The desired product (quantitative yield) was filtered under argon as an off-white solid: mp 50–60 °C. Anal. ($C_{19}H_{27}IN_2O_9$) C, H, I, N.

Radiolabeling. Chloramine-T solution of (50 μL, 1 mg/mL) was added to a mixture of BZM (50 μL, 1 mg/mL), sodium [^{125}I]iodide (10 μL, 1–5 mCi, no-carrier added, sp act. 2200 Ci/mmol), and pH 3 phosphate solution (0.3 mL) in a sealed vial. The reaction was allowed to proceed at room temperature for 1.5 min. The reaction was terminated by addition of sodium bisulfite (0.1 mL, 10 mg/mL) and neutralized with of 0.4 N sodium bicarbonate (0.5 mL). The product was extracted with ethyl acetate (3 × 1 mL). The combined organic layers were dried by passing through an anhydrous sodium sulfate column (0.2 cm × 5 cm). The organic solution was evaporated under a stream of nitrogen, and the residue was dissolved in absolute ethanol (50–200 μL). The desired product, [^{125}I]IBZM, was isolated from the unreacted BZM and a small amount of unknown radioactive impurities by HPLC on a reverse phase column (PRP-1, Hamilton Inc.), with an isocratic solvent system: 82:18 acetonitrile–pH 7.0 buffer (10 mM, 3,3-dimethylglutaric acid). After the appropriate fractions were collected, the solvent was evaporated under a stream of nitrogen, and the product was reextracted with ethyl acetate (1 × 3 mL). The solution containing the no-carrier-added product was condensed to dryness and redissolved in absolute ethanol (radiochemical purity >95%, overall yield 60%). This agent was used in the binding studies after dilution with saline.

Tissue Preparation. Male Sprague–Dawley rats (200–250 g) were decapitated, and the brains were removed and placed in ice. Striatal tissues were excised, pooled, and homogenized in 100 volumes (w/v) of ice-cold Tris·HCl buffer (50 mM), pH 7.4. The homogenates were centrifuged at 20000*g* for 20 min. The resultant pellets were rehomogenized in the same buffer and centrifuged again. The final pellets were resuspended in assay buffer containing 50 mM Tris buffer, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$.

Binding Assays. The binding assays were performed by incubating 50 μL of tissue preparations containing 40–60 μg of protein with appropriate amounts of [^{125}I]IBZM ligand and competitors in a total volume of 0.2 mL of the assay buffer. After an incubation period of 15 min at 37 °C (with stirring), the samples were rapidly filtered in the cell harvester (Brandel M-24R) under vacuum through Whatman GF/B glass fiber filters pretreated with 0.2% poly-L-lysine and washed with cold (4 °C) 50 mM Tris·HCl buffer, pH 7.4 (3 × 5 mL). The nonspecific binding was obtained in the presence of 10 μM spiperone. The filters were counted in a γ counter (Beckman 5500) at an efficiency of 70%.

Data Analysis. Both Scatchard and competition experiments, were analyzed by using the iterative nonlinear least-squares curve-fitting program LIGAND.[26]

Acknowledgment. This project is supported by grants from NIH (NS-24538) and Institute fur Diagonstikforschung, Schering-Freie Universitat Berlin.

Registry No. **1a**, 84226-06-2; [^{125}I]-**1a**, 113452-80-5; **1b**, 113452-78-1; [^{125}I]-**1b**, 113452-81-6; **1c**, 113452-77-0; **1d**, 113452-79-2; **2a**, 84226-04-0; **2b**, 113452-75-8; **2c**, 113474-70-7; **3**, 1466-76-8; **5a**, 96947-76-1; **5b**, 113531-33-2; **6**, 3147-64-6; **7**, 113452-76-9; (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine, 22795-99-9; (*R*)-(+)-*N*-ethyl-2-(aminomethyl)pyrrolidine, 22795-97-7.

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Synthesis and Biodistribution of ^{99m}Tc-Labeled Piperidinyl Bis(aminoethanethiol) Complexes: Potential Brain Perfusion Imaging Agents for Single Photon Emission Computed Tomography

S. M. N. Efange, H. F. Kung,* J. J. Billings, and M. Blau

Department of Nuclear Medicine, State University of New York at Buffalo, Buffalo, New York 14215.
Received September 10, 1987

In developing clinically useful ^{99m}Tc-labeled radiopharmaceuticals for the evaluation of regional cerebral perfusion with single photon emission computed tomography (SPECT), a number of substituted alkyl(aryl)piperidinyl bis(aminoethanethiol) ligands for chelating [^{99m}Tc]TcO(III) were synthesized. Each ligand forms two diastereomers, syn and anti, after reacting with a racemic mixture of the ligand. The diastereomers were separated by high-pressure liquid chromatography. In biodistribution studies conducted in rats, the diastereomers exhibit widely disparate brain uptake values; however, this disparity seems to diminish as the steric bulk of the substituent at the C-4 position of the piperidinyl moiety increases. Furthermore, all the complexes evaluated failed to show a prolonged retention in the rat brain, suggesting that further structural modification may be necessary to obtain clinically useful complexes from this class of compounds.

The basic thrust of the second generation of brain-imaging agents is the evaluation of changes of brain perfusion and metabolism in the living brain (with intact blood/brain barrier). The potential diagnostic utility of brain perfusion imaging lies in the recognition that functional imbalance, which may be manifested as a perfusion abnormality, generally precedes anatomic manifestations of disease. The ideal brain perfusion imaging agents are expected to exhibit characteristics that should include the following: (a) extraction, which is linearly proportional to a flow over a wide range of blood flow; (b) blood brain barrier penetrability (which in many cases is the result of lipid solubility); (c) high initial brain uptake; (d) prolonged brain retention; (e) a fixed regional distribution–no change from its original perfusion pattern; and (f) a high brain/blood ratio. In conjunction with tomographic modalities such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), a neutral, lipid-soluble, radiolabeled molecule could be utilized to evaluate regional cerebral perfusion. Such molecules have, in fact, been synthesized and evaluated. For SPECT, these are notably *N*,*N*,*N*^{1}-trimethyl-*N*^{1}-(2-hydroxyl-3-methyl-5-[^{123}I]iodobenzyl)-1,3-propanediamine ([^{123}I]HIPDM) and *N*-isopropyl-*p*-[^{123}I]iodoamphetamine ([^{123}I]IMP).[1-4] Both compounds have been used with SPECT in the evaluation

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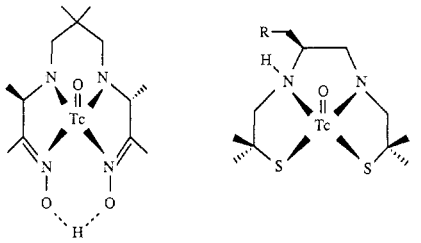


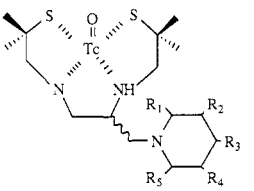
Tc-99m-d,l-HMPAO Tc-99m-BAT (SYN)

Figure 1. ^{99m}Tc-*d,l*-HMPAO and ^{99m}Tc-BAT.

of regional cerebral perfusion and have shown promise in the diagnosis of epilepsy, stroke, and Alzheimer's disease.[5-12] Although both compounds have demonstrated clinical utility and have progressed to phase III clinical trials, their widespread use is hampered by the limited availability and attendant high cost of pure cyclotron-produced ^{123}I. This, in part, has provided the impetus for the development of less expensive ^{99m}Tc-based radiopharmaceuticals for regional cerebral perfusion.

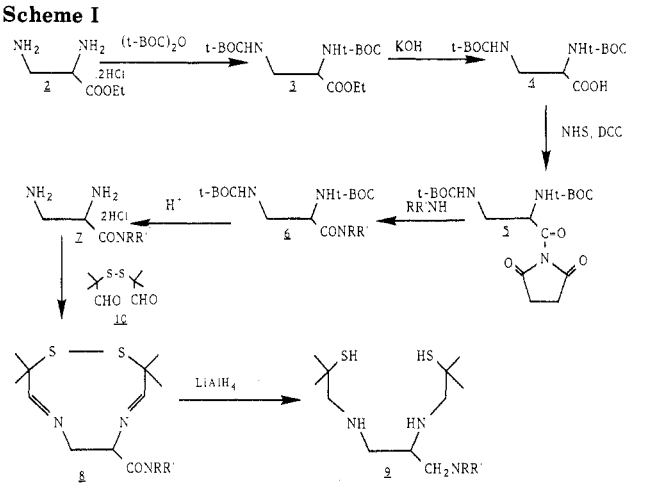
The past two years have witnessed the emergence of ^{99m}Tc-based lipid-soluble complexes for regional cerebral blood flow imaging (rCBF)-SPECT. Two ligand systems, propylenediamine oxime (PnAO) and bis(aminoethanethiol) (BAT) (Figure 1), have gained prominence in this area. ^{99m}Tc-*d,l*-HMPAO, a hexamethyl analogue of ^{99m}Tc-PnAO, has demonstrated utility in the assessment of (rCBF) and is now in clinical trials.[13-16] The retention of ^{99m}Tc-*d,l*-HMPAO is attributed to its in vitro instability. After entering the brain tissue from the cerebral vascular system, the agent apparently decomposed into a different chemical species, which shows irreversible bonding to the brain tissue. Other lipid-soluble complexes based on the BAT skeleton (Figure 1), some of which demonstrate varying degrees of brain retention in the monkey and baboon, have been reported by several workers[17-19] and by us.[20-24] This paper details part of our effort in this area.

Table I. ^{99m}Tc-Labeled Bis(aminoethanethiol) Complexes



compd	R_1	R_2	R_3	R_4	R_5
1a	H	H	H	H	H
1b	CH_3	H	H	H	H
1c	H	CH_3	H	H	H
1d	H	H	CH_3	H	H
1e	H	CH_3	H	CH_3	H
1g	CH_3	H	H	H	CH_3
1f	H	H	Ph	H	H

Scheme I



In the initial stages of the search for inexpensive, clinically efficacious ^{99m}Tc-labeled brain perfusion agents for SPECT, simple alkyl-substituted BAT complexes were synthesized and evaluated.[17-20] Although these studies were instrumental in demonstrating the blood/brain barrier penetrability of such complexes, the poor brain retention associated with these complexes, a characteristic which was attributed to the absence of a brain sequestration mechanism, led to the search for better agents in this class of compounds. Our subsequent efforts in this area have been based on the use of a working hypothesis on regional pH shifts as a trapping mechanism. According to this mechanism, a suitable basic arylalkyl or alkylamine would cross the blood/brain barrier in the neutral lipid soluble form; once inside the brain, a fraction of this amine would be protonated.[25] On the basis of the reported acidity of the intracellular brain medium relative to the extracellular environment, a higher fraction of the protonated amine would be found in the intracellular brain compartment. Owing to the reduced transport of such charged species across the blood/brain barrier, the protonated amine would be trapped in the brain. Since the radionuclide is associated with the amine-containing molecule, such trapping would be reflected in a high

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brain/blood ratio (concentration of radioactivity in brain/concentration of radioactivity in blood). Contrary to the retention mechanism proposed for ^{99m}Tc–HMPAO, this proposed mechanism of trapping is based on the ionization of the amine side chain; the ^{99m}Tc–BAT core should remain intact. On the basis of our working hypothesis, we synthesized and evaluated ^{99m}–BAT complexes containing simple alkylamine side chains.[22-24] Although the biodistribution and autoradiographic data on these complexes showed increased retention of radioactivity (in the rodent and primate brain) relative to the corresponding simple alkyl-substituted complexes, the brain retention in the monkey was less than optimum for SPECT imaging. While the increased brain retention associated with these alkylamine-substituted ^{99m}Tc–BAT complexes may not provide support for the pH-shift hypothesis, these results provided some support for the continued use of amine-containing side chains in our continuing search for suitable ligands. The following details the synthesis and biodistribution of some substituted ^{99m}Tc-labeled piperidinyl–BAT complexes. The target compounds are shown on Table I.

Results and Discussion

Chemistry. The ligands were synthesized following a general procedure (Scheme I). Ethyl 2,3-diaminopropionate dihydrochloride (obtained from the Pd-catalyzed reduction of ethyl cyanoglyoxalate oxime[26]) was converted to the protected ester **3** by refluxing with di-*tert*-butyl dicarbonate. Subsequent to saponification of the ester, the resulting acid **4** (obtained in 83% yield) was reacted with *N*-hydroxysuccinimide and *N,N*-dicyclohexylcarbodiimide to yield the activated ester **5** in 82% yield. The latter provided a versatile intermediate, which was subsequently reacted with several amines (in acetonitrile) to yield the protected amides **6a–g**. Acid-catalyzed deprotection of **6** (to yield **7**) followed by reaction of **7** with a slight excess of bis(2-methyl-2-mercaptopropanal), in the presence of triethylamine, yielded the heterocycles **8a–g**. The latter were characterized by IR, NMR, and elemental analyses, and found to be consistent with the structure indicated. A characteristic broad singlet at 6.90–7.055 (for the imine) was observed in all the NMR spectra of **8a–g**. The yields in this condensation ranged from 30–68%. These diimines were generally obtained as solids and were subsequently reduced to the desired ligands **9a–g** by refluxing with $LiAlH_4$ in THF. A characteristic –SH stretching frequency (λ) at 2550 cm^{-1} was observed in the IR spectra of these compounds. The ligands were subsequently converted to their corresponding hydrochlorides; the crystallizing ability of many of these was rather poor, and the poor elemental analyses obtained for some of them may be attributed in part to both the presence of more than one hydrochloride species and their hygroscopic nature. The yields after recrystallization ranged from 31% to 58%.

Radiolabeling. The radiolabeling to yield the target complexes **1a–g** was easily accomplished by reacting the ligands with sodium pertechnetate and stannous glucoheptonate in 0.09% saline. Following extraction of the complex into the organic layer and subsequent workup, the crude complex **1** was obtained; the labeling yields ranged from 45% to 85%. On reverse-phase HPLC, two major peaks of approximately equal intensity invariably appeared. Similar observations have been reported by other workers using the N_2S_2 system, and the presence of isomers has been used to explain these observations.[18,19,27]

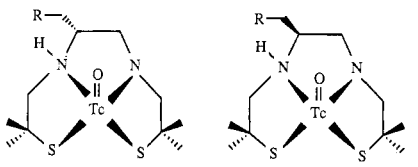

Figure 2. The syn and anti isomers of the ^{99m}Tc–BAT complex.

Table II. Biodistribution of the Parent ^{99m}Tc–BAT Complex **1a** in Rats (% Dose/Organ; Average of Three Rats)[a]

organ	2 min	15 min
	(1) Peak A (**1a**-anti)	
blood	5.35 (5.09–5.67)	2.03 (1.92–2.26)
muscle	12.8 (7.99–18.7)	16.0 (14.8–17.2)
heart	0.96 (0.79–1.04)	0.23 (0.21–0.25)
lung	10.3 (7.89–14.1)	2.03 (1.72–2.64)
liver	18.6 (15.4–23.2)	26.0 (24.5–28.4)
brain	1.08 (0.92–1.21)	0.27 (0.24–0.30)
brain/blood[b]	2.09	1.38
	(2) Peak B (**1a**-syn)	
blood	4.37 (3.76–4.94)	1.32 (1.28–1.36)
muscle	8.73 (8.50–9.07)	15.0 (12.6–19.0)
heart	1.59 (1.42–1.90)	0.23 (0.19–0.27)
lungs	17.9 (16.8–19.1)	2.53 (2.03–3.02)
liver	11.8 (10.2–12.6)	23.8 (22.1–24.9)
brain	2.34 (2.05–2.65)	0.40 (0.36–0.46)
brain/blood[b]	5.91	3.16

[a] The range is shown in parentheses. [b] Ratio (calculated from percent dose/gram values).

X-ray crystallographic data obtained for an analogous N_2S_2–Tc complex has shown that the BAT ligand forms a distorted square-planar complex with technetium (see Figure 2) as proposed by Davidson et al.[29] Furthermore, it has been proposed that substituents on the BAT skeleton can be oriented either syn or anti with respect to the TcO core, thereby yielding two isomers.[24] X-ray crystallographic studies (unpublished results) conducted on one set of the syn and anti isomers of our earlier complexes support the claims of these earlier workers. In most cases, the mixture of isomers could be easily separated on HPLC to give the pure diastereomers in greater than 90% purity. On the basis of the X-ray crystallographic studies, the more mobile of these complexes has been determined to be the anti isomer while the less mobile complex is the syn isomer (see Figure 2). These complexes were stable in saline for several hours as indicated by HPLC.

Biodistribution Studies. Biodistribution studies were carried out with male Sprague–Dawley rats. Table II shows that the syn isomer of the parent compound **1a**-syn exhibits an initial brain uptake of 2.34% with an attendant brain/blood ratio of 5.91; however, the brain retention, as shown by the data at 15-min postinjection (0.40%; brain/blood ratio = 3.16), is poor. A noticeable disparity exists between the two isomers with respect to brain uptake, and this can be seen by comparing the above data with that obtained for the anti isomer **1a**-anti (brain uptake: 1.08% at 2 min, 0.27% at 15 min; brain/blood ratio, 2.09 at 2 min and 1.38 at 15 min). This pattern is in agreement with both our earlier studies and those of other workers on other substituted ^{99m}Tc–N_2S_2 and –N_4 complexes.[16,19] This disparity may be attributed in part to

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Table III. Brain Uptake Values and Brain/Blood Ratios of ^{99m}Tc-Labeled Piperidinyl–BAT Complexes **1a–g** in Rats[a]

	syn		anti	
compd	2 min	15 min	2 min	15 min
1a	1.08	0.27	2.34	0.40
	(0.92–1.21)	(0.24–0.30)	(2.05–2.65)	(0.36–0.46)
ratio	5.91	3.16	2.09	1.38
1b	0.61	0.35	1.80	0.54
	(0.43–0.93)	(0.32–0.41)	(1.47–2.37)	(0.48–0.59)
ratio	1.06	1.84	4.96	3.84
1c	1.08	0.20	1.97	0.67
	(0.93–1.32)	(0.18–0.21)	(1.72–2.37)	(0.47–0.94)
ratio	1.89	0.93	3.78	3.17
1d	1.55	0.33	1.85	0.40
	(1.43–1.63)	(0.25–0.44)	(1.52–2.05)	(0.34–0.43)
ratio	2.71	1.40	4.10	1.81
1e	0.88	0.24	1.88	0.55
	(0.74–1.03)	(0.14–0.39)	(1.53–2.41)	(0.51–0.61)
ratio	1.43	0.93	2.52	2.40
1f	0.29	0.13	1.17	0.55
	(0.28–0.29)	(0.13–0.14)	(1.10–1.25)	(0.40–0.73)
ratio	0.44	0.51	2.76	2.44
1g	1.46	0.64	1.19	0.54
	(1.43–1.49)	(0.57–0.71)	(1.07–1.31)	(0.48–0.62)
ratio	2.56	2.61	1.98	2.07

[a] Numbers in parentheses are ranges for three rats. [b] Brain/blood ratio = (brain dose/gram)/(blood dose/gram).

interactions of the parent complex with the side chain; these interactions apparently depend on the orientation of the side chain (syn or anti) relative to the TcO core. However, it does appear that this disparity in biodistribution disappears when bulky substituents such as methyl or phenyl are introduced at the C-4 position of the piperidine ring (see Table III, compounds **1c**, **1g**). The foregoing suggests that substituents at the C-4 position of the piperidine ring constrain this ring system in a conformation that either diminishes the interaction of substituents with the TcO core (thereby eliminating differences) or subjects these substituents (be they disposed syn or anti) to similar interactions of like magnitude. Clearly, the disparate biological profiles of these isomers and their relative abundance in the mixture (50:50) severely compromise the clinical utility of these complexes, in view of the impracticality of HPLC separation in a clinical setting. One solution to this problem may be found in the design and synthesis of symmetrical complexes.

From their studies on N-substituted ^{99m}Tc–BAT complexes, Goldfarb et al.[28] have reported a significant increase in both the initial brain uptake and the brain retention following C_4-methyl substitution of the piperidine moiety in the side chain. In evaluating their results, they conclude that methyl substitution at this position in the side chain introduces molecular perturbations that alter the biological profile of the resulting ^{99m}Tc–BAT complex. In our case, however, methyl substitution at all positions of the piperidine ring, and phenyl substitution at C-4, appear to have introduced none of the desired changes. The foregoing suggests that the ^{99m}Tc–BAT skeleton may in fact be more sensitive to substitution at the nitrogen atoms than at the carbon atoms of the ethylene diamine fragment. This is clearly supported by the data in Table III, which shows, for all the analogues, at least a 50% reduction in the radioactivity in the brain after 15 min. However, the chemical nature of the fraction of radioactivity remaining in the brain is not known.

Finally, structural modifications carried out on the sidechain of the piperidine-substituted ^{99m}Tc–BAT complex have done little to increase the brain retention of these compounds; in most cases, the rate of efflux appears to have remained the same, suggesting either that this position is rather insensitive to structural modification, or that the modifications effected are insufficient to induce changes in the biological profile. The data suggests, contrary to our expectations, that the pH-shift mechanism may not play a significant role in increasing the fraction of radioactivity retained by the brain. This conclusion is only speculative, since the pH shift hypothesis is not tested. However the main objective of the research project is to develop ^{99m}Tc brain perfusion imaging agent, because the agents reported in this paper failed the first biological test; therefore, no further study is attempted to illucidate the pH hypothesis. Although the synthesis of symmetrical ^{99m}Tc–BAT complexes may largely eliminate the problem of isomers, the definition of the parameters that underly the optimization of brain retention will require more work. Such efforts are presently under way and will be the subject of future papers.

Experimental Section

General Procedures. Melting points were determined on a Nalge hot stage apparatus and are reported uncorrected. Elemental analyses were performed commercially. NMR spectra were recorded on a Varian EM 360 of T60A instrument (taken in either deuteriated chloroform or deuteriated DMSO, with tetramethylsilane as the internal standard). Infrared spectra were taken on a Perkin-Elmer 197 instrument. Spectral properties were consistent with the proposed structures. High-performance liquid chromatography (HPLC) was carried out on a Hamilton PRP-1 reverse-phase column eluted with acetonitrile/water (85:15); the radioactive eluent was detected by a sodium iodide detector and recorded on a multichannel analyzer.

2,3-Bis(*tert*-butoxycarbamoyl)propanoic Acid (4). Potassium hydroxide (1.29, 19.90 mmol) was added to a suspension of 6.01 g (18.09 mmol) of ethyl 2,3-bis(*tert*-butoxycarbamoyl)-propanoate, **3**, (prepared by reacting di-*tert*-butyl dicarbonate, following standard methods, with ethyl 2,3-diaminopropionate dihydrochloride, which latter was obtained from the Pd-catalyzed hydrogenation of ethyl cyanoglyoxalate 2-oxime[25]) in 50% aqueous ethanol (40 mL), and the mixture was stirred at room temperature. Upon completion of the reaction (monitored by TLC, 30% ethyl acetate/methylene chloride on silica gel TLC plates), the mixture was treated with 2 M HCl (12 mL), and the resulting solution was extracted with methylene chloride (2 × 100 mL). The organic extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to a colorless syrup. The latter crystallized from diethyl ether. The white solid was collected by filtration, washed with hexane, and dried to provide 4.57 g (82.9%) of the product **4**: mp 118–124 °C; ^{1}H NMR (acetone-d_6) δ 1.44 (s, 18, *tert*-butyl), 3.52 (m, 2, CONCH_2CHNCO), 4.30 (m, 1, CONCH$_2$CHNCO), 5.80–6.24 (m, 3, exchangeable); IR ($CHCl_3$) λ 3600–2500 (broad), 1710 cm^{-1}.

***N*-[[2,3-Bis(*tert*-butoxycarbamoyl)propanoyl]oxy]-succinimide (5).** To a cooled stirring solution of 2,3-bis(*tert*-butoxycarbamoyl)propanoic acid, **4** (prepared from 10.01 g, 48.81 mmol, of ethyl 2,3-diaminopropanoate dihydrochloride), in 75 mL of methylene chloride was added 5.64 g (48.91 mmol) of *N*-hydroxysuccinimide. *N*,*N*′-Dicyclohexylcarbodiimide (11.44 g, 55.42 mmol) was subsequently added. The ice bath was removed after 15 min, and stirring was continued for a total time of 1 h. The mixture was subsequently cooled and filtered. The precipitate was washed with a small amount of cold methylene chloride and discarded. The filtrate was then washed with water (80 mL) and saturated sodium bicarbonate (2 × 75 mL), respectively. The organic extract was subsequently dried over anhydrous Na_2SO_4 and concentrated to a syrup. The latter was treated with diethyl ether and cooled to yield the product as a crystalline material. The material was collected by filtration, washed with cold diethyl ether/hexane, and dried to give 16.21 g (82.2%) of the product: mp 108–114 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 18, *tert*-butyl), 2.83 (s, 4, COCH$_2$CH$_2$CO), 3.67 (m, 2, CONCH_2CHCON), 4.67 (br s, 1, CONCH$_2$CHCON), 5.17–6.00 (m, 2, CONH); IR ($CHCl_3$) λ_{max} 3400, 3358, 3280, 3146, 2984, 1812, 1783, 1749, 1713, 1530, 1452 cm^{-1}.

General Method for the Synthesis of the Heterocycles 8a–g. A 7-10-mmol portion of the amine was added to a stirring

solution of the activated ester **5** (5 mmol) in 200 mL of dry acetonitrile, and the resulting solution was stirred overnight (for hindered amines such as 2,6-dimethylpiperidine, the solution was refluxed for up to 24 h). At the end of this period, the solution was concentrated in vacuo, and the residue was dissolved in methylene chloride (200 mL). The resulting solution was subsequently washed consecutively with water (100 mL), cold 1.5 N HCl (2 × 75 mL), and water (100 mL). The organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo to yield the protected amide **6**. Preliminary characterization was done by IR (1650 ± 15 cm^{-1}).

The amide **6** was dissolved in 50% ether/ethyl acetate (150 mL) and dry HCl gas was bubbled through this solution for 45 min, with continuous stirring. Precipitation of the deprotected amide **7** (as the hydrochloride) occurred during this period. At the end of this period, the mixture was treated with diethyl ether (100 mL), and the crude product was collected by filtration. This material was subsequently used without further purification.

A mixture of the deprotected amide **7**, 7 mmol of 2,2′-bis(2-methyl-2-mercaptopropanal), anhydrous sodium sulfate (30 g), and triethylamine (60 mL) in toluene (150 mL) was refluxed (with stirring) for 2–3 h with azeotropic removal of water. During this period, the reaction was monitored by TLC on silica gel, with 15% or 30% ethyl acetate in methylene chloride. Upon completion of this reaction, the mixture was allowed to cool to room temperature and then filtered. The precipitate was washed repeatedly with methylene chloride and discarded. Subsequently, the filtrate was washed with a saturated solution of sodium bicarbonate (2 × 125 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo to a residue, which invariably crystallized on standing.

The crude heterocycle **8** was purified by recrystallization from diethyl ether to give a chromatographically homogeneous solid. **8a**: mp 173–176 °C; ^{1}H NMR ($CDCl_3$) δ 1.00–1.88 (m, 18 H), 3.0–3.7 (m, 5 H), 4.0–4.40 (m, 2 H), 6.95 (br s, 2 H). Anal. ($C_{16}H_{27}N_3OS_2$) C, H, N, S. **8b**: yield, 7.2 g (54%); mp 164–167 °C; ^{1}H NMR ($CDCl_3$) δ 1.00–1.88 (m, 21 H), 2.83 (m, 1 H), 3.35 (t, 1 H), 4.18 (m, 4 H), 6.97 (s, 2 H). Anal. ($C_{17}H_{29}N_3OS_2 \cdot {}^1/_2H_2O$) C, H, N. **8c**: yield, 9.1 g (69%); mp 158–161 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (m, 3 H), 1.43 (m, 20), 2.50 (m, 3), 3.33 (t, 1 H), 4.20 (m, 3 H), 6.95 (br s, 2 H). Anal. ($C_{17}H_{29}N_3OS_2$) C, H, N, S. **8d**: yield, 9.1 g (68%); mp 154–5–157 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (d, 3 H), 1.4 (d, 17 H), 2.08–3.02 (m, 2 H), 3.33 (t, 1 H), 4.20 (m, 4 H), 6.97 (s, 2 H). Anal. ($C_{17}H_{29}N_3OS_2$) C, H, N, S. **8e**: yield, 6.8 g (49%); mp 140–142 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 6 H), 1.16–2.90 (m, 18 H), 3.55 (m, 2 H), 4.33 (m, 3 H), 6.98 (d, 2 H). Anal. ($C_{18}H_{31}N_3OS_2$) C, H, N. **8f**: yield, 4.6 g (25%); mp 160–162 °C; ^{1}H NMR ($CDCl_3$) δ 1.05–1.80 (m, 24 H), 3.20–3.60 (m, 2 H), 4.0–4.40 (m, 3 H), 7.0 (s, 2 H). Anal. ($C_{18}H_{31}N_3OS_2$) C, H, N. **8g**: yield, 7.6 g (44%); mp 170–173 °C; ^{1}H NMR ($CDCl_3$) δ 0.97–2.07 (m, 16 H), 2.26–3.65 (m, 4 H), 4.22 (m, 3 H), 4.85 (m, 1 H), 7.05 (s, 2 H), 7.32 (s, 5 H). Anal. ($C_{22}H_{31}N_3OS_2$) C, H, N, S.

General Procedure for the Synthesis of the Ligands 9a–g. A solution of the heterocycle **8** (5 mmol) in dry THF (50 mL) was added dropwise (under nitrogen) to a stirring suspension of lithium aluminum hydride (50 mmol) in dry THF (70 mL). Subsequent to this addition, the reaction mixture was refluxed for 18–20 h and cooled in an ice bath. The reaction was quenched by careful addition of a saturated solution of Rochelle salt. The resulting mixture was concentrated in vacuo to a residue, and the latter was extracted with hot ethyl acetate (5 × 100 mL). The combined extracts were dried over anhydrous sodium sulfate and concentrated to give the free base form of **9** as an oil. The latter was characterized by IR and NMR spectroscopy. Conversion of the free base to the hydrochloride salt was accomplished by dissolving the oil in anhydrous ethanol (~20 mL), cooling in an ice bath, and bubbling dry HCl through this solution (with continued cooling) for several minutes. Crystalline material was then obtained from the appropriate solvent. **9a**: yield, 1.3 g (53%); mp 147–152 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.0–1.65 (m, 18 H), 2.0–2.9 (m, 17 H); IR (neat) λ 2550 cm^{-1} (SH). Anal. ($C_{16}H_{38}Cl_3N_3S_2$) C, H, N; Cl: calcd, 24.01; found, 23.37. **9b**: yield, 1.2 g (53%); mp 153–157 °C (ethanol/ether); ^{1}H NMR ($CDCl_3$) δ 0.75–1.8 (m, 21 H), 2.08 (s, 5 H), 2.57 (m, 11 H); IR (neat) λ 2550 cm^{-1} (SH). Anal. ($C_{17}H_{40}Cl_3N_3S_2$) C, H, N; Cl: calcd, 23.27; found, 22.55. **9c**: yield, 0.75 g (31%); mp 202–204 °C (acetone); ^{1}H NMR ($CDCl_3$) δ 0.8 (d, 3 H), 0.93–3.17 (m, 34 H); IR (neat) λ 2.550 cm^{-1} (-SH). Anal. ($C_{17}H_{40}Cl_3N_3S_2$) C, H, N. **9d**: yield, 1.0 g (39%); mp 131–134 °C (acetone); ^{1}H NMR ($CDCl_3$) δ 1.0–2.93 (m, 34 H), 1.88 (d, 3 H); IR (neat) λ 2550 cm^{-1} (SH). Anal. ($C_{17}H_{40}Cl_3N_3S_2$) Calcd: C, 44.68; H, 8.82; Cl, 23.27; N, 9.19. Found: C, 43.28; H, 8.95; Cl, 22.20; N, 8.75. **9e**: yield, 1.0 g (42%); mp 162–165 °C (acetone/ether); ^{1}H NMR ($CDCl_3$) δ 0.7 (d, 8 H), 1.23 (s, 12 H), 1.33–2.67 (m, 19 H); IR (neat) λ 2545 cm^{-1} (SH). Anal. ($C_{18}H_{42}Cl_3N_3S_2$) C, H, N; Cl: calcd, 22.58; found 21.98. **9f**: yield, 0.5 g (41%); mp 149–153 °C (acetone/ether); ^{1}H NMR ($CDCl_3$) δ 1.2 (m, 24 H), 2.43 (m, 12 H), 3.4 (m, 3 H); IR (neat) λ 2547 cm^{-1} (SH). Anal. ($C_{18}H_{42}Cl_3N_3S_2$) C, H, N; Cl: calcd, 22.58; found, 22.01. **9g**: yield, 0.5 g (23%); mp 146–150 °C (acetone/ether); ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 12 H), 1.73–3.23 (m, 22 H), 7.1 (s, 5 H); IR (neat) λ 2550 cm^{-1} (SH). Anal. ($C_{22}H_{42}Cl_3N_3S_2$) C, H, N; Cl: calcd, 20.49; found, 20.03.

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 ^{99m}Tc–BAT complex (0.5–20 μCi). At selected intervals following the injection, blood samples (1 mL each) were collected by cardiac puncture, and the rats were sacrificed immediately thereafter by cardiectomy. The organs on interest were subsequently excised, weighed, and counted in a dual-channel automatic γ counter. The percent dose/organ values were determined by comparison of the tissue radioactivity with suitable dilutions of the injected dose. The percent dose/gram values were computed from the percent dose/organ values and the corresponding mean organ weights (mean organ weights: heart, 0.85 g; brain, 1.65 g; blood, 18 g; liver, 9 g; kidneys, 1.9 g; lungs, 1.6 g). Finally, the blood/brain ratio was calculated from the corresponding percent dose/gram values.

Acknowledgment. We thank Elongia Farrell for her excellent technical assistance and Rebecca Hoffner and Joseph Machnica for preparation of this manuscript. This project is supported by a grant awarded by NIH, NINCDS, NS-18509.