Holman, B. L.; Madras, B. K. A technetium-99m SPECT imaging agent which targets the dopamine transporter in primate brain. *J. Med. Chem.* **1997**, *40*, 1835−1844.
(54) Meegalla, S. K.; Plössl, K.; Kung, M.-P.; Stevenson, D. A.; Liable-Sands, L. M.; Rheingold, A. L.; Kung, H. F. First example of a Tc-99m complex as a dopamine transporter imaging agent. *J. Am. Chem. Soc.* **1995**, *117*, 11037−11038.
(55) Meegalla, S. K.; Plössl, K.; Kung, M.-P.; Chumpradit, S.; Stevenson, D. A.; Kushner, S. A.; McElgin, W. T.; Mozley, P. D.; Kung, H. F. Synthesis and characterization of Tc-99m labeled tropanes as dopamine transporter imaging agents. *J. Med. Chem.* **1997**, *40*, 9−17.
(56) Meegalla, S. K.; Plössl, K.; Kung, M.-P.; Mu, M.; Hou, C.; Kung, H. F. Structure−activity relationship of [^{99m}Tc]TRODAT-1 derivatives as new dopamine transporter imaging agents. *Proceedings of the XIIth International Symposium on Radiopharmacol Chemistry*, Uppsala, Sweden, 1997; Abstract VI-13, pp 434−436.
(57) Kung, H. F.; Kim, H.-J.; Kung, M.-P.; Meegalla, S. K.; Plössl, K.; Lee, H.-K. Imaging of dopamine transporters in humans with technetium-99m TRODAT-1. *Eur. J. Nucl. Med.* **1996**, *23*, 1527−1530.
(58) Meegalla, S. K.; Plössl, K.; Kung, M.-P.; Chumpradit, S.; Stevenson, D. A.; Frederick, D.; Kung, H. F. Tc-99m labeled tropanes as dopamine transporter imaging agents. *Bioconjugate Chem.* **1996**, *7*, 421−429.
(59) Orpen, A. G.; Brammer, L.; Allen, F. H.; Kennar, O.; Watson, D. G.; Taylo, R. Tables of bond lengths determined by X-ray and neutron diffraction. Part 2. Organometallic compounds and coordination complexes of the *d*- and *f*-block metals. *J. Chem. Soc., Dalton Trans.* **1989**, S1−S83.
(60) O'Neill, J. P.; Wilson, S. R.; Katzenellenbogen, J. A. Preparation and structural characterization of monoamine-monoamide bis-(thiol) oxo complexes of technetium(V) and rhenium(V). *Inorg. Chem.* **1994**, *33*, 319−323.

(61) Lipowska, M.; Hayes, B. L.; Hansen, L.; Taylor, A. J.; Marzilli, L. G. Rhenium(V) oxo complexes of novel N_2S_2 dithiourea (DTU) chelate ligands: synthesis and structural characterization. *Inorg. Chem*. **1996**, *35*, 4227−4231.

(62) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Herrmann, W. A.; Artus, G.; Abram, U.; Kaden, T. A. Metal carbonyl syntheses XXII. Low-pressure carbonylation of $[MOCl_4]^-$ and $[MO_4]^-$: the technetium(I) and rhenium(I) complexes $[NEt_4]_2[MCl_3(CO)_3]$. *J. Organomet. Chem*. **1995**, *493*, 119−127.

(63) Kung, H. F.; Molnar, M.; Billings, J. J.; Wicks, R.; Blau, M. Synthesis and biodistribution of neutral lipid-soluble Tc-99m complexes that cross the blood-brain barrier. *J. Nucl. Med*. **1984**, *25*, 326−332.

(64) Kung, H. F.; Yu, C. C.; Billings, J. J.; Molnar, M.; Blau, M. Synthesis of new bis-aminoethanethiol (BAT) derivatives: possible ligands for Tc-99m brain imaging agents. *J. Med. Chem*. **1985**, *28*, 1280−1284.

(65) Kung, M.-P.; Essman, W. D.; Frederick, D.; Meegalla, S.; Goodman, M.; Mu, M.; Lucki, I.; Kung, H. F. IPT: a novel iodinated ligand for the CNS dopamine transporter. *Synapse* **1995**, *20*, 316−324.

JM970742B