Structure-Activity Relationship of ^{99m}Tc Complexes of Phenylenediamine-Thiol-Thioether Ligands (PhAT) to Brain Uptake in Rats

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A novel class of ligands, phenylenediamine-thiol-thioether (PhAT), was synthesized, and their ^{99m}Tc complexes were evaluated for potential use as a functional brain imaging agent. The ligands reacted with $Na^{99m}TcO_4$ and $SnCl_2$ to form single, stable, neutral, and lipophilic ^{99m}Tc complexes. Several of these complexes showed significant brain uptake and retention in rats. In particular, the S-ethyl, allyl, and propargyl derivatives had high initial brain uptake (0.88, 0.99, and 0.82% dose/g at 5 min, respectively) and good retention (0.71, 0.75, and 0.67% dose/g at 30 min). The structure-activity relationship of alkyl, alkenyl, and alkynyl thioether derivatives is reported.

The search for a brain perfusion imaging agent which accurately reflects regional cerebral blood flow (rCBF) in single photon emission computed tomography (SPECT) has been the subject of great interest in nuclear medicine. The first successful agents were the iodinated agents *N*-isopropyl-*p*-[^{123}I]iodoamphetamine ([^{123}I]IMP, 1980)[1] and *N,N,N'*-trimethyl-*N'*-(2-hydroxy-3-methyl-5-[^{123}I]-iodobenzyl)-1,3-propanediamine ([^{123}I]HIPDM, 1983).[2] Although these agents have proven useful in functional brain imaging,[3] the limited availability of cyclotron-produced ^{123}I has restricted their use. The favorable properties of ^{99m}Tc, availability from a ^{99}Mo-^{99m}Tc generator, short half-life (6.02 h), and 140 keV γ emission, prompted the search for ^{99m}Tc complexes which cross the blood-brain barrier (BBB). The requirements for a functional brain imaging agent are a high first-pass extraction into the brain and a distribution which remains fixed long enough to obtain a SPECT image. Ideally, for routine use, the ligand should form a single ^{99m}Tc complex in high yield and the ^{99m}Tc complex should be stable in vitro.

The first lipid-soluble ^{99m}Tc complexes to successfully penetrate the BBB were substituted oxines[5] and derivatives of structures based on the N_2S_2 core, termed bis(aminoethanethiol) (BAT)[6] and diaminodithiol (DADT).[7] Although the initial distribution of some of these agents accurately reflected rCBF, they often redistributed in the brain and were cleared too rapidly to be useful for clinical SPECT imaging.

Another class of ligands, propylenediamine oxime (PnAO),[8] was investigated and a derivative, technetium-99m-HMPAO,[9] was approved for use as a brain imaging agent in 1989. The ^{99m}Tc-HMPAO complex, however, is unstable in vitro, the brain/blood ratio is low,[9] and quantitative SPECT images underestimate blood flow at high flow rates.[10]

Technetium-99m-MRP20 [*N*-(1*H*-pyrrol-2-ylmethyl)-*N'*-(4-oxopenten-2-ylidene)ethane-1,2-diamine] is a neutral, lipophilic agent which crosses the blood-brain barrier. This agent is metabolized to a cationic species which may be trapped in the brain.[11]

An N_2S_2 derivative, ethyl cysteinate dimer (^{99m}Tc-ECD),[12] is showing promise as a brain imaging agent. ^{99m}Tc-ECD is hydrolyzed from a diethyl ester to the monoethyl ester by an enzyme in the brain. The monoester does not pass through the blood-brain barrier, thus

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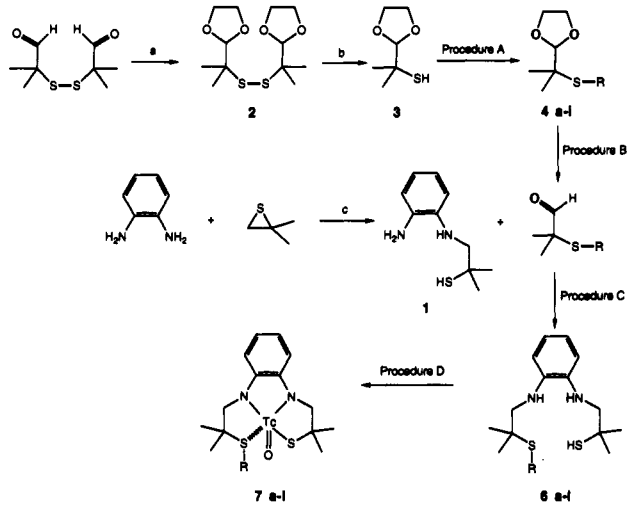
Scheme I[a]



[a] Reagents and conditions: (a) $HOCH_2CH_2OH$, *p*-TsOH, C_6H_6, reflux; (b) Na/NH_3, −78 °C; (c) sealed vessel, EtOH, 100 °C; (procedure A) NaH or K_2CO_3, alkyl, alkenyl, or alkynyl halide, THF or MeOH/H_2O; (procedure B) H_3O^+, THF, reflux; (procedure C) $NaBH_3CN$, HOAc, MeOH; (procedure D) $Na^{99m}TcO_4$, $SnCl_2$, EtOH, H_2O.

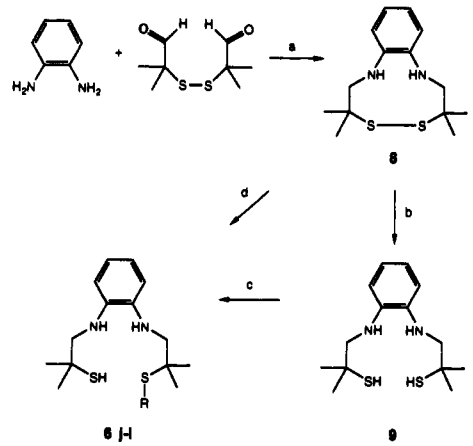
trapping the ^{99m}Tc in the brain. This drug therefore is not a simple blood flow imaging agent, as brain uptake depends not only on extraction into brain but hydrolysis by the enzyme in the brain cells as well. The ^{99m}Tc–ECD complex is stable in vitro and has a high brain/blood ratio, but the SPECT images may not accurately reflect rCBF at high flow rates.[13]

We chose to investigate phenylenediamine–thiol–thioether ligands **6** (phenylene–amine–thiols, "PhAT," Scheme I) similar to the amide–thiol–thioether ligands reported by Bryson et al.[14] The purpose of this report is to present the synthesis and structure–activity relationship of S-substituted alkyl, alkenyl, and alkynyl derivatives of **7** (Scheme I). The sulfur substituent was varied to determine the effect of chain length, branching, and unsaturation on the brain uptake and retention of the ^{99m}Tc complexes.

Chemistry

Compounds **6a–i** were synthesized in a convergent synthesis as outlined in Scheme I. 2,2′-Dithiobis(2-methylpropanal)[15] was protected as the bis-acetal and reduced by sodium in liquid ammonia to the thiol acetal **3**. Alkylation of the thiol with NaH in THF gave the S-substituted acetals **4a–i**. For the propargyl derivative **4b**, potassium carbonate was used because sodium hydride resulted in isomerization of the propargyl side chain to the corresponding allene. Acid hydrolysis of the acetals gave the aldehydes **5a–i**. 1,2-Phenylenediamine and isobutylene sulfide were reacted in a sealed vessel as described by Snyder[16] to give **1**. Reductive amination of the aldehydes with amine **1** gave the corresponding N_2S_2 ligands **6a–i**.

Scheme II[a]



[a] Reagents and conditions: (a) $NaBH_3CN$, HOAc, MeOH; (b) Na/NH_3, THF, −78 °C; (c) NaH, alkyl halide, THF; (d) vinylmagnesium bromide, reflux.

Compounds **6j–l** were synthesized as outlined in Scheme II. Reductive amination of 1,2-phenylenediamine with 2,2′-dithiobis(2-methylpropanal) gave disulfide **8**. Reaction of disulfide **8** directly with vinylmagnesium bromide gave the vinyl derivative **6l**. Alkylation of the thiol generated by hydride reduction of the disulfide gave **6j** and **6k**. This method allows for the rapid synthesis of ligands; however, disubstituted side products and unreacted dithiol make the isolation of the monosubstituted product difficult.

Results and Discussion

Reaction of the ligands with $Na^{99m}TcO_4$ and $SnCl_2$ in aqueous ethanol gave a single complex in 66–93% yield. Membrane filtration gave a 90% radiochemically pure complex, with the exception of the vinyl complex which required HPLC purification to obtain a similar purity. All ^{99m}Tc complexes were stable for at least 3 h at ambient temperature. All complexes stayed at the origin on electrophoresis, whereas a $Na^{99m}TcO_4$ control migrated 4 cm toward the anode, suggesting that the complexes were neutral.

The ligands form neutral ^{99m}Tc(V) complexes, where TcO presumably combines with the two nitrogens and two sulfurs (with the loss of the two NH and the SH protons) of the ligands to form the base of the square pyramidal complexes. The PhAT complexes form only one TcO isomer, unlike the BAT ligands, which form syn and anti ^{99m}TcO complexes.[5] The PhAT complexes are probably the anti structure, based on the observation by Bryson[14] and the X-ray crystallographic structure conducted on the rhenium propyl and allyl analogs (unpublished results).[17]

The ^{99m}Tc complexes displayed distinct retention times on HPLC and had different brain uptake and retention in rats (Table I), indicating that the *S*-alkyl groups are

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Table I. Characterization and Brain Uptake of ^{99m}Tc Complexes in Rats

R group	% purity[a]	HPLC[b] t_R(min)	brain (%dose/g)[c] 5 min	brain (%dose/g)[c] 30 min	brain/blood[d] 5 min	brain/blood[d] 30 min	retention ratio[e]
ethyl (**7i**)	98 (A)	3.8	0.88 (0.86–0.91)	0.71 (0.64–0.78)	6.3	6.2	0.80
propyl (**7c**)	96 (B)	3.9	0.75 (0.68–0.81)	0.69 (0.63–0.76)	2.1	3.1	0.92
isopropyl (**7d**)	91 (B)	4.2	0.73 (0.66–0.80)	0.67 (0.65–0.69)	2.1	2.4	0.92
butyl (**7k**)	92 (A)	4.4	0.41 (0.38–0.45)	0.43 (0.42–0.44)	0.5	1.2	1.00
sec-butyl (**7j**)	93 (B)	4.4	0.64 (0.58–0.71)	0.57 (0.56–0.58)	2.8	3.7	0.89
cyclopropylmethyl (**7g**)	98 (C)	4.9	0.54 (0.48–0.60)	0.47 (0.45–0.49)	2.6	4.2	0.87
vinyl (**7l**)	90 (B)	3.5	0.56 (0.51–0.61)	0.57 (0.47–0.66)	0.9	1.9	1.00
allyl (**7a**)	96 (B)	3.6	0.99 (0.98–0.99)	0.75 (0.75–0.76)	3.6	5.6	0.76
3-butenyl (**7e**)	95 (C)	3.9	0.80 (0.77–0.84)	0.69 (0.69)	2.2	3.8	0.87
(*E*)-2-butenyl (**7f**)	94 (C)	4.1	0.50 (0.39–0.61)	0.49 (0.46–0.51)	1.9	2.6	0.98
4-pentenyl (**7h**)	97 (D)	2.9	0.44 (0.41–0.47)	0.48 (0.45–0.51)	2.2	4.1	1.00
propargyl (**7b**)	93 (C)	3.3	0.82 (0.76–0.96)	0.67 (0.64–0.70)	1.8	3.5	0.82

[a] TLC was performed with the following solvent systems to obtain an R_f value of 0.4–0.8: A, 50% $Et_2O/CHCl_3$; B, 10–30% EtOAc/hexane; C, 30% EtOAc/hexane; D, EtOAc/MeOH/H_2O/concentrated NH_4OH (86/10/3/1). [b] HPLC was performed on a C-18 Radial-Pak cartridge with 90% EtOH/H_2O at a flow rate of 1.5 mL/min as described in the Experimental Section. [c] Mean of two animals (range). [d] Defined as the ratio of mean %dose/gram of brain to that of blood. [e] Defined as the ratio of mean %dose/gram of brain at 30 min to that at 5 min.

Table II. Biodistribution of ^{99m}Tc–AllylPhAT (**7a**) in Rats[a]

organ/tissue	organ uptake (%dose)[a] 5 min	organ uptake (%dose)[a] 30 min	organ/tissue	organ uptake (%dose)[a] 5 min	organ uptake (%dose)[a] 30 min
blood[b]	0.27	0.13	stomach	1.35	1.95
brain	1.62	1.30	gut	7.41	12.21
lungs	1.12	0.65	bladder	0.06	0.06
liver	40.95	42.27	remainder of body	39.13	35.54
heart	3.04	1.51			
kidneys	4.73	4.21			

[a] The values are expressed in %dose/organ and are the average of two animals. [b] The values for the blood are expressed in %dose/gram.

still present, unlike the ligands of Bryson,[14] where the alkyl groups were cleaved to give the parent compound. The biodistribution of allyl derivative (Table II) is typical of the biodistributions of ^{99m}Tc–PhAT complexes.

The brain uptake and retention of the ^{99m}Tc complexes were evaluated in pentobarbital-anesthetized female Sprague–Dawley rats (Table I). The brain uptake decreased as the chain length of the *S*-alkyl group increased (ethyl, propyl, and butyl had a brain uptake of 0.88, 0.75, and 0.41% dose/g at 5 min, respectively). Terminal olefinic compounds showed a similar trend and had a greater brain uptake than the corresponding saturated analogs (0.99, 0.80, and 0.44% dose/g at 5 min, for allyl, 3-butenyl, and 4-pentenyl, respectively).

Branching of the alkyl side chain increased brain uptake for the four-carbon side chain (0.64, 0.54, and 0.41% dose/g at 5 min for *sec*-butyl, cyclopropylmethyl, and *n*-butyl, respectively) but had no effect on the three-carbon derivatives.

The effect of unsaturation was studied using the propyl, allyl, and propargyl derivatives. The allyl compound had the highest initial brain uptake (0.99% dose/g at 5 min), followed by propargyl (0.82% dose/g) and propyl (0.75% dose/g).

The brain retention ratio (defined as percent dose/gram at 30 min/5 min) increased as the chain length of the saturated and olefinic substituents increased. The saturated compounds had greater retention ratios than the olefinic and propargyl compounds (0.92, 0.82, and 0.76 for propyl, propargyl, and allyl, respectively).

The ethyl, allyl, and propargyl derivatives exhibited high brain uptake and retention. These compounds were selected for further biological evaluation. Whole brain uptake of the allyl derivative (**7a**) in rat was 1.62% dose at 5 min and 1.30% at 30 min (Table II). Major uptake was observed in the liver (41% at 5 min, 42.3% at 30 min) and activity accumulated in the intestine (7.41% at 5 min increasing to 12.2% at 30 min), suggesting hepatobiliary excretion. Only negligible activity was found in the urinary bladder. The propargyl derivative (**7b**) was demonstrated to be suitable as a SPECT rCBF tracer in nonhuman primates.[18]

In summary, a novel class of N_2S_2 ligands has been synthesized that forms single, stable, lipophilic, neutral ^{99m}Tc complexes which have high brain uptake and retention, and thus show promise as functional brain imaging agents.[19]

Experimental Section

Chemistry and Radiolabeling. The 60-MHz ^{1}H nuclear magnetic resonance spectra were recorded on a Varian Model EM 360L spectrometer. Chemical shifts are reported in parts per million (ppm) down field from Me_4Si as an internal standard. High-field ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL200 or Varian XL400 super-conducting FT spectrometer. Mass spectra were performed using a V.G. 70E-HF (high-resolution) or V.G. ZAB-L1 (low-resolution) spectrometer. Melting points were determined in an open capillary tube with a Mel-Temp

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melting point apparatus. Infrared spectra were recorded on a Beckman Acculab 8 spectrophotometer. Elemental analyses were performed by University of California Analytical Services, Berkeley, CA. Analyses represented only by symbols of the elements were within ±0.4% of the theoretical values. Analytical HPLC was performed on a Waters system consisting of a Model 510 pump, U6K injector, 481 UV detector (254 nm), and a 4-μm Waters Radial Pak C-18 column. Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} aluminum-backed plates from E. Merck (Darmstadt, Germany), and the developed plates were visualized by UV quenching at 254 nm or by spraying with phosphomolybdic acid or 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). Flash chromatography was performed with silica gel (E. Merck, 230–400 mesh). Radial chromatography was performed on a Chromatotron (Harrison Research, Palo Alto, CA) using Merck silica gel 60 F_{254}. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl. All other solvents and reagents were used as received. Isobutylene oxide was obtained from American Tokyo Kasei (TCI), Portland, Oregon. All reactions were performed under nitrogen.

Sodium [^{99m}Tc] pertechnetate in physiological saline was obtained from a ^{99}Mo–^{99m}Tc generator (Cintichem, Tuxedo, NY). Electrophoresis was performed on Whatman 3MM filter paper impregnated and developed with 0.05 M NaH_2PO_4 buffer, pH 4.5, at 300 V for 30–45 min. TLC and electrophoresis strips were scanned with a NaI(Tl) scintillation detector.

Isobutylene Sulfide.[15] Yield: 82%. Bp: 87–88 °C (lit.[15] bp: 88–89 °C). ^{1}H NMR ($CDCl_3$): δ 1.5 (s, 6 H), 2.3 (s, 2 H).

***N*-(2-Mercapto-2-methylpropyl)-1,2-benzenediamine (1).** In a 200-mL Wheaton pressure bottle were dissolved 30 g (0.34 mol, 100 mol %) of isobutylene sulfide and 51 g (0.47 mol, 140 mol %) of 1,2-phenylenediamine in 20 mL of ethanol. The mixture was stirred in a 100 °C oil bath for 5 h. The vessel was allowed to cool to room temperature and the crude mixture was diluted with 200 mL of Et_2O. The ether layer was washed with 3 × 200 mL of 0.5 M NaOH. The base layers were combined and acidified to pH 1 with 40 mL of 12 M HCl. The acid layer was extracted with 3 × 200 mL of Et_2O. The pH of the acid layer was then adjusted to pH 4 with 9 g of NaOH pellets and extracted with 3 × 200 mL of Et_2O. The combined ether layers were washed with 100 mL of saturated NaCl and dried (Na_2SO_4). The solvent was then removed under reduced pressure to give 2.6 g (40% yield) of a waxy yellow solid. Mp: 38–40 °C. TLC (30% EtOAc/hexane): R_f 0.53 (DTNB+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (s, 6 H), 1.79 (s, 1 H), 3.11 (s, 2 H), 3.45 (br s, 2 H), 3.75 (br s, 1 H), 6.66–6.82 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 137.5, 134.8, 120.5, 119.1, 116.5, 112.5, 57.7, 45.3, 30.7. IR (film): 3390, 3320, 2960, 2910, 2850, 2540, 1615, 1590, 1505, 1450, 1380, 1360 cm^{-1}. MS (FAB$^+$, *m/z*): 196 (M$^+$), 163, 121 (100), 108, 92. Anal. ($C_{10}H_{16}N_2S$): C, H, N, S.

2,2'-Dithiobis(2-methylpropanal).[14] Yield: 23%. Bp: 115 °C/0.25 Torr) (lit.[14] bp: 92–93 °C/0.3 Torr). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.42 (s, 12 H), 9.12 (s, 2 H). Anal. ($C_8H_{14}O_2S_2$): C, H, S.

2,2'-[1,1-Dithiobis(1-methylethyl)]bis[1,3-dioxolane] (2). 2,2'-Dithiobis(2-methylpropanal) (4.12 g, 20 mmol, 100 mol %) and 5.0 mL (90 mmol, 448 mol %) of ethylene glycol were dissolved in 30 mL of benzene. *p*-Toluenesulfonic acid monohydrate (0.05 g, 0.02 mmol, 1.0 mol %) was then added, and a Dean–Stark trap and condenser were attached. The mixture was heated at reflux for 6 h. The reaction mixture was then diluted with 50 mL of Et_2O and washed with 30 mL of 0.5 M NaOH. The ether layer was then washed with 50 mL of saturated NaCl and dried (Na_2SO_4), and the solvent was removed under reduced pressure to give 5.5 g (94% yield) as a gold oil. TLC (50% Et_2O/hexane): R_f 0.47 (visualized by phosphomolybdic acid). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.42 (s, 12 H), 3.75–4.02 (m, 8 H), 4.72 (s, 1 H).

2-Mercapto-2-(1-methylethyl)-1,3-dioxolane (3). Compound 2 (2.4 g, 8.2 mmol, 100 mol %) was placed in a 250-mL round-bottom flask equipped with a cold-finger condenser. The reaction flask and cold finger were cooled to −78 °C (dry ice/acetone). Liquid NH_3 (40 mL) was condensed and dripped into the mixture. Hexane-washed sodium spheres (0.3 g, 13 mmol, 150 mol %) were added (the reaction turned dark blue). THF (20 mL) was added and the mixture was stirred for 3.5 h. Solid NH_4Cl was then added until the solution became colorless. The ammonia was evaporated in a 40 °C water bath and the residual white solid was dissolved in 100 mL of water. Concentrated H_3PO_4 (20 mL) was added until the solution was pH 2. The aqueous solution was extracted with 3 × 100 mL of Et_2O. The combined ether layers were washed with 50 mL saturated NaCl and dried (Na_2SO_4). The solvent was removed under reduced pressure to give 2.34 g (97% yield) of oil. TLC (50% Et_2O/hexane): R_f 0.66 (DTNB+). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.40 (s, 6 H), 1.92 (s, 1 H), 3.84–4.10 (m, 4 H), 4.75 (s, 1 H).

General Procedure A. Alkylation of Thiol Acetals. 2-[1-(Allylthio)-1-methylethyl]-1,3-dioxolane (4a). Compound 3 (10 g, 67 mmol, 100 mol %) was dissolved in 100 mL of THF. Sodium hydride (3.5 g, 74 mmol, 110 mol %) (50% mineral oil dispersion washed with 3 × 50 mL of hexane) was added and the reaction mixture was stirred at ambient temperature for 15 min. Allyl bromide (5.87 g, 67 mmol, 100 mol %) was then added and the mixture was stirred at ambient temperature for 2 h, and then 50 mL of 0.5 M HCl was added and the mixture was extracted with 3 × 50 mL of Et_2O. The combined ether layers were washed with 2 × 40 mL of saturated NaCl and dried (Na_2SO_4). The solvent was removed under reduced pressure to give 12.0 g (95% yield) as a gold oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (s, 6 H), 3.36 (d, 2 H, *J* = 6 Hz), 3.91–3.98 (m, 4 H), 4.83 (s, 1 H), 5.05 (m, 1 H), 5.18 (m, 1 H), 5.87 (m, 1 H). MS (FAB$^+$, *m/z*): 188 (M$^+$), 187, 147, 116, 115. Anal. ($C_9H_{16}O_2S$): C, H, S.

2-[1-(Propargylthio)-1-methylethyl]-1,3-dioxolane (4b). Compound 3 (1.50 g, 10 mmol, 100 mol %) was dissolved in 30 mL of MeOH and 30 mL of H_2O, then K_2CO_3 (1.38 g, 10 mmol, 100 mol %) and 0.89 g (12 mmol, 100 mol %) propargyl chloride were added, and the mixture was stirred at ambient temperature for 6 h. Water (100 mL) was added and the reaction mixture was extracted with 3 × 100 mL of EtOAc. The combined organic layers were washed with 100 mL of saturated NaCl and dried (Na_2SO_4). Removal of the solvent by rotary evaporation gave 1.26 g (68% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (s, 6 H), 2.20 (s, 1 H, *J* = 2.6 Hz), 3.47 (d, 2 H, *J* = 2.6 Hz), 3.88–4.0 (m, 4 H), 4.84 (s, 1 H). MS (FAB$^+$, *m/z*): 186 (M+), 147, 116, 113, 73 (100). Anal. ($C_9H_{14}O_2S$): C, H, S.

2-[1-(Propylthio)-1-methylethyl]-1,3-dioxolane (4c). Compound 3 (1.53 g, 10 mmol, 100 mol %) and 1.93 g (11.4 mmol, 110 mol %) of 1-iodopropane were reacted according to procedure A to give 2.10 g (100 % yield) as an oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.98 (t, 3 H, *J* = 7 Hz), 1.27 (s, 6 H), 1.60 (m, 2 H), 2.61 (t, 2 H, *J* = 7 Hz), 3.92 (s, 4 H), 4.73 (s, 1 H).

2-[1-[(1-Methylethyl)thio]-1-methylethyl]-1,3-dioxolane (4d). Compound 3 (1.62 g, 11 mmol, 100 mol %) and 2-iodopropane (2.05 g, 12 mmol, 110 mol %) were reacted according to procedure A to give 1.46 g (70% yield) as a colorless oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.01 (s, 6 H), 1.27 (s, 6 H), 2.65 (m, 1 H), 3.91–3.98 (m, 4 H), 4.80 (s, 1 H).

2-[1-(3-Butenylthio)-1-methylethyl]-1,3-dioxolane (4e). Compound 3 (7 g, 47 mmol, 100 mol %) and 4.80 mL (46 mmol, 100 mol %) of 4-bromo-1-butene were reacted according to procedure A to give 9.0 g (94% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (s, 6 H), 2.30 (m, 2 H), 2.74 (t, 2 H, *J* = 7 Hz), 3.89–3.97 (m, 4 H), 4.81 (s, 1 H), 5.01–5.08 (m, 2 H), 5.80–5.87 (m, 1 H). Anal. ($C_{10}H_{18}O_2S$): C, H, S.

2-[1-(*trans*-2-Butenylthio)-1-methylethyl]-1,3-dioxolane (4f). Compound 3 (2.15 g, 14.5 mmol, 100 mol %) and 1.64 mL (16.0 mmol, 110 mol %) of crotyl bromide were reacted according to procedure A to give 2.80 g (95% yield) as an oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.28 (s, 6 H), 1.67 (d, 3 H, *J* = 7 Hz), 3.30 (m, 2 H), 3.93 (br s, 4 H), 4.80 (s, 1 H), 5.50 (m, 2 H).

2-[1-[(Cyclopropylmethyl)thio]-1-methylethyl]-1,3-dioxolane (4g). Compound 3 (3.4 g, 23 mmol, 100 mol %) and 3.4 g (25 mmol, 110 mol %) of cyclopropylmethyl bromide were reacted according to procedure A to give 4.0 g (88% yield) as an oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.1–0.8 (m, 5 H), 1.24 (s, 6 H), 2.58 (d, 2 H, *J* = 9 Hz), 3.90 (s, 4 H), 4.78 (s, 1 H).

2-[1-(4-Pentenylthio)-1-methylethyl]-1,3-dioxolane (4h). Compound 3 (3.0 g, 20 mmol, 100 mol %) and 2.62 mL (24.3 mmol, 110 mol %) of 5-bromo-1-pentene were reacted according to procedure A to give 4.5 g (99% yield) as an oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.26 (s, 6 H), 1.43–1.83 (m, 4 H), 1.85–2.40 (m, 2 H), 2.53–2.83 (m, 2 H), 3.93 (m, 4 H), 4.82 (s, 1 H), 4.95–5.16 (m, 1 H), 5.45–6.12 (m, 1 H).

2-[1-(Ethylthio)-1-methylethyl]-1,3-dioxolane (4i). Compound 3 (2.0 g, 13.4 mmol, 100 mol %) and iodobenzene (2.3 g,

14.8 mmol, 110 mol %) were reacted according to procedure A to give 2.88 g (100% yield) as an oil. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.20 (t, 3 H, J = 7.5 Hz), 1.27 (s, 6 H), 2.69 (q, 2 H, J = 7.5 Hz), 3.93 (s, 4 H), 4.80 (s, 1 H).

General Procedure B. Hydrolysis of Acetals. 2-(Allylthio)-2-methylpropanal (5a). Compound **4a** (12.0 g, 64 mmol) was dissolved in 25 mL of THF and 25 mL of 0.5 M HCl. The mixture was heated at reflux for 2 h. The solution was extracted with 3 × 50 mL of Et_2O. The combined ether layers were washed with 50 mL of water and 50 mL of saturated NaCl and dried (Na_2SO_4). The solvent was removed under reduced pressure to give 7.0 g (77% yield) of a gold oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 6 H), 2.99 (d, 2 H, J = 5.8 Hz), 5.08–5.18 (m, 2 H), 5.75 (m, 1 H), 9.12 (s, 1 H). ^{13}C NMR ($CDCl_3$): δ 194.1, 133.5, 118.0, 52.1, 32.0, 21.2. Anal. ($C_7H_{12}OS$): C, H, S.

2-(Propargylthio)-2-methylpropanal (5b). Yield: 93%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.40 (s, 6 H), 2.25 (t, 1 H, J = 3 Hz), 3.05 (d, 2 H, J = 3 Hz), 9.20 (s, 1 H). IR (film): 3300, 2960, 2920, 2800, 2720, 2110, 1700 cm^{-1}. Anal. ($C_7H_{10}OS$): C, H, S.

2-(Propylthio)-2-methylpropanal (5c). Yield: 89%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.95 (t, 3 H, J = 7 Hz), 1.37 (s, 6 H), 1.52 (m, 2 H), 2.28 (t, 2 H, J = 7 Hz), 9.10 (s, 1 H).

2-[(1-Methylethyl)thio]-2-methylpropanal (5d). Yield: 85%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.25 (d, 3 H, J = 6 Hz), 1.37 (s, 6 H), 2.07 (m, 1 H), 9.17 (s, 1 H).

2-(3-Butenylthio)-2-methylpropanal (5e). Yield: 90%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.36 (s, 6 H), 2.26 (s, 6 H), 2.36 (m, 2 H), 5.02–5.06 (m, 2 H), 5.76 (m, 1 H). IR (film): 3080, 2960, 2920, 2800, 2700, 1705, 1630, 1460 cm^{-1}. Anal. ($C_8H_{14}OS$): C, H, S.

2-(*trans*-2-Butenylthio)-2-methylpropanal (5f). Yield: 97%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.32 (s, 6 H), 1.64 (d, 3 H, J = 7 Hz), 2.92 (d, 2 H, J = 7 Hz), 5.45 (m, 2 H), 9.12 (s, 1 H).

2-[(Cyclopropylmethyl)thio]-2-methylpropanal (5g). Yield: 98%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.1–0.8 (m, 5 H), 1.33 (s, 6 H), 2.33 (d, 2 H, J = 9 Hz), 9.12 (s, 1 H).

2-(4-Pentenylthio)-2-methylpropanal (5h). Yield: 83%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.35 (s, 6 H), 1.43–1.86 (m, 2 H), 2.0–2.4 (m, 6 H), 4.83–5.2 (m, 2 H), 5.40–6.12 (m, 1 H), 9.20 (s, 1 H).

2-(Ethylthio)-2-methylpropanal (5i). Yield: 100%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.21 (t, 3 H, J = 7 Hz), 1.33 (s, 6 H), 2.30 (q, 2 H, J = 7 Hz), 9.12 (s, 1 H).

General Procedure C. Reductive Amination. N^1-[2-(Allylthio)-2-methylpropyl]-N^2-(2-mercapto-2-methylpropyl)-1,2-benzenediamine (6a). Compounds 1 (3.5 g, 17.8 mmol, 100 mol %) and **5a** (3.5 g, 17.8 mmol, 100 mol %) were dissolved in 125 mL of MeOH. Acetic acid (2.06 mL, 36 mmol, 200 mol %) was then added, followed by 3.35 g (53.4 mmol, 300 mol %) of $NaBH_3CN$. The reaction was stirred at ambient temperature for 17 h, and then 100 mL of 0.5 M HCl was added. The acid solution was stirred for 15 min and extracted with 2 × 150 mL of Et_2O. The combined ether layers were washed with 60 mL of saturated NaCl and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude product was adsorbed onto 50 g of silica gel and filtered through a 270-g silica gel bed (10 × 10 cm, 10% Et_2O/hexane) to give 4 g (69% yield) as a gold oil. TLC (5% EtOAc/hexane): R_f 0.60 (DTNB+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 6 H), 1.50 (s, 6 H), 1.83 (s, 1 H), 3.05 (s, 2 H), 3.12 (s, 2 H), 3.14 (d, 2 H, J = 6.9 Hz), 4.01 (br s, 2 H), 5.02 (m, 1 H), 5.16 (m, 1 H), 5.82 (m, 1 H), 6.67–6.70 (m, 2 H), 6.79 (m, 2 H). ^{13}C NMR ($CDCl_3$): δ 137.9, 137.4, 134.8, 119.6, 119.0, 117.2, 112.4, 111.8, 57.8, 52.8, 46.7, 45.4, 31.3, 30.7, 27.7. IR (film): 3300, 3060, 2960, 2920, 2860, 2820, 2550, 1640, 1600, 1510, 1380, 1360, 1250, 1140, 1120, 980, 910 cm^{-1}. MS (FAB$^+$, m/z): 324 (M+), 290, 284, 249, 209, 207, 175, 163, 121 (100). Anal. ($C_{17}H_{28}N_2S_2$): C, H, N, S.

N^1-(2-Mercapto-2-methylpropyl)-N^2-[2-(propargylthio)-2-methylpropyl]-1,2-benzenediamine (6b). Compounds 1 (5.59 g, 28.5 mmol, 100 mol %) and **5b** (6.07 g, 42.8 mmol, 150 mol %) were reacted according to procedure C to give 6.8 g (75% yield) of oil. TLC (25% Et_2O/hexane): R_f 0.65 (DTNB+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.46 (s, 6 H), 1.49 (s, 6 H), 1.84 (s, 1 H), 2.09 (t, 1 H, J = 3 Hz), 3.11 (s, 2 H), 3.12 (s, 2 H), 3.21 (d, 2 H, J = 3 Hz), 3.92 (m, 2 H), 6.6–6.8 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 137.7, 137.5, 119.5, 119.2, 112.3, 112.1, 81.0, 70.8, 57.8, 52.8, 47.4, 45.4, 30.7, 27.3, 15.9. IR (film): 3280, 2950, 2920, 2850, 2360, 1595, 1520, 1380, 1360, 1140, 1120, 730 cm^{-1}. MS (FAB$^+$, m/z): 322 (M+), 289, 247, 209, 171, 121 (100), 119, 55, 39. Anal. ($C_{17}H_{26}N_2S_2$): C, H, N, S.

N^1-(2-Mercapto-2-methylpropyl)-N^2-[2-(propylthio)-2-methylpropyl]-1,2-benzenediamine (6c). Compounds 1 (0.40 g, 2.06 mmol, 100 mol %) and **5c** (0.45 g, 3.1 mmol, 150 mol %) were reacted according to procedure C to give 0.24 g (36% yield) of a colorless oil after purification by radial chromatography (2-mm silica gel plate, 20% EtOAc/hexane). TLC (5% Et_2O/petroleum ether): R_f 0.26 (DTNB+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (t, 3 H, J = 7.4 Hz), 1.42 (s, 6 H), 1.50 (s, 6 H), 1.54 (m, 2 H), 1.83 (s, 1 H), 2.42 (t, 2 H, J = 7.4 Hz), 3.01 (s, 2 H), 3.12 (s, 2 H), 3.87 (br s, 1 H), 4.16 (br s, 1 H), 6.65–6.71 (m, 2 H), 6.75–6.83 (m, 2 H). ^{13}C NMR ($CDCl_3$): δ 137.8, 137.3, 119.5, 118.9, 112.2, 111.5, 57.7, 52.6, 45.8, 45.4, 30.7, 29.5, 27.7, 23.0, 13.8. IR (film): 3310, 3055, 2984, 2968, 2940, 2920, 1600, 1518, 1440, 1260 cm^{-1}. MS (FAB$^+$, m/z): 326 (M+), 292, 284, 238, 209, 131, 121 (100), 119, 89, 55. Anal. ($C_{17}H_{30}N_2S_2$): C, H, N, S.

N^1-(2-Mercapto-2-methylpropyl)-N^2-[2-[(1-methylethyl)thio]-2-methylpropyl]-1,2-benzenediamine (6d). Compounds 1 (0.52 g, 2.65 mmol, 100 mol %) and **5d** (0.79 g, 5.31 mmol, 200 mol %) were reacted according to procedure C to give 0.24 g (28% yield) of a colorless oil after purification by radial chromatography (2-mm silica gel plate, 5% Et_2O/hexane). TLC (5% Et_2O/hexane): R_f 0.34 (DTNB+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (d, 6 H, J = 6.8 Hz), 1.43 (s, 6 H), 1.50 (s, 6 H), 1.83 (s, 1 H), 2.88 (m, 1 H, J = 7 Hz), 3.04 (s, 2 H), 3.12 (s, 2 H), 3.86 (br s, 1 H), 4.23 (br s, 1 H), 6.65 (m, 1 H), 6.69 (m, 1 H), 6.77 (m, 1 H), 6.80 (m, 1 H). ^{13}C NMR ($CDCl_3$): δ 137.9, 137.2, 119.5, 118.5, 112.1, 111.4, 57.7, 53.2, 47.1, 45.4, 32.5, 30.7, 28.3, 26.0. IR (film): 3300, 3040, 2975, 2920, 2860, 1595, 1510, 1435, 1260, 730 cm^{-1}. MS (FAB$^+$, m/z): 326 (M+), 292, 284, 238, 223, 209, 131, 121 (100), 119, 75. HPLC (90% EtOH/H_2O, 1.5 mL/min): t_R 4.91 min (87%).

N^1-[2-(3-Butenylthio)-2-methylpropyl]-N^2-(2-mercapto-2-methylpropyl)-1,2-benzenediamine (6e). Compounds 1 (3.50 g, 17.8 mmol, 100 mol %) and **6d** (4.23 g, 26.7 mmol, 150 mol %) were reacted according to procedure C to give 5 g (83% yield) of a yellow oil after filtration through 300 g of silica gel (10 × 10 cm, 10% Et_2O/hexane). TLC (5% EtOAc/hexane): R_f 0.40 (DTNB+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (s, 6 H), 1.50 (s, 6 H), 1.83 (s, 1 H), 2.27 (m, 2 H), 2.52 (t, 2 H, J = 7 Hz), 3.02 (s, 2 H), 3.12 (s, 2 H), 3.91–4.08 (m, 2 H), 4.98–5.06 (m, 2 H), 5.79 (m, 1 H), 6.63–6.8 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 138.0, 137.4, 136.7, 119.6, 119.0, 116.1, 112.3, 111.7, 57.9, 52.8, 46.1, 45.5, 33.8, 30.8, 27.7, 27.0. MS (FAB$^+$, m/z): 338 (M+), 304, 250, 209, 173, 143, 121 (100), 119, 55, 32. Anal. ($C_{18}H_{30}N_2S_2$): C, H, N, S.

N^1-[2-(*trans*-2-Butenylthio)-2-methylpropyl]-N^2-(2-mercapto-2-methylpropyl)-1,2-benzenediamine (6f). Compounds 1 (0.36 g, 1.84 mmol, 100 mol %) and **5f** (0.58 g, 3.67 mmol, 200 mol %) were reacted according to procedure C to give 0.55 g (88% yield) of a gold oil after purification by radial chromatography (4-mm silica plate, 10% Et_2O/hexane). TLC (25% Et_2O/hexane): R_f 0.65 (DTNB+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.41 (s, 6 H), 1.49 (s, 6 H), 1.56 (m, 3 H), 1.83 (s, 1 H), 3.01 (br s, 2 H), 3.03–3.08 (m, 2 H), 3.11 (br s, 2 H), 5.40–5.55 (m, 2 H), 6.60–6.77 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 140.0, 137.3, 128.2, 127.1, 119.6, 119.0, 112.3, 111.7, 57.8, 52.8, 46.6, 45.4, 30.6, 30.3, 27.7, 17.7. IR (film): 3300, 3010, 2960, 2920, 2860, 1600, 1515, 1465, 1435, 1385, 1365, 1140, 960, 740 cm^{-1}. MS (FAB$^+$, m/z): 338 (M+), 304, 250, 209, 121 (100), 55. Anal. ($C_{18}H_{30}N_2S_2$): C, H, N, S.

N^1-[2-[(Cyclopropylmethyl)thio]-2-methylpropyl]-N^2-(2-mercapto-2-methylpropyl)-1,2-benzenediamine (6g). Compounds 1 (0.43 g, 2.19 mmol, 100 mol %) and **5g** (0.82 g, 5.19 mmol, 237 mol %) were reacted according to procedure C to give 0.63 g (85% yield) of product after filtration through 300 g of silica gel (10 × 10 cm funnel, 20% Et_2O/hexane). TLC (25% Et_2O/hexane): R_f 0.60 (DTNB+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.10–0.21 (m, 2 H), 0.46–0.57 (m, 2 H), 0.78–0.92 (m, 1 H), 1.41 (s, 6 H), 1.50 (s, 6 H), 1.83 (s, 1 H), 2.37 (d, 2 H, J = 7 Hz), 3.00 (s, 2 H), 3.11 (s, 2 H), 3.8–4.2 (m, 2 H), 6.60–6.80 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 137.9, 137.3, 119.5, 118.9, 112.2, 111.5, 57.8, 52.8, 45.8, 45.3, 33.3, 30.7, 27.7, 10.8, 5.7. IR (film): 3300, 3090, 2970, 2920, 2860, 1705, 1600, 1515, 1440, 1385, 1365, 1260, 1145,

830, 730 cm^{-1}. MS (FAB$^+$, m/z): 338 (M+), 304, 209, 129, 121, 119, 55. Anal. ($C_{18}H_{30}N_2S_2$): C, H, N, S.

N^1-(2-Mercapto-2-methylpropyl)-N^2-[2-(4-pentenylthio)-2-methylpropyl]-1,2-benzenediamine (6h). Compounds 1 (0.155 g, 0.79 mmol, 100 mol %) and **5h** (0.30 g, 1.74 mmol, 270 mol %) were reacted according to procedure C to give 0.27 g (97% yield) of a gold oil after purification by radial chromatography (2-mm silica gel plate, 10% Et_2O/hexane). TLC (10% Et_2O/hexane): R_f 0.57 (DTNB+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.41 (s, 6 H), 1.50 (s, 6 H), 1.63 (m, 2 H), 1.82 (s, 1 H), 2.12 (m, 2 H), 2.45 (t, 2 H, J = 7 Hz), 3.01 (s, 2 H), 3.11 (s, 2 H), 3.94–4.04 (m, 2 H), 4.9–5.05 (m, 2 H), 5.73 (m, 1 H), 6.61–6.78 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 137.9, 137.6, 137.3, 119.6, 118.9, 115.3, 112.3, 111.6, 57.8, 52.7, 46.0, 45.4, 33.0, 30.7, 28.8, 27.7, 26.8. IR (film): 3300, 3040, 2960, 2920, 2850, 2550, 1640, 1590, 1510, 1430, 1380, 1360, 1300, 1140, 910, 730 cm^{-1}. MS (FAB$^+$, m/z): 352 (M+), 264, 209, 173, 121, 55. Anal. ($C_{18}H_{32}N_2S_2$): C, H, N, S.

N^1-[2-(Ethylthio)-2-methylpropyl]-N^2-(2-mercapto-2-methylpropyl)-1,2-benzenediamine (6i). Compounds 1 (0.43 g, 2.3 mmol, 100 mol %) and **5i** (0.59 g, 4.5 mmol, 200 mol %) were reacted according to procedure C to give 0.31 g (43% yield) of a gold oil after purification by radial chromatography (4-mm silica gel plate, 5% Et_2O/petroleum ether). TLC (20% EtOAc/hexane): R_f 0.72 (DTNB+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (t, 3 H, J = 7.5 Hz), 1.46 (s, 6 H), 1.54 (s, 6 H), 1.87 (s, 1 H), 2.52 (q, 2 H, J = 7.2 Hz), 3.06 (s, 2 H), 3.16 (s, 2 H), 3.90 (br s, 1 H), 4.20 (br s, 1 H), 6.68–6.83 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 138.0, 137.2, 119.6, 118.9, 112.2, 111.5, 57.8, 52.6, 46.0, 45.9, 30.6, 27.7, 21.5, 14.6. IR (film): 3300, 2980, 2940, 2880, 1600, 1510, 1450, 1270, 740 cm^{-1}. MS (FAB$^+$, m/z): 312 (M+), 278, 224, 209, 175, 121 (100), 117, 29. Anal. ($C_{16}H_{28}N_2S_2$): C, H, N, S.

1,2,3,6,7,8-Hexahydro-3,3,6,6-tetramethyl-4,5,1,8-benzodithiadiazacyclodecine (8). 1,2-Phenylenediamine (1.0 g, 10 mmol, 100 mol %) and 2.1 g (10 mmol, 100 mol %) of 2,2'-dithiobis-(2-methylpropanal) were dissolved in 30 mL of methanol, and then $NaBH_3CN$ (1.0 g, 30 mmol, 300 mol %) and 1.15 mL (20 mmol, 200 mol %) of acetic acid were added. The reaction mixture was stirred at ambient temperature for 15 h and then poured into a beaker containing 200 mL of 0.5 M HCl and stirred for 15 min. The resulting viscous brown oil was removed with a spatula. NaOH pellets were added to pH 13 and the solution was extracted with 2 × 100 mL of Et_2O. The combined ether layers were washed with 100 mL of NaCl and dried (Na_2SO_4). The solvent was removed under reduced pressure. Filtration through 250–300 g of silica gel (10 × 10 cm, 25% Et_2O/hexane) gave 0.69 g (25% yield) of a yellow solid. Mp: 107–108 °C. TLC (50% $CHCl_3$/Et_2O): R_f 0.75. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 6 H), 1.45 (s, 6 H), 2.82 (m, 2 H), 3.61 (m, 2 H), 4.69 (m, 2 H), 6.84 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 140.1, 121.7, 119.3, 57.3, 50.9, 28.6, 25.2. IR (film): 3330, 2960, 2910, 2850, 1595, 1500, 1380, 1360 cm^{-1}. MS (FAB$^+$, m/z): 282 (M+), 239, 218, 175, 133, 119. Anal. ($C_{14}H_{22}S_2N_2$): C, H, N, S.

N,N'-Bis(2-mercapto-2-methylpropyl)-1,2-benzenediamine (9). Compound 8 (51 mg, 1.8 mmol, 100 mol %) was reduced with 0.10 g (4.5 mmol, 250 mol %) of sodium metal in 30 mL of ammonia according to the procedure used to synthesize 3, to give 0.28 g (37% yield) of oil after filtration through silica gel (14 × 7 cm column, 50% $CHCl_3$/hexane). TLC (30% EtOAc/hexane): R_f 0.70 (DTNB+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.49 (s, 6 H), 1.80 (s, 2 H), 3.11 (s, 4 H), 4.00 (s, 2 H), 6.71 (m, 2 H), 6.80 (m, 2 H). ^{13}C NMR ($CDCl_3$): δ 137.6, 119.6, 112.6, 57.9, 45.4, 30.7. IR (film): 3300, 3030, 2940, 2820, 2550, 1600, 1510, 1400, 1275, 750 cm^{-1}. MS (FAB$^+$, m/z): 284 (M+), 195, 175, 133. Anal. ($C_{14}H_{24}S_2N_2$): C, H, N, S.

N^1-(2-Mercapto-2-methylpropyl)-N^2-[2-[(1-methylpropyl)thio]-2-methylpropyl]-1,2-benzenediamine (6j). Compound 9 (290 mg, 1.01 mmol, 110 mol %) and 162 mg (0.90 mmol, 100 mol %) of 2-iodobutane were treated with 40 mg (1.5 mmol, 166 mol %) of NaH in 25 mL of THF according to procedure A, to give 71 mg (21% yield) of oil after purification by radial chromatography (2-mm silica gel plate, 3% Et_2O/hexane). TLC (10% Et_2O/petroleum ether): R_f 0.48 (DTNB+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (t, 3 H, J = 7.4 Hz), 1.25 (d, 3 H, J = 6.9 Hz), 1.43 (s, 6 H), 1.50 (s, 6 H), 1.53 (m, 2 H), 1.83 (s, 1 H), 2.64 (m, 1 H), 3.02 (s, 2 H), 3.11 (s, 2 H), 3.95 (br s, 1 H), 4.15 (br s, 1 H), 6.64–6.70 (m, 2 H), 6.75–6.80 (m, 2 H). ^{13}C NMR ($CDCl_3$): δ 137.8, 137.2, 119.5, 118.8, 112.1, 111.5, 57.7, 53.3, 47.0, 45.4, 38.9, 31.7, 30.7, 28.3, 23.7, 11.4. MS (FAB$^+$, m/z): 340 (M+), 306, 252, 209, 175, 145, 121 (100), 119, 89. Anal. ($C_{18}H_{32}N_2S_2$): C, H, N, S.

N^1-[2-(Butylthio)-2-methylpropyl]-N^2-(2-mercapto-2-methylpropyl)-1,2-benzenediamine (6k). Compound 9 (170 mg, 0.06 mmol, 120 mol %) and 70 mg (0.05 mmol, 100 mol %) of 1-bromobutane were treated with 30 mg (0.07 mmol, 140 mol %) of NaH in 50 mL of THF according to procedure A to give 46 mg (23% yield) of a gold oil after purification by radial chromatography (1-mm silica gel plate, 5% EtOAc/hexane). TLC (20% EtOAc/hexane): R_f 0.60 (DTNB+). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3 H, J = 7 Hz), 1.41 (s, 6 H), 1.49 (s, 6 H), 1.43–1.47 (m, 4 H), 1.83 (s, 1 H), 2.40–2.47 (t, 2 H, J = 7 Hz), 3.00 (s, 2 H), 3.11 (s, 2 H), 4.0 (br s, 2 H), 6.66–6.79 (m, 4 H). ^{13}C NMR ($CDCl_3$): δ 137.9, 137.2, 119.5, 118.8, 112.2, 111.5, 57.7, 52.6, 45.8, 45.3, 31.6, 30.6, 29.6, 27.6, 27.2, 22.2, 13.7. MS (FAB$^+$, m/z): 340 (M+), 306, 252, 209, 175, 145, 121 (100). HPLC (90% EtOH/H_2O, 1.5 mL/min): t_R 5.60 min (92%).

N^1-(2-Mercapto-2-methylpropyl)-N^2-[2-(vinylthio)-2-methylpropyl]-1,2-benzenediamine (6l). Compound 8 (0.34 g, 1.2 mmol, 100 mol %) was placed in a flame-dried round-bottom flask. Vinylmagnesium bromide (1.0 M in THF, 13.6 mL, 13.6 mmol, 1133 mol %) was added and the solution was heated at reflux for 1.5 h. The reaction mixture was diluted with 150 mL of Et_2O, washed with 40 mL of saturated NaCl, and dried (Na_2SO_4). The solvent was removed under reduced pressure and the product was purified by radial chromatography (2-mm silica gel plate, 10% EtOAc/hexane) to give 0.15 g (40% yield) of oil. TLC (30% Et_2O/hexane): R_f 0.61 (DTNB+). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.46 (s, 6 H), 1.50 (s, 6 H), 1.80 (s, 1 H), 3.10 (s, 4 H), 3.90 (br s, 2 H), 5.28 (d, 1 H, J = 5 Hz), 5.50 (d, 1 H, J = 15 Hz), 6.2–6.9 (m, 5 H). IR (film): 3300, 3040, 2960, 2920, 2860, 2830, 1600, 1510, 1385, 1365 cm^{-1}. MS (FAB$^+$, m/z): 310 (M+), 115, 101, 89, 78. HPLC (90% EtOH/H_2O, 1.5 mL/min): t_R 4.2 min (90%).

General Procedure D. ^{99m}Tc Labeling of N_2S_2 Ligands. A sample of ligands **6a–l** (0.8–14 μmol) was dissolved in 1 mL of N_2-purged ethanol. Sodium [^{99m}Tc]pertechnetate (1.0 mL, 0.2–20 mCi) was added, and N_2(g) was bubbled through the solution for 3 min. Stannous chloride (0.2–1.0 μmol) was added and the mixture was shaken for 10 s. All complexes were characterized by HPLC, TLC, and electrophoresis.

General Procedure E. Purification of ^{99m}Tc Complexes by Filtration. The ^{99m}Tc complex solutions of **7a–l** were purified by filtration through a 0.22-μm cellulose acetate membrane filter (Millex GS, Millipore Division, Waters Corp., Milford, MA).

General Procedure F. HPLC Purification of ^{99m}Tc Complexes. A 150-μL sample of the ^{99m}Tc complex solution was injected into the U6K injector with a 200-μL injection loop, loaded onto the column, and eluted with 80% EtOH/H_2O. Fractions were collected manually in lead-shielded scintillation vials and assayed in an ionization chamber. The appropriate fractions were then combined and diluted to 50% EtOH/H_2O for biodistribution studies.

Biological Evaluation of ^{99m}Tc Complexes. Female Sprague–Dawley rats weighing 140–220 g (average, 170 g) were anesthetized with intraperitoneal sodium pentobarbital and were injected in a tail vein with 0.5–10 mCi of the ^{99m}Tc-labeled complex in a volume of 0.2–0.5 mL. Two rats were injected for each time point. The animals were sacrificed at 5 and 30 min after injection and 2–3 mL of blood was removed from the inferior vena cava. Selected organs were removed; the tails were discarded to avoid interference from the injection site. The radioactivity in each organ was measured at a standard geometry with a NaI(Tl) scintillation detector adjusted for the 140 keV emission of ^{99m}Tc. The organs were weighed to 0.01 g and the activity was calculated as percent administered dose per gram.

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