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

Bill J. McBride,^{*,†} Ronald M. Baldwin,[‡] Janice M. Kerr, Jiann-Long Wu, Lisa M. Schultze, Nilda E. Salazar, James M. Chinitz, and Edmund F. Byrne

Research and Development Laboratory, Medi-Physics, Inc., 5855 Christie Avenue, Emeryville, California 94608

Received June 30, 1992

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}TcO₄ and SnCl₂ 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-[¹²³I]iodoamphetamine ([¹²³I]IMP, 1980)¹ and N,N,N'-trimethyl-N'-(2-hydroxy-3-methyl-5-[123I]iodobenzyl)-1,3-propanediamine ([123I]HIPDM, 1983).2 Although these agents have proven useful in functional brain imaging,³ the limited availability of cyclotronproduced ¹²³I has restricted their use. The favorable properties of ^{99m}Tc, availability from a ⁹⁹Mo-^{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 oxines⁵ and derivatives of structures based on the N₂S₂ 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-(1H-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 N₂S₂ 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

[†] Present address: Diatech, Inc., 9 Delta Dr., Londonderry, NH 03053. [‡] Present address: Yale University, VA Medical Center, Psychiatry/ 116A2, 950 Campbell Ave., West Haven, CT 06516.

⁽¹⁾ Winchell, H. S.; Baldwin, R. M.; Lin, T. H. Development of I-123 Labeled Amines for Brain Studies: Localization of I-123 Iodophenylalkylamines in Rat Brain. J. Nucl. Med. 1980, 21, 940-946.

^{(2) (}a) Tramposch, K. M.; Kung, H. F.; Blau, M. Radioiodine Labeled N,N-dimethyl-N'-(2-hydroxy-3-alkyl-5-iodobenzyl)-1,3-propanediamines in Brain Perfusion Imaging. J. Med. Chem. 1983, 26, 121-125. (b) Kung, H. F.; Tramposch, K. M.; Blau, M. A New Brain Perfusion Imaging Agent: [¹²³]]HIPDM: N,N,N'-Trimethyl-N'-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine. J. Nucl. Med. 1983, 24, 66-72. (3) Holman, B. L.; Hill, T. C.; Polak, J. F.; Lee, R. G.; Royal, H. D.

⁽³⁾ Holman, B. L.; Hill, T. C.; Polak, J. F.; Lee, R. G.; Royal, H. D. Functional Brain Imaging with I-123 Labeled Amines. In *Radionuclide Imaging of the Brain*; Holman, B. L., Ed.; Livingstone: New York, 1985; pp 163-184.

⁽⁴⁾ Oldendorf, W. H. Need for New Radiopharmaceuticals. J. Nucl. Med. 1978, 19, 1181-1182.

⁽⁵⁾ Loberg, M. D.; Corder, E. H.; Fields, A. T.; Callery, P. S. Membrane Transport of Tc-99m Labeled Radiopharmaceuticals I. Brain Uptake by Passive Transport. J. Nucl. Med. 1979, 20, 1181–1188.

^{(6) (}a) Efange, S. M. N.; Kung, H. F.; Billings, J. J.; Blau, M. Synthesis and Biodistribution of Tc-99m Labeled Piperidinyl bis(aminoethanethiol) Complexes: Potential Brain Perfusion Imaging Agents for Single Photon Emission Computed Tomography. J. Med. Chem. 1988, 31, 1043–1047.
(b) Kung, H. F.; Molnar, M.; Billings, J. J.; Wicks, R.; Blau, M. Synthesis and Biodistribution of Neutral Lipid Soluble Tc-99m Complexes that Cross the Blood Brain Barrier. J. Nucl. Med. 1984, 25, 326–332. (c) Efange, S. M. N.; Kung, H. F.; Billings, J. J.; Guo, Y.-Z.; Blau, M. Technetium-99m bis(aminoethanethiol) Complexes with Amine Side Chains-Potential Brain Perfusion Imaging Agents for SPECT. J. Nucl. Med. 1987, 28, 1012–1019. (d) Kung, H. F.; Guo, Y.-Z.; Yu, C. C.; Mach, R. H.; Efange, S. M. N.; Blau, M. Mc. Tc-99m Complexes Based on N₂S₂ Ligands. J. Nucl. Med. 1986, 27, 1051.

⁽⁷⁾ Lever, S. Z.; Burns, H. D.; Kervitsky, T. M.; Goldfarb, H. N.; Woo, D. V.; Wong, D. F.; Epps, L. A.; Kramer, A. V.; Wagner, H. N. Design, Preparation, and Biodistribution of a Technetium-99m Triaminedithiol Complex to Assess Regional Cerebral Blood Flow. J. Nucl. Med. 1985, 26, 1287-1294.

⁽⁸⁾ Volkert, W. A.; Hoffmann, T. J.; Seger, R. M.; Troutner, D. E.; Holmes, R. A. Tc-99m Propyleneamine Oxime (Tc-99m PnAO): A Potential Brain Radiopharmaceutical. *Eur. J. Nucl. Med.* 1984, 9, 511– 516.

⁽⁹⁾ Neirinckx, R. D.; Canning, L. R.; Piper, I. M.; Nowotnik, D. P.; Pickett, R. D.; Holmes, R. A.; Volkert, W. A.; Forster, A. M.; Weisner, P. S.; Marriot, J. A.; Chaplin, S. B. Technetium-99m d.]-HM-PAO: A New Radiopharmaceutical for SPECT Imaging of Regional Cerebral Blood Perfusion. J. Nucl. Med. 1987, 28, 191-202.
(10) Mathias, C. J.; Welch, M. J.; Lich, L.; Raichle, M. E.; Volkert, W.

⁽¹⁰⁾ Mathias, C. J.; Welch, M. J.; Lich, L.; Raichle, M. E.; Volkert, W. A.; Hung, J. C. Single Pass Extraction of d,l and meso Tc-99m HM-PAO in Primate Brain. J. Nucl. Med. 1988, 29, 747–748.

^{(11) (}a) Morgan, G. F.; Deblaton, M.; Clemens, P.; Van Den Broeck, P.; Bossuyt, A.; Thornback, J. R. Technetium-99m-MRP20, a Potential Brain Perfusion Agent: In Vivo Biodistribution and SPECT Studies in Non-Primate Animals. J. Nucl. Med. 1991, 32, 500-505. (b) Morgan, G. F.; Deblaton, M.; Van Den Broeck, P.; Bastin, B.; Pirotte, R.; Michel, P.; Clemens, P.; Abram, U.; Thornback, J. R. Synthesis and Biological Studies of Neutral Technetium(V) Complexes Containing NNOS Donor Sets. Nucl. Med. Biol. 1991, 19, 65-72.

Scheme I^{*}



^a Reagents and conditions: (a) HOCH₂CH₂OH, *p*-TsOH, C₆H₆, reflux; (b) Na/NH₃, -78 °C; (c) sealed vessel, EtOH, 100 °C; (procedure A) NaH or K₂CO₃, alkyl, alkenyl, or alkynyl halide, THF or MeOH/ H₂O; (procedure B) H₃O⁺, THF, reflux; (procedure C) NaBH₃CN, HOAc, MeOH; (procedure D) Na^{99m}TcO₄, SnCl₂, EtOH, H₂O.

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 Ssubstituted 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(2methylpropanal)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 Scheme II⁴



 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.

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 Snyder¹⁶ to give 1. Reductive amination of the aldehydes with amine 1 gave the corresponding N_2S_2 ligands 6a-i.

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 61. 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 Bryson¹⁴ 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

⁽¹²⁾ Vallabhajosula, S.; Zimmerman, R. E.; Picard, M.; Stritzke, P.; Mena, I.; Hellman, R. S.; Tikofsky, R. S.; Stabin, M. G.; Morgan, R. A.; Goldsmith, S. J. Technetium-99m ECD: A New Brain Imaging Agent: in vivo Kinetics and Biodistribution Studies in Normal Human Subjects. J. Nucl. Med. 1989, 30, 599-604.

<sup>J. Nucl. Med. 1989, 30, 599-604.
(13) (a) Mathias, C. J.; Welch, M. J.; Green, M. A.; McGuire, A. H.;</sup> Perry, D. J.; Raichle, M. E.; Lich, L. L.; Zinn, K. R. PET Cerebral Blood Flow Imaging with Copper Labeled PTSM. J. Nucl. Med. 1989, 30, 791.
(b) Walovitch, R. C.; Platts, S. H.; Walsh, R. A.; Orlandi, C. Regional Cerebral Distribution of Microspheres and Tc-99m ECD in Nonhuman Primates. J. Nucl. Med. 1989, 30, 792.
(14) Bryson, N.; Dewan, J. C.; Lister-James, J.; Jones, A. G.; Davison,

⁽¹⁴⁾ Bryson, N.; Dewan, J. C.; Lister-James, J.; Jones, A. G.; Davison, A. Neutral Technetium(V) Complexes of Amide Thiol Thioether Chelating Ligands. *Inorg. Chem.* 1988, 27, 2154–2161.

<sup>Ligands. Inorg. Chem. 1988, 27, 2154-2161.
(15) (a) Corbin, J. L.; Work, D. E. 1-Alkyl- (or aryl-) amino-2-methylpropane-2-thiols. Some Bi- and Tetradentate Nitrogen-Sulfur Ligands from Schiff's Base Disulfides. J. Org. Chem. 1976, 41, 489-491.
(b) Kung, H. F.; Molnar, K.; Billings, J.; Wicks, R.; Blau, M. Synthesis and Biodistribution of Neutral Lipid-Soluble Tc-99m Complexes that Cross the Blood-Brain Barrier. J. Nucl. Med. 1984, 25, 326-332.</sup>

⁽¹⁶⁾ Snyder, H. R.; Stewart, J. M.; Ziegler, J. B. The Synthesis of Amino Mercaptans from Olefin Sulfides. J. Am. Chem. Soc. 1947, 69, 2672– 2674.

⁽¹⁷⁾ Schultze, L. M.; Todaro, L. J.; Byrne, E. F.; Baldwin, R. M.; McBride, B. J. Unpublished results.

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

	%	HPLC ^b	brain (% dose/g)°		brain/blood ^d		retention
R group	purity ^a	$t_{\rm R}({\rm min})$	5 min	30 min	5 min	30 min	ratioe
ethyl (7i)	98 (A)	3.8	0.88	0.71	6.3	6.2	0.80
propyl (7c)	96 (B)	3.9	(0.86-0.91) 0.75 (0.68-0.81)	(0.64 - 0.78) 0.69 (0.63 - 0.76)	2.1	3.1	0.92
isopropyl (7 d)	91 (B)	4.2	0.73 (0.66-0.80)	0.67	2.1	2.4	0.92
butyl (7 k)	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	0.57	2.8	3.7	0.89
cyclopropylmethyl (7g)	98 (C)	4.9	0.54	0.47	2.6	4.2	0.87
vinyl (71)	90 (B)	3.5	$(0.46 \ 0.00)$ $(0.56 \ (0.51-0.61)$	0.57	0.9	1.9	1.00
allyl (7a)	96 (B)	3.6	0.99	0.75 (0.75-0.76)	3.6	5.6	0.76
3-butenyl (7e)	95 (C)	3.9	(0.80 - 0.80) (0.77 - 0.84)	0.69	2.2	3.8	0.87
(E)-2-butenyl (7f)	94 (C)	4.1	0.50	(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% Et₂O/CHCl₃; B, 10–30% EtOAc/hexane; C, 30% EtOAc/hexane; D, EtOAc/MeOH/H₂O/concentrated NH₄OH (86/10/3/1). ^b HPLC was performed on a C-18 Radial-Pak cartridge with 90% EtOH/H₂O 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		organ/	organ uptake (% dose)ª		
	5 min	30 min	tissue	5 min	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	39.13	35.54	
heart	3.04	1.51	of body			
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/gat 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 ¹H 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 Me₄Si as an internal standard. High-field ¹H and ¹³C 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

⁽¹⁸⁾ Taylor, T. F.; Frey, K. A.; Baldwin, R. M.; Papadopoulis, S. M.; Petry, N. A.; Rogers, W. L.; McBride, B. J.; Kerr, J. M.; Kuhl, D. E. [^{99m}Tc]N¹-(2-Mercapto-2-methylpropyl)-N²-(2-propargylthio-2-methylpropyl)-1,2-benzenediamine (T691): Preclinical Studies of a Potential New Tracer of Regional Cerebral Perfusion. J. Nucl. Med. 1992, 33, 1836-1842.

⁽¹⁹⁾ McBride, W. J.; Baldwin, R. M.; Kerr, J. M.; Schultze, L. M.; Salazar, N.; Chinitz, J. M. Hydrocarbylphenyl Diaminedithiol Derivatives. Eur. Patent No. 0 432 988 A1, 1-30, 1991.

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 $\pm 0.4\%$ 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 F₂₅₄ 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 99m Tc generator (Cintichem, Tuxedo, NY). Electrophoresis was performed on Whatman 3MM filter paper impregnated and developed with 0.05 M NaH₂PO₄ 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). ¹H NMR (CDCl₃): 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 Et₂O. 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 Et₂O. The pH of the acid layer was then adjusted to pH 4 with 9 g of NaOH pellets and extracted with $3 \times 200 \,\mathrm{mL}$ of Et₂O. The combined ether layers were washed with 100 mL of saturated NaCl and dried (Na₂SO₄). 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+). ¹H NMR (400 MHz, CDCl₃): § 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). $^{13}\mathrm{C}$ NMR (CDCl₃): 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). ¹H NMR (60 MHz, CDCl₃): δ 1.42 (s, 12 H), 9.12 (s, 2 H). Anal. (C₈H₁₄O₂S₂): 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 Et₂O and washed with 30 mL of 0.5 M NaOH. The ether layer was then washed with 50 mL of saturated NaCl and dried (Na₂-SO₄), and the solvent was removed under reduced pressure to give 5.5 g (94% yield) as a gold oil. TLC (50% Et₂O/hexane): R_{f} 0.47 (visualized by phosphomolybdic acid). ¹H NMR (60 MHz, CDCl₃): 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 NH₃ (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 NH₄Cl 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 H₃-PO₄ (20 mL) was added until the solution was pH 2. The aqueous solution was extracted with 3×100 mL of Et₂O. The combined ether layers were washed with 50 mL saturated NaCl and dried (Na₂SO₄). The solvent was removed under reduced pressure to give 2.34 g (97% yield) of oil. TLC (50% Et₂O/hexane): R_{f} 0.66 (DTNB+). ¹H NMR (60 MHz, CDCl₃): 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 $3 \times 50 \text{ mL}$ of $\text{Et}_2 O$. The combined ether layers were washed with 2×40 mL of saturated NaCl and dried (Na₂SO₄). The solvent was removed under reduced pressure to give 12.0 g (95%) yield) as a gold oil. ¹H NMR (400 MHz, CDCl₃): 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, 4 H)1 H), 5.18 (m, 1 H), 5.87 (m, 1 H). MS (FAB⁺, m/z): 188 (M⁺), 187, 147, 116, 115. Anal. (C₉H₁₆O₂S): 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 H₂O, then K₂CO₃ (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 6h. 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 (Na₂SO₄). Removal of the solvent by rotary evaporation gave 1.26 g (68% yield) as an oil. ¹H NMR (400 MHz, CDCl₃): 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. (C₉H₁₄O₂S): 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. ¹H NMR (60 MHz, CDCl₃): 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. ¹H NMR (60 MHz, CDCl₃): 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. ¹H NMR (400 MHz, CDCl₃): 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. (C₁₀H₁₈O₂S): 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. ¹H NMR (60 MHz, CDCl₃): 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. ¹H NMR (60 MHz, CDCl₃): 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. ¹H NMR (60 MHz, CDCl₃): 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. ¹H NMR (60 MHz, CDCl₃): 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 Et₂O. The combined ether layers were washed with 50 mL of water and 50 mL of saturated NaCl and dried (Na₂SO₄). The solvent was removed under reduced pressure to give 7.0 g (77% yield) of a gold oil. ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): δ 194.1, 133.5, 118.0, 52.1, 32.0, 21.2. Anal. (C₇H₁₂OS): C, H, S.

2-(Propargylthio)-2-methylpropanal (5b). Yield: 93%. ¹H NMR (200 MHz, CDCl₃): 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. (C₇H₁₀OS): C, H, S.

2-(Propylthio)-2-methylpropanal (5c). Yield: 89%. ¹H NMR (60 MHz, CDCl₃): 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%. ¹H NMR (60 MHz, CDCl₃): 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%. ¹H NMR (60 MHz, CDCl₃): 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. (C₈H₁₄OS): C, H, S.

2-(trans-2-Butenylthio)-2-methylpropanal (5f). Yield: 97%. ¹H NMR (60 MHz, CDCl₃): 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%. ¹H NMR (60 MHz, CDCl₃): 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%. ¹H NMR (60 MHz, CDCl₃): 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%. ¹H NMR (60 MHz, CDCl₃): 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]-N²-(2-mercapto-2-methylpropyl)-1,2-benzenediamine (6a). Compounds 1 (3.5g, 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 NaBH₃CN. 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 $2 \times 150 \text{ mL}$ of Et₂O. 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 $(10 \times 10 \text{ cm}, 10\% \text{ Et}_2\text{O}/\text{hexane})$ to give 4 g (69% yield) as a gold oil. TLC (5% EtOAc/hexane): R₁ 0.60 (DTNB+). ¹H NMR $(400 \text{ MHz}, \text{CDCl}_3): \delta 1.43 (s, 6 \text{ H}), 1.50 (s, 6 \text{ H}), 1.83 (s, 1 \text{ H}), 3.05$ (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). ¹³C NMR (CDCl₃): 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.

N¹-(2-Mercapto-2-methylpropyl)-N²-[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% Et₂O/hexane): R_f 0.65 (DTNB+). ¹H NMR (200 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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. (C₁₇H₂₆N₂S₂): C, H, N, S.

*N*¹-(2-Mercapto-2-methylpropyl)-*N*²-[2-(propylthio)-2methylpropyl]-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 (2mm silica gel plate, 20% EtOAc/hexane). TLC (5% Et₂O/ petroleum ether): R_f 0.26 (DTNB+). ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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, 288, 209, 131, 121 (100), 119, 89, 55. Anal. (C₁₇H₃₀N₂S₂): 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% Et₂O/hexane). TLC $(5\% \text{ Et}_2\text{O}/\text{hexane}): R_f 0.34 \text{ (DTNB+}).$ ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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/H₂O, 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% Et₂O/hexane). TLC (5% EtOAc/hexane): R_{f} 0.40 (DTNB+). ¹H NMR (200 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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. (C₁₈H₃₀N₂S₂): C, H, N, S.

 N^{-} [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% Et₂O/hexane). TLC (25% Et₂O/hexane): R_{f} 0.65 (DTNB+). ¹H NMR (200 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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. (C₁₈H₃₀N₂S₂): C, H, N, S.

N¹-[2-[(Cyclopropylmethyl)thio]-2-methylpropyl]-N²-(2mercapto-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% Et₂O/hexane). TLC (25% Et₂O/hexane): R_f 0.60 (DTNB+). ¹H NMR (200 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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. (C₁₈H₃₀N₂S₂): C, H, N, S.

N¹-(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% Et₂O/hexane). TLC (10% Et₂O/hexane): R_f 0.57 (DTNB+). ¹H NMR (200 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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. (C₁₉H₃₂N₂S₂): C, H, N, S.

*N*¹-[2-(Ethylthio)-2-methylpropyl]-*N*²-(2-mercapto-2-methylpropyl)-1,2-ben zenediamine (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% Et₂O/petroleum ether). TLC (20% EtOAc/hexane): R_f 0.72 (DTNB+). ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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. (C₁₆H₂₈N₂S₂): 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 NaBH₃CN (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 Et₂O. The combined ether layers were washed with 100 mL of NaCl and dried (Na₂SO₄). The solvent was removed under reduced pressure. Filtration through 250–300 g of silica gel (10 \times 10 cm, 25% Et₂O/hexane) gave 0.69 g (25% yield) of a yellow solid. Mp: 107-108 °C. TLC (50% CHCl₃/Et₂O): R_f 0.75. ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): δ140.1, 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% CHCl₃/hexane). TLC (30% EtOAc/hexane): R_{f} 0.70 (DTNB+). ¹H NMR (600 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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/2): 284 (M+), 195, 175, 133. Anal. (C₁₄H₂₄S₂N₂): C, H, N, S.

N⁻(2-Mercapto-2-methylpropyl)-N²-[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 silicagel plate, 3% Et₂O/hexane). TLC (10% Et₂O/petroleum ether): R_f 0.48 (DTNB+). ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃):

 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. (C₁₈H₃₂N₂S₂): C, H, N, S.

*N*¹-[2-(Butylthio)-2-methylpropyl]-*N*²-(2-mercapto-2methylpropyl)-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_1 0.60 (DTNB+). ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃): 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/ H₂O, 1.5 mL/min): t_R 5.60 min (92%).

N¹-(2-Mercapto-2-methylpropyl)-N²-[2-(vinylthio)-2methylpropyl]-1,2-benzenediamine (61). Compound 8 (0.34 g, 1.2 mmol, 100 mol %) was placed in a flame-dried roundbottom 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 Et₂O, washed with 40 mL of saturated NaCl, and dried (Na₂SO₄). 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% Et₂O/hexane): R_f 0.61 (DTNB+). ¹H NMR (60 MHz, CDCl₃): 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/H₂O, 1.5 mL/min): t_R 4.2 min (90%).

General Procedure D. ^{99m}Tc Labeling of N₂S₂ Ligands. A sample of ligands 6a-l (0.8-14 μ mol) was dissolved in 1 mL of N₂-purged ethanol. Sodium [^{99m}Tc]pertechnetate (1.0 mL, 0.2-20 mCi) was added, and N₂(g) was bubbled through the solution for 3 min. Stannous chloride (0.2-1.0 μ mol) was added and the mixture was shaken for 10 s. All complexes were characterized by HPLC, TLC, and electrophoresis.

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

General Procedure F. HPLC Purification of ^{99m}Tc Complexes. A 150- μ L sample of the ^{99m}Tc complex solution was injected into the U6K injector with a 200- μ L injection loop, loaded onto the column, and eluted with 80% EtOH/H₂O. 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/H₂O for biodistribution studies.

Biological Evaluation of ⁹⁹mTc 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.

Acknowledgment. We wish to thank Dr. Wolfgang Benz and Dr. Thomas Williams of Roche Research Center, Nutley, NJ, for mass spectra and high-field NMR. We also thank Douglas White and James Rego for their technical assistance.