Technetium-99m *N,N'*-Bis(2-mercapto-2-methylpropyl)-2-aminobenzylamine: Technetium-99m Complexes of a Novel Bis(aminoethanethiol) Ligand

Lynn C. Francesconi,† Yun Y. Yang, Mei-Ping Kung, Xiao X. Zhang, Jeffrey J. Billings, Yu-Zhi Guo, and Hank F. Kung*

Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and Department of Chemistry, Hunter College of the City University of New York, New York City, New York 10021

Received April 14, 1994®

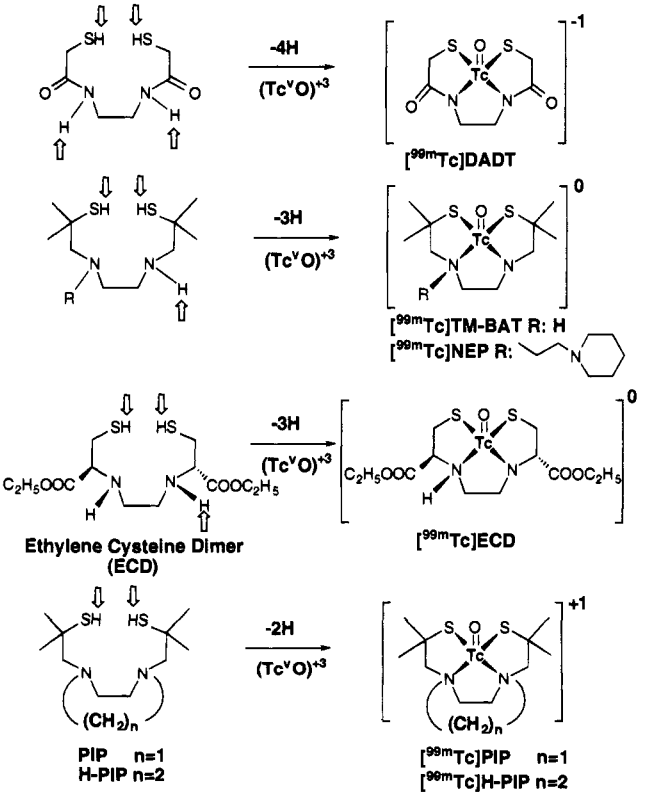
A new N_2S_2 ligand system, *N,N'*-bis(2-mercapto-2-methylpropyl)-2-aminobenzylamine, U-BAT, **1**, containing uneven amine groups (two amine groups with different pK_a values) for complexing $[Tc^VO]^{3+}$, was prepared. The reaction of this novel ligand with [^{99m}Tc]pertechnetate, in the presence of stannous tartrate as the reducing agent, produces the neutral and lipid-soluble [^{99m}Tc]Tc^VO(U-BAT), $TcS_2C_{15}H_{23}N_2O$, **2**. However, when the same reaction was carried out at a higher pH, 9–10, and with 30 min of heating (100 °C), a second neutral but more lipid-soluble complex, [^{99m}Tc]Tc^VO(OU-BAT), $TcS_2C_{15}H_{21}N_2O$, **3**, was isolated. The X-ray crystallography data of the ^{99}Tc complexes show square pyramidal coordination with N_2S_2 as the base and the Tc=O in the apical position. Compound **3** can be derived from **2** by an oxidation of the ligand to form an imine. After iv injection into rats, the neutral and lipid-soluble technetium-99m complexes showed significant brain uptake, 1.54 and 1.07% dose/organ at 2 min for [^{99m}Tc]Tc^VO(U-BAT) and Tc^VO(OU-BAT), respectively. The novel Tc chemistry of this new ligand system may provide a useful foundation for designing Tc complexes with a built-in redox mechanism.

Introduction

The most commonly used radionuclide in diagnostic nuclear medicine is technetium-99m. Due to the physical characteristics of technetium-99m ($t_{1/2}$ = 6 h and γ energy 140 keV) and the convenient availability from the ^{99}Mo/^{99m}Tc generator, most of the currently used scanning devices are optimized for detecting this radionuclide. There is a strong interest in developing technetium-99m chemistry in order to refine the biological properties of technetium-99m-labeled compounds. One of the ligand systems which has proven useful for forming a variety of stable $[Tc^VO]^{3+}$ complexes is the bis(aminoethanethiol), BAT (N_2S_2), ligand system[1–4] (see Scheme 1).

The BAT ligands are very robust and versatile in their coordination chemistry. Neutral,[5,6] cationic,[7] and anionic[8,9] $[Tc^VO]^{3+}$ complexes of N_2S_2 ligands have been formed. In addition, N_2S_2 ligands form neutral $^{67}Cu^{2+}$ complexes and also complex with Ga^{3+} [10,11] and In^{3+} [12] to form monocationic compounds.[13] The overall net charges of the complexes are determined by the charge of the metal ion and also by the ionization at four possible sites, two S-H and two N-H (either as amines or amides) groups. It is important to recognize that the ionizability of the S-H and the amine or amide N-H groups determines the final net charge and, therefore, the ultimate biodistribution of these imaging agents. Scheme 1 illustrates the variable ionization sites for a number of recently reported N_2S_2 ligands and the corresponding $Tc(O)N_2S_2$ complexes. The diamide dithiol ligand (DADT)[8,14,15] forms a complex with $[Tc^VO]^{3+}$ which is negatively charged, due to complete ionization of the two S-H and two amide N-H groups; amide groups

Scheme 1. Comparison of $[Tc^VO]^{3+}$ Complexes of Various N_2S_2 (BAT) Ligands



are more readily ionized compared to amines. Ligands with amine N-H groups form Tc complexes which are neutral and lipid-soluble.[13,16] The S-H groups (pK_a = 6–8) are ionized upon complexation with $[Tc^VO]^{3+}$; however, the amine N-H groups are usually not ionized or only partially ionized. The N_2S_2 (BAT) ligand forms

* Address correspondence to: Hank F. Kung, Ph.D., Department of Radiology, University of Pennsylvania, Room 305, 3700 Market St., Philadelphia, PA 19104. Tel.: (215) 662–3096. Fax: (215) 349–5035.
† Hunter College of the City University of New York.
® Abstract published in *Advance ACS Abstracts,* September 1, 1994.

0022-2623/94/1837-3282$04.50/0

a neutral complex with $[Tc^{V}O]^{3+}$ because three, two S-H and one N-H, groups are ionized.[1]

One neutral N_2S_2 (BAT) complex, [^{99m}Tc]ECD (ethylene cysteine dimer), developed by NEN/DuPont, is a clinically useful regional brain perfusion imaging agent.[3,17-20] [^{99m}Tc]ECD is a diester derivative of a neutral $[Tc^{V}O]^{3+}$ complex of a N_2S_2 ligand, which contains two optical centers (see Scheme 1). The L,L-isomer shows both the desired brain uptake and prolonged retention. The D,D-isomer also displays high initial uptake, but the brain retention is disappointing.[21] The proposed brain retention mechanism for [^{99m}Tc]ECD is based on the specific hydrolysis of one of the ester groups to an acid, probably by intracellular esterases; consequently, the water-soluble acid is trapped in the brain. The hydrolysis is an enzymatic process, which is stereospecific to the L,L-isomer and occurs predominantly in the brains of primates (monkeys and humans), less prominently in rats or rabbits.[21] The specific localization mechanism is of note because the regional perfusion images obtained by this agent most likely are a combination of perfusion (delivery) and the specific enzyme reaction (trapping).[19,20]

In developing technetium-99m complexes as potential myocardial imaging agents, a new series of ligands based on a conformationally restricted N_2S_2 (BAT) system was investigated. Two of these N_2S_2 ligands, with an additional ethylene or propylene bridge between the two nitrogen donor atoms to provide structural rigidity, were synthesized. Examination of the [^{99}Tc]$Tc^{V}O$ complexes of these conformationally restricted ligands confirmed that the complexes contain a $Tc^{V}O$(III) center core and are monocationic. The data suggest that by alkylating the nitrogens and restricting the conformation of the ligand it is possible to form cationic complexes based on the N_2S_2 ligand system.[7]

The main objective of this report is to demonstrate the feasibility of developing N_2S_2 (BAT) ligands with N-H groups of differing pK_a values, in which the final net charge of the complex can be controlled and the complex can undergo a novel redox reaction. It is well known that the pK_a for the ionization of the N-H bond of alkylamines is high (i.e., very basic). For example, the pK_a value for NH_3 and alkyl-NH_2 is 33-35, while the pK_a drops to 27 for arylamines (Ph-NH_2). For amides (-CONH-), the pK_a of the N-H group drops even lower, to 16. It is obvious that the pK_a of the ionizable groups can be adjusted and the final Tc complexes could be neutral, positively charged, or negatively charged. By varying substitution groups on the phenyl ring, it is also possible to modify the overall stability and the lipid solubility of the final complex. We report herein the synthesis of an N_2S_2 (BAT) ligand with uneven amine groups (two amine groups which have different pK_a values) (U-BAT), the characterization of the corresponding Tc [^{99}Tc, ^{99m}Tc] complexes, and the evaluation of the biodistribution in rats. In addition, a novel reversible redox reaction between $Tc^{V}O$(U-BAT), **2**, and the oxidized form, $Tc^{V}O$(OU-BAT), **3**, is demonstrated.

Experimental Section

General. *Caution*: The isotope ^{99}Tc is a weak β-emitter with a half-life of 2×10^5 years. Appropriate radiochemical precautions should be taken to avoid contamination. ^{99}Tc, as $NH_4{}^{99}TcO_4$, was obtained from Oak Ridge National Laboratory, Oak Ridge, TN. (30% H_2O_2 was added to an aqueous solution of $NH_4{}^{99}TcO_4$ to oxidize any $^{99}TcO_2$ present.) The ammonium pertechnetate solution was standardized prior to use, as previously described.[22] The starting reagents, $N(C_4H_9)_4[TcOCl_4]$ and Na[TcO(ethylene glycol)$_2$], were prepared as described recently.[23,24] Saline solutions of $Na^{99m}TcO_4$ were obtained from a ^{99m}Tc/^{99}Mo generator (NEN/DuPont, North Billerica, MA). Melting points were determined with a Meltemp instrument (Laboratory Devices) and are reported uncorrected. Infrared spectra were obtained with a Mattson Polaris FT-IR spectrometer. NMR spectra were determined with a Varian EM 360A and a Picker 500 mHz spectrometer. UV-visible spectra were recorded in acetonitrile on a Beckman DU-7 spectrophotometer at ambient temperatures. The positive ion fast atom bombardment mass spectra (FABMS) were measured on a MAT 731 high-resolution mass spectrometer equipped with an Ion Tech B11N FAB gun, with xenon used as the neutral gas. The samples were dissolved in methylene chloride, and the matrix was *m*-nitrobenzyl alcohol. Elemental analyses were performed by Atlantic Microlabs, Inc., of Norcross, GA. High-performance liquid chromatography (HPLC) measurements were made on a PRP-1 column (25 cm; Hamilton) at a flow rate of 1 mL/min; the mobile phase consisted of acetonitrile/5 mM 3,3-dimethylglutaric acid, pH = 7 (90/10). All of the chemicals were of reagent grade and used without further purification.

2-Aminobenzylamine (4). To a solution of 2-aminobenzamide (10.2 g, 74.9 mmol) in anhydrous tetrahydrofuran (100 mL) was slowly added a 1 M solution of borane-THF complex in tetrahydrofuran (300 mL, 300 mmol), and the reaction mixture was stirred at reflux overnight. A 6 N HCl solution (100 mL) was added to the reaction mixture under cooling, and the mixture was refluxed for 1 h. The tetrahydrofuran was then removed *in vacuo*. After basification under cooling, the reaction mixture was extracted with chloroform (250 mL × 3). The combined organic layers were washed with water (150 mL × 3), dried over sodium sulfate, and evaporated *in vacuo* to give the free base as a solid. Recrystallization from MeOH and $CHCl_3$ afforded the desired product **4** (7.50 g, 82%): mp 58-59 °C; ^{1}H-NMR (CD_3OD) δ 3.59 (2H, s, CH_2), 4.48 (4H, s, NH_2), 6.43-7.13 (4H, m, ArH); IR (KBr) 3340, 3200, 3000, 2850, 1610, 1590, 1500, 1450, 1370, 1320, 1140, 750 cm^{-1}. Anal. ($C_7H_{10}N_2$) C, H, N.

***N,N'*-Bis[2-[(*p*-methoxybenzyl)thio]-2-methylpropionyl]-2-aminobenzylamine (5).** A solution of 2-[(*p*-methoxybenzyl)thio]-2-methylpropionic acid[7] (3.63 g, 15.11 mmol) in thionyl chloride (30 mL) was refluxed for 3 h. The solvent was then removed *in vacuo*. The residue was dissolved in dry chloroform (5 mL), which was added dropwise to a solution of 2-aminobenzylamine (**4**) (0.803 g, 6.57 mmol) and triethylamine (2.11 mL, 15.11 mmol) in dry chloroform (30 mL). The resultant reaction mixture was stirred overnight at ambient temperature and then washed with 2 N NaOH (30 mL × 2), 3 N HCl (30 mL × 3), and water (35 mL × 3). The organic phase was dried over sodium sulfate and evaporated *in vacuo* to give an oil which was purified by column chromatography (silica gel) with ethyl acetate and hexane (1/1, v/v) to afford the pure compound **5** (3.12 g, 84%): ^{1}H-NMR ($CDCl_3$) δ 1.57 (12H, d, CH_3), 3.45 (2H, s, NCH_2Ar), 3.71 (6H, d, OCH_3), 4.15 (4H, d, SCH_2Ar), 6.52-7.70 (12H, m, ArH); IR (oil) 3350, 2950, 2900, 2800, 1670, 1650, 1600, 1580, 1510, 1450, 1400, 1370, 1300, 1250, 1180, 1140, 1040, 840, 750 cm^{-1}. Anal. ($C_{31}H_{38}N_2O_4S_2$) C, H, N.

***N,N'*-Bis[2-[(*p*-methoxybenzyl)thio]-2-methylpropyl]-2-aminobenzylamine (6).** Compound **6** was prepared using a borane-THF reduction procedure similar to that for compound **4**. Purification by column chromatography (silica gel) with ethyl acetate and hexane (1/1, v/v) afforded the pure compound **6** (1.47 g, 70%): ^{1}H-NMR ($CDCl_3$) δ 1.32 (12H, d, CH_3), 2.42 (2H, s, $NCH_2C(Me)_2$), 3.10 (2H, s, $ArNCH_2C(Me)_2$), 3.65 (12H, m, $NCH_2Ar + OCH_3 + SCH_2Ar$), 6.40-7.30 (12H, m, ArH); IR (oil) 3300, 2970, 2940, 2840, 1620, 1580, 1520, 1465, 1300, 1250, 1175, 1040, 840, 750 cm^{-1}. Anal. ($C_{31}H_{42}N_2O_2S_2$) C, H, N.

***N,N'*-Bis[2-mercapto-2-methylpropyl]-2-aminobenzylamine, UBAT (1).** To a solution of S-protected amine **6** (1.02 g, 1.89 mmol) and anisole (0.61 g, 5.67 mmol) in tri-

fluoroacetic acid (18.9 mL) was added methanesulfonic acid (5.67 mL), and the reaction mixture was stirred for 3 h at ambient temperature. Volatile components were removed *in vacuo* to give a residue that was dissolved in water (60 mL) and washed with ether (30 mL × 3). The pH of the aqueous layer was adjusted to neutral and the layer extracted with chloroform (30 mL × 3). The combined chloroform layers were washed with water (30 mL × 3), dried over sodium sulfate, and concentrated *in vacuo* to give the free base as an oil which was converted to HCl salt 1 (577 mg, 82%): ^{1}H-NMR (free base in $CDCl_3$) δ 1.32 (12H, d, CH_3), 2.52 (2H, s, $NCH_2C(Me)_2$), 3.12 (2H, s, $ArNCH_2C(Me)_2$), 3.70 (2H, s, NCH_2Ar), 6.40–7.10 (4H, m, ArH); IR (oil, free base) 3300, 2980, 2950, 2860, 2840, 1610, 1590, 1520, 1460, 1375, 1360, 1320, 1250, 1125, 750 cm^{-1}. Anal. ($C_{15}H_{28}Cl_2N_2S_2$) C, H, N.

Preparation of [^{99}Tc]Tc^{V}O(UBAT) (2) and [^{99}Tc]Tc^{V}O-(OU-BAT) (3). The hydrochloride salt of ligand 1 (33 mg, 0.089 mmol) was dissolved in 2 mL of methanol at room temperature with stirring to give a clear, colorless solution. A 2 mL methanolic solution of $[N(C_4H_9)_4][Tc^VOCl_4]$ (42 mg, 0.09 mmol) was added to the ligand solution at room temperature. Immediately, the color changed to brown-red. A solution of $NaOCH_3$ in methanol (0.5 mL, 0.5 M, 8.5 mmol) was added to the stirring solution, and the reaction mixture was refluxed for 15 h. The color of the resulting solution was wine-red. The reaction solution was evaporated under reduced pressure to a film and dissolved in 15–20 mL of CH_2Cl_2. A white solid was filtered off. The volume of the solution was reduced to ca. 1 mL and loaded onto a silica gel column (1 × 20 cm) prepared in CH_2Cl_2. The column was eluted with CH_2Cl_2. The first band to elute was red, and the second was brown. Both bands were collected in approximately a 5–10 mL volume; ethanol was added to each, and the solutions were evaporated slowly. The first fraction, **3**, yielded 7 mg, 0.017 mmol, 19% of a red crystalline solid. The second fraction, **2**, yielded 9 mg, 0.022 mmol, 25% of a brown crystalline solid. Anal. ($TcS_2C_{15}H_{23}N_2O \cdot {}_2CH_3OH$) **2**, C, H, N, S; ($TcS_2C_{15}H_{21}N_2O$) **3**, C, H, N, S. IR: **2**, 940 (Tc=O), 3300 cm^{-1} (NH); **3**, 940 (Tc=O), 1600 cm^{-1} (C=N). ^{1}H-NMR ($CDCl_3$): δ **2**, 3.44 (dd, 1, CH_2), 2.62 (t, 1, CH_2), 4.41 (d, 1, CH_2), 4.00 (d, 1, CH_2), 3.89 (1H, d, CH_2), 2.98 (1H, t, CH_2), 4.94 (1H, s, NH), 1.76 (3H, s, CH_3), 1.68 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.53 (3H, s, CH_3), 6.82 (1H, m, aromatic), 7.09 (1H, m, aromatic), 7.21 (1H, m, aromatic), 7.39 (1H, m, aromatic); **3**, 1.50 (3H, s, CH_3), 1.55 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.66 (3H, s, CH_3), 4.00 (1H, d, CH_2), 4.14 (1H, d, CH_2), 4.65 (2H, m, CH_2), 6.91 (1H, m, aromatic), 7.40 (1H, m, aromatic), 7.59 (2H, m, aromatic), 8.71 (1H, s, imine-H). UV–visible (CH_3CN) (λ, nm; ϵ, M^{-1} cm^{-1}): **2**, 405.0 (7572), 344.0 (12 600), 286.5 (29 500); **3**, 467.0 (3100), 396.5 (4950), 285.5 (20 900), 267.0 (22 670), 244.0 (sh) (21 922), 222.0 (26 000). MS (FAB+): **2** $(M + H)^+$, m/z = 411; **3** $(M + H)^+$, m/z = 409.

Conversion of [^{99}Tc]Tc^{V}O(U-BAT) (2) to [^{99}Tc]Tc^{V}O-(OU-BAT) (3). Approximately 1 mg, 0.0024 mmol, of TcO-(U-BAT), **2**, was dissolved in 10 mL of $CHCl_3$. Activated carbon, 50 mg, was added to the yellow-brown solution, and the mixture was stirred at room temperature for 15 h and then filtered to obtain a red-brown solution which was evaporated to a red solid. HPLC analysis, based on retention time and proton NMR data, showed quantitative conversion to **3**.

Conversion of [^{99}Tc]Tc^{V}O(OU-BAT) (3) to [^{99}Tc]Tc^{V}O-(U-BAT) (2). Approximately 2 mg, 0.005 mmol, of **3** was dissolved in 2 mL of ethanol. $NaBH_4$ in ethanol (100 μL, 0.1 M, 0.01 mmol) was added. Immediately the color of the solution changed to yellow. After 10 min, quantitative conversion to **2** (based on HPLC retention time) was observed.

Preparation of [^{99m}Tc]TcO(U-BAT) (2). The hydrochloride salt of ligand 1 (0.5 mg) was dissolved in 200 μL of water. To this solution was added sodium [^{99m}Tc]TcO_4 (0.2 mL, 5 mCi) followed by 25 μL of saturated stannous tartrate solution. The resulting solution was mixed and allowed to stand at room temperature for 15 min. The solution was loaded onto a Sep-Pak cartridge (C-18) conditioned with ethanol. The cartridge was washed with water and 50/50 (v/v) ethanol/water and eluted with ethanol. The ethanol solution was evaporated to dryness, redissolved in 20 μL of ethanol, and injected onto the reverse phase HPLC. The 4.6 min peak was collected (90/10 CH_3CN/5 mM DMGA, pH = 7). The resulting solution was evaporated under reduced pressure to dryness and redissolved in $CHCl_3$, and any undissolved particles were filtered off. The $CHCl_3$ solution was evaporated to dryness under reduced pressure, and the activity was redissolved in ethanol.

Preparation of [^{99m}Tc]TcO(OU-BAT) (3). The hydrochloride salt of ligand 1 (0.5 mg) was dissolved in 200 μL of water. To this solution was added $^{99m}TcO_4$ (0.2 mL, 5 mCi) followed by 25 μL of saturated stannous tartrate solution. The pH was adjusted to 9 by the addition of a few drops of a 1 M $NaHCO_3$ solution. The solution was heated for 30 min, cooled, and loaded onto a Sep Pak cartridge (C-18) conditioned with ethanol. The cartridge was washed with water and 50/50 (v/v) ethanol/water and eluted with ethanol. The ethanol solution was evaporated to dryness, redissolved in 20 μL of ethanol, and injected onto the reverse phase HPLC. The 7.8 min peak was collected (90/10 CH_3CN/5 mM DMGA, pH = 7). The resulting solution was evaporated under reduced pressure to dryness and redissolved in $CHCl_3$, and any undissolved particles were filtered off. The $CHCl_3$ solution was evaporated to dryness under reduced pressure, and the activity was redissolved in ethanol.

Conversion of [^{99m}Tc]TcO(U-BAT) (2) to [^{99m}Tc]TcO(OU-BAT) (3). Activated carbon was added to a $CHCl_3$ solution of [^{99m}Tc]**2** (100 μL) and the resulting suspension heated in air at 40 °C for 2 h. Quantitative conversion to the imine species, [^{99m}Tc]**3**, HPLC retention time = 7.8 min (90/10 CH_3CN/5 mM DMGA, pH = 7), was observed.

Conversion of [^{99m}Tc]TcO(OU-BAT) (3) to [^{99m}Tc]-TcO(U-BAT) (2). To a solution of 100 μL of [^{99m}Tc]**3** in ethanol, prepared as above, was added 25 μL of a 0.1 M $NaBH_4$ solution, and the solution was heated for 1 h. HPLC analysis showed quantitative conversion to a peak, with retention time at 4.6 min, corresponding to [^{99m}Tc]**2** (90/10 CH_3CN/5 mM DMGA, pH = 7).

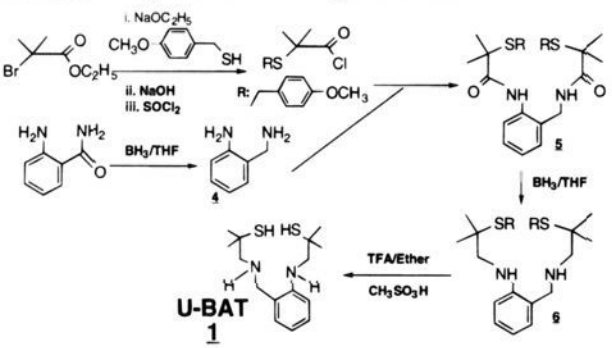
Collection and Reduction of X-ray Crystallography Data. Compound **2**, [^{99}Tc]$TcS_2C_{15}H_{23}N_2O$, crystallizes in the monoclinic space group $P2_1/n$ (systematic absences $0k0$, k = odd, and $h0l$, $h+l$ = odd) with a = 11.253(2), b = 21.823(2), and c = 14.392(2) Å, β = 95.00(1)°, V = 3521(1) Å^3, Z = 8, and d_{calcd} = 1.545 g/cm.^{3} The cell constants were determined from a least-squares fit of the setting angles for 25 accurately centered reflections. X-ray intensity data were collected on an Enraf-Nonius CAD4 diffractometer employing graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) and using the ω - 2θ scan technique. A total of 5019 reflections were measured over the ranges: 4° ≤ 2θ ≤ 45°, 0° ≤ h ≤ 12°, 0° ≤ k ≤23°, −15° ≤ l ≤ 15°. Three standard reflections, measured every 3500 s of X-ray exposure, showed no intensity decay over the course of data collection. The intensity data were corrected for Lorentz and polarization effects, and an empirical absorption correction was performed. Of the reflections measured, a total of 1417 unique reflections with $F^2 > 3\sigma(F^2)$ were used during subsequent structure refinement. The structure was solved by standard heavy atom Patterson techniques followed by weighted Fourier syntheses. Hydrogen atoms were found from difference Fourier maps calculated after anisotropic refinement. Refinement was by full-matrix least-squares techniques based on F to minimize the quantity $\Sigma_w(|F_o| - |F_c|)^2$ with $w = 1/\sigma^2(F)$. Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were refined isotropically. Refinement converged to R_1 = 0.038 and R_2 = 0.041.

Compound **3**, [^{99}Tc]$TcS_2C_{15}H_{21}N_2O$, crystallizes in the monoclinic space group $P2_1/c$ (systematic absences $0k0$, k = odd, and $h0l$, l = odd) with a = 6.922(1), b = 18.597(3), and c = 13.802(2) Å, β = 90.95(1)°, V = 1776.5(8) Å^3, Z = 4, and d_{calcd} = 1.527 g/cm.^{3} The cell constants were determined from a least-squares fit of the setting angles for 25 accurately centered reflections. X-ray intensity data were collected on an Enraf-Nonius CAD4 diffractometer employing graphite-monochromated Mo Kα radiation (λ=0.71073Å) and using the ω − 2θ scan technique. A total of 4522 reflections were measured over the ranges: 4° ≤ 2θ ≤ 55°, 0° ≤ h ≤ 8°, 0° ≤ k ≤ 24°, −17° ≤ l ≤ 17°. Three standard reflections, measured every 3500 s of X-ray exposure, showed no intensity decay over the course of data collection. The intensity data were corrected for

Table 1. Summary of Structure Determinations of [^{99m}Tc]Tc^{V}O(U-Bat), **2**, and Tc^{V}O(OU-BAT), **3**

	[^{99m}Tc]Tc^{V}O(U-BAT) (**2**)	Tc^{V}O(OU-BAT) (**3**)
formula	$TcS_2C_{15}H_{23}N_2O$	$TcS_2C_{15}H_{21}N_2O$
formula weight	409.48042	407.46454
crystal class	monoclinic	monoclinic
space group	$P2_1/n$ (No. 14)	$P2_1/c$ (No. 14)
Z	8	4
cell constants		
a (Å)	11.253(2)	6.922(1)
b (Å)	21.823(2)	18.597(3)
c (Å)	14.392(2)	13.802(2)
β (deg)	95.00(1)	90.95(1)
V (Å^3)	3521(1)	1776.5(8)
μ (cm^{-1})	10.26	10.17
trans. (min, max, avg, %)	85.5, 99.8, 93.5	94.2, 99.9, 97.2
d_{calcd} (g/cm^3)	1.545	1.527
F(000)	1680	836
radiation	Mo Kα (λ = 0.71073 Å)	Mo Kα (λ = 0.71073 Å)
θ range	2.0−22.5°	2.0−27.5°
scan mode	$\omega - 2\theta$	$\omega - 2\theta$
h, k, l collected	12, 23, ±15	8, 24, ±17
no. of reflections measured	5019	4522
no. of unique reflections	4586	4053
no. of reflections used in refinement	1417 ($F^2 > 3.0\sigma$)	2661 ($F^2 > 3.0\sigma$)
no. of parameters	379	190
data parameter ratio	3.7	14.0
R_1	0.038	0.037
R_2	0.041	0.044
GOF	1.109	1.284

Scheme 2. Synthesis of the Ligand UBAT



Lorentz and polarization effects, and an empirical absorption correction was performed. Of the reflections measured, a total of 2661 unique reflections with $F^2 > 3\sigma(F^2)$ were used during subsequent structure refinement. The structure was solved by the same method described above. Refinement converged to $R_1 = 0.037$ and $R_2 = 0.044$.

Animal Distribution Studies. Male Sprague−Dawley rats (200−300 g) were injected intravenously (under ether anesthesia) with 0.2 mL of a saline solution containing the technetium-99m complex (2−5 μCi). At selected intervals following the injection, blood samples were collected by cardiac puncture, and the rats were sacrificed immediately thereafter by cardiectomy. The organs of interest were subsequently excised, weighed, and counted in a dual-channel automatic gamma counter (Beckman 5500). The percent dose/organ values were determined by comparison of the tissue radioactivity with suitable dilutents of the injected dose. The percent dose/g values were computed from the percent dose/organ values and the corresponding organ weights.

Results

Ligand Synthesis. By reducing 2-aminobenzamide with diborane−THF to the corresponding 2-aminobenzylamine (**4**) and reacting the diamine with 2-[(*p*-methoxybenzyl)thio]-2-methylpropionyl chloride, the desired diamide, **5**, was achieved. After another diborane−THF reduction and deprotection of the thiols from the *p*-methoxybenzyl group, the final U-BAT ligand, **1**, was obtained.

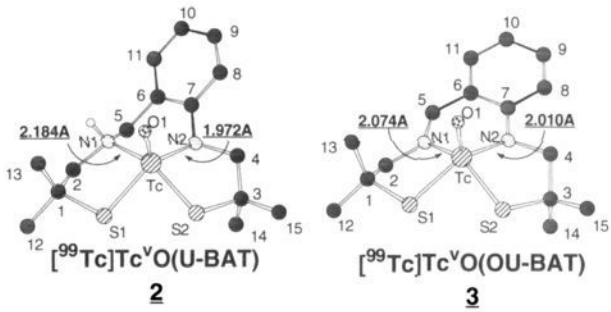


Figure 1. X-ray crystallography structures of ^{99}Tc U-BAT and OU-BAT.

Radiolabeling of 1 with Technetium-99m. Radiolabeling with technetium-99m was achieved by reacting the ligand **1** with sodium [^{99m}Tc]pertechnetate in the presence of stannous tartrate. At room temperature and a pH of 3−4, a neutral and lipid-soluble product, [^{99m}Tc]Tc^{V}O(U-BAT) (**2**), was formed in greater than 90% yield. However, in the presence of 1 M sodium bicarbonate, pH = 9, and heating (85 °C for 30 min) in air, about 50% of the oxidized [^{99m}Tc]Tc^{V}O(U-BAT-imine) (**3**), was produced. Both **2** and **3** are neutral and lipid-soluble complexes and are stable for 5 h in saline solution at room temperature. [^{99m}Tc]**3** can be converted quantitatively to [^{99m}Tc]**2** by reduction with sodium borohydride. [^{99m}Tc]**2** can be converted to [^{99m}Tc]**3** quantitatively by oxidation in air using activated charcoal as a catalyst.

Synthesis of [^{99}Tc]Tc^{V}O(U-BAT) (2) and [^{99}Tc]-Tc^{V}O(OU-BAT) (3). The [^{99}Tc]Tc^{V}O(U-BAT), **2**, and [^{99}-Tc]Tc^{V}O(OU-BAT), **3**, complexes have been prepared by the ligand exchange reaction of N(C_4H_9)[Tc^{V}OCl_4] with the hydrochloride salt of the ligand under basic conditions. Another [Tc^{V}O]$^{3+}$ starting material, Na[Tc^{V}O-(ethylene glycol)$_2$] can also be used in this reaction. With both [Tc^{V}O]$^{3+}$ starting materials, a white solid was isolated from the reaction. The HPLC profile and IR spectrum indicate that unreacted ligand is present in this solid. HPLC and TLC analyses of the reaction solution indicate the presence of many byproducts of this reaction, necessitating the use of column chromatography to purify the two isolated complexes. Both products elute with CH_2Cl_2; the imine (oxidized) species, **3**, elutes in a red band followed by the amine species, **2**. Slow evaporation of CH_2Cl_2/ethanol solutions resulted in crystalline solids of [^{99}Tc]Tc^{V}O(U-BAT), **2**, and [^{99}Tc]-Tc^{V}O(OU-BAT), **3**.

X-ray Crystallography. The X-ray crystallographic structures for [^{99}Tc]Tc^{V}O(U-BAT), **2**, and [^{99}Tc]Tc^{V}O-(OU-BAT), **3**, are presented in Figure 1. TcO(U-BAT) crystallized in the centrosymmetric space group $P2_1/n$. Two independent molecules were found in the unit cell; the enantiomers exist and are related by a center of symmetry. The two independent molecules vary only slightly in their metric parameters, and for simplicity, one will be discussed in consideration of the structure of this species. TcO(OU-BAT) (**3**) crystallized in the centrosymmetric space group $P2_1/c$ with four molecules/unit cell. One independent molecule was found; the

Table 2. Bond Distances of TcO(U-BAT), **2**, and TcO(OU-BAT), **3** (Å)

		TcO(U-BAT), **2**			
Tc—S1	2.311(4)	C1—C12	1.508(17)	C5—N1	1.489(15)
Tc—S2	2.265(4)	C1—C13	1.546(19)	C6—C7	1.421(19)
Tc—N1	2.184(10)	C2—N1	1.501(16)	C6—C11	1.384(19)
Tc—N2	1.972(10)	C3—C4	1.436(21)	C7—C8	1.418(20)
Tc—O	1.653(8)	C3—C14	1.470(22)	C7—N2	1.390(17)
S1—C1	1.822(13)	C3—C15	1.573(21)	C8—C9	1.350(21)
S2—C3	1.835(14)	C4—N2	1.480(18)	C9—C10	1.399(23)
C1—C2	1.510(18)	C5—C6	1.479(19)	C10—C11	1.351(24)
		TcO(OU-BAT), **3**			
Tc—S1	2.303(1)	C1—C12	1.513(7)	C5—N1	1.291(6)
Tc—S2	2.263(1)	C1—C13	1.508(7)	C6—C7	1.419(6)
Tc—N1	2.074(3)	C2—N1	1.465(6)	C6—C11	1.422(6)
Tc—N2	2.010(4)	C3—C4	1.512(7)	C7—C8	1.402(6)
Tc—O	1.661(3)	C3—C14	1.524(7)	C7—N2	1.371(5)
S1—C1	1.849(5)	C3—C15	1.510(7)	C8—C9	1.366(7)
S2—C3	1.843(5)	C4—N2	1.476(6)	C9—C10	1.385(7)
C1—C2	1.499(7)	C6—C5	1.401(6)	C10—C11	1.357(8)

others are related by the symmetry operations of the space group.

The structures of (**2**) and (**3**) are shown in Figure 1. Selected bond distances and bond angles comparing the two molecules are given in Table 2. It is clear that there are two orientations of the phenyl ring with respect to the $N_2S_2Tc^VO^{3+}$ core in these two molecules. The phenyl ring in molecule **3** forms a plane with N1, C5, and N2 of the six-membered Tc chelate ring. Atoms N1, C5, and N2 and the atoms of the phenyl ring deviate less than 0.08 Å from the least-squares plane calculated from these atoms. Such a planar arrangement is not seen in molecule **2**, presumably due to the tetrahedral amine nitrogen (N1) atom. The bond angles about N1 in **2** are consistent with sp^3 hybridization about the nitrogen atom, and the Tc atom is in a distorted square pyramidal coordination environment. In contrast, the bond angles about N1 in Tc^VO(OU-BAT) (**3**) are consistent with sp^2 hybridization about the nitrogen atom, and the Tc atom experiences a less distorted square pyramidal coordination geometry with the oxygen atom in an apical position. The bond distances of Tc—N1 (2.184 and 2.074 Å for molecules **2** and **3**, respectively) are consistent with amine—Tc bonding for **2**[7,25-30] and imine—Tc bonding for **3**.[31,32]

The Tc—N2 (amide nitrogen) bond distance in **2** is 1.972(10) Å. The corresponding $Tc—N_{amide}$ bond in Tc^VO(OU-BAT) (**3**) is 2.010(4) Å. Both are at the high end of the range generally seen for $Tc—N_{amide}$ bonds.[29,33,34] Finally, the N1—C5 bond is typical of a N—C single bond for **2**; the N1—C5 bond in compound **3** is typical of an imine N=C bond.

Infrared spectroscopy data, taken as KBr pellets, show features which are consistent with the molecular structures. For **2**, a strong band is observed at 940 cm^{-1} assigned to the Tc=O stretching frequency. A strong band at 3300 cm^{-1} assigned to the N—H stretching frequency is also observed. For **3**, no NH stretch is observed; rather a strong band at 1600 cm^{-1} attributed to the C=N frequency is observed. The Tc=O stretching frequency of **3** is found at 940 cm^{-1}. FAB mass spectroscopy data (positive ions) show molecular ion peaks at $(M + H)^+$ of 411 and 409, consistent with molecular weights of 410 and 408 for molecules **2** and **3**, respectively.

HPLC measurements, made on a Hamilton PRP-1 column, 25 cm, with the mobile phase 80/20 acetonitrile/5 mM DMGA, pH = 7.0, show that [^{99}Tc]Tc^VO(U-BAT), **2**, has a retention time of 10 min and [^{99}Tc]Tc^VO-(OU-BAT), **3**, a retention time of 17 min. Figure 2 shows the HPLC profiles of [^{99m}Tc]- and [^{99}Tc]Tc^VO(U-BAT), **2**, and [^{99m}Tc]- and [^{99}Tc]Tc^VO(OU-BAT), **3**. It is clear from the coelution of these species that the ^{99m}Tc species are chemically the same as the ^{99}Tc species in these solutions.

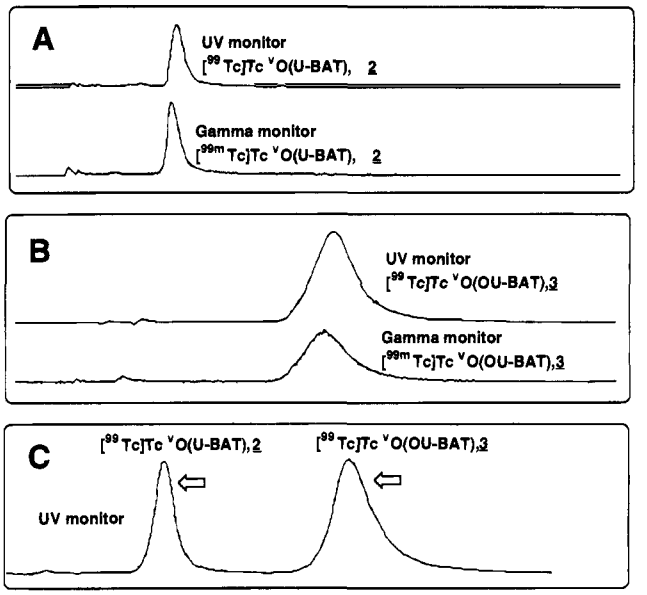
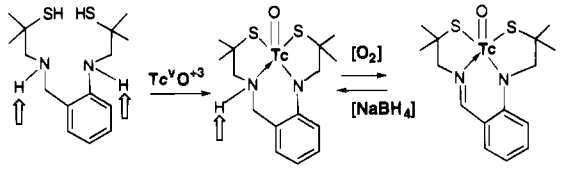


Figure 2. HPLC profiles of [^{99}Tc]- and [^{99m}Tc]Tc^VO(U-BAT) (**2**) and -Tc^VO(OU-BAT) (**3**): (A) simultaneous injection of [^{99}Tc]- and [^{99m}Tc]Tc^VO(U-BAT) (**2**), (B) simultaneous injection of [^{99}Tc]- and [^{99m}Tc]Tc^VO(OU-BAT) (**3**), and (C) simultaneous injection of [^{99}Tc]Tc^VO(U-BAT) (**2**) and the oxidized form, [^{99}Tc]-Tc^VO(OU-BAT) (**3**). HPLC conditions: reverse phase column (PRP-1 column), acetonitrile/buffer, pH = 7.0 (90/10, v/v), at 1 cc/min.

Scheme 3. Reversible Ligand-Based Redox Reaction Interconverting the TcO(U-BAT), **2**, Species and the TcO(OU-BAT), **3**, Species



Tc = ^{99m}Tc, ^{99}Tc

Biodistribution in Rats. As expected, both [^{99m}Tc]-Tc^VO(U-BAT), **2**, and Tc^VO(OU-BAT), **3**, are neutral and capable of penetrating the intact blood—brain barrier. Initial brain uptake in rats at 2 min i.v. postinjection

Table 3. Biodistribution Data in Rats (average of three rats, % dose/organ ± SEM)

	TcO(U-BAT), **2**			TcO(OU-BAT), **3**	
organ	2 min	15 min	60 min	2 min	30 min
blood	4.37 ± 0.36	2.00 ± 0.08	1.27 ± 0.10	8.12 ± 0.94	3.22 ± 0.54
heart	1.28 ± 0.27	0.53 ± 0.13	0.28 ± 0.06	1.15 ± 0.06	0.24 ± 0.04
muscle	20.77 ± 0.14	16.50 ± 3.82	20.08 ± 5.28	10.24 ± 0.02	13.52 ± 1.62
lung	1.79 ± 0.30	0.91 ± 0.14	0.50 ± 0.08	4.47 ± 0.38	1.98 ± 0.55
kidney	3.58 ± 0.74	1.54 ± 0.33	0.93 ± 0.06	3.30 ± 0.18	1.33 ± 0.19
spleen	0.64 ± 0.14	0.72 ± 0.10	0.68 ± 0.01	0.68 ± 0.49	0.30 ± 0.11
liver	23.58 ± 1.86	26.50 ± 2.27	20.75 ± 3.62	22.76 ± 2.54	22.65 ± 2.53
skin	13.48 ± 9.59	10.45 ± 6.31	16.50 ± 4.85	3.94 ± 0.74	11.94 ± 1.42
brain	1.54 ± 0.42	0.72 ± 0.22	0.21 ± 1.01	1.07 ± 0.05	0.28 ± 0.08

was 1.54 and 1.07% dose/organ for [^{99m}Tc]Tc^{V}O(U-BAT), **2**, and Tc^{V}O(OU-BAT), **3**, respectively. Since there is no built-in trapping mechanism, both compounds washed out from the rat brain at later intervals.

Discussion

In this paper, we report an unique ligand system (U-BAT) based on the N_2S_2 chelating group containing a set of uneven amine groups with different ionizability. There are several advantages of the U-BAT ligand system compared to other BAT ligands. The U-BAT ligand system has amine groups of differing pK_a values, one anilinic and one benzylic; therefore the ionization of the N-H protons is selectively controlled by the pK_a value of the various amine groups. By controlling the ionization of the N-H groups, it is possible to design ligands to form only neutral $[Tc^{V}O]^{3+}$ complexes. This is an essential property for penetrating the intact blood–brain barrier or normal cell membranes. Recently, reports[2,35] on the design of new technetium-99m-labeled BAT-estrogen derivatives as potential receptor specific technetium-99m-based imaging agents show that another N_2S_2 (BAT) ligand system with one amine and one amide group resulted in the formation of a neutral Tc^{V}O complex. In these ligands, the amide N-H group, with a lower pK_a value, deprotonated upon complexation to the $[Tc^{V}O]^{3+}$ unit. Attachments of Tc complexes to CNS receptor ligands that maintain specificity and high receptor affinity are important considerations for developing technetium-99m-based CNS receptor imaging agents. Several recent papers on Tc-labeled QNB[36] and vesamicol[37] have suggested that the N_2S_2 ligand system may be potentially useful for designing the technetium-99m-labeled CNS receptor imaging agents. The modified N_2S_2 ligand, U-BAT, may provide improvements over the recently reported ligands because Tc-U-BAT will have less stereoisomers and predictable configurations of the final Tc complexes. Several such Tc-labeled CNS receptor-directed agents are currently being investigated in our laboratory.

The TcO(U-BAT) and TcO(OU-BAT) complexes display unique reversible ligand-based redox reactions. During the course of these redox reactions, the Tc atom remains firmly bound to the nitrogen and sulfur donor atoms; the reduction and oxidation occurs on periphery atoms of the ligand not directly involving chelation with the Tc^{V}O core. These redox reactions are easily achievable in both no carrier and carrier-added conditions. These reactions underscore the potential of using the TcO(U-BAT) N_2S_2 core in designing future imaging agents based on oxidation and reduction of the ligand bound to the Tc, under *in vivo* conditions. We are currently investigating the redox potential *in vitro* and *in vivo* in order to use the U-BAT system in such a fashion. Recently, technetium-99m-labeled PnAO derivatives containing a 2-nitroimidazole moiety, another technetium-99m agent which can undergo a redox reaction, were reported as potential imaging agents for hypoxic cells or tissues.[38]

The U-BAT ligand can also be derivatized through substitutions on the phenyl ring to further modify the redox potential as well as the lipophilicity, both of which may be essential for fine tuning the properties of the technetium-99m complexes. Substitution on the phenyl ring will not induce the formation of stereoisomers, which may be a considerable advantage in simplifying the evaluation of structure–activity relationships.

Another unique feature of molecules **2** and **3** is that the Tc atom is incorporated into a phenyl system with a compact, stable $Tc^{V}O^{3+}$ center core, with two different orientations of the TcO group relative to the phenyl group. The phenyl group is canted up syn to the TcO group in molecule **2**, whereas in molecule **3**, the phenyl group and atoms N1, N2, and C5 form a plane with the Tc atom above the plane. Reaction chemistry can be done on the ligand coordinated to the Tc without altering the stable pentacoordinate technetium bound to the N_2S_2 donor atoms. These molecules are readily adaptable for designing new biologically selective technetium-99m BAT complexes for targeting receptor binding or other tissues, i.e., tumors. We are currently investigating the feasibility of using this approach to design technetium-99m imaging agents for CNS receptors.

Acknowledgment. The authors thank Catherine Cartwright, Karen Loomis, and Susan West for their assistance in preparing this mauscript. The authors are grateful to Dr. C. E. Costello and Mr. C.-W. Zheng of the MIT Mass Spectrometry Facility for providing mass spectral (FAB) data (NIH Grant for Mass Spectrometry Facility No. RR00317 to K. Biemann), to Ms. Grazyna Graczyk of the Department of Biophysics, University of Pennsylvania, for providing NMR data, and to Dr. Patrick Carroll, Chemistry Department, University of Pennsylvania, for solving the crystal structures. L.C.F. is grateful for a Eugene Lang Faculty Development Award and a PSC-CUNY research award. This work is supported by a grant awarded by NIH (NS-18509).

Supplementary Material Available: Refined positional parameters for compounds **2** and **3** (3 pages). Ordering information is given on any current masthead page.

References

(1) Kung, H. F. New Technetium 99m-Labeled Brain Perfusion Imaging Agents. *Semin. Nucl. Med.* **1990**, *20*, 150–158.

(2) 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.
(3) Walovitch, R. C.; Hill, T. C.; Garrity, S. T.; Cheesman, E. H.; Burgess, B. A.; et al. Characterization of Technetium-99m-L,L-ECD For Brain Perfusion Imaging, Part 1: Pharmacology Of Technetium-99m ECD In Non-Human Primates. *J. Nucl. Med.* **1989**, *30*, 1892–1901.
(4) Lever, S. Z. Design, Preparation and Biodistribution of a Technetium-99m Triaminedithiol Complex To Assess Regional Cerebral Blood Flow (correction). *J. Nucl. Med.* **1987**, *28*, 1064–1065.
(5) Scheffel, U.; Goldfarb, H. W.; Lever, S. Z.; Gungon, R. L.; Burns, H. D.; Wagner, H. N., Jr. Comparison of Technetium-99m Aminoalkyl Diaminodithiol (DADT) Analogs As Potential Brain Blood Flow Imaging Agents. *J. Nucl. Med.* **1988**, *29*, 73–82.
(6) Mahmood, A.; Halpin, W. A.; Baidoo, K. E.; Sweigart, D. A.; Lever, S. Z. Structure of a Neutral N-Alkylated Diaminedithiol (DADT) 99TcV Complex: Syn[99TcO(NEt-tmdadt)]. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1991**, *47*, 254–257.
(7) Ohmomo, Y.; Francesconi, L. C.; Kung, M.-P.; Kung, H. F. New Conformationally Restricted Tc-99m N_2S_2 Complexes As Myocardial Perfusion Imaging Agents. *J. Med. Chem.* **1992**, *35*, 157–162.
(8) Jones, A.; Davison, A.; DePamphilis, B. Preparation and Crystal Structure of Tetraphenylarsonium Oxotechnetiumbis(2-Mercaptoethanolate). *Inorg. Chem.* **1981**, *20*, 1617–1618.
(9) Kasina, S.; Fritzberg, A. R.; Johnson, D. L.; Eshima, D. Tissue Distribution of Technetium-99m-Diamide-Dimercaptide Complexes and Potential Use As Renal Pharmaceuticals. *J. Med. Chem.* **1986**, *29*, 1933.
(10) Kung, H. F.; Liu, B. L.; Mankoff, D.; Kung, M. P.; Billings, J. J.; Francesconi, L. C.; Alavi, A. A New Myocardial Imaging Agent: Synthesis, Characterization, and Biodistribution of Gallium-68-BAT-TECH. *J. Nucl. Med.* **1990**, *31*, 1635–1640.
(11) Francesconi, L. C.; Liu, B.-L.; Billings, J. J.; Carroll, P. J.; Graczyk, G.; Kung, H. F. Synthesis, Characterization and Solid State Structure of a Neutral Ga(II) Aminothiolate Complex: A Potential Radiopharmaceutical For PET Imaging. *J. Chem. Soc., Chem. Commun.* **1991**, *2*, 94–95.
(12) Liu, B.-L.; Kung, H. F.; Jin, Y. T.; Zhu, L.; Meng, M. A New Myocardial Imaging Agent: Synthesis, Characterization and Biodistribution of [^{113m}In]TE-BAT. *J. Nucl. Med.* **1989**, *30*, 367–373.
(13) Kung, H. F.; Guo, Y.-Z.; Yu, C.-C.; Billings, J. J.; Subramanyam, V.; Calabrese, J. New Brain Perfusion Imaging Agents Based on Tc-99m Bis-Amino-Ethanethiol (BAT) Complexes: Stereoisomers and Biodistribution. *J. Med. Chem.* **1989**, *32*, 433–437.
(14) Fritzberg, A. R.; Abrams, P. G.; Beaumier, P. L.; Kasina, S.; Morgan, A. C.; Rao, T. N.; Reno, J. M.; Sanderson, J. A.; Srinivasan, A.; Wilbur, D. S.; et al. Specific and Stable Labeling of Antibodies With Technetium-99m With a Diamide Dithiolate Chelating Agent. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4025–4029.
(15) Rao, T. N.; Adhikesavalu, D.; Camerman, A.; Fritzberg, A. R. Technetium(V) and Rhenium(V) Complexes of 2,3-Bis(Mercaptoacetamido)Propanoate. Chelate Ring Stereochemistry and Influence on Chemical and Biological Properties. *J. Am. Chem. Soc.* **1990**, *112*, 5798–5804.
(16) 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.
(17) Walovitch, R. C.; Cheesman, E. H.; Maheu, L. J.; Hall, K. M. Studies of the Retention Mechanism of the Brain Perfusion Imaging Agent ^{99m}Tc-Bicisate (^{99m}Tc-ECD). *J. Cereb. Blood Flow Metab.* **1994**, *1*, S4–S11.
(18) Waldemar, G.; Walovitch, R. C.; Andersen, A. R.; Hasselbalch, S. G.; Bigelow, R.; Joseph, J. L.; Paulson, O. B.; Lassen, N. A. ^{99m}Tc-Bicisate (Neurolite) SPECT Brain Imaging and Cognitive Impairment in Dementia of the Alzheimer Type: A Blinded Read of Image Sets From a Multicenter SPECT Trial. *J. Cereb. Blood Flow Metab.* **1994**, *1*, S99-S105.
(19) Knudsen, G. M.; Andersen, A. R.; Somnier, F. E.; Videbaek, C.; Hasselbalch, S.; Paulson, O. B. Brain Extraction and Distribution of ^{99m}Tc-Bicisate In Humans and In Rats. *J. Cereb. Blood Flow Metab.* **1994**, *1*, S12–S18.
(20) Lassen, N. A.; Sperling, B. ^{99m}Tc-Bicisate Reliably Images CBF In Chronic Brain Diseases But Fails to Show Reflow Hyperemia In Subacute Stroke: Report of a Multicenter Trial of 105 Cases Comparing ^{133}Xe and ^{99m}Tc-Bicisate (ECD, Neurolite) Measured by SPECT on Same Day. *J. Cereb. Blood Flow Metab.* **1994**, *1*, S44–S48.
(21) Walovitch, R. C.; Franceschi, M.; Picard, M.; Cheesman, E. H.; Hall, K. M.; Makuch, J.; Watson, M. W.; Zimmerman, R. E.; Watson, A. D.; Ganey, M. V. Metabolism of ^{99m}Tc-L,L-Ethyl Cysteinate Dimer In Healthy Volunteers. *Neuropharmacology* **1991**, *30*, 283–292.
(22) Boyd, G. E. Technetium and Promethium. *J. Chem. Educ.* **1959**, *36*, 3.
(23) Linder, K. E. Aminocarboxylate Complexes of Technetium. Thesis, Massachusetts Institute of Technology, MA, 1986.
(24) Brenner, D.; Davison, A.; Lister-James, L.; Jones, A. Synthesis and Characterization of a Series of Isometric Oxotechnetium (V) Diamido Dithiolates. *Inorg. Chem.* **1984**, *23*, 3793.
(25) Zuckman, S. A.; Freeman, G. M.; Troutner, D. E.; Volkert, W. A.; Holmes, R. A.; Van Derveer, D. G.; Barefield, E. K. Preparation and X-Ray Structure of Trans-Dioxo(1,4,8,11-Tetraazacyclotetradecane)Technetium(V) Perchlorate Hydrate. *Inorg. Chem.* **1981**, *20*, 2386–2389.
(26) Kastner, M. E.; Lindsay, M. J.; Clarke, M. J. Synthesis and Structure of Trans-$[O_2(en)_2Tc^V]^+$. *Inorg. Chem.* **1982**, *21*, 2037-2040.
(27) Thomas, R. W.; Estes, G. W.; Elder, R. C.; Deutsch, E. Technetium Radiopharmaceutical Development. 1. Synthesis, Characterization, and Structure of Dichloro[Hydrotris(1-Pyrazolyl)-Borato]Oxotechnetium(V). *J. Am. Chem. Soc.* **1979**, *101*, 4581–4585.
(28) Francesconi, L. C.; Graczyk, G.; Wehril, S.; Shaikh, S. N.; Mc Clinton, D.; Liu, S.; Zubieta, J.; Kung, H. F. Synthesis and Characterization of Neutral M^VO (M = Tc, Re) Amine-Thiol Complexes Containing a Pendant Phenylpiperidine Group. *Inorg. Chem.* **1993**, *32*, 3114–3124.
(29) John, C. S.; Francesconi, L. C.; Kung, H. F.; Wehrli, S.; Graczyk, C.; Carroll, P. Synthesis and Characterization of Neutral Oxotechnetium (V) Bisaminoethanethiol Complexes: Potential Brain Imaging Agents. *Polyhedron* **1992**, *11*, 1145.
(30) Mahmood, A.; Halpin, W. A.; Baidoo, K. E.; Swigart, D. A.; Lever, S. Z. Synthesis and Characterization of N-Ethyl-Diaminedithiol Oxotechnetate (V): A Potential Lung Imaging Agent. In *Technetium and Rhenium In Chemistry and Nuclear Medicine;* Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Raven Press: New York, 1990; pp 113.
(31) Treher, E. N.; Francesconi, L. C.; Gougoutas, J. Z.; Malley, M. F.; Nunn, A. D. Monocapped Tris(Dioxime) Complexes of Technetium (III): Synthesis and Structural Characterization of TcX-(Dioxime)3B-R (X = Cl, Br; Dioxime = Dimethylglyoxime, Cyclohexanedionedioxime; R = CH_3, C_4H_9). *Inorg. Chem.* **1989**, *28*, 3411.
(32) Jurisson, S. S.; Sclemper, E. O.; Troutner, D. E.; Canning, L. R.; Nowotnik, D. P.; Neirinckx, R. D. Synthesis, Characterization and X-Ray Structural Determinations of Technetium(V)-Oxo-Tetradentate Amine Oxime Complexes. *Inorg. Chem.* **1986**, *25*, 543.
(33) Mach, R. H.; Kung, H. F.; Guo, Y.-Z.; Yu, C.-C.; Subramanyam, V.; Calabrese, J. Synthesis, Characterization and Biodistribution of Neutral and Lipid-Soluble ^{99m}Tc-PAT-HM and ^{99m}Tc-TMR For Brain Imaging. *Int. J. Nucl. Med. Biol.* **1989**, *16*, 828–837.
(34) Bandoli, G.; Gerber, T. I. A. Synthesis and Characterization of Tetrabutylammonium Trans-Bis(2-Amidobenzene-Thiolato(2–)-S,N,) Oxotechnetate(V) and Its Reduction By 2-Aminobenzenethiol. *Inorg. Chim. Acta* **1987**, *126*, 205.
(35) 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 Beta-Substituted Progestin Labeled With Technetium-99 and Rhenium-186. *J. Nucl. Med.* **1992**, *33*, 558–569.
(36) Lever, S. Z.; Baidoo, K. E.; Mahmood, A.; Matsumura, K.; Scheffel, U.; Wagner, H. N. Novel Technetium Ligands With Affinity for the Muscarinic Cholinergic Receptor. *Nucl. Med. Biol.* **1994**, *21*, 157–164.
(37) 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.
(38) Linder, K. E.; Chan, Y.-W.; Cyr, J. E.; Malley, M. F.; Nowotnik, D. P.; Nunn, A. D. TcO(PnAO-1-(2-Nitroimidazole)) [BMS-181321], A New Technetium-Containing Nitroimidazole Complex For Imaging Hypoxia: Synthesis, Characterization, and Xanthine Oxidase-Catalyzed Reduction. *J. Med. Chem.* **1994**, *37*, 9–17.