and finally with 1-[(4-methylbenzyl)thio]-1-(carboxymethyl)-cyclohexane, cleaved, cyclized, and purified. Peptide 15 (35 mg) was recovered as pure product after purification.

Radioiodination of Peptides. A 0.2 N AcOH solution (50 μL) containing 20 μg of 8 was placed in a microfuge vial (polyethylene, 250 μL) coated with 5 μg of Iodogen.[38] After the addition of ca. 1 mCi of $Na^{125}I$ (Amersham, not completely carrier free) in 10 μL of aqueous solution, the vial was centrifuged and left for 30 min. With a disposable syringe containing 200 μL of 1 N KI and Na_2SO_3 the vial content was aspirated and rinsed twice with distilled water. The syringe content was injected onto a C-1.8 Sep-pack cartridge (Waters), washed with 5 mL of water and eluted with a gradient of 20%-80% acetonitrile in aqueous 0.05% trifluoroacetic acid. The radioactive fraction containing ^{125}I-8 was collected, pooled, partially evaporated, diluted with water, and injected onto reversed-phase analytical HPLC column, eluted with the same gradient. In the selected main fraction, ca. 300 μCi of labeled 8 were collected. The specific radioactivity of ^{125}I-8 was determined by Scatchard analysis and total binding capacity,[39] generally in the range of 900-400 Ci/mmol and adjusted hereafter according to the decay of ^{125}I. The radiolabeling of the other compounds to ^{125}I-7, ^{125}I-9, and ^{125}I-15 was carried out in an identical manner.

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New Conformationally Restricted ^{99m}Tc N_2S_2 Complexes as Myocardial Perfusion Imaging Agents

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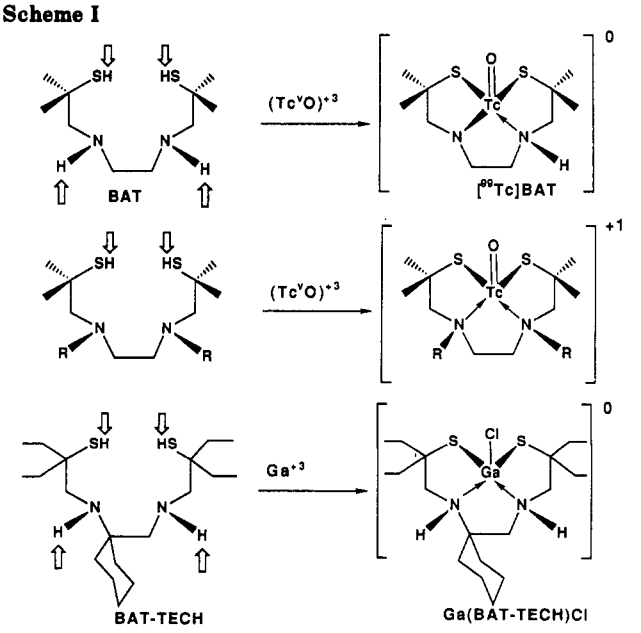
In developing ^{99m}Tc complexes as potential myocardial imaging agents, a new series of ligands based on a conformationally restricted N_2S_2 system were investigated. Using piperazine or homopiperazine as the starting material, two N_2S_2 ligands (**4a** and **4b**) with additional conformation restriction between the two nitrogen donor atoms were synthesized. The ^{99m}Tc complexes were prepared by a direct labeling method with tin(II) tartrate as the reducing agent for [^{99m}Tc]pertechnetate. The resulting ^{99m}Tc complexes were purified through a sulfonpropyl Sephadex column and further purified by HPLC with a reverse-phase column eluting with a solvent system of acetonitrile/buffer. Biodistribution studies in rats showed initial uptake in the heart (0.21%, 0.42% dose/order for [^{99m}Tc]**4a** and **4b** at 2 min postinjection). Carrier-added preparation of [^{99m}Tc]**4b** was successful. NMR, IR, UV, crystallographic, and elemental analysis of the [^{99}Tc]**4b** complex suggest that it contains a $Tc^{V}O^{3+}$ center core and is 1+ charged. The results suggest that this series of 1+ charged ^{99m}Tc complexes may have potential as myocardial imaging agents, and further study of the complexes is warranted.

Imaging of regional myocardial perfusion to separate ischemic from infarcted tissue is one of the most important diagnostic procedures for nuclear cardiology. The procedure is commonly performed with ^{201}Tl ($T_{1/2}$ = 73 h, 70-90 keV) in conjunction with a rest and stress test.[1,2] Based on the size/charge ratio, it is generally accepted that the thallium ion (Tl^{1+}) is a close analogue of potassium (K^+), and the ions are transported across the cell membrane by a Na^+/K^+-ATPase pump.[3] After iv injection, the agent displays a high initial first-pass extraction (>85%) into the myocardium, and distribution reflects regional myocardial blood flow. However, there are several disadvantages associated with ^{201}Tl. First of all, the γ-rays (70-90 keV, Hg X-rays) are too low for optimal γ-detection; a significant amount of the γ-rays is attenuated. Scattering also increases the noise level of the final images, both of which prevent quantitative interpretation of the images. Secondly, the physical half-life is too long; therefore, only a limited quantity (1-3 mCi) can be used for routine procedure. Finally, the isotope is relatively expensive. For these reasons, an agent labeled with ^{99m}Tc and possessing both a shorter half-life ($T_{1/2}$ = 6 h) and an optimal γ-ray energy (140 keV) would be preferable.

Scheme I



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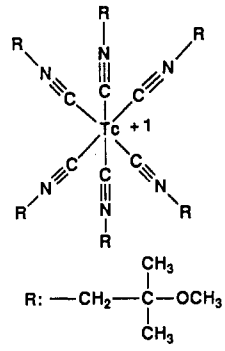
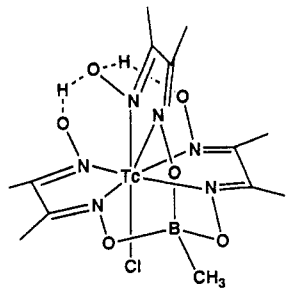
Several new myocardial imaging agents (both neutral and 1+ charged) labeled with ^{99m}Tc have been developed.[4,5]



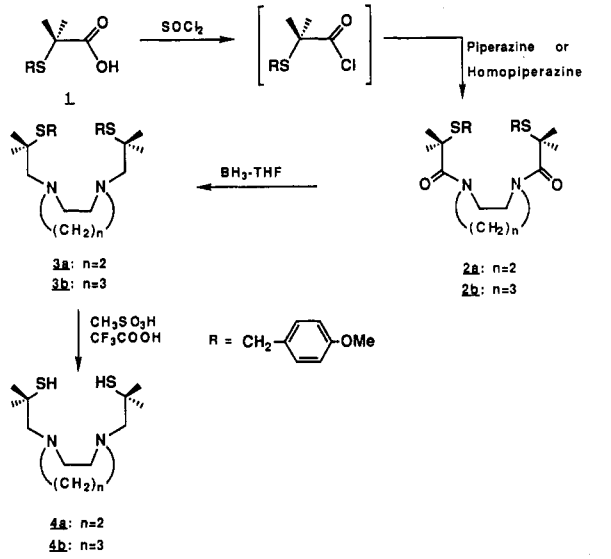
[^{99m}Tc]MIBI



[^{99m}Tc]Teboroxime

Of these, [^{99m}Tc]MIBI [hexakis(2-methoxyisobutyl isocyanide)[^{99m}Tc]technetium][6] and Teboroxime (boric acid adducts of technetium trisdioxime; BATO complex)[7] are FDA approved and are currently being used as ^{201}Tl replacements for clinical studies. [^{99m}Tc]MIBI is a 1+ charged, highly lipid-soluble complex. After iv injection, the compound crosses the cell membrane by a simple diffusion mechanism. Once inside the cell, the agent binds to a cytoplasmic protein with very little washout.[3,8] Initial extraction of the agent is high (~60%), and heart uptake in humans is 1–1.5% of the injected dose. Liver and lung uptake are markedly clear within 1 h. The retention time of [^{99m}Tc]MIBI permits imaging to be carried out after maximum heart-to-lung and heart-to-blood ratios have been established (normally at 1–2 h postinjection).[9] [^{99m}Tc]Teboroxime, on the contrary, is a neutral Tc^{V} complex which displays a higher initial first-pass extraction (90%). The high initial uptake (3% dose) of ^{99m}Tc-labeled Teboroxime in the myocardium is followed by rapid washout. Imaging studies of this agent in humans is initiated at 2 min postinjection. Due to the rapid clearance, a repeat study either at rest or in the exercise state can be carried out within 2 h.[10] Several other new 1+ charged

Scheme II



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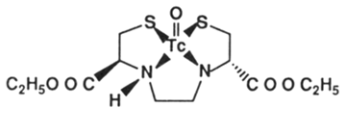
^{99m}Tc complexes, including phosphine, mixed phosphine, and salicylamides, have been reported.[4,11] However, their clinical utility in humans have not been established.

Neutral and lipid-soluble complexes based on the $[Tc^{V}ON_2S_2]$ system have been reported, and one of the most promising agents, [^{99m}Tc]L,L-ECD (ethylene cysteine



[^{99m}Tc] ECD

dimer), is currently under clinical trial as a brain perfusion imaging agent.[12] The $[Tc^{V}ON_2S_2]$ complexes are unique. Normally, there are four ionizable atoms attached to the N_2S_2 ligand: two S–H and two N–H. After complexing, three of the four ionizable hydrogens are ionized, leaving one N–H group intact. Serendipitously, the center core is $Tc^{V}O^{3+}$. The positive charges on Tc and the negative charges from the ligand cancel each other out, resulting in the formation of a neutral complex (Scheme I). When both of the nitrogens are alkylated, the ionization process normally associated with the complex formation with $Tc^{V}O^{3+}$ no longer exists; therefore, the overall net charge of the complex is 1+ (Scheme I). Initial evaluation of these types of complexes was reported.[13]

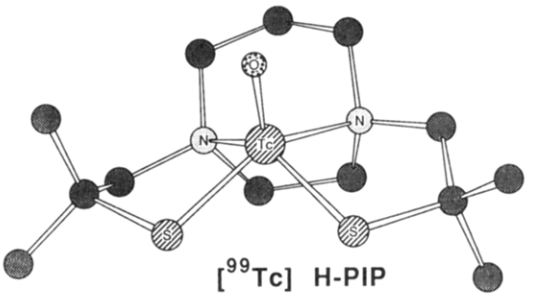
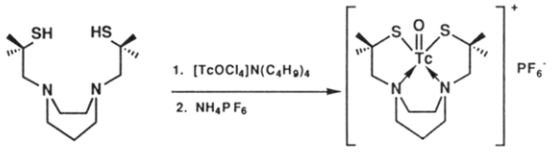


Figure 1. X-ray crystallographic structure of [$^{99}Tc^{V}$O**4b**]PF_6.

Scheme III



Recently, synthesis, characterization, and biodistribution of the [^{68}Ga]BAT-TECH complex as a myocardial imaging agent was reported. Gallium formed a 1+ charged cation with the N-unalkylated N_2S_2 ligand. Apparently, after complexing with Ga^{3+}, only the SH groups of N-unalkylated N_2S_2 ligand were ionized. Therefore, the resulting complex is a 1+ charged. Structure determinations, including UV, FT-IR, NMR, and X-ray crystallography, confirm the proposed structure for Ga(BAT-TECH).[14] As expected, [^{68}Ga]BAT-TECH displayed high myocardial uptake in rats and monkeys.

In order to investigate the structural versatility of this series of ligands, a new series of ligands based on a conformationally restricted N_2S_2 system was investigated. These ligands were chosen because the nitrogen donor atoms are tertiary amines (N-alkylated) which are unionizable; therefore, after complex formation, the $Tc^{V}O^{3+}$ complexes will be 1+ charged. The lipid-soluble, 1+ charged ^{99m}Tc complexes may potentially be useful for myocardial imaging.

Chemistry

Using piperazine or homopiperazine as the starting material, two N_2S_2 ligands, **4a** and **4b**, with additional conformation restriction between the two nitrogen donor atoms are synthesized (Scheme II). The starting material, 2-[(*p*-methoxybenzyl)thio]-2-methylpropionic acid (**1**), was prepared by reacting *p*-methoxybenzyl mercaptan and ethyl 2-bromo-2-methylpropionate in the presence of sodium ethoxide. The ethyl ester was hydrolyzed in sodium hydroxide solution to give the desired propionic acid (**1**).

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The acid was converted to the acyl chloride by thionyl chloride in refluxing chloroform. The active acyl chloride was used in situ with the piperazine or homopiperazine to give the corresponding amides (**2a** and **2b**) in excellent yield (79% and 82%, respectively). After reduction reaction with borane–tetrahydrofuran complex, the reduced products (**3a** and **3b**) were obtained. The final ligands, **4a** and **4b**, were produced by deprotecting the *p*-methoxyphenyl group, and the ligands were isolated as hydrochloride salts. Overall yields of the reaction sequences are high and they are flexible and can be adapted for preparation of other N_2S_2 ligands.

Radiolabeling and Characterization

The radiolabeling of **4a** and **4b** with ^{99m}Tc was accomplished (at a tracer concentration of 10^{-8} M) using stannous tartrate as the reducing agent. The radiochemical purity of the complexes (with HPLC purification for the **4a** complex) were analyzed by HPLC and determined to be greater than 95%.

Preparation of carrier-added [^{99}Tc^{V}O**4b**][PF_6] was accomplished by reacting the ligand **4b** with [$TcOCl_4$][N-(C_4H_9)$_4$] (Scheme III). The reaction mixture was condensed and passed through an LH-20 Sephadex column and eluted sequentially with acetone and acetone/methanol (90:10). The fraction containing the desired product was treated with aqueous NH_4PF_6. After condensing the solution, the [^{99}Tc^{V}O**4b**][PF_6] was precipitated (yield, 50%, based on the ligand used). Crystals were grown by evaporation of a methylene chloride/ethanol solution. Reverse-phase HPLC profiles of coinjected [^{99m}Tc] and [^{99}Tc]**4b** complexes suggest that carrier-added (UV detector) and no-carrier-added (γ-detector) complexes exhibited an identical chromatographic profile, and it is likely that the same chemical structure was obtained under both reaction conditions. A distinct Tc=O^{3+} absorption peak at approximately 960 cm^{-1} in the FT-IR spectra was observed. The UV spectra exhibited a strong absorption peak at 308 (ϵ = 3406) and 276 nm (ϵ = 3788) for [^{99}Tc]**4b**. The [^{99}Tc]**4b** complex also afforded correct elemental analyses.

The X-ray crystallographic data for [^{99}Tc^{V}O**4b**][PF_6] confirmed the proposed structure (Figure 1). The coordination geometry about the cationic complex [^{99}Tc^{V}O**4b**][PF_6] is square pyramidal. The deviation of the nitrogens and sulfurs from the plane defined by the N1, N2, S1, S2 nitrogen and sulfur atoms is less than 0.1 Å. This is in contrast to heavily distorted square pyramidal Tc coordination environment in the neutral TcO(BAT) complexes, where one nitrogen is an amine and one nitrogen is a deprotonated amide and the deviations of the nitrogens and sulfurs from the plane are ca. 0.2–0.3 Å.[15] For [^{99}Tc^{V}O**4b**][PF_6], the Tc is 0.793 Å above the N_2S_2 plane. The atoms in the propylene bridge are 1.010, 0.879, and 0.810 Å above the N_2S_2 plane, and the atoms in the ethylene bridge are 1.426 and 1.293 Å below the N_2S_2 plane. Proton and carbon-13 NMR data (CD_3CN) show that two conformers are present (90% and 10%) in solution. The TcO bond length, 1.667 Å, is in the range observed for TcO bonds. The Tc–N bond lengths (2.118, 2.139 Å) are both in the range of Tc–N single bonds. The Tc–S bond distances (2.24, 2.246 Å) are in the range observed for Tc–S thiolate bonds. The N1–Tc–N2 bond angle is 76.4°, which is less than that found in other structures with ethylene (BAT-TE) or propylene (PAT) diamine backbones, for example, TcO(BAT-TE) (N–Tc–N = 79.83°) or TcO(PAT) (89.7°), presumably due to the conformational restriction of this ligand. As expected both sulfur atoms undergo ionization during complexation. This pattern of ionization, of only the hydrogens of sulfur atoms but not the alkylated groups on nitrogen atoms, leads to the formation of a 1+ charged complex, which is different from that occurring with the [^{99m}Tc]BAT complexes.[16]

Table I. Biodistribution of [^{99m}Tc]**4a** and [^{99m}Tc]**4b** in Rats

organ	% dose/organ		
	2 min	15 min	65 min
	[^{99m}Tc]**4a**		
blood	7.16 ± 0.32	2.00 ± 0.26	0.70 ± 0.05
heart	0.21 ± 0.02	0.10 ± 0.02	0.07 ± 0.01
muscle	10.67 ± 0.88	10.12 ± 3.80	5.06 ± 0.18
lung	0.62 ± 0.08	0.23 ± 0.02	0.12 ± 0.005
kidney	15.71 ± 0.11	9.43 ± 1.39	1.09 ± 0.19
spleen	0.57 ± 0.09	0.25 ± 0.05	0.08 ± 0.01
liver	31.81 ± 1.64	29.63 ± 6.64	20.23 ± 0.76
skin	6.65 ± 0.96	14.13 ± 5.27	7.13 ± 2.21
brain	0.03 ± 0.005	0.01 ± 0.001	0.003 ± 0.001
heart/blood[b]	0.29	0.50	1.0
	[^{99m}Tc]**4b**		
blood	3.57 ± 0.64	1.14 ± 0.38	0.77 ± 0.04
heart	0.42 ± 0.04	0.38 ± 0.03	0.37 ± 0.02
muscle	9.08 ± 0.86	6.98 ± 1.39	8.66 ± 2.02
lung	0.56 ± 0.10	0.28 ± 0.006	0.23 ± 0.02
kidney	19.37 ± 0.69	15.90 ± 1.81	4.26 ± 0.60
spleen	0.41 ± 0.10	0.26 ± 0.04	0.22 ± 0.03
liver	25.92 ± 3.28	27.48 ± 8.04	15.23 ± 2.80
skin	7.45 ± 0.84	5.16 ± 1.21	6.80 ± 2.07
brain	0.04 ± 0.01	0.01 ± 0.001	0.007 ± 0.001
heart/blood[b]	1.18	3.33	4.80

[a] IV injection, average of three rats. [b] % dose/gram ratio.

The 2-min post iv injection data indicated that the initial heart uptake of [^{99m}Tc]**4b** is twice that observed for [^{99m}Tc]**4a** (Table I). This is most likely due to the slightly higher lipid solubility of **4b** in turn caused by the presence of an additional methylene group and is consistent with the reverse-phase HPLC retention times of the two compounds. [^{99m}Tc]**4b** had a retention time of 6.8 min, whereas the retention time of [^{99m}Tc]**4a** was 6.3 min. However, the partition coefficients (PC) of the two compounds are similar. In the presence of a buffer containing 0.1 M NaCl, the PC values are 1.09 and 1.19 for [^{99m}Tc]**4a** and [^{99m}Tc]**4b**, respectively. In a buffer containing 0.1 M NH_4PF_6, the PC values are 10.75 and 11.50 for [^{99m}Tc]**4a** and [^{99m}Tc]**4b**, respectively. The heart:blood ratio for [^{99m}Tc]**4a** and [^{99m}Tc]**4b** at 2 min postinjection was 0.29 and 1.18, respectively. At 30 min, the concentration of [^{99m}Tc]**4b** remained relatively constant (0.38% dose). This indicates that the diffusion of the chelates to the myo-

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cardium is a reversible process for [^{99m}Tc]**4a** and is dependent on the relative concentration gradient between the blood and the tissue. However, the [^{99m}Tc]**4b** can be localized in the myocardial tissue and it appears to remain in the tissue for up to 1 h without significant washout. The initial data on heart uptake are encouraging; however, compared to the uptake of [^{99m}Tc]MIBI or [^{99m}Tc]Teboroxime in the heart (both showed ~3% dose), the uptake of [^{99m}Tc]**4b** is not sufficiently high for clinical use. The liver is the organ with high uptake for these two compounds. Heart to liver ratios were low. As expected, the cationic ^{99m}Tc compounds showed little brain uptake uptake and retention. The biodistribution data suggest that this type of complex may be potentially useful. With increase of the intrinsic lipophilicity by adding more nonpolar functional groups, this series of complexes may serve as a core structure for developing useful myocardial imaging agents.

In conclusion, the two new conformationally restricted N_2S_2 ligands, **4a** and **4b**, described above possess desirable characteristics, forming stable, lipid-soluble, 1+ charged complexes with ^{99m}Tc. The novel N_2S_2 ligands contain additional linkage between the two nitrogen donor atoms, which prevents ionization after complex formation. Spectroscopic studies of the carrier-added ^{99}Tc complex of **4b** confirm that this ligand forms a 1+ charged complex with a $Tc^{V}O^{3+}$ center core. Biodistribution studies in rats showed initial uptake in the heart for [^{99m}Tc]**4a** and **4b**. The results suggest that this series of 1+ charged ^{99m}Tc complexes may have potential as myocardial imaging agents, and further study of the complexes is warranted.

Experimental Section

Melting points were determined with a Meltemp (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 spectrometer. Elemental analyses were performed by Atlantic Microlabs, Inc., of Atlanta, GA. All of the chemicals were of reagent grade and used without further purification.

2-[(*p*-Methoxybenzyl)thio]-2-methylpropionic Acid (1). To a solution of sodium (1.2 g, 0.05 mol) in dry ethanol (100 mL) was added slowly *p*-methoxybenzyl mercaptan (7.7 g, 0.05 mol) followed by ethyl 2-bromo-2-methylpropionate (9.8 g, 0.05 mol). The reaction mixture was stirred at reflux for 3 h. The precipitates were filtered off, and a 5 N NaOH solution (20 mL) was added to the filtrate, which was then refluxed for another hour. The reaction mixture was acidified with concentrated HCl with cooling. After removal of the ethanol in vacuo, the residue was taken up with chloroform (50 mL) and washed with water (30 mL × 2). The organic layer was dried over sodium sulfate and evaporated in vacuo. Recrystallization from hexane afforded the desired compound (1) (9.8 g, 82%): mp 88–89 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 (6 H, s, CH_3), 3.73 (3 H, s, OCH_3), 3.82 (2 H, s, CH_2S), 6.70 (2 H, d, aromatics), 7.17 (2 H, d, aromatics), 9.84 (1 H, br, COOH); IR (KBr) 3000, 1690, 1620, 1520, 1300, 1250 cm^{-1}. Anal. for $C_{12}H_{16}O_3S$: C, H.

***N*,*N*′-Bis[2-[(*p*-methoxybenzyl)thio]-2-methylpropionyl]piperazine (2a).** To a solution of 2-[(*p*-methoxybenzyl)thio]-2-methylpropionic acid (1) (5.3 g, 0.022 mol) in dry chloroform (30 mL) was added thionyl chloride (11.9 g, 0.1 mol). The reaction mixture was refluxed for 3 h; then the solvent was removed in vacuo. The residue was dissolved in dry methylene chloride (10 mL) which was added dropwise to a solution of piperazine (0.86 g, 0.01 mol) and triethylamine (2.2 g, 0.022 mol) in dry methylene chloride (40 mL). The resultant reaction mixture was stirred for 3 h at ambient temperature and then washed with 1 N NaOH (50 mL × 2) and water (50 mL × 2). The organic phase was dried over sodium sulfate and evaporated in vacuo. Recrystallization from hexane and methylene chloride gave the desired amide **2a** (4.2 g, 79%): mp 183–184 °C; ^{1}H NMR ($CDCl_3$) δ 1.55 (12 H, s, CH_3), 3.60 (6 H, s, OCH_3), 3.63 (4 H, s, CH_2S), 3.67 (8 H, s, NCH_2), 6.70 (4 H, d, aromatics), 7.10 (4 H, d, aromatics); IR (KBr) 1620, 1520, 1410, 1250 cm^{-1}. Anal. for $C_{28}H_{38}N_2O_4S_2$: C, H, N.

***N*,*N*′-Bis[2-[(*p*-methoxybenzyl)thio]-2-methylpropionyl]homopiperazine (2b).** Compound **2b** was prepared using a procedure similar to that described for compound **2a** to yield 82%.

***N*,*N*′-Bis[2-[(*p*-methoxybenzyl)thio]-2-methylpropyl]piperazine (3a).** To a suspension of amide **2a** (3.9 g, 7.3 mmol) in anhydrous tetrahydrofuran (50 mL) was slowly added a 1 M solution of borane–tetrahydrofuran complex in tetrahydrofuran (50 mL, 50 mmol). The reaction mixture was stirred at reflux for 16 h and quenched by dropwise addition of water (10 mL) with cooling. Volatile components were removed in vacuo. The residue was dissolved in a 6 N HCl solution (100 mL) and the mixture was refluxed for 1 h. After bacification with cooling, the reaction mixture was extracted with chloroform (50 mL × 3). The combined organic layers were washed with water (50 mL × 3), dried over sodium sulfate, and evaporated in vacuo to give an oil which was converted to its HCl salt. Recrystallization from ethanol and water gave the diamine dihydrochloride salt **3a** (3.2 g, 76%): mp (HCl salt) 209–210 °C; ^{1}H NMR (free base in $CDCl_3$) δ 1.27 (12 H, s, CH_3), 2.40 (4 H, s, $NCH_2C(Me)_2$), 2.58 (8 H, s, NCH_2CH_2N), 3.72 (10 H, overlap, OCH_3 + CH_2S), 6.72 (4 H, d, aromatics), 7.15 (4 H, d, aromatics); IR (HCl salt, KBr) 3000, 2550, 1620, 1520, 1450, 1260 cm^{-1}. Anal. for $C_{28}H_{42}N_2O_2S_2$·2HCl: C, H, N.

***N*,*N*′-Bis[2-[(*p*-methoxybenzyl)thio]-2-methylpropyl]homopiperazine (3b).** Compound **3b** was prepared using a procedure similar to that described for compound **3a** to yield 81%.

***N*,*N*′-Bis(2-mercapto-2-methylpropyl)piperazine (4a).** To a solution of S-protected amine **3a** (0.5 g, 1 mmol) and anisole (0.32 g, 3 mmol) in trifluoroacetic acid (10 mL) was added methanesulfonic acid (3 mL). The reaction mixture was stirred for 1 h at ambient temperature. Volatile components were removed in vacuo to give a residue that was dissolved in water (30 mL) and washed with ether (15 mL × 2). The pH of the aqueous layer was adjusted to neutral and the layer was extracted with chloroform (15 mL × 3). The combined chloroform layers were washed with water (20 mL × 2), dried over sodium sulfate, and concentrated in vacuo to give a free base as an oil which was converted to HCl salt. Recrystallization from ethanol and water afforded the desired product **4a** (0.3 g, 90%): mp (HCl salt) 220–222 °C dec; ^{1}H NMR (free base in $CDCl_3$) δ 1.28 (12 H, s, CH_3), 2.26 (2 H, br, SH), 2.34 (4 H, s, $NCH_2C(Me)_2$), 2.60 (8 H, s, NCH_2CH_2N); IR (HCl salt, KBr) 3400, 3000, 2650, 2600, 2500, 1450, 1420, 1370, 1280 cm^{-1}. Anal. for $C_{12}H_{28}N_2S_2$·2HCl: C, H, N.

***N*,*N*′-Bis(2-mercapto-2-methylpropyl)homopiperazine (4b).** Compound **4b** was prepared by a procedure similar to that described for compound **4a** to yield 94%: mp (HCl salt) 180–182 °C; ^{1}H NMR (free base in $CDCl_3$) δ 1.28 (12 H, s, CH_3), 1.74 (2 H, m, $CH_2CH_2CH_2$), 2.17 (2 H, br, SH), 2.56 (4 H, s, $NCH_2C(Me)_2$), 2.86 (8 H, overlap, NCH_2CH_2N and $NCH_2CH_2CH_2N$); IR (HCl salt, KBr) 3400, 3000, 2600, 2450, 1470, 1420, 1400, 1380, 1320 cm^{-1}. Anal. for $C_{13}H_{28}N_2S_2$·2HCl: C, H, N.

Radiolabeling. Ligands **4a** and **4b** (1.0 mg in 0.4 mL of 50% ethanol solution) were mixed with ~5 mCi of sodium [^{99m}Tc]-pertechnetate (in 0.2 mL saline) and 0.2 mL of saturated stannous tartrate solution. The mixture was heated for 15 min at 90 °C (heating block). After cooling, the mixtures were passed through a small sulfonpropyl Sephadex column (cation exchange) and the column was washed with water and eluted with saline. The saline eluent of [^{99m}Tc]**4a** was further purified on HPLC (PRP-1 column, 70:30 acetonitrile/dimethyl glutarate buffer, 5 mM, pH 7.0) with a flow rate of 1.0 mL/min. The radiochemical purity of the purified [^{99m}Tc]**4a** complex was ≥95% with a retention time of 6.3 min in the HPLC system (radiochemical yield, 50%). Saline elute of the [^{99m}Tc]**4b** complex was analyzed on HPLC under the same conditions which gave a major peak (≥95% pure) with a retention time of 6.8 min. No further HPLC purification for the [^{99m}Tc]**4b** complex was required (radiochemical yield, 70%). After dilution with saline, both labeled complexes were used for animal biodistribution studies.

Preparation of [Tc^{V}O4b][PF_6]. The ligand **4b**·2HCl (15.5 mg, 0.044 mmol) was dissolved in 2 mL of methanol. To the stirring ligand solution was added a solution of [$TcOCl_4$]($N(C_4H_9)_4$)

(24.4 mg, 0.048 mmol) dropwise.[17] Immediately upon addition of the Tc solution, the color of the resulting solution turned orange. The solution was heated at 40 °C for 30 min and then stirred for 10 more hours. The orange solution was evaporated to a film and redissolved in 10 mL of acetone. A light solid was filtered off. The acetone solution was reduced by evaporation to a volume of 0.5 mL and loaded onto a Sephadex LH-20 column prepared with acetone. The column was eluted with acetone. A green-yellow diffuse band eluted first. A tight orange band remained at the top of the column. This band was eluted with 10% methanol and 90% acetone, and 50 mL of a 6 M aqueous solution of NH_4PF_6 was added along with 200 μL of water. The resulting solution was reduced in volume until an orange solid precipitated. The solid was filtered, washed with water (5 × 1 mL), isopropyl alcohol (3 × 1 mL), and ether (3 × 1 mL), and dried in vacuo to yield 12 mg (0.022 mmol, 50% based on **4b**): IR 960 (Tc=O), 840 cm^{-1} (PF_6); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.777 (6 H, CH_3), 1.805 (6 H, CH_3), 2.432 (4 H, CH_2), 3.455 (2 H, CH_2), 3.618 (4 H, CH_2), 3.790 (2 H, CH_2), 4.156 (2 H, CH_2); UV (acetonitrile) 308 (ε 3406), 276 nm (ε 3788). Anal. for $C_{13}H_{26}N_2S_2TcOPF_6$: C, H, N, S.

Crystal Data. $TcC_{13}H_{26}N_2S_2OPF_6$, ^{99}TcO**4b**·$PF_6$, dark red, block, monoclinic, $P2_1/n$ (#14) (systematic absences 0k0: k = odd and h0l: h + l = odd), a = 7.774 (3) Å, b = 23.157 (5) Å, c = 12.120 (2) Å, b = 105.62 (3)°. The cell constants were determined from a least-squares fit of the setting angles for 25 accurately centered reflections. V = 2101 (2) Å^3, Z = 4, FW = 533.46, D_c = 1.686 g/mL, μ(Mo) = 9.90 cm^{-1}.

Data Collection and Treatment. X-ray intensity data were collected on an Enraf-Nonius CAD4 diffractometer employing graphite-monochromated MoKα radiation (λ = 0.71073 Å) and using the ω–2θ scan technique. A total of 4083 reflections were measured over the ranges $4 \le 2\theta \le 50°$, $0 \le h \le 9$, $0 \le k \le 27$, $-14 \le l \le 14$. 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 for absorption by the empirical ψ-scan method (minimum, maximum, and average transitions 75.2%, 99.9%, 86.3%). Of the reflections measured, a total of 1899 unique reflections with $F^2 > 3\sigma(F^2)$ were used during subsequent structure refinement.

Solution and Refinement. The structure was solved by standard heavy-atom Patterson methods followed by weighted Fourier syntheses. 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 included as constant contributions to the structure factors and were not refined. Refinement converged to R_1 = 0.056 and R_2 = 0.061. Goodness of fit: 1.589.

Animal Distribution Studies. Male Sprague–Dawley rats (200–300 g) were injected intravenously (under anesthesia) with 0.2 mL of a saline solution containing the ^{99m}Tc 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 γ-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/gram values were computed from the percent dose/organ values and the corresponding organ weights. Finally, the blood:brain ratio was calculated from the corresponding percent dose/gram values.

Determination of Partition Coefficients. The partition coefficients were determined by mixing the [^{99m}Tc]**4a** and **4b** complexes with 3 g each of 1-octanol and buffer (50 mM phosphate, pH 7.4 containing either 0.1 M NaCl or 0.1 M NH_4PF_6) in several test tubes. The tubes were vortexed three times for 1 min at room temperature and subsequently centrifuged for 5 min to separate the layers. Two weighed samples from the octanol and the aqueous layers were then counted in a γ-counter. The partition coefficient was calculated as the ratio of the counts per gram in the octanol layer divided by that in the aqueous layer.

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Registry No. 1, 136847-16-0; 2, 136847-17-1; **2b**, 136847-18-2; **3a**, 136847-19-3; **3a**·2HCl, 136847-20-6; **3b**·HCl, 136847-21-7; **4a**, 68219-41-0; **4a**·2HCl, 136847-22-8; **4b**, 136847-23-9; **4b**·2HCl, 136847-24-0; [^{99}Tc^{V}O**4b**][PF_6], 136847-26-2; [^{99m}Tc]**4a**, 136847-27-3; [^{99m}Tc]**4b**, 136847-28-4; [^{99}TcOCl_4]($N(C_4H_9)_4$), 92622-25-8; *p*-methoxybenzyl mercaptan, 6258-60-2; ethyl 2-bromo-2-methylpropionate, 600-00-0; piperazine, 110-85-0; sodium[^{99m}Tc]pertechnetate, 23288-60-0; homopiperazine, 505-66-8.

Supplementary Material Available: X-ray crystallography data including four tables giving refined positional parameters, refined thermal parameters, bond distances and bond angles for compound [^{99}Tc]**4b** (8 pages). Ordering information is given on any current masthead page.

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