

A New, General Synthetic Route to Multidentate N₂S₂ Ligands for Use in Technetium-99m Radiopharmaceuticals. Preparation of Diamido Disulfur, Diamino Dithiol, and Tripodal N₃S₃ Prototypes. Comparative Biodistributions of [^{99m}Tc^vO-DADS]⁻ Analogues Which Contain 5,5,5- and 5,7,5-Membered Chelate Ring Systems

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A new, versatile synthetic route to a variety of tetradentate N₂S₂ and hexadentate N₃S₃ ligands for use in technetium-99m radiopharmaceuticals has been developed. The key reaction employs 1-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) for coupling an appropriate di- or triamine with S-protected thioglycolic acid. Selected DADS (diamido disulfur) and DADT (diamino dithiol) and analogues derived from ethanediamine-1,2 and butanediamine-1,4, as well as a tripodal N₃S₃ analogue, were synthesized in high yield. The labeling of DADS analogue ligands derived from butanediamine-1,4 (DADS-bn) with ^{99m}Tc results in a single radiochemical product, or the expected number of stereoisomeric products. FAB MS analysis, using ⁹⁹Tc, indicates that DADS-bn ligands form a tetradentate 5,7,5-membered ring chelate system with the monooxo Tc(V) core: [TcO-DAD-bn]⁻. In rat biodistribution studies the DADS-en and DADS-bn complexes show very similar biological activity. Phenyl substituents on the 7-membered ring give rise to a ^{99m}Tc complex of increased lipophilicity, increased liver uptake, and minimal hepatobiliary clearance. Thus, new classes of DADS ligand analogues which contain a 5,7,5-membered chelate ring system are readily synthesized and labeled with ^{99m}Tc to form stable complexes. The presence of the 7-membered chelate ring allows for facile introduction of pendant groups into the ligand system, and thus for a ready route to control the biodistribution of the resulting ^{99m}Tc radiopharmaceutical. The ^{99m}Tc labeling of the DADT analogues derived from butanediamine-1,4 provided to be erratic, despite the use of a variety of labeling techniques; the larger ring system of DADT-bn apparently does not favor the monooxo Tc(V) core and appears to generate a mixture of mono- and dioxo Tc(V) cores.

Two classes of tetradentate N₂S₂ ligands of the general structures shown in Figure 1 are of interest in the design and development of ^{99m}Tc radiopharmaceuticals. In currently used terminology, the first class consists of diamido disulfur (DADS) ligands and the second consists of diamino dithiol (DADT) ligands. Both types of ligands form stable complexes of ^{99m}Tc in high purity and yield, and this makes them very attractive for bifunctional chelate applications.^{1,2} Moreover, the tetradentate N₂S₂ system is amenable to variations both in the size of the chelate rings and in the nature of the substituents attached to the chelate rings; these variations make it possible to delineate important structure-activity relationships.

The overall net charge of the ^{99m}Tc complexes formed from these ligands, and thus the possible applications of these complexes, is determined by the oxidation state and composition of the ^{99m}Tc core and by the ionization of hydrogen atoms at the four ligating atoms. The ^{99m}Tc-DADS complexes are anionic because all four hydrogen atoms are ionized and the Tc(V) core is [Tc^vO]³⁺.³ DADT ligands form neutral complexes with the same [Tc^vO]³⁺ core by deprotonation of two thiols and one amine. Since the resulting ^{99m}Tc-DADT complexes are lipophilic and uncharged at physiological pH, they have the necessary chemical requirements for brain perfusion imaging.⁴⁻⁷ Consequently, neutral ^{99m}Tc-DADT complexes have been proposed for both scintigraphic evaluation of brain perfusion and the detection of neurological disorders based on receptor- or enzyme-related defects.⁸

For the reasons outlined above, there is currently a considerable level of interest in the design of new ligands based on the DADS and DADT series. A variety of substituted ^{99m}Tc-DADS and ^{99m}Tc-DADT complexes⁷⁻¹¹ based on ethanediamine-1,2 have now been synthesized and their biodistributions evaluated. These studies clearly show that the biological behavior of the complexes is de-

pendent on the nature and position of substituents on the N₂S₂ ligand. In this work we extend these observations

- (1) Fritzberg, A. R.; Kuni, C. C.; Klingensmith, W. C.; Stevens, J.; Whitney, W. P. Synthesis and Biological Evaluation of Tc-99m N,N'-bis(mercaptoacetyl)-2,3-diaminopropanoate: A Potential Replacement for ¹³¹I o-Hippurate. *J. Nucl. Med.* 1982, 23, 592.
- (2) Burns, H. D.; Dannals, R. F.; Dannals, T. E.; et al. *J. Labeled Comp. Radiopharm.* 1981, 18, 54.
- (3) Kasina, S.; Fritzberg, A. R.; Johnson, D. L.; Eshima, D. Tissue Distribution Properties of Technetium-99m-Diamide-Dimercaptide Complexes and Potential Use as Renal Radiopharmaceuticals. *J. Med. Chem.* 1986, 29, 1933.
- (4) Kung, H. F.; Molnar, M.; 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.
- (5) Kung, H. F.; Yu, C. C.; Billings, J.; Molnar, M.; Blau, M. Synthesis of New Bis(aminoethanethiol) (BAT) Derivatives: Possible Ligands for ^{99m}Tc Brain Imaging Aspects. *J. Med. Chem.* 1985, 28, 1280.
- (6) Lever, S. Z.; Burns, H. D.; Kervitsky, T. M.; Goldfarb, H. W.; Woo, D. W.; Wong, D. F.; Epps, L. A.; Kramer, A. Y.; Wagner, H. N. Design, Preparation and Biodistribution of a Technetium-99m Triaminodithiol Complex to Assess Regional Cerebral Blood Flow. *J. Nucl. Med.* 1985, 26, 1287.
- (7) Efang, S. M. N.; Kung, H. F.; Billings, J. J.; Blau, M. Synthesis and Biodistribution of ^{99m}Tc-Labeled Piperidinyl Bis(aminoethanethiol) Complexes. *J. Med. Chem.* 1988, 31, 1043.
- (8) Eisenhut, M.; Brandan, W.; Missfeldt, M. Synthesis and In Vivo Testing of a Bromobutyl Substituted 1,2-Dithia-5,9-diazacycloundecane: a Versatile Precursor for New ^{99m}Tc-Bis(aminoethanethiol) Complexes. *Nucl. Med. Biol.* 1989, 16, 805.
- (9) Mach, R. H.; Kung, H. F.; Guo, Y.-Z.; Yu, C.-C.; Subramanyam, V.; Calabrese, J. C. Synthesis, Characterization and Biodistribution of Neutral and Lipid-soluble ^{99m}Tc-PAT-HM and ^{99m}Tc-TMR for Brain Imaging. *Nucl. Med. Biol.* 1989, 16, 829.
- (10) Epps, L. A.; Burns, H. D.; Lever, S. Z.; Goldfarb, H. W.; Wagner, H. N., Jr. Brain Imaging Agents: Synthesis and Characterization of (N-Piperidinylethyl)Hexamethyl Diaminodithiolate Oxo Technetium(V) Complexes. *Appl. Radiat. Isot.* 1987, 38, 661.

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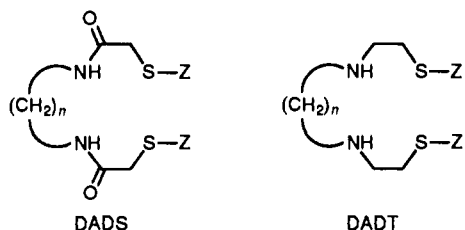


Figure 1. General structure of DADS and DADT ligands ($n = 2, 3$; Z is a protecting group, often benzyl or benzoyl).

by synthesizing selected DADS and DADT ligands with butanediamine-1,4 backbones (i.e., DADS-bn and DADT-bn). Our primary goal was to determine if N_2S_2 chelates containing a 7-membered ring form anionic ^{99m}Tc -DADS-bn and neutral ^{99m}Tc -DADT-bn complexes, analogous to those based on ethanediamine-1,2. Secondly, we wished to determine if the enhanced lipophilicity and increased size of the Tc coordination sphere expected to be generated by the 4-membered carbon backbone would influence the biological behavior of the corresponding ^{99m}Tc complexes.

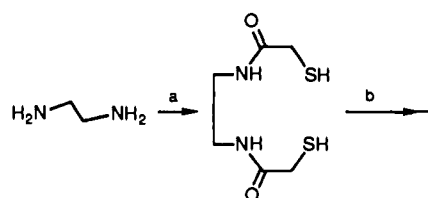
To achieve this goal we developed a new, versatile synthetic route which allows the preparation of a variety of DADS and DADT ligands. The proposed reaction route derives from peptide chemistry and uses *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) as a coupling agent.¹² Since the introduction of EEDQ in 1968, several papers have described its use in the successful syntheses of peptides¹³⁻¹⁵ as well as bile acid conjugates.^{16,17} To demonstrate the generality of the route reported herein, the EEDQ coupling procedure was also extended to the synthesis of a tripodal hexadentate N_3S_3 ligand.

Results and Discussion

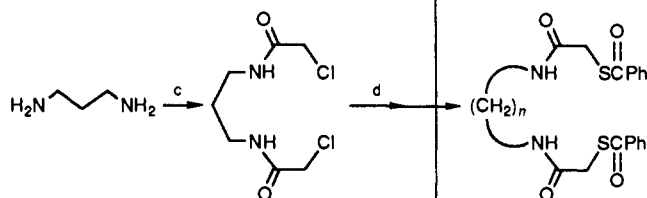
Synthesis of Ligands: Background. There are only a few published synthetic routes to DADS ligands.^{3,18,19} Two of these routes start with the corresponding substituted ethylene- or propylenediamine, and by series of reactions convert the diamines to the diamido dimercaptide N_2S_2 system. Specifically, diamines are brought into reaction with methyl thioglycolate to give the bis(mercaptides), and then the sulfhydryls are protected via thio ester

Scheme I^a

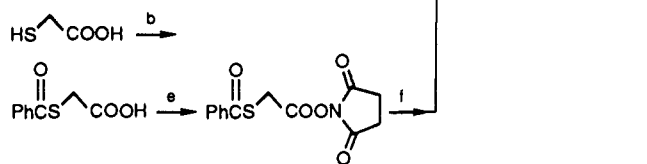
Route 1



Route 2



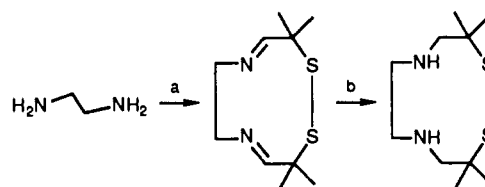
Route 3



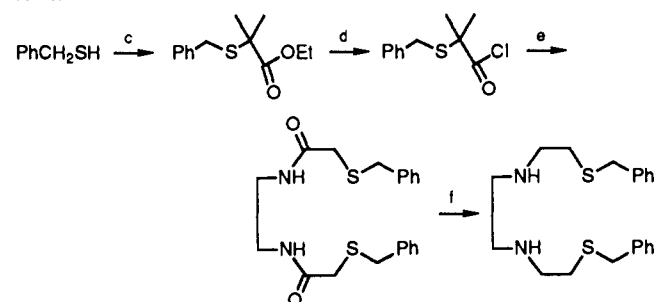
^a (a) $\text{SHCH}_2\text{COOMe}$; (b) PhCOCl ; (c) ClCOCH_2Cl ; (d) KSCOPh ; (e) NHS , DCC ; (f) $\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}_2$.

Scheme II^a

Route 4



Route 5



^a $(\text{CH}_3)_2\text{C}(\text{CHO})\text{S}-\text{SC}(\text{CHO})(\text{CH}_3)_2$; (b) NaBH_4 or LiAlH_4 ; (c) $\text{BrC}(\text{CH}_3)_2\text{COOEt}$; (d) NaOEt , SOCl_2 ; (e) $\text{NH}_2(\text{CH}_2)_2\text{NH}_2$; (f) $\text{BH}_3\cdot\text{THF}$.

derivatization (Scheme I, route 1),¹⁸ or diamines are condensed with chloroacetyl chloride to intermediate bis(chloroacetamides), and then the DADS product is formed by nucleophilic substitution with thiobenzoate (Scheme I, route 2).³ The reported yields are relatively high. However, these routes have two disadvantages. First, in some cases the starting, substituted diamines must be synthesized by multistep procedures, often with low yields. To expose these relatively scarce diamines to all the reaction steps mentioned above causes additional losses. Second, the unprotected thiol functionality is easily oxidized, and this allows byproduct formation.

Alternatively, the DADS system can be synthesized by condensation of the *N*-hydroxysuccinimide-activated ester

- Bryson, N.; Dewan, J. C.; ListJames, J.; Jones, A. G.; Davison, A. Neutral Technetium(V) Complexes with Amide-Thiol-Thioester Chelating Ligands. *Inorg. Chem.* 1988, 27, 2154.
- Bellau, B.; Malek, G. A. New Convenient Reagent for Peptide Syntheses. *J. Am. Chem. Soc.* 1968, 90, 1651.
- Bodanszky, M. *Peptide Chemistry*; Springer-Verlag: Berlin, 1988; Chapter V.
- Izumiya, N.; Muraoka, M. A. Racemization Test in Peptide Synthesis. *J. Am. Chem. Soc.* 1969, 91, 2391.
- Dermott, J. R. Mc.; Benoiton, N. L. *N*-Methylamino Acids in Peptide Synthesis. *Can. J. Chem.* 1973, 51, 2562.
- Tserng, K. Y.; Hachey, D. I.; Klein, P. D. An Improved Procedure for the Synthesis of Glycine and Taurine Conjugates of Bile Acids. *Lipid Res.* 1977, 18, 404.
- Huijghebaert, S. M.; Hofmann, A. F. Pancreatic Carboxypeptidase Hydrolysis of Bile Acid-Amino Acid Conjugates: Selective Resistance of Glycine and Taurine Amides. *Gastroenterology* 1986, 90, 306.
- Fritzberg, A. R.; Klingensmith, W. C.; Whitney, W. P.; Kuni, C. C. Chemical and Biological Studies of Tc-99m *N,N'*-Bis(mercaptoacetamido)ethylenediamine: A Potential Replacement for I-131 Iodohippurate. *J. Nucl. Med.* 1981, 22, 258.
- Schneider, R. F.; Subramanian, G.; Feld, T. A.; McAfee, J. G.; Zapf-Longo, C.; Palladino, E.; Thomas, F. D. *N,N'*-bis(S-benzoylmercaptoacetamido)Ethylenediamine and Propylenediamine Ligands as Renal Function Imaging Agents. *J. Nucl. Med.* 1984, 25, 223.

Table I. Data for the Preparation of 3-7 from the Reaction of 1 with Various Amines

compd	mole ratio 1:EEDQ:amine	solvent	recrystallization solvent	mp, °C (lit. mp)
3	2.05:2.05:1	THF	MEK	195-196 (192-194) ^a
4	2.05:2.05:1	THF	MEK	199-200
5	2.1:2.1:1	THF	- ^b	142-143
6	2.05:2.05:1	AcOEt/ CH ₂ Cl ₂	CH ₂ Cl ₂ /AcOEt	190-191
7	4:4:1	THF	CH ₂ Cl ₂ /ligroine	159.5-160

^aReference 3. ^bNot recrystallized.**Table II.** Comparison of Yields for Syntheses Performed by Different Reaction Routes

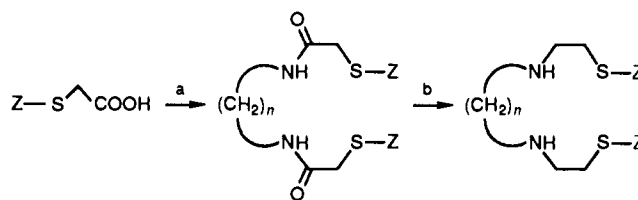
compd	yield (%) ^a		
	EEDQ route	route 1	route 3
3	74	65 (94 crude)	70 (72.1)
4	70	15	61.1
5	83	- ^b	- ^b
6	65.2	- ^b	- ^b
7	75	- ^b	- ^b

^aLiterature data are given in parentheses. ^bSynthesis not performed.

of *S*-benzoylthioglycolate with suitable diamines (Scheme I, route 3).¹⁹ This route uses the diamine only in the last step, but still there are a total of three reaction steps necessary. The dicyclohexylcarbodiimide (DCC), used in this route, is not an ideal reagent because it causes allergic reactions and the dicyclohexylurea formed as a byproduct is often difficult to completely remove by filtration despite its poor solubility in most solvents.

The classical method of synthesis of the DADT framework involves condensation of a primary diamine with dithia dialdehyde to give the corresponding cyclobis(imine), which is further reduced by a mild reducing agent such as sodium borohydride to yield 1,2-dithia-5,8-diazacyclodecane⁴ or 1,2-dithia-5,9-diazacycloundecane (Scheme II, route 4).^{8,9} Moderate yields in the range of 25-69% are reported for the condensation step. The disulfide bond provides protection for the thiol functionality; this protection can be removed by different methods depending on the steric hindrance associated with the carbon atom adjacent to the sulfur atom. It has been shown by Joshua²⁰ that sodium borohydride reduction of the bis(imine) results mostly in intramolecular reductive cyclization to provide bicyclic imidazolines. Under the reaction conditions studied by Joshua, the isolated yields of bicyclic products range from 57% to 70% while those of the desired monocyclic products range from trace amounts to 38%. Alternatively, both DADT and multidentate aminethiol ligands have been obtained from appropriate di- or triamine and benzyl mercaptans (Scheme II, route 5).^{21,22}

- (20) Joshua, A. V.; Scott, J. R.; Sondhi, S. M.; Ball, R. G.; Lown, J. W. Transannular Cyclization of 1,2-Dithia-5,8-diazacyclodeca-4,8-dienes during Borohydride Reduction. *J. Org. Chem.* 1987, 52, 2447.
- (21) John, C. S.; Ohmomo, Y.; Kung, H. F.; Paik, C. H.; Reba, R. C. A New Versatile Method for the Synthesis of Aminethiol Ligands. *J. Labelled Compd. Radiopharm.* 1991, 30, 26.
- (22) John, C. S.; Schlemper, E. O.; Hossain, P.; Cioloca, L.; Paik, C. H.; Reba, R. C. Preparation and Characterization of Multidentate Aminethiol Ligands Labeled with Technetium-99m. Reference 21, p 18.
- (23) Haverback, B. J.; Dyce, B.; Bundy, H.; Edmoudson, H. A.; Trypsin, Trypsinogen and Trypsin Inhibitor in Human Pancreatic Juice. *Am. J. Med.* 1960, 29, 424.

Scheme III^a^a(a) H₂N(CH₂)_nNH₂, EEDQ; (b) BH₃-SMe₂.

Synthesis of Ligands: Existing Routes. Standard literature routes (1 and 3) were used to prepare analogues 3 and 4. Using ethanediamine-1,2 no special problems occurred although the product obtained by route 1 was less pure than that obtained from the EEDQ procedure and additional effort was required to purify it. The spectral data of all three purified samples of 3 (obtained by routes 1, 3, and the EEDQ procedure)^{18,19} agreed well with those reported in the literature.^{18,19}

However, attempts to obtain the diamine derivative of butanediamine-1,4 according to route 1 failed. With some special efforts a relatively pure product could be obtained, but only in very low yield (Table II). This is not unexpected since erratic behavior during the synthesis of bis-amides by the reaction of a diamine with aliphatic esters has been reported earlier for cases wherein the number of carbon atoms between two amine groups exceed two.²⁴ In addition, high melting, polymeric species were always observed in high amounts. The desired product could be obtained via the *N*-hydroxysuccinimide active ester route (route 3) but it is more readily obtained by the route presented now (Scheme III).

Several attempts to condense butanediamine-1,4 with dithia dialdehyde in order to obtain DADT-bn were unsuccessful. TLC analysis showed that the reaction mixture was always multicomponent and no individual compound could be isolated either by distillation or by flash chromatography. For some isolated fractions, the molecular ion peak of cyclobis(imine) is observed by FAB-MS analysis, and the ¹H NMR spectra of these fractions show the presence of CH=H (br s, 7.4), CH₂N (m, 3.4), CH₂C (m, 1.3), and CH₃ (s, 1.4) protons. Nevertheless, TLC analysis of these isolated fractions shows the presence of several components. It is possible that some of them are macrocyclic compounds with structures similar to the one described earlier⁹ or are linear polymers.

Synthesis of Ligands: New Procedure. In our hands the classical synthesis of DADT-bn from butanediamine-1,4 failed. Now, we wish to report a simple and versatile synthetic procedure for both classes of DADS and DADT ligands (Scheme III). In practice this route yields diamido dimercaptide ligands in essentially one step since the thiol group of the starting thioglycolic acid can be benzoyl- or benzyl-protected and the thus protected thioglycolic acid can be stored for a prolonged time; thus the synthetic procedure involves a single reaction between a protected thioglycolic acid and the appropriate diamine. The corresponding amido groups can be reduced with borane to yield the diamino dithiol ligands. In this work the general utility of the method proposed for coupling of a protected thioglycolic acid with different di- and triamines has been established.

The reactivity of *S*-benzoylthioglycolic acid toward EEDQ was determined by monitoring the reaction of equimolar amounts of these reagents in the absence of an amino component. The quinoline is recovered from the

(24) Deutsch, E.; et al., unpublished results.

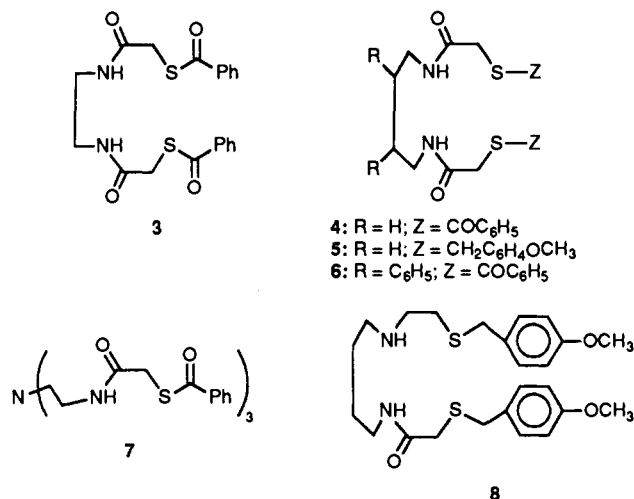


Figure 2. Compounds synthesized by the EEDQ route.

postraction mixture in almost theoretical yield. The part of the reaction mixture which is soluble in organic solvents consists of two components which are identified by spectral methods as *S*-benzoylthioglycolic acid ethyl ester and benzoic acid anhydride in a molar ratio 2:1. These observations show that the reaction proceeds typically²³ except that deprotection of the thiol functionality takes place to some extent. For reactions with polyamines this could lead to bis(benzamides) as byproducts. Fortunately, the presence of amino components in the reaction mixture efficiently prevents deprotection, and no such byproducts are detected for reaction with di- or triamines. For safety reasons, especially with amines with electron-withdrawing substituents on the carbon backbone, it is better to use *S*-benzoylthioglycolic acid and EEDQ in slight excess. The compounds synthesized by the general reaction procedure described in the Experimental Section are shown in Figure 2.

The reaction between *S*-protected thioglycolic acid and butanediamine-1,4 yields the corresponding DADS ligand with high yield and purity. Introduction of phenyl substituents onto the carbon backbone of the butanediamine-1,4 or the use of systems with more than two amino groups does not adversely effect the reaction course and the yields are still high (Table II).

The additional advantage of this method is that the final product crystallizes directly from the reaction mixture in high purity; recrystallization does not change the melting point. It is important to note that the pure final products show very low solubility in most organic solvents, even under reflux.

The two amido groups undergo reduction with borane under mild conditions to directly provide the desired DADT derivative. Benzyl protection of the thiol functionality is a good choice for those amido compounds intended for reduction to amine derivatives. It can be expected that other protecting groups could be used as well. Thus, the route proposed seems to be a general and easy way to obtain a variety of ligands in both the DADS and DADT series. Conversion of the tripod amine, tris(2-aminoethyl)amine, to the hexadentate N₃S₃ tripod shows that this route can be applied to the synthesis of a variety of useful N,S chelates simply by choosing the appropriate amino precursor.

Radiolabeling. The labeling of the various ligands with ^{99m}Tc proceeds by standard techniques described in the Experimental Section. The progress and success of these labeling reactions is monitored by HPLC with radiometric detection. The radiochromatogram of ^{99m}Tc-labeled 6

shows two main peaks at 4.7 and 5.2 min (mobile phase B, see the Experimental Section), resulting from the two possible diastereoisomers. These diastereoisomers are produced in a 1:3 ratio with an overall yield of 80–85%; the recovery of injected radioactivity is close to 100%. The two diastereoisomers can be further separated by decreasing the strength of the mobile phase. When labeling ligands 3 and 4 with ^{99m}Tc, only a single peak is observed (6.2 and 9.3 min, respectively; mobile phase A, see the Experimental Section). Both complexes are formed in 95% yield. When mobile phase A is applied to the analysis of ^{99m}Tc-6 only a broad peak with a severe tailing (retention time 20 min) is observed. Thus, a stronger organic phase and a faster flow rate (1.4 mL/min) has to be used for ^{99m}Tc-6, indicating that this complex is more lipophilic than ^{99m}Tc-3 or ^{99m}Tc-4.

FAB MS analyses (negative mode) of "carrier added" preparations of the ^{99m}Tc-3 and ^{99m}Tc-DADS-bn complexes: ^{99m}Tc-4 and ^{99m}Tc-6 show strong parent peaks at 319, 347, and 499 amu, respectively. In addition, single-crystal X-ray structural analysis of ^{99m}Tc-4 conclusively establishes the presence of the [Tc=O]³⁺ core and the 5,7,5-membered chelate ring structure.²⁴ This indicates that the starting ligands 3, 4, and 6 lose two benzoyl protecting groups upon coordination to a [Tc^vO]³⁺ center to form anionic complexes containing a 5- or 7-membered ring, respectively. These three complexes are stable toward dilution with saline or water, as evidenced by the observation that HPLC peak shapes, retention times, and recovered yields are unchanged upon dilution. The ^{99m}Tc-6 complex is stable in air for at least 5–6 h, but 24 h after preparation it suffers about 25% decomposition. The complexes formed with ligands 3 and 4 are much more stable; no decomposition is observed several days after preparation with "carrier added" technetium.

Upon labeling with ^{99m}Tc, the hexadentate triamido trithiol (TATT) (7) ligand yields mainly two different complexes with retention times 2.8 and 3.6 min (mobile phase A, see the Experimental Section). The more lipophilic complex is formed as a major product in varying yields by use of dithionite as a pertechnetate-^{99m} reducing agent, and it seems to be unstable in air. The less lipophilic complex is the major product resulting from the ligand-exchange synthetic procedure, but the results are not very reproducible.

While the literature reports reproducibly high yields in labeling DADT derivatives which contain ethanediamine-1,2 and propanediamine-1,3 backbones, our attempts to obtain stable complexes of the DADT-bn ligand were generally not successful. Different pertechnetate-^{99m} reducing agents were used, but under all conditions studied 50% or more of the ^{99m}TcO₄⁻ introduced appears to be reduced to TcO₂. The recovery of the radioactivity injected onto C-18 HPLC columns varies from 5% for Na₂S₂O₄ and NaBH₄ to about 30% for SnCl₂. Moreover, the reproducibility is poor and the ratio of the two main HPLC peaks changes with the time and temperature of the labeling reaction. Best results were obtained using the ligand-exchange labeling procedure. The recovery of the injected radioactivity is still poor (40%), but the results are relatively reproducible. Three main species are observed, characterized by retention times 2.4, 2.8, and 3.3 min (mobile phase C, see the Experimental Section) in a ratio of 27:39:31.

Biodistribution Studies. From the biodistribution data in rats (Table III), it is seen that the Tc-6 complex is strongly absorbed by the liver and does not undergo significant hepatobiliary clearance. One minute after in-

Table III. Rat Biodistribution Data: Average Percent Injected Dose/Organ ($n = 3$)^a

time, min	blood	brain	heart	kidneys	liver
[^{99m} TcO(4)] ⁻					
1	31.08 (22)	0.06 (0)	0.38 (5)	16.50 (69)	17.44 (18)
5	9.52 (9)	0.03 (0)	0.18 (5)	5.40 (16)	6.96 (10)
15	4.62 (4)	0.01 (0)	0.08 (2)	5.04 (32)	3.84 (4)
30	0.98 (1)	0.00 (0)	0.02 (0)	1.92 (21)	0.88 (1)
60	0.56 (1)	0.00 (0)	0.01 (0)	1.14 (7)	0.56 (0)
[^{99m} TcO(6)] ⁻					
1	24.64 (26)	0.05 (0)	0.17 (5)	2.20 (27)	66.32 (41)
5	5.18 (8)	0.01 (0)	0.10 (5)	1.60 (13)	61.60 (28)
15	3.64 (5)	0.01 (0)	0.10 (0)	1.20 (10)	71.36 (70)
30	2.52 (2)	0.01 (0)	0.04 (0)	1.00 (6)	61.12 (48)
60	2.10 (2)	0.00 (0)	0.03 (0)	0.92 (5)	47.68 (20)

^a Standard deviation of last significant digit(s) given in parentheses.

jection, the liver uptake is 8.3% /g and an hour later is still as high as 6.0% /g. There is a small amount of kidney uptake but no heart or brain uptake.

The ^{99m}Tc-4 complex has much lower liver uptake and higher kidney uptake. The in vivo difference between the ^{99m}Tc-4 and ^{99m}Tc-6 analogues may be due to the difference in their lipophilicity; ^{99m}Tc-6 is much more lipophilic than ^{99m}Tc-4, so it has a higher affinity for liver tissue, while the less lipophilic ^{99m}Tc-4 complex displays higher affinity for the kidney and a faster renal excretion.

The biodistribution in rats of ^{99m}Tc-3 has been reported by Jones et al.²⁵ A comparison with the rat biodistribution data for ^{99m}Tc-4 (Table III) shows that despite the incorporation of a 7-membered ring into ^{99m}Tc-4, it exhibits a biodistribution that is generally similar to that of ^{99m}Tc-3. Both agents are cleared rapidly from the blood and exhibit relatively rapid renal uptake and excretion.

Conclusion

Davison and co-workers have previously shown that 5,5,5- and 5,6,5-membered diamido disulfur N₂S₂ ligands from anionic complexes with the TcO³⁺ core.²⁶ The results presented herein indicate that the same type of TcO(N₂S₂)⁻ complex is formed with the 5,7,5-membered DADS-bn chelate structure. Moreover, expanding the 5,5,5-membered chelate ring structure of DADS-en to the 5,7,5-membered chelate ring structure of DADS-bn engenders only minimal effects on the efficiency of ^{99m}Tc labeling and on the biodistribution of the resulting ^{99m}Tc complex. This observation creates considerable synthetic flexibility in the design of new ^{99m}Tc agents since the four-carbon backbone of the 7-membered ring allows for the introduction of a variety of pendant functionalities.

Even though the 5,5,5- and 5,7,5-amido derivatives DADS-en and DADS-bn are both efficiently labeled with ^{99m}Tc, the 5,5,5- and 5,7,5-amino derivatives DADT-en and DADT-bn are labeled with quite different efficiencies. Expanding the 5,5,5-membered ring system of DADT-en to the 5,7,5-membered ring system of a DADT-bn markedly degrades the labeling efficiency and leads to several products. This is possibly due to the delicate balance which exists between the stabilities of the TcO³⁺ and

TcO₂⁺ cores^{27,28} and the acidities of the N-bonded protons of the noncoordinated ligand. In both amido ligands the N-H bonds are acidic; the ligands readily lose four protons and thus stabilize the monooxo core. However, in the amino ligands the N-H bonds are less acidic and thus the system is more delicately balanced with respect to stabilization of the monooxo and dioxo cores.²⁸ The smaller ring system of DADT-en apparently favors loss of three protons and coordination to the monooxo core to form a neutral complex; the larger ring system of DADT-bn apparently does not greatly favor the monooxo core and appears to generate a mixture of mono- and dioxo cores.

Experimental Section

General. All melting points are uncorrected. Elemental analyses were performed commercially (Galbraith Laboratories, Knoxville, TN). Infrared spectra were determined on a Perkin-Elmer 580B spectrophotometer over the range 4000–200 cm⁻¹ in Nujol mulls. ¹H NMR spectra were obtained using either a Bruker WP80-MHz or 300 MHz Nicolet spectrometer with (CH₃)₄Si as an internal standard. The mass spectral analyses were conducted on a VG 30-250 quadrupole mass spectrometer (VG Mass lab) fitted with a FAB source. Xenon was used as the primary gas, and the ion gun was operated at 8 keV and 100 mA. Data were collected in the positive ion mode for ligands and in the negative ion mode for "carrier added" ^{99m}Tc complexes. The purity of all products was checked by TLC on silica gel (DC-Plastikfolien Kieselgel 50 F₂₅₄ from Merck); benzene-ethanol (9:1) was used as a developing solvent and bromocresol green, ninhydrin, or nitroprusside were used to visualize the spots. HPLC was carried out on Perkin-Elmer 400 system with use of a 5-μm reverse-phase C-18 column. Spectrophotometric detection at 254 nm was accomplished by Beckman Model-153 module. Radiometric detection of the effluent from the column was performed by Beckman Model 170 radioisotope detector and analyzed by Shimadzu C-R3A Chromatopac integrator. The mobile phases used were as follows: (A) 40% MeOH and 60% 0.05 M NH₄OAc; (B) 40% acetonitrile and 60% 0.05 M NH₄OAc; (C) 72% MeOH and 28% 0.05 M NH₄OAc.

Ethanediamine-1,2, butanediamine-1,4, and tris(2-aminoethyl)amine were dried over potassium hydroxide and distilled prior to use. 2,3-Diphenylbutanediamine-1,4 was obtained by a multistep synthesis detailed elsewhere.²⁹ Impure ^{99m}Tc was purchased in the form of NH₄TcO₄ (containing TcO₂) from Oak Ridge National Laboratory, Oak Ridge, TN. Purification was performed as follows: 100 mg of black ^{99m}Tc (containing NH₄TcO₄ and TcO₂) was mixed with 100 mL of H₂O and 100 mL of CH₃OH; the above suspension was stirred at 50 °C, and 10 drops of 30% H₂O₂ and 10 drops of concentrated NH₃·H₂O were added. The reaction mixture was stirred overnight at 50 °C to evaporate the solvent and to remove the excess of ammonia. The dried, white NH₄TcO₄ was collected the next day and used to prepare an aqueous 10 mg NH₄TcO₄/mL solution. ^{99m}TcO₄⁻ was obtained by elution of a commercially available ⁹⁹Mo/^{99m}TcO₄ generator (Mallinckrodt, Inc.) with 0.9% aqueous NaCl solution provided by the generator manufacturer. All other chemicals were of reagent grade.

Syntheses of Ligands. *S*-Benzoylthioglycolic acid (1) was obtained by a published procedure¹⁹ except that it was recrystallized twice using benzene-ligroin instead of ethyl acetate.

S-(4-Methoxybenzyl)thioglycolic acid (2) (mp 55–56 °C) was obtained according to a literature procedure which describes the reaction of thiols with benzyl chlorides.³⁰

- (25) Jones, A. G.; Davison, A.; LaTegola, M. R.; Brodack, J. W.; Orwig, C.; Sohn, M.; Toothaker, A. K.; Lock, C. J.; Franklin, K. J.; Costello, C. E.; Car, S. A.; Biemann, K.; Kaplan, M. R. Chemical and In Vivo Studies of the Anion oxo[N,N'-ethylenebis(2-mercaptoacetimido)] Technetate(V). *J. Nucl. Med.* 1982, 23, 801.
- (26) Davison, A.; Jone, A. J.; Orwig, C.; Sohn, M. A New Class of Oxotechnetium(5+) Chelate Complexes Containing a TcON₂S₂ Core. *Inorg. Chem.* 1981, 20, 1629.

- (27) Jurisson, S.; Aston, K.; Fair, C. K.; Schlemper, E. O.; Sharp, P. R.; Troutner, D. A. Effect of Ring Size on Properties of Technetium Amine Oxime Complexes. X-ray Structures of TcO₂Pent(AO)₂, Which Contains an Unusual Eight-Membered Chelate Ring, and of TcOEn(AO)₂. *Inorg. Chem.* 1987, 26, 3576.
- (28) Deutsch, E.; Libson, K.; Jurisson, S.; Lindoy, L. F. Technetium Chemistry and Technetium Radiopharmaceuticals. *Prog. Inorg. Chem.* 1983, 30, 75.
- (29) Belletire, J. L.; Fry, D. F. A Useful Approach to Primary Amines. *Synth. Commun.* 1988, 18, 29.

General Procedure (3-7). To a solution of 1 or 2 and *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) in an appropriate solvent was added a solution of the amine component. The suspension thus formed was stirred at room temperature for 15-30 min and then refluxed overnight. The precipitate which formed upon cooling the reaction mixture was removed by filtration, washed with water and organic solvent, and then recrystallized. Table I lists the other conditions used.

***N,N'*-Bis((*S*-benzylthio)acetamido)ethanediamine-1,2 (3):** IR (cm⁻¹) 3270, 1640, 1660. ¹H NMR (CDCl₃/DMSO) δ 3.1-3.45 (m, 2 H), 3.8 (s, 2 H), 7.3-8.1 (aromatic H), 8.2 (br s, 1 H); FAB-MS (M + 1)⁺ 417.

***N,N'*-Bis((*S*-benzoylthio)acetamido)butanediamine-1,4 (4):** ¹H NMR (DMSO/CDCl₃) δ 1.3-1.55 (m, 2 H), 3.0-3.2 (m, 2 H), 3.85 (s, 2 H), 7.5-8.2 (m, aromatic H and CONH); FAB-MS (M + 1)⁺ 445. Anal. (C₂₂H₂₄N₂O₄S₂) C, H, N, S.

***N,N'*-Bis((*S*-(4-methoxybenzyl)thio)acetamido)butanediamine-1,4 (5):** ¹H NMR (CDCl₃/DMSO) δ 1.4-1.7 (m, 2 H), 3.1 (s, 2 H), 3.1-3.4 (m, 2 H), 3.7 (s, 2 H), 3.8 (s, 3 H), 7.0 (center of q, aromatic H); FAB-MS (M + 1)⁺ 477. Anal. (C₂₄H₃₂N₂O₄S₂) C, H, N, S.

***N,N'*-Bis((*S*-benzoylthio)acetamido)-2,3-diphenylbutanediamine-1,4 (6):** IR (cm⁻¹) 3360, 1645, 1660; ¹H NMR (CDCl₃/DMSO) δ 3.0-3.2 (m, 3 H), 3.55 (s, 2 H), 7.5-8.2 (m, aromatic H); FAB-MS (M + 1)⁺ 597. Anal. (C₃₄H₃₂N₂O₄S₂) C, H, N, S.

Tris[2-((*S*-benzoylthio)acetamido)ethyl]amine (7): ¹H NMR (CDCl₃) δ 2.45-2.70 (m, 2 H), 3.15-3.40 (m, 2 H), 3.8 (s, 2 H), 7.0-8.1 (m, aromatic H); FAB-MS (M + 1)⁺ 681. Anal. (C₃₃H₃₆N₄O₆S₃) C, H, N.

***N,N'*-Bis((*S*-(4-methoxybenzyl)thio)ethyl)butanediamine-1,4 (8).** The experimental procedure used for this reaction was similar to one described earlier.³¹ To a stirred solution of 5 (0.953 g) in 20 mL of dry THF was added dropwise 7 mL of a 2 M solution of BH₃·SMe₂ in THF at room temperature under nitrogen. The reaction mixture was stirred at room temperature for 1 h and then refluxed for 2 h. After cooling to room temperature again, 10 mL of 1 M HCl in ethanol was added dropwise with stirring to destroy the excess of boron reagent. The reaction mixture was stirred 1 additional h, and after that all solvents were evaporated under vacuum; 15 mL of H₂O and 50 mL of ethyl acetate were added to the residue which was then shaken vigorously. The resulting white precipitate was removed by filtration and recrystallized twice from methanol: yield 66%; mp 276 °C dec; ¹H NMR (DMSO) δ 1.55-1.75 (m, 2 H), 2.5-3.0 (m, 8 H), 2.75 (s, 3 H), 6.8-7.4 (q, aromatic H); FAB-MS (M + 1)⁺ 449.

Labeling with ^{99m}Tc. Ligand-Exchange Procedure. A vial containing a lyophilized mixture of 1 mg of SnCl₂ and 50 mg of sodium gluconate was reconstituted with 1 mL of deoxygenated water, and then 0.1 mL of this solution was mixed with 0.75 mL of pertechnetate in a previously evacuated vial; 0.5 mL of a solution of 7 (4 mg/mL water) or 0.5 mL of a solution of 8 (3 mg/mL acetate buffer, pH 7.0) was added to the resulting ^{99m}Tc-gluconate complex, and the reaction mixture was heated at 80-100 °C for 10-30 min.

Labeling of DADS Ligands. Three to four milligrams of ligand (3, 4, 6, or 7) was mixed with 0.5 mL of EtOH and 0.2 mL of 1 N NaOH to form a suspension which became a transparent solution after being heated at 95 °C for 5-10 min. About 3-4 mCi of pertechnetate and saline were added to make a total volume of 2.8 mL. Finally, 0.2 mL of freshly prepared Na₂S₂O₄ solution (10 mg/mL) was added to the above reaction mixture which was heated at 95 °C for 30 min. In "carrier-added" experiments for ^{99m}Tc-3, ^{99m}Tc-4, and ^{99m}Tc-6 complexes, 20-30 μL of NH₄^{99m}TcO₄ aqueous solution (10 mg/mL) were introduced into the reaction mixture (described above) followed by addition 0.4-0.6 mL of freshly prepared Na₂S₂O₄ solution (10 mg/mL). Other conditions used were the same as for "carrier-free" experiments. Complexes prepared under "carrier added" conditions were analyzed by FAB-MS.

Labeling of DADT-bn (8). (a) Na₂S₂O₄ as Reducing Agent. The mixture of 8 (3 mg) and 0.1 mL of trifluoroacetic acid was heated at 70 °C for 10 min and then neutralized with 0.025 N phosphate buffer (pH 7) and finally filtered through 0.45-μm Aerodisc film. To the filtrate was added 0.3 mL of 2 N NaOH, about 3-4 mCi of pertechnetate-99m, and 0.3 mL of freshly prepared Na₂S₂O₄ solution (10 mg/mL). The resulting mixture was heated at 90 °C for 10-30 min.

(b) NaBH₄ as Reducing Agent. To a filtrate obtained after deprotection of the ligand by TFA (as described above) were added 15 mg of NaBH₄ and 3-4 mCi of pertechnetate-99m. The mixture was kept at room temperature for 1 h.

(c) SnCl₂ as Reducing Agent. Three milliliters of a SnCl₂ solution (10 mg SnCl₂·2H₂O/mL 0.1 N HCl) was mixed with 250 mg of sodium pyrophosphate, diluted to 25 mL with water, and purged with argon. To the filtrate obtained after TFA deprotection of the ligand (as described above) and purging with argon was added 3-4 mCi of pertechnetate-99m and 0.5 mL of Sn-pyrophosphate solution. The mixture was heated for 80 °C for 10-30 min.

Biodistribution. Fifteen female Sprague-Dawley rats of 200-g average weight were divided into five groups of three rats each. After anesthetization with Metofane (Pigman-Moore), 0.2 mL of the radiopharmaceutical containing 50 μCi of ^{99m}Tc were administered by means of jugular vein injection. The rats were sacrificed by cervical dislocation for the early time points and by CO₂ asphyxiation for the later time points. The first group of three rats was sacrificed 1 min after injection and the remaining four groups were killed 5, 15, 30, and 60 min after injection, respectively. One milliliter of blood, the brain, the heart, a sample of liver, and one kidney of each rat were collected, rinsed with saline (except for blood), weighed, and counted (window: 100-150 keV) vs appropriate standards and blanks for calculation of percent uptake per gram of tissue.

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Registry No. 1, 6398-74-9; 2, 34722-37-7; 3, 75948-92-4; 4, 103668-21-9; 5, 137823-59-7; 6, 137823-60-0; 7, 137823-61-1; 8, 137823-62-2; EEDQ, 16357-59-8; NH₂(CH₂)₂NH₂, 107-15-3; NH₂(CH₂)₄NH₂, 110-60-1; NH₂CH₂CH(Ph)CH(Ph)CH₂NH₂, 26709-33-1; NH₄^{99m}TcO₄, 34035-97-7; ^{99m}Tc, 14133-76-7; tris(2-aminoethyl)amine, 4097-89-6.

(30) Frankel, M.; Gertner, D.; Jakobson, H.; Zilkha, A. Syntheses of Poly-S-alkyl-L-cysteines. *J. Chem. Soc.* 1960, 1390.

(31) Kornet, M. J.; Thio, P. A.; Tan, S. I. The Borane Reduction of Amido Esters. *J. Org. Chem.* 1968, 33, 3637.