Synthesis of New Bis(aminoethanethiol) (BAT) Derivatives: Possible Ligands for ^{99m}Tc Brain Imaging Agents

H. F. Kung,*† C. C. Yu,† J. Billings,† M. Molnar,† and M. Blau‡

Department of Nuclear Medicine, SUNY—Buffalo and VA Medical Center, Buffalo, New York 14215, and Department of Radiology, Harvard Medical School, Boston, Massachusetts 02115. *Received July 23, 1984*

In developing new brain perfusion imaging agents for single photon emission computed tomography (SPECT), five ^{99m}Tc-labeled neutral bis(aminoethanethiol) (BAT) derivatives capable of crossing the blood–brain barrier are reported. The five ligands are prepared by two versatile synthetic methods that can specifically introduce substituents on one of the carbons between two nitrogens. These ligands formed stable and neutral complexes with the reduced ^{99m}Tc, using either Sn(II) or sodium borohydride to reduce sodium [^{99m}Tc]pertechnetate. The biodistribution in rats was evaluated with [^{125}I]iodoantipyrine, a freely diffusible tracer, as the internal reference. Compounds with a free hydroxyl group, **6** and **15**, showed lower brain uptake. High initial brain uptake was observed for compounds **10** and **14**, 1.28 and 2.30% dose/organ, respectively. Compounds of this type may be used as a basis for future structural modification to improve brain uptake and retention.

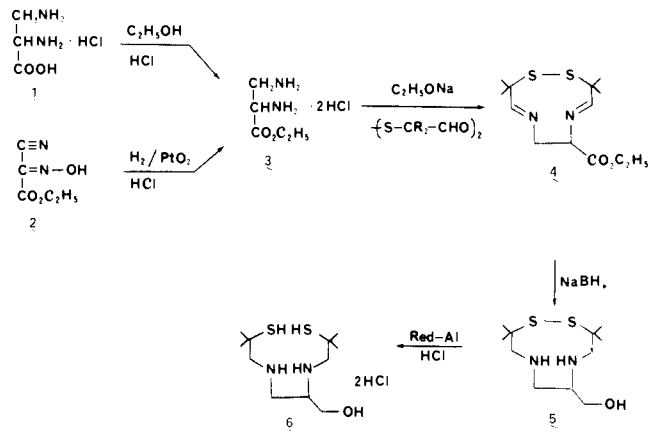
In developing brain perfusion imaging agents for single photon emission computed tomography (SPECT), we reported a group of ^{75}Se- and ^{123}I-labeled tertiary diamines[1-4] that can cross the blood–brain barrier (BBB) and will maintain a fixed brain distribution pattern reflecting regional perfusion. One of the ^{123}I-labeled diamines, HIPDM (*N,N,N'*-trimethyl-*N'*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine),[4] is currently under clinical trial. A monoamine, IMP (*N*-isopropyl-*p*-[^{123}I]iodoamphetamine), also showed similar brain uptake and retention.[5,6] Several recent reports have validated qualitatively and quantitatively the clinical usefulness of these two agents as local cerebral blood flow indicators.[7-11]

The major drawback of the various iodinated brain imaging agents for SPECT is the high cost and limited supply of uncontaminated ^{123}I ($T_{1/2}$ = 13 h, γ ray 159 keV), which currently requires a 70-MeV cyclotron to produce. A ^{99m}Tc- ($T_{1/2}$ = 6 h, γ ray 140 keV) labeled agent with similar biological properties would be more desirable and practical for routine clinical application.

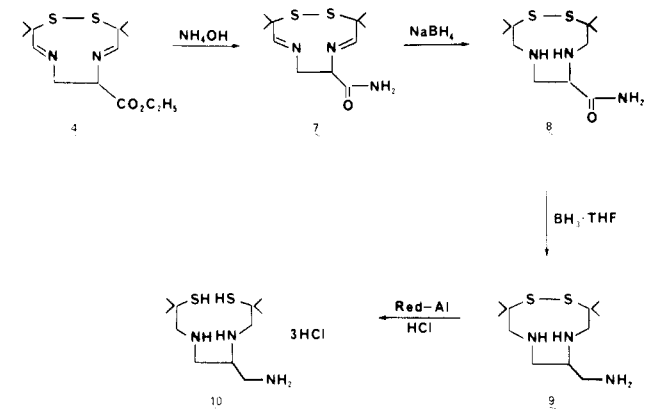
Three ^{99m}Tc-labeled neutral BAT (bis(aminoethanethiol), a N_2S_2 ligand) chelates that are capable of crossing the blood–brain barrier (BBB) have been reported.[12] Biodistribution in rats showed a significant brain uptake (1–3%/whole brain) at 2 min (iv), but at 15 min the uptake dropped to about one-tenth of the original level. This rapid brain washout indicates free passage (via a simple diffusion mechanism) in both directions across the BBB for the ^{99m}Tc complexes. This group of ^{99m}Tc BAT complexes clearly exhibited the ability to cross the BBB after an iv injection. The neutral lipid-soluble ^{99m}Tc-labeled 3,3'-(1,3-propanediyldiimino)bis(3-methyl-2-butanone) dioxime (^{99m}TcPnAO) has also been shown to penetrate the BBB by passive diffusion.[13] It also showed high initial brain uptake (1%/dose at 1 min) and fast washout (0.22%/dose at 15 min) in rats after iv injection.

These types of lipid-soluble ^{99m}Tc brain agents, showing high initial uptake and fast washout, are probably not very useful clinically because the retention time may not be long enough for data collection in SPECT imaging. Currently, the SPECT systems, based on single- or dual-head γ cameras, require 15-min to 1-h imaging time. Therefore, brain imaging agents that exhibit both a high initial uptake and a fixed regional distribution of 15 min or more are essential for measuring local cerebral blood flow. In order to prepare BAT derivatives with longer brain retention we have developed two versatile synthetic methods that can specifically introduce substitution groups on one of the carbons between the two nitrogens of the N_2S_2 ligand. By avoiding substitution on the N or S positions, the ability

Scheme I



Scheme II



† SUNY–Buffalo and VA Medical Center.
‡ Harvard Medical School.

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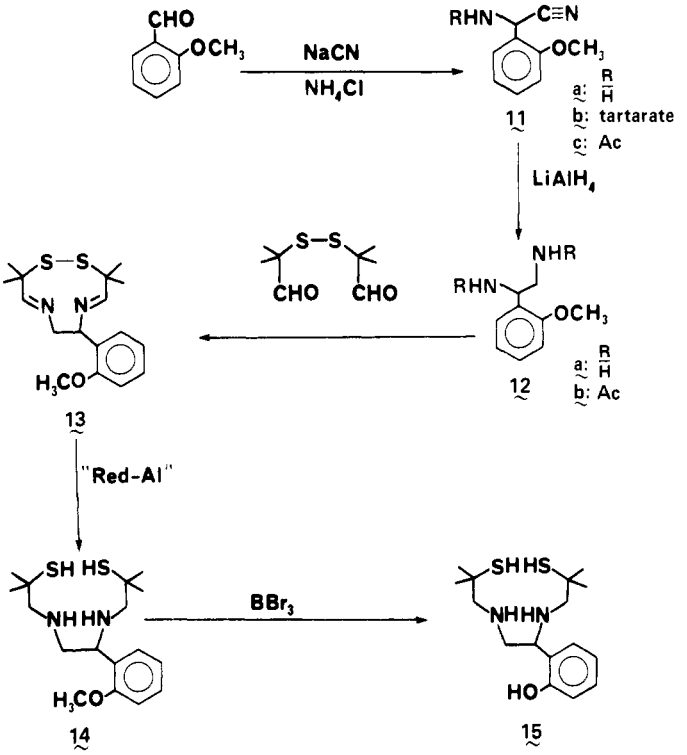
Scheme III



of these derivatives to form stable and neutral ^{99m}Tc complexes would, in theory, be unchanged. The new BAT ligands were successfully labeled with ^{99m}Tc, and the biodistribution of the ^{99m}Tc complexes was evaluated in rats.

Chemistry. Ethyl 2,3-diaminopropionate (**3**) was prepared either by esterification of 2,3-diaminopropionic acid (**1**) (method A[14]) or by a simultaneous catalytic reduction of the cyano and oxime groups on ethyl cyanoglyoxylate 2-oxime (**2**) (method B[15]). The latter is the preferred reaction because it uses a starting material, **2**, that is less expensive and is available in a larger quantity. Ethyl 2,3-diaminopropionate (**3**) was reacted with 2,2′-dithiobis(2-methylpropanal) to give a 10-membered ring diimine, **4**. When the diimine was treated with sodium borohydride at 50 °C, the free BAT hydroxyl derivative, **5**, was obtained (Scheme I). The diimine **4** can also be converted to the amide **7** by reaction with concentrated ammonia at room temperature (Scheme II). Subsequent reduction of the amide **7** with diborane gave the free amine **9**. All of the reactions in Scheme I and II gave excellent yields (80–90%), except the diborane reduction step which yielded 60%.

The second route employs the Strecker reaction for preparing α-amino nitriles (Scheme III). Using 2-methoxybenzaldehyde as the starting material for the α-amino nitrile (**11a**), derivatives can be prepared. After reducing the amino nitrile, **11a**, with lithium aluminum hydride, the diamine, **12a**, was condensed with the dialdehyde to give the corresponding diimine, **13**. The dimercapto and diimine bonds were reduced simultaneously by Red-Al to give the desired BAT compound, **14**. Compound **14** was treated with boron tribromide, a demethylating agent, to give a phenolic compound **15**.

Using tin(II) pyrophosphate (PPi) or sodium borohydride as the reducing agent, the BAT derivatives were labeled successfully with [^{99m}Tc]pertechnetate. The radiochemical purity of the ^{99m}Tc BAT complexes was >95% (HPLC, reversed-phase column, acetonitrile–pH 7.0 dimethylglutaric acid buffer (85:15). The partition coefficients (PC) of these ^{99m}Tc complexes were measured in a mixture of 1-octanol and pH 7.0 phosphate buffer. Partition coefficient (PC) and protein binding data for ^{99m}Tc complexes of compounds **6**, **10**, **14**, and **15** are presented in Table I. All of the ^{99m}Tc compounds are lipid soluble. Compound **10** showed the lowest PC (46.8), and compound **14** showed the highest PC (260). The protein binding of these ^{99m}Tc compounds is inversely related to the lipid solubility (PC).

Table I. Partition Coefficients and Protein Binding of ^{99m}Tc Complexes

compd	partition coeff (pH 7.0)	protein binding: % free
6	64.5 ± 0.56	42.5 ± 0.69
10	46.8 ± 4.06	46.2 ± 0.86
14	260 ± 15.4	23.4 ± 1.92
15	248 ± 33.4	14.7 ± 1.23

Biological Results. In order to accommodate for differences between individual rats, the biodistribution in rats after an iv injection was evaluated with use of [^{125}I]-iodoantipyrine (IAP), a freely diffusible tracer, as the internal reference.[16]

The ^{99m}Tc complex **14** showed the highest brain uptake (2.3% dose/organ) in rats 2 min after the iv injection. This value is 43% higher than that for [^{125}I]IAP. The complex is very lipid soluble (PC = 260 at pH 7.0), which may explain the high initial brain uptake. The ^{99m}Tc-labeled compound **15**, which is the O-demethylated analogue of compound **14**, showed similar lipid solubility (PC = 248 at pH 7.0), but the brain uptake was much lower (0.41% dose/organ, at 2 min). It seems possible that the presence of a free hydroxyl group may decrease the in vivo stability of the ^{99m}Tc complex; as a consequence, the decomposed compound cannot pass through the BBB. Compound **6**, which has a free hydroxy group, also showed low brain uptake. It may also be due to the poor in vivo stability of the ^{99m}Tc complex.

Discussion

In the past few years the development of new ^{99m}Tc brain imaging agents has attracted a lot of attention.[17-20] In conjunction with single photon emission computed tomography (SPECT), these agents will be useful to image and evaluate the regional cerebral blood flow (rCBF) and/or cerebral metabolism.

In this paper, two substituted ^{99m}Tc BAT complexes with high initial brain uptake in rats are reported. However, high initial brain uptake without prolonged brain

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retention is not very useful clinically, because the imaging time required for SPECT is in the range of 15–60 min.

The schemes reported in this paper provide convenient and versatile synthetic routes for preparing BAT derivatives that form neutral and lipid-soluble ^{99m}Tc complexes. Some of the ^{99m}Tc complexes showed significant initial brain uptake, but the retention time in the brain was too short. Compounds of this type may be useful as a basis for further structure–activity relationship studies to develop agents with optimal brain uptake and retention properties suitable for SPECT imaging.

Experimental Section

Melting points were determined on a Nalge hot stage and are reported uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and all values are within ±0.4% of theoretical numbers. NMR spectra were recorded on a Varian T-60A spectrometer, taken in either deuterated chloroform or dimethyl sulfoxide with tetramethylsilane as the internal standard. Infrared spectra were determined on a Perkin-Elmer Model 727B spectrophotometer as KBr pellets. Mass spectroscopy was performed by the Department of Chemistry, Cornell University, Ithaca, NY. Spectral properties were consistent with the proposed structures. Radioactivity was determined using a dual-channel Beckman Automatic Gamma Counter (Model 4000). High-performance liquid chromatography (Varian 5000) was done on a Hamilton PRP-1 reversed-phase column eluted with acetonitrile–3,3-dimethylglutaric acid buffer (0.01 M, pH 7.0) (85:15).

Preparation of Ethyl 2,3-Diaminopropionate Dihydrochloride (3). Method A. Dry hydrogen chloride gas was bubbled into a solution of 2.8 g (0.02 mol) of 2,3-diaminopropionic acid hydrochloride (1) in 300 mL of absolute ethanol for 30 min. The reaction mixture was refluxed under nitrogen for 2 days. After cooling to room temperature, the solvents were evaporated to give a dry solid. Crystallization in 95% ethanol gave 3.08 g (yield 75%) of pure product: mp 160–161 °C (lit.[15] mp 164–165 °C); IR (KBr) 1750 cm^{-1} (–$COOC_2H_5$).

Method B. A solution of 14.2 g (0.1 mol) of ethyl cyanoglyoxylate 2-oxime in 200 mL of absolute ethanol was saturated with hydrogen chloride gas and transferred to a hydrogenation bottle, and 0.3 g of PtO_2 catalyst was added. The hydrogenation was performed under H_2 (initial pressure 45 psi). When the hydrogenation was completed, 900 mL of ethanol was added to the reaction mixture that was then refluxed to digest the gray solid material. The black solid was filtered, and the filtrate was concentrated to half of its original volume. Upon cooling, it gave 12.1 g of pure compound **3**. Further treatment of the mother liquor gave 4.3 g. The overall yield is 16.4 g (yield 80%). The physical properties of this compound are identical with those of the authentic sample prepared by method A as described above.

Preparation of Ethyl 3,3,10,10-Tetramethyl-1,2-dithia-5,8-diazacyclodeca-4,8-diene-6-carboxylate (4). To a mixture of 10.25 g (0.05 mol) of ethyl 2,3-diaminopropionate dihydrochloride (3) in 200 mL of ethanol was added a solution of 2.3 g (0.1 mol) of sodium in 100 mL of ethanol. After filtering the insoluble solid material, the solution was concentrated to one-third of its original volume and 10.3 g (0.05 mol) of 2,2′-dithiobis(2-methylpropanal) was added dropwise. The reaction mixture was heated at 40–50 °C for 20 min. The solvent was removed under vacuum, and the reaction residue was treated with 200 mL of dichloromethane. The solid material was filtered, and the dichloromethane filtrate was evaporated to give a light yellow residue. Upon standing under vacuum, it was crystallized to give 14.1 g (yield 94%) of compound **4**. Recrystallization in hexane provided 13.3 g (yield 88%) of the pure product: mp 100–101 °C; IR (KBr) 1750 (–COO), 1660 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.1–1.7 (m, 15 H, CH_3), 3.2 (t, 1 H), 3.8–4.6 (m, 4 H), 6.9–7.0 (m, 2 H, CH=N); mass spectrum (CI) 305 (10), 304 (17), 303 (100, M^+ + H), 239 (8), 238 (15). Anal. ($C_{13}H_{22}N_2O_2S_2$) C, H, N.

Preparation of 6-(Hydroxymethyl)-3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (5a,b). To a solution of 8.7 g (2.86 mmol) of compound **4** in 200 mL of ethanol was added 12 g (3.16 mmol) of sodium borohydride in several portions. The reaction mixture was well stirred and slightly heated at 40 °C for 3 days. After cooling to room temperature, to the reaction mixture was added 200 mL of H_2O and the resultant solution was extracted with CH_2Cl_2 (3 × 300 mL). The solvents were evaporated, and the reaction residue was chromatographed on silica gel (EtOAc as eluent) to give 6.9 g (91%) of compound **5a** (mp 52–53 °C) and 0.45 g (6%) of compound **5b** (mp 111–112 °C). Compounds **5a** and **5b** may be stereoisomers. However, only **5a**, the major product, was used for the next reduction step.

Compound 5a: ^{1}H NMR ($CDCl_3$) 1.1–1.5 (m, 12 H, CH_3), 2.2–3.8 (m, 12 H) including a singlet at 2.45 (–NH); mass spectrum (CI) 265 (M^+ + H, 10), 264 (14), 263 (100), 188 (17), 157 (7). Anal. ($C_{11}H_{24}N_2OS_2$) C, H, N.

Compound 5b: ^{1}H NMR ($CDCl_3$) 1.0–1.7 (m, 12 H, CH_3), 2.5–4.2 (m, 12 H) including a singlet at 2.5 (–NH); mass spectrum (CI) 265 (M^+ + H, 8), 264 (14), 263 (100), 188 (21). Anal. ($C_{11}H_{24}N_2OS_2$) C, H, N.

Preparation of 1-(Hydroxymethyl)-*N*,*N*′-bis(2-methyl-2-mercaptopropyl)ethylenediamine Dihydrochloride (6). To a solution of 5 mL of Red-Al (70% in toluene) diluted with 20 mL of benzene was added 1.75 g (7.3 mmol) of compound **5a** in 20 mL of benzene. After the addition, the reaction mixture was refluxed for $^1/_2$ h and then cooled to room temperature and stirred for another 2 h. The reaction mixture was treated with 35 mL of 1 N HCl solution, and the pH of the aqueous layer was adjusted to 10. The organic layer was separated, and the aqueous layer was extracted with 30 mL of benzene. The combined organic solution was dried over $MgSO_4$ and then evaporated to dryness. The residue was dissolved in 50 mL of ether and caused to precipitate by bubbling with dry HCl gas through the solution. The white solid product was collected, washed with ether, and dried under vacuum to give 0.4 g (18%) of compound **6**, mp 150–155 °C (dec). Anal. ($C_{11}H_{28}Cl_2N_2OS_2$) C, H, N.

Preparation of 3,3,10,10-Tetramethyl-1,2-dithia-5,8-diazacyclodeca-4,8-diene-6-carboxamide (7). To a solution of 1.70 g (5.6 mmol) of compound **4** in 20 mL of ethanol was added 25 mL of concentrated NH_4OH solution. The reaction mixture was well stirred at room temperature for 1 day and then concentrated to half of its original volume. After an addition of 25 mL of water, the resulting solution was extracted with dichloromethane (2 × 200 mL). The organic layers were combined and evaporated to give a solid material. Crystallization in hexane–$CHCl_3$ afforded 1.1 g (yield 80%) of pure compound **7**: mp 188–190 °C (dec); IR (KBr) 3150 and 3350 (–NH_2), 1690 ($CONH_2$), 1660 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$) 1.4 and 1.5 (d, 12 H, CH_3), 3.0 (t, 1 H), 3.85 and 4.0 (two sets of d, 1 H), 4.45 and 4.6 (two sets of quartets, 1 H), 6.4 and 6.8 (br, 2 H, $CONH_2$), 6.95 (s, 2 H, CH=N); mass spectrum (CI) 276 (11), 275 (16), 274 (100, M^+ + 1), 273 (9), 209 (5), 167 (5). Anal. ($C_{11}H_{19}N_3OS_2$) C, H, N.

Preparation of 3,3,10,10-Tetramethyl-1,2-dithia-5,8-diazacyclodecane-6-carboxamide (8). To a solution of 1.37 g (5 mmol) of compound **7** in 40 mL of ethanol was added 1.9 g (5 mmol) of sodium borohydride in portions, and the reaction mixture was well stirred at 40 °C for 1 day. After addition of 20 mL of water, the solution was extracted with dichloromethane (2 × 300 mL). Evaporation of solvents gave 1.3 g (94%) of compound **8**. Recrystallization in ether–hexane provided the analytical sample: mp 125–126 °C; IR (KBr) 3400 and 3150 (m, $CONH_2$), 3300 (m, NH), 1670 cm^{-1} (s, $CONH_2$); ^{1}H NMR ($CDCl_3$) 1.1–1.6 (m, 12 H, CH_3), 2.45 (s, 2 H, NH), 2.5–4.0 (m, 7 H), 6.3 and 7.0 (br, 2 H, $CONH_2$); mass spectrum (CI) 278 (12, M^+ + 1), 277 (18), 276 (100), 275 (9), 201 (8), 157 (6). Anal. ($C_{11}H_{23}N_3OS_2$) C, H, N.

Preparation of 6-(Aminomethyl)-3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (9). A solution of 4.0 g (14 mmol) of compound **8** in 50 mL of THF was added dropwise into 100 mL of 1 M borane–THF complex solution cooled in an ice bath. After the addition, the reaction mixture was refluxed under nitrogen for 4 days and then cooled to room temperature. To the solution was added 10 mL of ethanol, and the resultant mixture was acidified by bubbling with dry HCl gas. Evaporation of solvents gave a white solid material, which was dissolved in 50 mL of water and treated with NaOH pellets to adjust to pH 10. The solution was extracted with ether (2 × 250 mL), and the ether solution was dried over $NaSO_4$. Evaporation of the solvent gave a clear residue, which was chromatographed on silica gel (EtOH–Et_3N (95:5)) to give 2.21 g (60%) of compound **9**: IR (neat) 3375, 3300, 1580 cm^{-1} (m, NH and NH_2); ^{1}H NMR ($CDCl_3$) 1.0–1.6

(m, 12 H, CH_3), 2.1 (s, 4 H, NH and NH_2), 2.25–3.9 (m, 9 H); mass spectrum (CI) 266 (10), 264 (100, $M^+ + 1$), 89 (8), 61 (38). Anal. ($C_{11}H_{25}N_3S_2$) C, H, N.

Preparation of *N*,*N*'-Bis(2-methyl-2-mercaptopropyl)-3-aminopropane-1,2-diamine Hydrochloride (10). To a mixture of 44 mL of Red-Al (70% in toluene, about 0.15 mol) in 50 mL of benzene was added dropwise a solution of 2.63 g (0.01 mol) of compound **9** in 60 mL of benzene. The reaction mixture was refluxed for 2 h and then was cooled to room temperature. The solution was added slowly to 100 mL of 10% hydrochloric acid, and the aqueous layer was then adjusted to pH 10 by adding NaOH pellets. The organic layer was separated, and the aqueous phase was extracted with benzene (2 × 50 mL). The combined organic solution was dried over magnesium sulfate and evaporated to give an oily residue that was dissolved in 100 mL of ether. A precipitate was obtained by bubbling dry hydrogen chloride gas through the solution. The white precipitate was collected, washed with ether, and dried to yield 1.46 g (yield 43%) of compound **10**. Recrystallization in C_2H_5OH–ether gave the analytical sample, mp 135–138 °C. Anal. ($C_{11}H_{29}Cl_2N_3S_2$) C, H, N: calcd, 12.43; found, 10.46.

Preparation of α-Amino-2-methoxybenzeneacetonitrile (11a). To a solution of 50.0 g (1.0 mol) of sodium cyanide, 53.5 g (1.0 mol) of ammonium chloride, and 60 mL of concentrated ammonium hydroxide in 400 mL of water was added 125 g (0.92 mol) of *o*-anisaldehyde in 400 aromatic C=C); of dry methanol. After the mixture was stirred for 3 h at 23 °C, the methanol was removed under reduced pressure, and the residual solution containing the crude product was diluted with 500 mL of water and extracted with methylene chloride (2 × 300 mL). The organic extract was washed with water (2 × 400 mL), dried over sodium sulfate, and filtered; the solvent was subsequently removed under vacuum to give an orange oil. By means of column chromatography on silica gel using ethyl acetate as the eluent, the α-amino nitrile compound, **11a**, was separated from the starting material and the side products: IR (neat) 2240 (w, –C≡N), 1600, 1500 cm^{-1} (s, aromatic C=C); NMR ($CDCl_3$) 1.03 (2 H, s, NH_2), 3.90 (3 H, s, OCH_3), 5.05 (1 H, s, CH), 7.15 (4 H, m, ArH). The α-amino nitrile (yellow oil) was unstable as a free base; therefore, it was either used immediately for the next reaction or converted to the tartarate salt.

Preparation of D-α-Amino-2-methoxybenzeneacetonitrile *d*-Hemitartarate (11b). To a solution of crude compound **11a** prepared freshly from 62.5 g (0.46 mol) of *o*-anisaldehyde in 1 L of benzene–methanol (4:1) was added 60 g (0.40 mol) of *d*-tartaric acid dissolved in 400 mL of methanol. The resultant flocculent precipitate was filtered, washed with benzene–methanol (2:1), suspended in carbon tetrachloride, filtered, and dried to produce 80 g (0.26 mol, 55.7%) of dense white powder, **11b**, mp 218 °C.

A small sample was recrystallized twice from methanol for elemental analysis: mp 218 °C (dec). Anal. ($C_{13}H_{16}N_2O_7 \cdot {}^1/_2CH_3OH$) C, H, N.

Preparation of α-(Acetylamino)-2-methoxybenzeneacetonitrile (11c). Compound **11b** (28.0 g, 89.7 mmol) dissolved into 250 mL of aqueous sodium bicarbonate, pH 8.0, was extracted with methylene chloride (2 × 150 mL). The combined methylene chloride solution was dried over sodium sulfate (anhydrous), filtered, and reduced to ~50 mL of light yellow solution under vacuum. This solution was slowly added to 14.6 g (142 mmol) of acetic anhydride at 0 °C and then stirred for 2 h at room temperature, after which excess volatiles (CH_2Cl_2, HOAc, Ac_2O) were removed under vacuum. The residue was recrystallized 2 times from ethyl acetate–hexane (1:1) to give 10.65 g (48.1 mmol) of white microcrystalline powder, **11c**; mp 139–140 °C; IR 3450 (m, N-H), 1695 (s, amide), 1600, 1500 (aromatic C=C), 2250 cm^{-1} (w, C≡N); NMR ($CDCl_3$) 2.00 (3 H, s, O=CCH_3), 3.93 (3 H, s, OCH_3), 6.13 (1 H, d, J_1 = 9 Hz, CH), 7.10 (5 H, m, ArH + NH). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N, O.

Preparation of α-Amino-2-methoxybenzeneethanamine (12). To a cold (0 °C) solution of 34.0 g (0.90 mol) of $LiAlH_4$ in 300 mL of dry THF under N_2 was added dropwise crude dry compound **11a** dissolved in 200 mL of dry THF, freshly prepared from 125 g (0.92 mol) of *o*-anisaldehyde. After the mixture was stirred 12 h at room temperature, the excess hydride was decomposed with 1 L of wet THF–ether. The alumina was filtered off, and the organic solvents were evaporated under vacuum to produce approximately 200 mL of an orange oil. The crude product was azeotropically dried with benzene and then fractionally distilled under vacuum (0.5–0.25 torr). The clear oil (distilled at 96–124 °C), which represented the majority of the distillate, was found to be primarily 2-(aminomethyl)anisole. The minor fraction (distilled at 124–140 °C), approximately 15 mL, was used without further purification: IR (disappearance) 2250 cm^{-1}; NMR ($CDCl_3$) 1.57 (4 H, s, NH_2), 2.83 (2 H, m, NCH_2), 3.77 (3 H, s, $ArOCH_3$), 4.12 (1 H, t, J_1 = 6 Hz, NCH), 7.05 (4 H, m, ArH). Anal. ($C_9H_{14}N_2O$) C, H, N, O.

Preparation of α-(Acetylamino)-2-methoxybenzeneethanacetamide (12b). To a cold (0 °C) solution of 1.0 g (9.8 mmol) of acetic anhydride in 50 mL of ethyl acetate was slowly added approximately 1 mL (5 mmol) of enriched distillate of compound **12a**. After the mixture was stirred for 2 h at room temperature, the volatile organics were removed under vacuum to leave a white solid. The residue was recrystallized from ethanol–ethyl acetate to yield 1.0 g (66%) of white crystals, **12b**: mp 199 °C; IR (KBr) 3310 (s, N-H), 1640 cm^{-1} (s, C=O, amide 1 band); NMR ($CDCl_3$) 1.97 (6 H, d, J_1 = 3 Hz, NAc), 3.47 (2 H, m, NCH_2), 3.85 (3 H, s, $ArOCH_3$), 5.27 (1 H, m, NCH), 6.20 (1 H, m, NH), 7.03 (5 H, M, ArH + NH). Anal. ($C_{13}H_{18}N_2O_2$) C, H, N, O.

Preparation of 3,3,10,10-Tetramethyl-1,2-dithia-7-(2-methoxyphenyl)-5,8-diazacyclodeca-4,8-diene (13). To a solution of 6.10 g (29.6 mmol) of 2,2'-dithiobis(2-methylpropanal) in 25 mL of absolute ethanol was added 5.0 g (30.0 mmol) of distilled compound **12a**. The solution was stirred at 50 °C for 30 min and subsequently allowed to stand for 12 h at 4 °C, after which a precipitate formed. The precipitate was washed with cold methanol and dried to yield 6.52 g (19.4 mmol, 65.5%) of white powder, **13**. An analytical sample was recrystallized once from ethyl acetate: mp 121 °C; IR (KBr) 1650 (s, C=N), 1600, 1495 cm^{-1} (w, Ar); NMR ($CDCl_3$) 1.43 (12 H, d, J = 10 Hz, $C(CH_3)_2$), 2.90 (1 H, t, J_1 = 9 Hz, NCH), 3.83 (3 H, s, OCH_3), 4.58 (2 H, m, NCH_2), 7.00 (5 H, m, ArH + N=CH), 7.77 (1 H, m, ArH). Anal. ($C_{17}H_{24}N_2OS_2$) C, H, N.

Preparation of 2,9-Dimethyl-2,9-dimercapto-5-(2-methoxyphenyl)-4,7-diazadecane Hydrochloride (14). To a solution of 45 mL (153 mmol) of Red-Al (Aldrich Chemical Co.) in 200 mL of dry benzene under N_2 was added dropwise 7.0 g (20.8 mmol) of compound **14** dissolved in 10 mL of dry benzene. After refluxing for 1 h, the solution was chilled to 0 °C and excess hydride decomposed by the slow addition of 50 mL of concentrated HCl. The pH was adjusted to 10 with concentrated aqueous NaOH. The solids were removed via filtration and the benzene evaporated under vacuum to produce a foul-smelling purple oil. The oil was dissolved into 60 mL of absolute ethanol at 0 °C, and compound **14** was caused to precipitate by bubbling dry HCl gas. The precipitate was filtered, rinsed with ethanol, and dried to produce 5.95 g (68.8%) of white powder **14**. An analytical sample was prepared by recrystallization once from ethanol: mp 182–188 °C; IR (neat) 3300 (w, NH st), 2550 cm^{-1} (w, SH str); NMR ($CDCl_3$) (free base) 1.37 (12 H, s, $C(CH_3)_2$), 2.00 (4 H, s, SH, NH), 2.65 (6 H, m, NCH_2), 3.82 (3 H, s, $ArOCH_3$), 4.13 (1 H, m, NCH), 7.13 (4 H, m, ArH). Anal. ($C_{17}H_{30}N_2OS_2$) C, H, N, S.

Preparation of 2,9-Dimethyl-2,9-mercapto-5-(2-hydroxyphenyl)-4,7-diazadecane (15). To a suspension of compound **14**, 5.95 g (14.3 mmol) in 60 mL of absolute ethanol was added a solution of 0.6 g (26.8 mmol) of sodium in 20 mL of absolute ethanol. After the mixture was stirred for 30 min, the sodium chloride was filtered off and the solvent removed under vacuum to produce the free-base form of compound **14** as a clear oil in quantitative yield. The oil (4.89 g, 14.3 mmol) was dissolved into 50 mL of CH_2Cl_2 at 0 °C and added dropwise to boron tribromide (72 mmol in 72 mL of CH_2Cl_2) at 0 °C under N_2. After addition, the solution was refluxed for 12 h, then allowed to cool to room temperature, and treated with 200 mL of water. The aqueous phase was adjusted to pH 8 with $NaHCO_3$, and the methylene chloride layer was separated, dried over Na_2SO_4, and filtered; the solvent was evaporated under vacuum to produce a crimson oil (5.3 g) that solidified upon standing for 12 h at room temperature. The crude solid was washed with hexane–2-propanol (2 × 5 mL), and multiple recrystallizations yielded a foul-smelling white powder, **15**: yield 0.484 g (10.3%); mp 85 °C; IR (CCl_4) 3370 (w, NH str), 1600 cm^{-1} (m, Ar str); NMR ($CDCl_3$) 1.40 (14 H, s,

Table II. Biodistribution of ^{99m}Tc Complexes in Rats[a]

organ	% dose organ (^{99m}Tc/[^{125}I]IAP ratio)			
	6	10	14	15
		2 min		
brain	0.240 (0.24)	1.28 (0.96)	2.30 (1.43)	0.408 (0.25)
blood	18.1 (1.07)	13.6 (0.93)	7.81 (0.52)	14.5 (0.82)
muscle	13.4 (0.61)	9.19 (0.73)	7.09 (0.58)	8.66 (0.62)
heart	0.887 (1.04)	1.08 (1.18)	4.46 (4.18)	3.31 (3.05)
lungs (2)	.27 (1.26)	3.08 (1.67)	5.49 (1.05)	4.94 (2.76)
spleen	0.349 (0.89)	0.274 (0.88)	0.149 (0.62)	0.407 (0.96)
kidneys (2)	2.21 (1.18)	3.57 (1.65)	3.23 (1.65)	4.75 (1.99)
stomach	1.12 (0.72)	2.23 (1.61)	1.07 (1.1)	1.61 (1.05)
small intest	4.46 (0.84)	5.09 (1.01)	4.47 (1.18)	6.24 (1.08)
liver	16.7 (0.83)	21.7 (1.63)	11.9 (0.97)	29.0 (1.76)
skin	6.72 (0.60)	8.84 (0.85)	5.21 (0.74)	7.45 (0.62)
thyroid	0.088 (0.55)	0.124 (0.74)	0.292 (1.58)	0.189 (1.07)
brain/blood[b] ratio	0.145	0.970	2.79	0.289
		15 min		
brain	0.150 (0.45)	0.288 (0.52)	0.297 (0.67)	0.229 (0.44)
blood	9.14 (0.83)	8.07 (0.78)	2.56 (0.29)	4.77 (0.44)
muscle	14.9 (0.49)	18.5 (0.53)	15.1 (0.51)	12.4 (0.36)
heart	0.300 (0.79)	0.375 (0.79)	0.395 (0.97)	0.441 (0.97)
lungs (2)	1.31 (1.50)	1.57 (1.40)	0.877 (1.05)	1.52 (1.41)
spleen	0.309 (1.49)	0.206 (0.78)	0.197 (0.82)	0.323 (1.00)
kidneys (2)	2.39 (1.91)	2.47 (1.94)	1.10 (1.14)	1.73 (1.44)
stomach	0.850 (0.47)	5.24 (2.25)	3.16 (1.55)	5.46 (2.52)
small intest	19.5 (4.12)	11.8 (3.04)	12.5 (4.04)	18.2 (4.41)
liver	16.8 (2.51)	22.7 (3.63)	17.4 (3.24)	25.4 (3.35)
skin	6.53 (0.40)	9.43 (0.58)	11.8 (0.84)	8.17 (0.50)
thyroid	0.053 (0.22)	0.045 (0.27)	0.063 (0.42)	0.057 (0.29)
brain/blood[b] ratio	0.180	0.378	1.31	0.512

[a] Data are the average of three rats. Dual isotope experiments, [^{125}I]IAP and ^{99m}Tc complexes were injected (iv) simultaneously. [b] Brain (dose/g)/blood (dose/g).

$C(CH_3)_2$ + NH), 2.67 (4 H, d, J_1 = 1.5 Hz, NCH_2), 2.93 (2 H, d, J_1 = 3 Hz, NCH_2), 3.72 (1 H, t, J_1 = 8 Hz, NCH), 7.00 (4 H, m, ArH). Anal. ($C_{16}H_{28}N_2OS_2$) C, H, N, S.

Radiolabeling. Sodium [^{99m}Tc]pertechnetate (1–10 mCi, 0.3–0.5 mL) was added to a test tube containing the BAT ligand (2–4 mg) and sodium borohydride (15 mg). The mixture was vortexed and kept at room temperature for 0.5 h. To this solution was added 1 mL each of saline and hexane (or hexane–ethyl acetate (50:50)). The solution was vortexed, and the hexane layer was separated. This extraction process was repeated three times, and the combined hexane extracts were dried over anhydrous sodium sulfate. The filtered solution was then condensed to dryness with a stream of nitrogen. The residue was redissolved in absolute ethanol: yields 30–50%.

For labeling the ligands using Sn(II) as the reducing agent, a stock solution of Sn(II)/PPi was prepared by mixing 250 mg of sodium pyrophosphate and 3 mL of stannous chloride solution ($SnCl_2 \cdot 2H_2O$, 10 mg/mL of 0.1 N HCl) in 25 mL of water. A mixture of BAT ligand (2–4 mg), sodium [^{99m}Tc]pertechnetate, and 0.1–1.0 mL of the stock solution was heated in a water bath at 80 °C for 15 min. The rest of the procedure was the same as described above. The yields were similar: 30–50%.

Animal Distribution Study. Sprague–Dawley male rats (220–300 g) under halothane or ether anesthesia were injected intravenously with 0.2 mL of a saline ethanol (1:1) solution containing 0.5–20 μCi of the ^{99m}Tc BAT compound and 0.5–5 μCi of [^{125}I]IAP. At different time periods after the iv injection, rats were killed by cardiectomy. The organs of interest were excised, weighed, and counted in a dual-channel Beckman automatic γ-counter (Model 4000).

The percent dose/organ was determined by comparison of tissue radioactivity levels to suitably diluted aliquots of the injected dose. The spillover counts into each window were corrected by a computer program. The Tc-99m/I-125 IAP ratio was calculated by percent dose for ^{99m}Tc divided by percent dose for I-125 in each organ. The brain to blood concentration ratio was calculated from the percent dose/gram of wet tissue. Data are presented in Table II.

Partition Coefficients. The partition coefficient was measured by mixing the ^{99m}Tc BAT compound with 3 g each of 1-octanol and buffer (pH 7.0 or 7.4, 0.1 M phosphate) in a test tube. This test tube was vortexed 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 octanol to that of buffer. Samples from the octanol layer were repartitioned until consistent partition coefficient values were obtained. Usually the measurement was repeated three times.

Protein Binding. The binding of the ^{99m}Tc BAT compounds to human serum proteins was determined by equilibrium dialysis. Human serum (0.4 mL, pooled) and 0.4 mL of phosphate buffer (0.15 M, pH 7.4) containing the test compound (~0.025 μCi) were separated by a dialysis membrane. The dialysis cells were rotated in a water bath at 37 °C for 18 h. At the end of the incubation, aliquots from both sides were weighed and counted. The percent free of protein binding was determined by calculating the radioactivity concentration ratio of buffer to serum multiplied by 100. To determine possible membrane binding, the membrane was counted at the end of the experiment. Usually less than 5% of the original activity was found on the membrane.

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Registry No. **1**, 6018-55-9; **2**, 3849-21-6; **3**, 6020-57-1; **4**, 96929-37-2; **5** (isomer 1), 96929-38-3; **5** (isomer 2), 96929-39-4; **6**, 96929-40-7; **7**, 96929-41-8; **8**, 96929-42-9; **9**, 96929-43-0; **10**, 96929-44-1; **11a**, 96929-45-2; **11b**, 96929-47-4; **11c**, 96929-48-5; **12a**, 96929-49-6; **12b**, 96929-50-9; **13**, 96929-51-0; **14**, 96929-52-1; **14** (free base), 96929-54-3; **15**, 96929-53-2; 2,2′-dithiobis(2-methylpropanal), 15581-80-3; *o*-anisaldehyde, 135-02-4; technetium, 14133-76-7.