First Example of a ^{99m}Tc Complex as a Dopamine Transporter Imaging Agent

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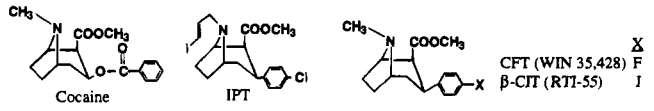
Radiolabeled dopamine transporter ligands are useful tools for in vivo and in vitro studies of Parkinson's disease (PD), which is characterized by a selective loss of dopamine neurons in the basal ganglia and substantia nigra areas of the brain. In vivo imaging of dopamine transporters, using radiolabeled cocaine analogs [^{11}C]CFT, [^{123}I]-β-CIT and [^{123}I]IPT, provides a powerful tool for studying their function in normal or disease states.[1−3] However, carbon-11 and iodine-123 are cyclotron-



produced radionuclides, which are expensive and are not readily available, unlike technetium-99m[4,5] ($t_{1/2}$ = 6 h, γ-ray 140 keV), which is the most commonly used radionuclide for diagnostic nuclear medicine. A comparable agent that could be labeled with ^{99m}Tc would be highly desirable for routine clinical study.

Several recent reports demonstrate that it is possible to incorporate [Tc^{V}O]$^{3+}$ 2,2′-iminobis[ethanethiol] complexes into potential receptor-selective imaging agents for muscarinic receptors,[6] vesamicol sites,[7] and steroid hormone receptors.[8−13] Surprisingly, these ^{99m}Tc steroid analog complexes[8,9] are neutral, lipophilic, and quite stable in vivo and in vitro, properties essential for any brain imaging agent to pass through the intact blood−brain barrier. Recently, new mixed ligands, consisting of iminobis[ethanethiols] and monothiols that form neutral Tc complexes with high brain uptake and retention in animals, were reported.[14−16] To develop ^{99m}Tc-based dopamine transporter imaging agents, we have investigated a novel series of *N*-ethanethiol tropane complexes containing a neutral Tc^{V}O iminobis[ethanethiol] unit and the monothiol complex moiety. [^{99m}Tc]-**4** is the first example of a ^{99m}Tc complex as a potential dopamine transporter imaging agent, reported herein.

Synthesis of an *N*-ethanethiol derivative of tropane **2** was achieved by alkylating the nortropane derivative **1** with 2-tritylthioethyl bromide, followed by removal of the trityl group of the resulting product with Hg(OAc)$_2$. The tridentate aminodithiol ligand **3** was synthesized according to the literature.[17] Radiolabeling with ^{99m}Tc was carried out by reacting the mixed ligands **2b** and **3** with sodium [^{99m}Tc]pertechnetate (no carrier added) in the presence of tin(II) glucoheptonate at room temperature. The labeled compound [^{99m}Tc]-**4** was purified by HPLC (40% radiochemical yield, radiochemical purity > 95%).[18] The complex is quite stable; after 4 h at room temperature, the radiochemical purity was >95%. The lipophilicity, as measured by partition coefficient in *n*-octanol/pH 7.0 buffer, was 307. The characterization of ^{99m}Tc-labeled compounds is often done with ^{99}Tc, a β emitter ($t_{1/2}$ = 2.1 × 10^5 years). However, ^{99}Tc requires an extensive setup approved for handling long-lived β-emitters, which is unavailable at this time. Since the chemistry of Tc and Re are quite similar, the corresponding Re-**4** (rhenium, [methyl-3-(4-chlorophenyl)-8-(2-mercaptoethyl)-8-azabicyclo[3.2.1]octane-2-carboxylato-*S*][[2,2′-(methylimino)-bis[ethanethiolato]](2−)-*N*,*S*,*S*′]oxo) was prepared as a surrogate molecule for more detailed chemical characterization.

Formation of the Re complex, Re-**4**, was carried out as previously reported.[15,16] A CH_3CN solution of *N*-(mercaptoethyl)tropane derivative **2b** and iminobis[ethanethiol] **3** in 1.5:1 molar ratio was added to a methanolic solution of $Bu_4NReOCl_4$ under argon at 0 °C, in the presence of Et_3N as an acid scavenger, to produce the Re-**4** as dark green crystals, in 53% isolated yield (Scheme 1).[19] X-ray crystallography of the mixed-ligand complex Re-**4** displayed an expected square pyramidal structure with the Re=O at the apex and an *N*-methyl group at the anti position to the Re=O functionality (Figure 1).[20] The compounds [^{99m}Tc]-**4** and Re-**4** behaved similarly under identical HPLC conditions (coinjection). On a C-18 column (Partisil 10-ODS-3, 250 mm × 4.6 mm) with MeOH/NH_4HCO_3 (0.1 M, pH 7.0, ratio 8:2) as eluent, the retention times were 14.9 and

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(18) Purified by HPLC on a 250 mm × 4.1 mm PRP-1 column eluted with acetonitrile/5 mM dimethylglutaric acid buffer, pH 7 (80:20); flow rate, 1 mL/min; retention time, 16.6 min.

(19) FT-IR: Re=O 960 cm^{-1}. $C_{22}H_{32}O_3N_2S_3ClRe$ high-resolution mass spectra: *m/z* 690.0822; M$^+$ + 1 = 691.0906. Anal. Calcd: C, 38.26; H, 4.63; N, 4.05. Found: C, 38.38; H, 4.62; N, 4.05.

(20) Crystal data for Re-**4**: $C_{22}H_{32}ClN_2O_3ReS_3$, fw = 690.3, orthorhombic, $P2_12_12_1$, a = 6.5260(10), b = 13.690(2), and c = 29.160(6) Å, V = 2607.9(8) Å^3, Z = 4, D_x = 1.758 g cm^{-3}, μ(Mo Kα) = 50.28 cm^{-1}, T = 296 K. Of 4837 data collected ($2\theta_{max}$ = 58°), 2344 were independent and observed ($4\sigma F_o$). The ethylene bridges formed by carbon atoms 1, 2, 4, and 5 are disordered over two equally occupied sites. The pairing chosen for the figure is arbitrary. All ordered non-hydrogen atoms were anisotropically refined. $R(F)$ = 3.33 and $R(F)_w$ = 3.73.

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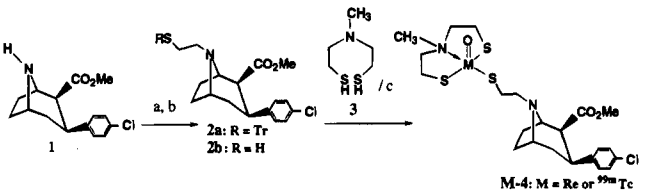
Scheme 1[a]



[a] Conditions: (a) $BrCH_2CH_2STr$/KI/dioxane/reflux. (b) (i) $Hg(OAc)_2$/CF_3COOH; (ii) H_2S/EtOH. (c) (i) $Bu_4N^+ReOCl_4^-$/CH_3CN/MeOH/RT/12 h or (ii) $Na^{99m}TcO_4$/Sn-glucoheptonate.

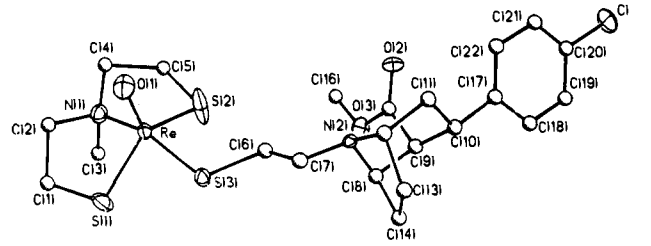


Figure 1. Molecular structure and labeling diagram for Re-**4**. Thermal ellipsoids are drawn at the 35% probability level. Bond distances (Å) and angles (deg): Re−S(1), 2.269(4); Re−S(2), 2.269(4); Re−S(3), 2.291(4); Re−O(1), 1.688(11); Re−N(1), 2.227(11); S(1)−Re−S(2), 128.8(2); S(1)−Re−S(3), 84.4(2); S(1)−Re−O(1), 116.6(5); S(1)−Re−N(1); 82.7(3); S(2)−Re−S(3), 90.7(2); S(2)−Re−O(1), 113.8(5); S(2)−Re−N(1), 81.6(3); S(3)−Re−O(1), 105.6(4); S(3)−Re−N(1); 155.8(3); N(1)−Re−O(1), 98.5(4).

15.3 min for [^{99m}Tc]-**4** and Re-**4**, respectively. On a PRP-1 column (Hamilton, 250 mm × 4.1 mm) with CH_3CN/dimethyl glutarate buffer (5 mM, pH 7.0, ratio 9:1) as eluent, the retention times were 10.2 and 8.9 min for [^{99m}Tc]-**4** and Re-**4**, respectively. An in vitro binding study in rat striatal membrane homogenates using a comparable compound, [^{125}I]IPT, as the radioligand showed that Re-**4** has an excellent binding affinity. The inhibition constant (K_i) of Re-**4** was 0.31 ± 0.03 nM, which was comparable to the equilibrium binding constant (K_d) of the radioligand, [^{125}I]IPT (K_d = 0.2 nM).[21]

The in vivo biodistribution of [^{99m}Tc]-**4** was evaluated in male Sprague-Dawley rats. The brain uptake of the complex was relatively low (0.1% dose at 2 min post-iv injection, compared to 1.0% dose at 2 min for [^{123}I]IPT). However, the ^{99m}Tc complex displayed specific uptake in the striatum, where dopamine transporters are highly concentrated (striatum/cerebellum ratio = 3.5 at 60 min postinjection; the cerebellum area contains no dopamine transporters and is used as the background region). The ratio of ST/CB observed with this complex is lower than those for other reported dopamine transporter ligands (16 and 8 for IPT and RTI-55, respectively).[3,22] The specific uptake of the complex in striatum can be blocked (ST/CB close to 1.0) by pretreating the rats with a dose of β-CIT (1 mg/kg iv, 5 min prior to injection of [^{99m}Tc]-**4**), a dopamine transporter ligand, but not with a dose of haloperidol (1 mg/kg iv), an agent with a mixed pharmacological profile (binding to various CNS receptors but not to dopamine transporters).

In conclusion, the results presented indicate that complex **4**, a ^{99m}Tc-labeled mixed ligand consisting of iminobis[ethanethiol] and monothiol, can be prepared, and it displays dopamine transporter specific brain uptake in rats after an iv injection. The specific uptake in the striatum can be blocked by a specific dopamine transporter ligand. This is the first example of a ^{99m}Tc complex which displays selective dopamine transporter binding. Further studies are warranted to fully characterize this new ^{99m}Tc complex, which may be very important as a tool for early detection of Parkinson's disease.

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Supporting Information Available: Characterization data for Re-**4**, including X-ray structure determination summary and tables of atomic coordinates, isotropic and anisotropic displacement coefficients, and bond lengths and angles, as well as experimental details for synthesis of compounds **1**−**4** (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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