between EtOAc (200 mL) and 2 N NaOH (100 mL). The combined organic layers were extracted with 6 N HCl (3 × 100 mL). The combined acid extracts were washed with Et_2O (2 × 100 mL), basified with Na_2CO_3, and extracted with Et_2O (2 × 200 mL). The combined ethereal layers were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give **9** (3 g, 88%) as a yellow solid. The **9** oxalate crystallized from EtOAc–EtOH and had mp 139–140 °C. Anal. ($C_{19}H_{25}NO_5$) C, H, N.

The base had a $[\alpha]^{20}_D$ value of −100° (*c* 0.5, 95% EtOH). The hydrochloride crystallized from EtOAc–EtOH and had mp 205–207 °C.

Acknowledgment. We thank the Association of Commonwealth Universities for the award of an Academic Staff Fellowship to V. K. Kapoor. Thanks also to Dr. A. E. Jacobson, of the National Institutes of health, Bethesda, MD, for analgesic evaluations and to Dr. J. H. Woods, The University of Michigan, for the rat brain membrane binding and mouse vas deferens assays.

Registry No. **1**, 5965-16-2; **2**, 101155-88-8; **3**, 102977-05-9; **4**, 102977-06-0; **4**·oxalate, 103064-28-4; **4**·HCl, 103064-35-3; **5**, 102977-07-1; **5**·HCl, 103064-29-5; **6**, 102977-08-2; **6**·HCl, 103064-30-8; **7a**, 102977-09-3; **7a**·HCl, 103064-31-9; **7b**, 102977-14-0; **8**, 102977-10-6; **8**·HCl, 103064-32-0; **9**, 102977-11-7; **9**·oxalate, 103064-34-2; **9**·HCl, 103064-36-4; **10**, 102977-12-8; **11**, 102977-13-9; **11**·HCl, 103064-33-1; **12**, 103066-55-3; **12**·HCl, 102977-15-1; **12** (phenyl ethyl ether), 103002-53-5; (−)-dihydrocodeinone, 125-29-1; allyl bromide, 106-95-6; phenylethyl bromide, 103-63-9; 5-chloro-1-phenyl-1*H*-tetrazole, 14210-25-4.

Tissue Distribution Properties of Technetium-99m–Diamide–Dimercaptide Complexes and Potential Use as Renal Radiopharmaceuticals

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A series of new ligands and the corresponding technetium-99m chelates based on diamide dimercaptide donor groups were synthesized as derivatives of technetium-99m 1,2-bis(2-thioacetamido)ethane, a complex shown to be excreted by renal tubular secretion. Chelation with ^{99m}Tc resulted in single radiochemical products or the expected numbers of stereoisomers. They were purified by high-performance liquid chromatography (HPLC) and evaluated in mice as potential renal tubular function agents. The in vivo properties were sensitive to the presence of functional groups, the positional isomerism of the carboxylate group functionality, and the chelate ring stereochemistry of the ligand. The presence of methyl groups slowed renal transit and decreased renal specificity. Cyclohexyl rings fused to the ethylene bridge of the center chelate ring decreased renal excretion while aromatic rings essentially abolished renal excretion. Slow hepatobiliary clearance was observed as an alternate mode of excretion. Polar groups, such as hydroxyl, carboxylate, and carboxamide, increased renal excretion rates and specificity in a stereochemically dependent manner. ^{99m}Tc chelates of 1,3-bis(2-thioacetamido)-2-hydroxypropane, 3,4-bis(2-thioacetamido)butanoate and 1,8-dimercapto-2,7-dioxo-3,6-diazanonanoate were identified as promising new renal radiopharmaceuticals.

Technetium-99m diethylenetriaminepentaacetate (DTPA) and *o*-iodohippurate (OIH) are both currently clinically used for the evaluation of renal function.[1] Renal perfusion is evaluated by rapid serial imaging during the first circulation after bolus injection of about 15 mCi of ^{99m}Tc-DTPA. Normally, OIH cannot be used for the same purpose since the ^{131}I limits the amount of radioactivity that can be injected, because of increased radiation dose, to about 300 μCi. Renal clearance can be conveniently evaluated with either ^{99m}Tc DTPA or OIH. However, ^{99m}Tc DTPA is excreted solely by glomerular filtration and thus has a maximum renal extraction efficiency of about 20% in humans. This low extraction efficiency results in a low kidney-to-background ratio and poor-quality images in patients with impaired renal function. In contrast, OIH is secreted by the tubular cells in addition to some filtration and thus has a higher extraction efficiency of about 75% as measured in dogs.[2,3] The extraction efficiency of OIH increases the kidney-to-background image ratio and thus increases the sensitivity of OIH for detection and evaluation of renal disease in patients with poor kidney function. Since the excretion of OIH involves active transport by the renal tubular cells, the uptake and excretion reflect levels of cellular function.[4]

Because of the reasons outlined above, there is a need to provide radiopharmaceuticals for the evaluation of renal function that do not contain iodine-131 yet exhibit a high specificity for renal tubular excretion equal to or greater than levels obtained with iodine-131-labeled *o*-iodohippurate. In 1979 Davison and co-workers[5] introduced a new class of tetradentate chelating agents for technetium based on amide and mercaptide donor groups. In a later report, the authors presented structural data for the characterized compounds.[6] The initial report described the rapid renal excretion in animals of technetium-99m 1,2-bis(2-thioacetamido)ethane (**1**). This was independently corroborated by animal[7,8] and clinical studies.[1] With an interest in developing an improved ^{99m}Tc replacement for ^{131}I OIH, we reported[9] the synthesis of technetium-99m

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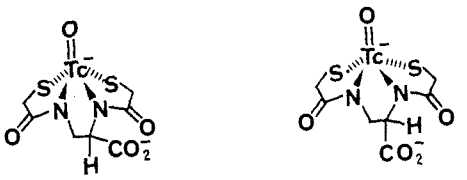
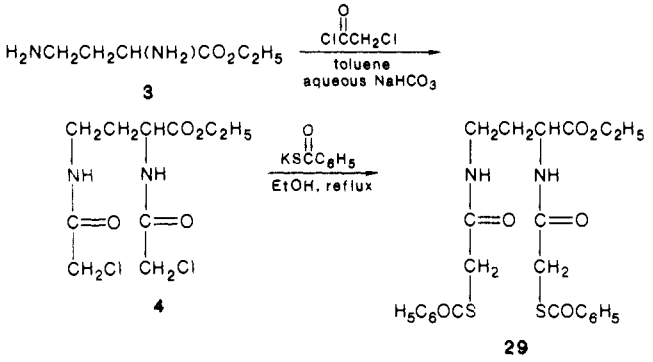


Figure 1. Structures of technetium-99m 2,3-bis(2-thioacetamido)propanoate (**2A** and **2B**) indicating chelate ring epimeric relationship of carboxylate and technetium oxo groups.

Scheme I

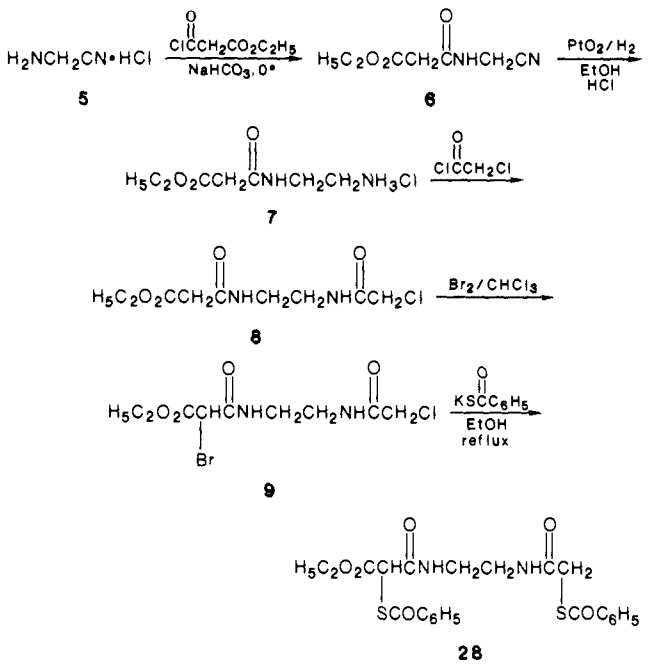


2,3-bis(2-thioacetamido)propanoate (**2A** and **2B**). The A and B components of ^{99m}Tc-**2** are designations indicating the order of elution on reversed-phase HPLC. The components have been shown to be stereoisomers resulting from the syn and anti disposition of the carboxylate group on the center ethylene bridge chelate ring with respect to the technetium oxo bond[10] (Figure 1). Mouse biodistribution studies of the two high-performance liquid chromatography (HPLC) components resulting from chelation of ^{99m}Tc and ligand **2** indicated that component A slightly exceeded OIH in renal excretion while component B was excreted more slowly than OIH. The goals of the present study were to develop a new ^{99m}Tc tubular function radiopharmaceutical and to further understand the relationship between structural features and renal handling by synthesizing additional derivatives of ligand **1**. The synthesis, chelation with ^{99m}Tc, and preliminary evaluation of these agents in mice are described in this paper. Other derivatives that have evaluated the effect of positional isomerism of amide carbonyl and carboxylate groups have been reported with respect to chemistry[11] and biological evaluation.[12]

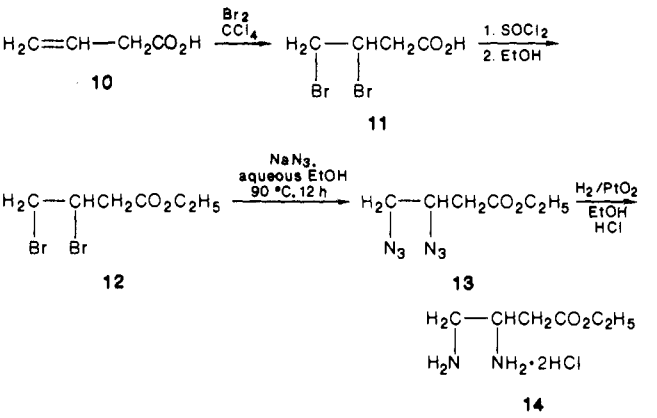
Results

Synthesis of Ligands. The general synthetic route for preparation of the N_2S_2 diamide dimercaptide ligands from

Scheme II



Scheme III



diamines using ethyl 2,4-diaminobutanoate dihydrochloride (**3**) as an example is outlined in Scheme I. Condensation of **3** with chloroacetyl chloride gave the intermediate bis(chloroacetamide) **4** in good yield. The chloroacetamide derivative gave the final sulfhydryl-protected ligands upon nucleophilic substitution with thiobenzoate. Diamino acids were esterified by conventional methods due to the ease in isolation of their condensation products as esters. The syntheses of some of these ligands by condensation of the *N*-hydroxysuccinimide activated ester of benzoylthioglycolate with suitable diamines has been reported.[13] Alternatively, diamines can be reacted with methyl thioglycolate to give the bis(mercaptoacetamides) followed by sulfhydryl protection by thio ester derivatization.[7] In this series ligand **20** was synthesized by the latter route.

In the case of cyclohexane ligand derivatives **24** and **25**, the vicinal diaminocyclohexane used for condensation was a mixture of cis and trans isomers. The mixture was acylated with chloroacetyl chloride to give the cis and trans isomers of the bis(chloroacetamides), which were separated by differential solubility in absolute ethanol. The final ligands from each of the precursor compounds were sep-

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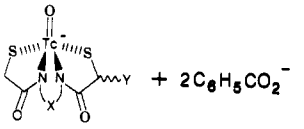
Table I. Properties of Diamide Dimercaptide Ligands and ^{99m}Tc Complexes



ligand	X	Y	R	analyses	mp, °C (lit. mp)	HPLC retention volume: ligand (mL)	HPLC retention volume: Tc chelates (mL)
1	CH_2CH_2	H		ref 5, 6	192–194 (202.5–204)[15]	4.05[a]	5.33[b]
2	$CH_2CH(CO_2R)$	H	C_2H_5	ref 9	129.5–131 (133–136)[15]	6.48	4.32, 6.05[c]
19	$CH_2CH(CH_3)$	H		C, H, N, S	196–197 (191–193)[15]	5.33	28.10, 43.02[d]
20	$CH_2CH(OH)CH_2$	H		C, H, N, S	175–176 (173–175)[15]	3.88	5.17, 9.82[b]
21	CH_2COCH_2	H		C, H, N, S	191–192 (185–187.5)[15]	4.45	
22	C_6H_4	H		C, H, N, S	169–170 (155–156.6)[15] (164–165)[5]	7.98	23.92[e]
23	$C_6H_3CO_2R$	H	C_2H_5	C, H, N, S	188–189	5.63	4.41[c]
24	*cis*-C_6H_{10}	H		C, H, N, S	178–179	10.75[h]	8.97, 16.61[e]
25	*trans*-C_6H_{10}	H		C, H, N, S	217–218	13.02[h]	16.48[e]
26	$C_{10}H_6$	H		C, H, N, S	210–211	13.22	7.0[j]
27	C_4H_8	H		C, H, N, S	192–193	5.98	
28	CH_2CH_2	CO_2R	C_2H_5	C, H, N, S	143–144	5.57	5.0, 8.20[f]
29	$CH_2CH_2CH(CO_2R)$	H	C_2H_5	C, H, N, S	141–142	5.75	3.81, 5.14[c]
30	$CH_2H(CH_2CO_2R)$	H	C_2H_5	C, H, N, S	135–136	6.65	8.30, 9.60[g]
31	$CH_2CH(CONH_2)$	H		C, H, N, S	192–193	3.48	5.83, 6.89[b]
32	$CH_2CH(CONHCH_2CO_2R)$	H	C_2H_5	C, H, N, S	173–174	4.69	7.15, 8.45
33	$CH(CH_3)CH(CO_2R)$	H	C_2H_5	C, H, N, S	204–205	10.50[h]	13.8, 15.0[g]
34	$CH(CH_3)CH(CO_2R)$	H	C_2H_5			11.20[h]	7.60, 14.70[c]
35	$CH(CO_2R)CH(CO_2R)$	H	CH_3	C, H, N, S	165–167	5.92	2.25[i]

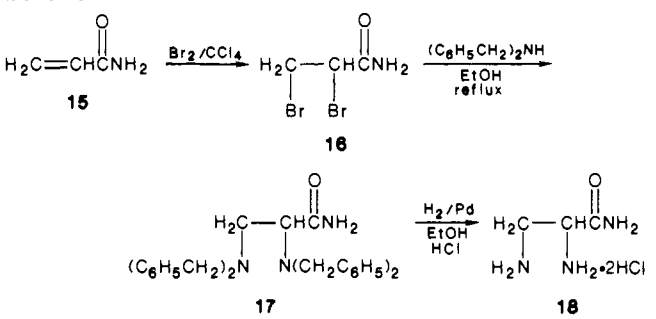
[a] Five micrometer ODS column, 60% acetonitrile/40% phosphate, 0.01 M, pH 2.4. [b] Five micrometer ODS column, 15% EtOH/85% phosphate, 0.01 M. [c] Five micrometer ODS column, 5% EtOH/95% phosphate, 0.01 M. [d] Five micrometer ODS column, 10% EtOH/90% phosphate, 0.01 M. [e] Five micrometer ODS column, 20% EtOH/80% phosphate, 0.01 M. [f] Five micrometer ODS column, 1.25% EtOH/98.75% phosphate, 0.01 M. [g] Ten micrometer anion exchange column, 0.01 M sulfate/0.01 M phosphate, pH 6. [h] 45% EtOH/55% phosphate, 0.01 M, pH 2.4. [i] Five micrometer ODS column, 0.01 M phosphate, pH 6. [j] 40% EtOH/60% phosphate 0.01 M.

arately prepared by the general procedure described in Scheme I. Ligands **24** and **25** with TLC R_f values of 0.6 and 0.7 in ethyl acetate solvent, respectively, were identified as *cis*-**24** and *trans*-**25** by comparison of their NMR spectra and melting points with those of material synthesized from *trans*-1,2-diaminocyclohexane.[14]

The synthesis of ligand **28** with the carboxylate α to the sulfur is shown in Scheme II. The route utilized aminoacetonitrile (**5**), which was condensed with ethyl malonyl chloride to give *N*-cyanomalonamide (**6**) in high yield. The nitrile was then catalytically reduced to the corresponding amino derivative **7** in good yield. The aminoethyl derivative **7** was condensed with chloroacetyl chloride to give the chloroacetamide derivative **8**. Bromine was then introduced at the active methylene carbon of the malonyl portion of the molecule to give **9** in moderate yield. Both the halides were subsequently displaced with thiobenzoate to give the sulfhydryl-protected form of ligand **28**.

The synthesis of the diamino precursor used to prepare the acetate derivative ligand **30** is shown in Scheme III. The route began with bromination of vinylacetic acid (**10**) to give **11** followed by reaction with thionyl chloride to give dibromobutanoyl chloride. Esterification of the acid chloride with absolute ethanol gave **12** followed by reaction with sodium azide to give ethyl diazidobutanoate (**13**) in moderate yield. The diazido compound was then catalytically reduced to the corresponding diamine (**14**).

Scheme IV



Conversion to ligand **30** from the diamine was carried out as in Scheme I.

Synthesis of the diamino precursor to the carboxamide ligand **31** was performed according to Scheme IV. Bromination of acrylamide (**15**) to give **16** followed by alkylation with dibenzylamine afforded the bis(dibenzylamino)propanamide **17** in high yield. The benzyl groups were removed by catalytic hydrogenation. The resulting diaminopropanamide **18** was converted to **31** as in Scheme I.

The diamino precursor to glycine conjugate derivative, ligand **32**, was synthesized from 2,3-diaminopropionic acid by carbobenzoxy protection and glycine conjugation similarly to the method described by Poduska et al.[15] The conjugation of glycine, however, was performed with dicyclohexylcarbodiimide, and removal of the carbobenzoxy

(14) *trans*-1,2-Diaminocyclohexane was obtained from Aldrich Chemical Co., Milwaukee, WI. The NMR spectrum and the melting point of the *N,N'*-bis[(benzoylthio)acetyl]-1,2-cyclohexanediamine product were identical with those of the higher R_f material, protected ligand **9**.

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Table II. Biodistribution Data of Non-Carboxylate Technetium-99m–Diamide–Dimercaptide Complexes in Mice[a]

^{99m}Tc complex	time, min	blood	liver	kidney	stomach	intestines	urine	urine % ^{99m}Tc/ % ^{131}I
1	10	4.41 ± 0.26	4.49 ± 0.22	4.73 ± 0.31	0.16 ± 0.01	5.37 ± 0.15	64.66 ± 3.26	93.82 ± 1.00
	120	0.02 ± 0.00	0.06 ± 0.00	0.06 ± 0.02	0.21 ± 0.10	3.89 ± 0.31	94.59 ± 0.46	98.19 ± 0.48
19A	10	5.78 ± 0.33	16.15 ± 0.39	29.00 ± 2.09	0.23 ± 0.02	7.30 ± 0.45	33.13 ± 2.25	49.85 ± 2.38
	120	0.04 ± 0.01	0.12 ± 0.03	0.13 ± 0.03	0.15 ± 0.07	6.58 ± 1.07	93.19 ± 0.46	103.10 ± 1.21
19B	10	8.26 ± 0.40	8.47 ± 0.60	13.57 ± 1.28	0.26 ± 0.02	5.00 ± 0.38	49.26 ± 1.78	76.47 ± 2.77
	120	0.07 ± 0.03	0.14 ± 0.04	0.20 ± 0.04	0.14 ± 0.05	3.87 ± 0.25	95.03 ± 1.25	103.00 ± 1.31
20A	10	4.04 ± 0.27	3.12 ± 0.79	5.01 ± 1.01	0.28 ± 0.02	5.40 ± 0.54	70.21 ± 3.10	103.30 ± 1.16
	120	0.39 ± 0.03	0.94 ± 0.07	1.21 ± 0.11	0.26 ± 0.07	6.07 ± 0.54	91.47 ± 1.05	99.34 ± 0.81
20B	10	3.81 ± 0.24	7.97 ± 0.93	5.72 ± 0.54	0.32 ± 0.04	8.19 ± 0.82	61.47 ± 3.00	94.47 ± 1.27
	120	0.31 ± 0.02	2.80 ± 0.07	1.03 ± 0.09	0.24 ± 0.05	12.28 ± 1.02	84.27 ± 0.98	93.04 ± 1.35
22	10	59.19 ± 0.98	9.34 ± 0.32	2.51 ± 0.08	0.74 ± 0.07	6.19 ± 0.13	0.05 ± 0.03	0.07 ± 0.04
	120	37.62 ± 0.95	6.23 ± 0.15	1.64 ± 0.06	0.73 ± 0.03	8.86 ± 0.43	0.19 ± 0.04	0.20 ± 0.05
24A	10	12.86 ± 0.86	36.96 ± 2.05	17.47 ± 1.52	0.40 ± 0.03	5.75 ± 0.60	8.68 ± 1.79	11.41 ± 2.07
	120	2.08 ± 0.38	6.08 ± 1.54	2.27 ± 0.41	0.28 ± 0.09	7.36 ± 0.81	76.64 ± 3.65	81.42 ± 3.15
24B	10	12.40 ± 0.48	55.76 ± 1.88	6.43 ± 0.27	0.43 ± 0.04	4.17 ± 0.27	3.41 ± 0.97	4.77 ± 1.28
	120	4.49 ± 0.26	23.29 ± 1.07	2.93 ± 0.29	0.86 ± 0.41	8.43 ± 0.25	50.95 ± 1.28	54.91 ± 1.68
25	10	33.88 ± 1.35	28.90 ± 1.69	4.93 ± 0.40	0.52 ± 0.02	6.03 ± 0.24	6.69 ± 0.83	8.65 ± 1.01
	120	7.68 ± 0.96	7.36 ± 0.88	1.37 ± 0.08	0.42 ± 0.05	9.18 ± 0.79	59.40 ± 3.49	62.38 ± 3.62
26	10	15.13 ± 0.89	59.13 ± 2.12	1.41 ± 0.04	0.52 ± 0.03	6.00 ± 0.08	0.42 ± 0.30	0.96 ± 0.45
	120	12.03 ± 0.40	44.47 ± 1.66	1.24 ± 0.02	1.40 ± 0.25	21.31 ± 1.40	1.39 ± 0.23	1.48 ± 0.25

[a] Values are mean ± SEM for five or six mice.

groups was carried out on the ethyl ester instead of the free acid. Conversion of ethyl 2,3-diaminopropionylglycine to ligand **32** was accomplished according to Scheme I. The synthesis of the vicinal methyl carboxylate derivatives, ligands **33** and **34**, was carried out as described for **30** except that the starting olefin used for bromination was ethyl crotonate. The two possible diastereoisomers of the final methyl carboxylate ligand were separated by differential solubility in absolute ethanol.[16] The unequivocal structural assignment of their stereoisomers was not attempted due to the low renal excretion performance of the ^{99m}Tc chelates in mice as shown in Table II.

Properties of ^{99m}Tc Complexes. Structural characterization of technetium compounds by classical means requires the use of long-lived ^{99}Tc ($t_{1/2}$ = 200 000 years). In the case of diamide dimercaptide complexes, the structure of Tc-**1** has been determined by X-ray crystallography and the molecular weight confirmed by mass spectrometry.[5] The structural assignment of Tc-**2A** and Tc-**2B** as epimeric isomers was based on mass spectral determination of a molecular weight of 363 for each HPLC component as prepared on carrier-added ^{99}Tc scale.[10] Our assignment of the expected structures of the ^{99m}Tc complexes for the remainder of the ligands in this study is based on correlation of lipophilicity (HPLC retention volumes; solvent systems) and obtaining the number of components expected from chelate ring stereochemical considerations (Table I). For ligands **22**, **23**, **25**, **26**, and **35**, added groups are planar or, in the case of trans cyclohexyl and dicarboxylate, enantiomeric products result from possible modes of chelation. For these compounds single chelate products were expected. For ligands **2**, **14**, **20**, **24**, and **28–34**, chelate ring substituents result in epimers syn and anti to the technetium oxo bond. Pairs of stereochemical products were thus expected, and these were found to be separable by HPLC. The lipophilicity of the ligands studied varied significantly as indicated by the percentage of the organic modifier ethanol necessary to achieve retention and separations. In two cases, ^{99m}Tc-**30** and -**33**, superior resolution of epimers was obtained on anion-exchange HPLC.

Technetium complexes of ligands **21** and **27** were not obtained. In the case of **21**, the keto group bond angle may not allow the second amide nitrogen to bond to the metal. Ligand **27** is a piperazine derivative with tertiary amide nitrogens. Without the presence of protons that can be lost in metal amide nitrogen bond formation, the metal amide nitrogen interactions are apparently too weak for chelation.

Biological Studies. Biodistribution values of percent injected dose per organ at 10 and 120 min postinjection are presented in Tables II and III. The urine and kidney values at 10 min indicate rate of renal clearance while the 120-min values indicate specificity.

The compounds in the tables are separated into two general groups. Table II includes alkyl, hydroxyl, and fused hydrocarbon systems. Table III includes carboxylate derivatives based on positional isomerism, chain extension, and amide derivatization. The parent compound, ^{99m}Tc-**1**, was excreted rapidly but with some hepatobiliary excretion. The hepatobiliary excretion increased in rats without kidney function[17] and in patients with elevated creatinine levels.[1] Addition of a methyl group to the center chelate ring (^{99m}Tc-**19**) and chelation with ^{99m}Tc resulted in the formation of two radiochemical components. Differences were observed, with peak A showing a high uptake (29%) in the kidneys at 10 min. Peak B exhibited 14% kidney uptake at 10 min, and both were nearly completely excreted in the urine by 2 h. Use of a propyl-bridged derivative with a hydroxyl group, ^{99m}Tc-**20**, resulted in the formation of two components, both excreted at rates comparable to that of ^{131}I OIH at 10 min. The benzo derivative, ^{99m}Tc-**22**, was a single-component chelate and showed only very slow blood disappearance and hepatobiliary excretion. Renal excretion was negligible at 2 h. The benzocarboxylate, ^{99m}Tc-**23**, was a single component and exhibited slow renal excretion as well as hepatobiliary

(16) The HPLC system used for ligand purity analysis, 60% CH_3CN/40% phosphate, 0.01 M, pH 2.4, on 5 μm ODS, 4.6 × 250 mm column, was not able to separate the diastereomers. However, 45% EtOH/55% phosphate, 0.01 M, pH 2.4, resulted in separation, and retention volumes of 10.50 and 11.20 mL and peak proportions indicated ligand **19** as a 70:30 mixture of **19** and **18**. NMR integration of peaks indicated a similar value of 80:20 for the mixture.

(17) Jones, A. G.; Davison, A.; LaTegola, M. R.; Brodack, J. W.; Orvig, C.; Sohn, M.; Toothaker, A. K.; Lock, C. J. L.; Franklin, K. J.; Costello, C. E.; Carr, S. A.; Biemann, K.; Kaplan, M. L.; *J. Nucl. Med.* **1982**, *23*, 801.

Table III. Biodistribution Data of Carboxylate Technetium-99m Diamide Dimercaptide Complexes in Mice[a]

^{99m}Tc complex	time, min	blood	liver	kidney	stomach	intestines	urine	urine % ^{99m}Tc/ % ^{131}I
2A	10	3.10 ± 0.19	4.26 ± 0.32	2.23 ± 0.31	0.11 ± 0.00	0.82 ± 0.05	67.97 ± 0.97	105.00 ± 0.68
	120	0.21 ± 0.04	0.78 ± 0.20	0.25 ± 0.05	0.04 ± 0.01	1.46 ± 0.19	86.57 ± 0.92	105.00 ± 1.59
2B	10	5.07 ± 0.31	10.57 ± 0.64	5.49 ± 0.56	0.20 tu 0.03	1.27 ± 0.09	53.38 ± 3.38	87.87 ± 2.21
	120	0.09 ± 0.01	2.70 ± 0.89	0.54 ± 0.21	0.05 ± 0.01	0.33 ± 0.04	88.67 ± 1.67	100.10 ± 0.86
23	10	29.70 ± 1.36	15.47 ± 0.85	6.85 ± 0.59	0.61 ± 0.08	12.97 ± 1.28	17.27 ± 0.61	24.59 ± 1.15
	120	4.61 ± 0.49	2.13 ± 0.30	0.51 ± 0.03	1.04 ± 0.18	38.36 ± 3.01	43.79 ± 3.50	45.46 ± 3.60
28A	10	7.87 ± 0.40	2.48 ± 0.24	5.88 ± 0.77	0.22 ± 0.01	1.32 ± 0.14	59.86 ± 3.80	85.20 ± 0.96
	120	0.12 ± 0.07	0.05 ± 0.02	0.28 ± 0.11	0.01 ± 0.00	0.21 ± 0.02	97.60 ± 0.62	101.80 ± 0.61
28B	10	7.54 ± 0.39	3.82 ± 0.31	5.68 ± 0.41	0.21 ± 0.01	1.31 ± 0.09	57.40 ± 4.21	85.00 ± 1.87
	120	0.07 ± 0.02	0.11 ± 0.02	0.35 ± 0.15	0.06 ± 0.62	0.44 ± 0.04	90.72 ± 1.42	102.40 ± 1.00
29A	10	3.01 ± 0.39	3.03 ± 0.45	3.66 ± 0.73	1.68 ± 1.58	1.29 ± 0.21	69.66 ± 2.75	105.60 ± 1.27
	120	0.10 ± 0.03	0.24 ± 0.02	1.27 ± 1.08	0.17 ± 0.12	1.39 ± 0.10	91.29 ± 1.45	101.20 ± 1.60
29B	10	6.43 ± 0.84	9.80 ± 0.69	9.13 ± 0.57	0.21 ± 0.02	1.65 ± 0.25	46.65 ± 3.83	74.45 ± 2.44
	120	0.07 ± 0.02	0.13 ± 0.04	0.31 ± 0.15	0.07 ± 0.03	0.90 ± 0.23	86.02 ± 4.83	103.92 ± 0.80
30A	10	2.31 ± 0.25	2.50 ± 0.36	2.03 ± 0.19	0.12 ± 0.02	4.16 ± 0.44	75.49 ± 2.42	100.60 ± 0.87
	120	0.02 ± 0.00	0.20 ± 0.04	0.15 ± 0.02	0.17 ± 0.11	3.82 ± 0.49	90.74 ± 1.00	97.67 ± 0.53
30B	10	3.86 ± 0.05	1.60 ± 0.22	3.56 ± 0.52	0.14 ± 0.02	1.33 ± 0.15	71.12 ± 2.45	98.69 ± 2.10
	120	0.09 ± 0.01	0.11 ± 0.01	0.60 ± 0.16	0.05 ± 0.00	0.87 ± 0.04	95.06 ± 0.98	101.20 ± 0.19
31A	10	2.93 ± 0.21	1.50 ± 0.16	3.58 ± 0.45	0.16 ± 0.02	3.66 ± 0.17	77.46 ± 3.68	94.70 ± 1.20
	120	0.34 ± 0.11	1.85 ± 0.25	0.42 ± 0.05	0.20 ± 0.06	8.52 ± 0.33	87.50 ± 1.05	95.68 ± 1.71
31B	10	3.18 ± 0.22	2.34 ± 0.19	1.90 ± 0.14	0.17 ± 0.02	1.93 ± 0.08	68.09 ± 0.22	88.60 ± 1.40
	120	0.17 ± 0.00	0.98 ± 0.05	0.17 ± 0.02	0.04 ± 0.01	5.01 ± 0.33	91.77 ± 0.75	100.70 ± 0.47
32A	10	1.97 ± 0.16	8.24 ± 0.80	3.58 ± 1.34	0.28 ± 0.13	9.80 ± 0.13	57.06 ± 1.76	88.11 ± 0.51
	120	0.30 ± 0.18	1.43 ± 0.44	0.21 ± 0.18	0.22 ± 0.11	12.37 ± 1.01	69.07 ± 2.47	88.20 ± 1.15
32B	10	2.27 ± 0.40	21.89 ± 1.22	2.77 ± 0.36	0.20 ± 0.05	3.23 ± 0.38	59.03 ± 2.23	79.93 ± 1.12
	120	0.08 ± 0.00	7.40 ± 1.05	0.50 ± 0.29	0.27 ± 0.14	8.49 ± 0.56	76.08 ± 0.98	85.17 ± 1.56
33A	10	5.37 ± 0.42	5.45 ± 0.35	4.29 ± 0.33	0.28 ± 0.05	1.30 ± 0.07	60.99 ± 1.21	87.34 ± 1.02
	120	0.05 ± 0.02	0.16 ± 0.04	1.94 ± 0.68	0.03 ± 0.01	0.55 ± 0.02	94.11 ± 1.80	101.80 ± 0.26
33B	10	3.62 ± 0.23	4.12 ± 0.32	4.96 ± 0.31	0.17 ± 0.02	1.15 ± 0.07	71.77 ± 0.92	99.97 ± 0.72
	120	0.04 ± 0.00	0.13 ± 0.02	0.48 ± 0.36	0.06 ± 0.01	0.73 ± 0.13	92.69 ± 1.78	101.98 ± 0.41
34A	10	4.32 ± 0.27	5.74 ± 0.56	3.64 ± 0.23	0.17 ± 0.01	1.02 ± 0.12	64.44 ± 4.64	89.33 ± 1.02
	120	0.13 ± 0.10	0.19 ± 0.06	0.50 ± 0.59	0.09 ± 0.06	1.13 ± 0.10	97.62 ± 0.35	101.20 ± 0.31
34B	10	17.75 ± 0.76	10.08 ± 0.60	4.71 ± 0.32	0.49 ± 0.03	3.49 ± 0.19	24.09 ± 1.75	33.00 ± 1.53
	120	1.02 ± 0.31	1.12 ± 0.28	0.23 ± 0.04	0.15 ± 0.06	4.79 ± 0.48	85.67 ± 2.30	93.17 ± 1.78
35	10	12.01 ± 0.41	5.80 ± 0.34	4.51 ± 0.24	0.53 ± 0.02	2.70 ± 0.10	27.80 ± 1.58	42.88 ± 1.00
	120	0.68 ± 0.06	4.12 ± 0.60	2.66 ± 0.18	0.24 ± 0.17	0.68 ± 0.27	85.62 ± 1.50	92.48 ± 0.94

[a] Values are mean ± SEM for five or six mice.

excretion. The cis and trans cyclohexyl derivatives, ^{99m}Tc-**24** and -**25**, formed two products for the cis and one for the trans. The latter component of *cis*-^{99m}Tc-**8** had a greater liver affinity, and both had slow renal excretion. The latter component of ^{99m}-Tc-**24** had a similar HPLC retention volume to the single component of *trans*-**25**. However, both the liver uptake of *trans*-^{99m}Tc-**25** and the blood clearance were slower.

Complexes ^{99m}Tc-**28** and -**29** had carboxylate groups on chelate rings, and both resulted in pairs of components. In contrast to ^{99m}Tc-**2A** and **2B**, ^{99m}Tc-**28A** and -**28B** were identical in percent renal excretion in mice at 10 min. Ligand **19** contained a propyl bridge, which was expected to afford greater conformational flexibility and thus less renal handling difference between epimers. However, ^{99m}Tc-**19A** and -**19B** were nearly identical in biological behavior with ^{99m}Tc-**2A** and -**2B**.

Extension of the carboxylate group as an acetate, ^{99m}Tc-**30**, resulted in incomplete separation of epimers by reversed phase but complete separation on anion-exchange HPLC. Both epimers were rapidly excreted with high specificity. Conversion of the carboxylate group to carboxamide (^{99m}Tc-**31**) slowed renal excretion and decreased differences between epimers. The glycine derivative, ^{99m}Tc-**32**, was evaluated since it provides a structural similarity to the side chain of hippurate (benzoylglycine). However, renal excretion rates were slower and biliary excretion was significant. All epimer products of ^{99m}Tc-**33** and -**34** were of high specificity, but renal excretion rates varied from 33% to 100% of ^{131}I OIH at 10 min. Finally, ligand **35**, a dicarboxylic acid derived from *d,l*-diaminosuccinic acid, formed a single ^{99m}Tc chelate as expected and exhibited renal excretion at a rate slower than ^{99m}Tc DTPA, which reflects glomerular filtration rate.

Discussion

Earlier work on ^{99m}Tc-**1** showed it to be a small, monoanionic complex with the technetium oxo group surrounded by the tetradentate amide nitrogen and sulfur donor atoms in a basal plane.[6] Our work on its biological handling showed it to be transported by the renal proximal tubular cells.[7,8] The work described here indicates the sensitivity in in vivo behavior of the ^{99m}Tc N_2S_2 ligand system to structural modifications. Exploration of hydrocarbon-group effects demonstrated significant loss of renal excretion efficiency for fused cyclohexyl derivatives and nearly complete loss of renal excretion for aromatic derivatives. Interestingly, addition of a methyl group resulted in maintenance of renal specificity but markedly increased renal cell transit times. Increased renal excretion efficiency resulted from a polar but uncharged hydroxyl-group addition. Carboxylate derivatives that are dianions at physiological pH showed increased specificity. However, renal excretion rates depended on chelate ring epimer and positional isomer relationships. The dicarboxylate derivative studied showed reduced excretion rates, which suggests that trianions of this class of metal complex are not easily handled by the renal tubular cells. Extensions from the chelate ring as in acetate or glycinate showed decreased epimer differences but increased hepatobiliary excretion, which indicates that renal excretion specificity maintenance is quite restrictive.

The results presented herein on biodistribution in mice suggests further evaluation of ^{99m}Tc complexes of **20**, **28**, and **30** as potential renal tubular function radiopharmaceuticals on the basis of efficient renal excretion, high specificity, and minimal differences between chelate ring epimers.

Experimental Section

Proton NMR spectra were obtained on either a Varian 360A or 390 spectrometer with Me_4Si as an internal standard. Melting points were obtained on an Electrothermal apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Analysis of synthetic organic intermediates was performed by TLC with use of precoated 0.2-mm plastic sheets from E. Merck. HPLC was carried out on Beckman-Altex systems with use of 5 μm ODS Ultrasphere columns for reversed-phase conditions and Ultrasil AX columns for ion exchange. Spectrophotometric detection was carried out on Beckman Model 153 fixed-wavelength or 155-40 variable-wavelength spectrophotometers. Radiometric detection on column outflow was performed either by crystal scintillation with use of Canberra electronics or with a GM survey meter (Eberline) equipped with ratemeter output.

A general method for the synthesis of *N,N'*-bis[(benzoylthio)acetyl]diamines (ligands **1**, **2**, **19–27**, **29**, and **35**) from starting diamino precursors is described with ethyl DL-*N,N'*-bis[(benzoylthio)acetyl]-2,4-diaminobutanoate (**29**) as an example.

Ethyl DL-2,4-Diaminobutanoate Dihydrochloride (3). DL-2,4-Diaminobutanoic acid dihydrochloride (5.0 g, 0.026 mol) was dissolved in 300 mL of absolute ethanol. After HCl gas was bubbled into the solution, the solution was refluxed for 12 h. Removal of solvent left 5.70 g of ester, which was used without purification.

Ethyl DL-2,4-Bis(chloroacetamido)butanoate (4). Ethyl DL-2,4-diaminobutanoate (5.50 g, 0.025 mol) was combined with 200 mL of toluene and cooled in an ice bath. While the mixture was stirred, 250 mL of saturated $NaHCO_3$ solution was added. Then 15.0 g (0.133 mol) of chloroacetyl chloride dissolved in 50 mL of toluene was added dropwise over a 1-h period to the cooled, stirred mixture. During this period additional solid $NaHCO_3$ was occasionally added to the reaction mixture. After completion of addition, the reaction mixture was allowed to come to room temperature, and stirring was continued for an additional 15 h. The layers were separated, and the aqueous layer was extracted several times with ethyl acetate. The extracts and the toluene layer were combined and dried (Na_2SO_4), and the solvent was removed to give 7.0 g (93%) of product: NMR ($CDCl_3$) δ 1.27 (t, 3, CH_3), 1.55–4.0 (m, 4, β-CH_2 and γ-CH_2), 4.12 (s, 2, CH_2Cl), 4.20 (s, 2, CH_2Cl), 4.25 (q, 2, OCH_2), 4.50–4.90 (m, 1, α-CH), 7.0–7.75 (m, 2, CONH). The product was used without purification.

Ethyl DL-2,4-Bis[(benzoylthio)acetamido]butanoate (29). Ethyl DL-2,4-bis(chloroacetamido)butanoate (4) (5.0 g, 0.017 mol) was dissolved in 100 mL of absolute ethanol. To the stirred solution under nitrogen was added an ethanolic solution of potassium thiobenzoate (prepared from 1.30 g (0.033 mol) of potassium in 25 mL of absolute ethanol to prepare potassium ethoxide, which in turn was reacted with 4.60 g (0.033 mol) of thiobenzoic acid). The mixture was refluxed for 3 h. The reaction mixture was filtered while hot to remove precipitate, and the filtrate was concentrated under reduced pressure. The residue was dried and then washed with 50 mL of $CHCl_3$. The $CHCl_3$ suspension was filtered again, and $CHCl_3$ was removed to yield a light pink product. The product was purified by silica gel chromatography with use of $CHCl_3$ and AcOEt as successive eluting solvents. The final yield was 8.0 g (95%). An analytical sample was obtained from ethanol: mp 141–142 °C; NMR ($CDCl_3$) δ 1.20 (t, 3, CH_3), 1.40–4.35 (m, 4, α-CH_2 and γ-CH_2), 3.70 (s, 2, $COCH_2S$), 3.80 (s, 2, $COCH_2S$), 4.10 (q, 2, OCH_2), 4.35–4.90 (m, 1, CH), 6.80–8.40 (m, aromatic H and CONH). Anal. ($C_{26}H_{26}O_6S_2$) C, H, N, S.

Ethyl 2,9-Bis(benzoylthio)-3,8-dioxo-4,7-diazanonanoate (28). Aminoacetonitrile hydrochloride (5) (5.0 g, 0.054 mol) was dissolved in 300 mL of toluene. After the mixture was cooled in an ice bath, 300 mL of saturated $NaHCO_3$ was added with stirring. Ethyl malonyl chloride (15.0 g, 0.10 mol) dissolved in 50 mL of toluene was added dropwise with rapid stirring. During the addition, dry $NaHCO_3$ was occasionally added. After addition, the reaction mixture was allowed to come to room temperature, and stirring was continued for 15 h. Layers were separated, and the aqueous phase was extracted several times with AcOEt. The extracts were combined with the toluene phase, washed with water and brine, and dried (Na_2SO_4). Removal of solvent left 8.80 g (96%) of ethyl *N*-(cyanomethyl)malonamide (**6**), which was used without purification: NMR ($CDCl_3$) δ 1.35 (t, 3, CH_3), 3.50 (s, 2, $COCH_2CO$), 4.30 (d, 2, CH_2CN), 4.32 (q, 2, CH_2), 7.90–8.35 (t, 1, NH).

The ethyl *N*-(cyanomethyl)malonamide (**6**) (4.0 g, 0.024 mol) obtained above was dissolved in 50 mL of absolute EtOH. To the solution was added 2.5 mL of concentrated HCl. After the mixture was purged with N_2 for 10 min, 0.40 g of Adams' catalyst was added. The mixture was purged for another 10 min with N_2 and then hydrogenated at 50 psi for 12 h. After filtration, the solvent was removed to give 4.80 g (97%) of crude **7**: NMR (Me_2SO-d_6) δ 1.25 (t, 3, CH_3), 2.70–3.80 (m, 4, CH_2CH_2N), 3.35 (s, 2, CH_2), 4.15 (q, 2, CH_2), 7.0–8.5 (3, NH_3^+), 8.50–9.0 (t, 1, NH).

The product ethyl *N*-(2-aminoethyl)malonamide hydrochloride (**7**) (5.0 g, 0.024 mol) was dissolved in 200 mL of toluene. After the mixture was cooled in an ice bath, 200 mL of saturated $NaHCO_3$ was added. While the mixture was stirred rapidly, 15.0 g (0.133 mol) of chloroacetyl chloride in 50 mL of toluene was added dropwise over a 1-h period. The reaction mixture was allowed to come to room temperature, and stirring was continued for 15 h. The layers were separated, and the aqueous layer was extracted with AcOEt. The extracts and toluene layer were combined and dried (Na_2SO_4), and the solvent was removed to give 4.30 g (72%) of white solid **8** that was used without purification: NMR ($CDCl_3$) δ 1.29 (t, 3, CH_3), 3.45–3.70 (m, 4, CH_2), 3.40 (s, 2, CH_2), 4.20 (s, 2, $COCH_2Cl$), 4.22 (q, 2, CH_2), 7.4–8.1 (br t, 2, CONH).

Ethyl *N*-[2-(chloroacetamido)ethyl]malonamide (**8**) (1.50 g, 0.0060 mol) was dissolved in 100 mL of $CHCl_3$. While the mixture was stirred, a solution of 1.0 g (0.0060 mol) of Br_2 in 15 mL of $CHCl_3$ was added. After 10 min the reaction mixture color changed to pale yellow. After 30 min, the solution was washed with water and dried (Na_2SO_4), and the solvent was removed. The solid residue contained a mixture of mono- and dibromo products that had R_f values of 0.5 and 0.7 in TLC (AcOEt, silica gel), respectively. The desired monobromo product was purified by silica gel column chromatography eluted with AcOEt. Recovery of **9** was 1.40 g (70%): NMR ($CDCl_3$) δ 1.32 (t, 3, CH_3), 3.30–3.70 (m, 4, CH_2CH_2), 4.10 (s, 2, CH_2Cl), 4.30 (q, 2, OCH_2), 4.75 (s, 1, COCHBrCO), 6.70–7.70 (br t, 2, NHCO).

Ethyl *N*-[2-(chloroacetamido)ethyl]bromomalonamide (**9**) (0.80 g, 0.0024 mol) was dissolved in 50 mL of absolute EtOH. To a stirred solution under N_2 was added a solution of potassium thiobenzoate (prepared from 0.155 g (0.004 mol) of potassium in 25 mL of absolute EtOH, the product of which was reacted with 0.57 g (0.004 mol) of thiobenzoic acid). The reaction mixture was heated at 70–75 °C for 3 h. After filtration while hot to remove precipitate, the solvent was removed to yield 1.40 g of gummy residue. The residue was dried and then extracted several times with AcOEt. Material extracted was purified by passage through a silica gel column packed in $CHCl_3$ and eluted with AcOEt. The desired product (0.75 g, 63%) had an R_f value of 0.5 in AcOEt in silica gel TLC. Crystallization from EtOH gave an analytically pure sample of **28**: mp 143–144 °C; NMR ($CDCl_3$) δ 1.35 (t, 3, CH_3), 3.45–3.65 (m, 4, CH_2CH_2), 3.80 (s, 2, $COCH_2S$), 4.29 (q, 2, OCH_2), 5.10 (s, 1, COCH(S-)CO), 6.60–8.30 (m, 12, aromatic H and CONH). Anal. ($C_{24}H_{24}N_2O_6S_2$) C, H, N, S.

2,3-Bis[(benzoylthio)acetamido]propanamide (31). Acrylamide (10.0 g, 0.141 mol) was suspended in CCl_4. While the mixture was stirred and cooled in an ice bath, Br_2 (24.0 g, 0.15 mol) in 50 mL of CCl_4 was added dropwise. After completion of addition, the reaction mixture was stirred at room temperature overnight. Removal of solvent left 32.0 g (98%) of white solid. Recrystallization from $CHCl_3$ gave white needles of 2,3-dibromopropanamide (**16**): NMR (Me_2SO-d_6) δ 3.70–4.10 (m, 2, β-CH_2), 4.40–4.85 (m, 1, α-CH), 7.30–8.35 (2, $CONH_2$).

2,3-Dibromopropanamide (10.2 g, 0.044 mol) and dibenzylamine (35.0 g, 0.178 mol) was dissolved in 200 mL of dry EtOH. The

reaction was maintained at reflux for 1.5 h. After the reaction mixture was cooled, precipitated amine salt was removed by filtration. Removal of solvent left a light yellow residue, which was extracted with $CHCl_3$. Removal of solvent and suspension in ether yielded 10.5 g (89%) of crystalline **17**: NMR ($CDCl_3$) δ 2.80–3.10 (m, 2, β-CH_2), 3.40–3.90 (m, 1, α-CH), 3.40 (s, 2, CH_2), 3.60 (s, 2, CH_2), 3.65 (s, 2, CH_2), 3.80 (s, 2, CH_2), 5.70–6.10 (2, $CONH_2$).

2,3-Bis(dibenzylamino)propanamide (**17**) (6.50 g, 0.024 mol) was dissolved in 200 mL of EtOH. After addition of 4.0 mL of concentrated HCl, the solution was purged with N_2 for 10 min. Then 1.40 g of 10% palladium on activated carbon was added, and purging was continued for an additional 10 min. The mixture was subjected to 60 psi of H_2 for 45 h. Afterward, the mixture was filtered and the filtrate removed under reduced pressure to give 4.10 g (91%) of 2,3-diaminopropanamide dihydrochloride (**18**): NMR (Me_2SO-d_6) δ 2.30–4.10 (m, 3, α-CH and β-CH_2), 4.30–6.20 (2, $CONH_2$), 6.90–8.0 (6, NH_3^+).

Conversion of the 2,3-diaminopropanamide to the final product was carried out as described in the general procedure. The resulting 2,3-bis[(benzoylthio)acetamido]propanamide was crystallized in EtOH: NMR ($CDCl_3$) δ 3.30–4.0 (m, 4, β-CH_2 and SCH_2CO), 4.30–4.90 (m, 1, α-CH), 6.20–8.50 (m, 14, aromatic H, CONH). Anal. ($C_{21}H_{21}N_3O_5S_2$) C, H, N, S.

Ethyl 2,3-Bis[(benzoylthio)acetamido]propanoylglycinate (32). A 5.40-g (0.05 mol) sample of 2,3-diaminopropionic acid monohydrochloride was dissolved in 37 mL of 2.0 N NaOH and cooled to 0 °C. From separate addition funnels were added 75.0 mL of 2.0 N NaOH and 27.3 g (0.16 mol) of benzyl chloroformate dissolved in 35 mL of toluene. After completion of addition, the reaction mixture was allowed to slowly warm. After the mixture was stirred at room temperature for an additional 3 h, it was acidified with concentrated HCl. The precipitate that resulted was filtered and washed several times with ice-cold water. The product was dried to yield 12.50 g (67%) of the carbobenzoxy derivative: NMR ($CDCl_3$, Me_2SO-d_6) δ 3.40–3.80 (m, 2, β-CH_2), 4.15–4.55 (m, 1, α-CH), 5.10 (s, 4, $PhCH_2$), 6.20–6.80 (m, 1, NH), 7.10–7.70 (m, 1, NH), 7.40 (s, 10, aromatic), 9.20–9.65 (m, 1, COOH).

In a 250-mL three-neck flask, 7.50 g (0.020 mol) of 2,3-bis(carbobenzoxyamino)propionic acid and 2.30 g (0.022 mol) of glycine ethyl ester were dissolved in 75 mL of CH_2Cl_2. After the reaction mixture was cooled in an ice bath while being stirred, 4.60 g (0.022 mol) of solid dicyclohexylcarbodiimide was added. After the mixture was stirred at 0 °C for 2.0 h and then for 18 h at room temperature, the precipitate of cyclohexylurea was filtered. The precipitate was washed with 10 mL of CH_2Cl_2. The solvent from the filtrate and wash was removed under reduced pressure to yield 9.10 g (99%) of the crude product: NMR ($CDCl_3$) δ 1.17 (t, 3, CH_3), 3.48 (m, 2, β-CH_2), 3.95 (d, 2, $NHCH_2$), 4.20 (q, 2, CH_2CH_3), 4.40–4.70 (m, 1, α-CH), 5.15 (s, 4, CH_2Ph), 5.50–6.0 (m, 1, NH), 6.0–6.5 (m, 1, NH), 7.0–7.65 (m, 1, NH), 7.45 (s, 10, aromatic).

Ethyl *N*-[2,3-bis(carbobenzoxyamino)propanoyl]glycinate (5.0 g, 0.01 mol) was dissolved in 150 mL of EtOH. To the solution was added 5.0 mL of 12.0 N HCl, and the solution was purged with nitrogen gas for 10 min. Then 0.5 g of palladium on activated carbon (10%) was added, and purging was continued for an additional 10 min. Hydrogenation at 60 psi was carried out for 15 h. Afterward, the solution was filtered and the solvent from the filtrate removed under reduced pressure to yield 2.40 g (83%) of product. The crude product gave a positive reaction with ninhydrin (5% solution in ethanol). The compound was used for the condensation reaction without further purification: NMR (Me_2SO-d_6) δ 1.27 (t, 3, CH_3), 3.20–3.60 (m, 2, β-CH_2), 3.60–4.70 (m, 5, α-CH, $NHCH_2$, CH_2CH_3), 8.30–9.70 (br m, 7, NH_3 and CONH).

The remainder of the synthesis of **32** was carried out as in Scheme I.

Ethyl 2,3-Bis[(benzoylthio)acetamido]butanoate (33, 34). Ethyl crotonate (20.0 g, 0.175 mol) was dissolved in 150 mL of $CHCl_3$ at 0 °C. A solution of 30.0 g (0.188 mol) of Br_2 in 75 mL of chloroform was added dropwise with stirring. After the mixture was stirred overnight, the solvent was removed under reduced pressure to yield 47.6 g (99%) of ethyl 2,3-dibromobutanoate: NMR ($CDCl_3$) δ 1.28 (t, 2, CH_3), 1.82–1.95 (m, 3, CH_3), 4.10–4.70 (m, 4, α-H, β-H, and CH_2).

Sodium azide (9.75 g, 0.15 mol) dissolved in 40 mL of water was added dropwise to a warm solution of 13.70 g (0.05 mol) of ethyl 2,3-dibromobutanoate in 20 mL of EtOH over a 3.0-h period. The mixture was heated at 90 °C for an additional hour. After removal of EtOH under reduced pressure, the residual aqueous layer was extracted several times with benzene. The solvent was removed in vacuo, and the crude product was vacuum distilled at 85–90 °C to yield 6.50 g (66%) of ethyl 2,3-diazidobutanoate: NMR ($CDCl_3$) δ 1.20–1.60 (superimposed multiplets, 6, γ-CH_3 and CH_2CH_3), 3.65–4.70 (complex, 4, α-CH, β-CH, and CH_2CH_3).

Ethyl 2,3-diazidobutanoate (4.5 g, 0.023 mol) was dissolved in 100 mL of 95% EtOH, and then 40 mL of 12.0 N HCl was added. After the mixture was purged with nitrogen gas for 10 min, 0.50 g of Adams' catalyst was added. Purging was continued for an additional 10 min. The reaction mixture was then hydrogenated at 60 psi for 15 h. After filtration, the solvent was removed under reduced pressure to yield 5.40 g (98%) of ethyl 2,3-diaminobutanoate dihydrochloride: NMR (Me_2SO-d_6) δ 1.0–1.60 (superimposed multiplets, 6, CH_3 and CH_2CH_3), 3.30–4.90 (complex, 4, α-CH, β-CH, and CH_2CH_3), 8.0–9.80 (br, 6, NH_3).

Ethyl 2,3-diaminobutanoate dihydrochloride (2.0 g, 0.009 mol) was dispersed in 150 mL of toluene. To the cooled suspension under nitrogen was added 200 mL of saturated $NaHCO_3$ solution. While the mixture was vigorously stirred, chloroacetyl chloride (15.0 g, 0.133 mol) dissolved in 50 mL of toluene was added dropwise over a period of 1.0 h. During the course of the reaction, additional solid $NaHCO_3$ was added occasionally to the reaction mixture. After being stirred for an additional 15.0 h, the two layers were separated. The aqueous phase was extracted with EtOAc several times. The combined organic layers were washed with distilled water and brine solution and dried ($MgSO_4$). Removal of solvent under reduced pressure yielded 2.60 g (95%) of solid product: NMR δ 1.0–1.50 (m, 6, γ-CH_3 and CH_2CH_3), 2.40–2.80 (d, 1, β-CH), 3.50–4.80 (complex, 7, α-CH, CH_2CH_3, $COCH_2Cl$), 7.20–8.19 (br t, 2, NH).

Ethyl 2,3-bis(chloroacetamido)butanoate (3.0 g, 0.010 mol) was dissolved in 25 mL of absolute EtOH. To the stirred solution under nitrogen was added sodium thiobenzoate in absolute EtOH (prepared from 0.57 g (0.025 mol) of sodium in 25 mL of EtOH to initially prepare sodium ethoxide, which in turn was reacted with 3.67 g (0.27 mol) of thiobenzoic acid). The reaction mixture was heated under reflux for 3.0 h. The precipitate was filtered, and the solvent from the filtrate was removed under reduced pressure. To the residue was added 25 mL of $CHCl_3$, and the precipitate was filtered again. After removal of solvent, the dried residue was chromatographed on silica gel with use of $CHCl_3$ as eluting solvent. Crystallization from absolute EtOH yielded 1.50 g (45%) of cottony substance: mp 201–202 °C; NMR ($CDCl_3$) δ 1.0–1.35 (d and t, 6, γ-CH_3 and ester CH_3), 3.70–3.80 (s, 4, SCH_2), 4.40–4.90 (m, 2, α-CH and β-CH), 6.60–8.20 (m, 12, aromatic and amide H). Anal. ($C_{26}H_{26}O_6S_2$) C, H, N, S.

The solvent from the above mother liquor was removed under reduced pressure to yield 1.20 g (36%) of a mixture of **33** and **34** with a melting point range of 150–160 °C. No attempt was made to separate the second isomer in a pure form from the mixture. NMR ($CDCl_3$) δ 1.0–1.40 (d and t, 6, γ-CH_3 and CH_2CH_3), 3.65–3.85 (s, 4, CH_2S), 4.0–4.35 (2, CH_2CH_3), 3.90–5.0 (m, 2, α-CH and β-CH), 6.50–8.30 (complex, 12, aromatic and amide H).

Ethyl 3,4-Bis[(benzoylthio)acetamido]butanoate (30). Vinylacetic acid (12.5 g, 0.145 mol) was dissolved in 75 mL of CCl_4. While the mixture was cooled in an ice bath, Br_2 (20.0 g, 0.125 mol) in 24 mL of CCl_4 was added dropwise over a 1-h period. After addition was completed, stirring was continued for an additional 15.0 h. Removal of solvent under reduced pressure gave 32.05 g (90%) of viscous **11**: NMR ($CDCl_3$) δ 2.70–3.40 (m, 1, α-CH), 3.40–4.10 (m, 2, CH_2), 4.20–4.75 (m, 1, β-CH), 12.80 (s, 1, COOH).

The crude dibromobutanoic acid (**11**) (15.0 g, 0.016 mol) and $SOCl_2$ (20.0 g, 0.168 mol) were combined in a flask and heated under reflux for 2.0 h. The excess $SOCl_2$ was removed under reduced pressure to yield 12.50 g (77.5%) of crude product. The absence of the carboxylic acid proton together with the downfield shift of the α-methylene protons in the NMR indicated conversion to the acid chloride. To the crude 3,4-dibromobutanoyl chloride (10.0 g, 0.038 mol) was added dropwise 200 mL of absolute EtOH. After completion of the addition, the reaction mixture was heated under reflux for 2.0 h. The solvent was removed under reduced

pressure to yield 9.5 g (92%) of the crude ester **12**: NMR ($CDCl_3$) δ 1.29 (t, 3, CH_3), 2.60–3.30 (m, 1, α-CH_2), 3.40–4.80 (m, 5, β-CH, γ-CH_2, CH_2CH_3).

Sodium azide (19.50 g, 0.30 mol) in 78 mL of water was added slowly over a period of 3 h to 27.40 g (0.10 mol) of the crude ethyl 3,4-dibromobutanoate in 40 mL of EtOH. The reaction mixture was heated at 90 °C for 12 h. The EtOH was removed under reduced pressure, and the residual aqueous layer was extracted several times with benzene to give 15.0 g (76%) of crude ethyl 3,4-diazidobutanoate. The crude product was fractionally distilled under vacuum to give colorless liquid **13**: bp 80–85 °C; NMR ($CDCl_3$) δ 1.32 (t, 3, CH_3), 2.50–2.75 (d, 2, α-CH_2), 3.35–3.60 (d, 2, γ-CH_2), 3.70–4.50 (m, 1, β-CH), 4.40–4.50 (q, 2, CH_2CH_3).

Ethyl 3,4-diazidobutanoate (**13**) (5.0 g, 0.025 mol) was dissolved in 100 mL of 95% EtOH to which 3.4 mL of concentrated HCl was added. After the reaction mixture was purged with nitrogen gas for 10 min, 0.5 g of Adams' catalyst (PtO_2) was added and purging continued with nitrogen for an additional 10 min. The reaction mixture was then hydrogenated at 60 psi for 15 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to yield a gummy viscous **14** weighing 4.50 g (81%): NMR (Me_2SO-d_6) δ 1.25 (t, 3, CH_3), 2.80–3.10 (d, 2, α-CH_2), 3.10–3.40 (d, 2, γ-CH_2), 3.30–4.50 (m, 1, β-CH), 7.30–9.70 (br m, 6, NH_3). The crude product gave a positive reaction with ninhyrin and was converted to **30** by the method in Scheme I without further purification.

General Method for Synthesis of ^{99m}Tc Complexes. Approximately 1 mg of the sulfur protected ligand under study was dissolved in 0.01 mL of ethanol with warming. Then 20 μL of 5 N NaOH was added, and the mixture was heated at 95 °C for 5 min to hydrolyze ester groups. Radioactivity as technetium-99m pertechnetate in generator saline was added as needed in volumes of 1–3 mL. One milligram of freshly dissolved sodium dithionite (50 μL of a 20 mg/mL solution) was added, and the mixture was heated for 5–15 min depending on effect on chelate epimer distribution. Finally, about 20 μL of 5 N HCl was added to neutralize the reaction mixture.

Ligands that did not require alkyl ester hydrolysis could be easily chelated via exchange from sodium gluconate. In general, they required technetium-99m pertechnetate radioactivity added to a mixture of 20 mg of sodium gluconate, 1.0 mg of the thiobenzoate-protected ligand, and 20 μg of $SnCl_2 \cdot 2H_2O$, pH 5.5. The mixture was heated for 5–10 min at 95 °C. During this time the initially formed technetium-99m gluconate complex exchanges to the N_2S_2 chelate.

HPLC Purification of ^{99m}Tc Chelates. The preparations from one of the above methods were injected into either reversed-phase ODS or anion-exchange columns as required with use of a 250-μL injection loop. The HPLC eluate was collected as radioactivity was detected at expected retention volumes. Ethanol was used as the organic modifying solvent in reversed-phase HPLC because of its known biological dosage effects. The HPLC eluate was diluted with saline for mice and rat studies to ethanol levels corresponding to less than 1% of a low-level pharmacologic dose, 250 mg/kg. Columns, solvent systems, and retention volumes are given in Table I for the ^{99m}Tc chelates studied.

Biological Studies. The biodistribution studies were performed in groups of five or six Swiss Webster Albino outbred strain of mice. Each mouse was injected intravenously through a tail vein with 0.10 mL containing 1 μCi of the ^{99m}Tc chelate and 0.2 μCi of ^{131}I OIH. The mice were then placed in metabolic cages for the collection of excreted urine. At 10 or 120 min postinjection, the urethras were ligated and the mice anesthetized with chloroform. Various organs of interest (blood, liver, kidney, stomach, intestines, urinary bladder, and tail) were then removed and counted in a dual-channel gamma well counter with appropriate energy settings for ^{131}I and ^{99m}Tc. Corrections were made for ^{131}I crossover into the ^{99m}Tc channel. Corrections were also made for extravasation of the dose in the tail.

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Registry No. **1**, 75948-92-4; Tc-**1**, 103639-37-8; (±)-**2**, 103773-03-1; Tc-**2A**, 103729-78-8; Tc-**2B**, 103639-44-7; (±)-**3**, 103668-08-2; (±)-4, 103668-09-3; **5**, 6011-14-9; **6**, 103668-10-6; **7**, 103668-11-7; **8**, 103668-12-8; **9**, 103668-13-9; **11**, 16507-32-7; **12**, 59006-06-3; **13**, 103668-14-0; **14**, 103668-15-1; **16**, 15102-42-8; **17**, 103668-16-2; **18**, 103668-17-3; (±)-**19**, 103668-18-4; Tc-**19A**, 103639-38-9; Tc-**19B**, 103728-98-9; (±)-**20**, 90236-51-4; Tc-**20A**, 103639-39-0; Tc-**20B**, 103728-99-0; **21**, 90236-52-5; **22**, 75948-96-8; Tc-**22**, 103639-40-3; **23**, 103668-19-5; Tc-**23**, 103639-41-4; **24**, 90236-60-5; Tc-**24A**, 103639-42-5; Tc-**24B**, 103729-00-6; **25**, 90236-53-6; Tc-**25**, 103729-01-7; **26**, 103668-20-8; Tc-**26**, 103639-43-6; **27**, 103668-21-9; **28**, 75948-92-4; Tc-**28A**, 103639-45-8; Tc-**28B**, 103729-02-8; (±)-**29**, 103668-22-0; Tc-**29A**, 103639-46-9; Tc-**29B**, 103729-03-9; (±)-**30**, 103668-23-1; Tc-**30A**, 103639-47-0; Tc-**30B**, 103729-04-0; (±)-**31**, 103668-24-2; Tc-**31A**, 103639-48-1; Tc-**31B**, 103729-05-1; (±)-**32**, 103668-25-3; Tc-**32A**, 103639-49-2; Tc-**32B**, 103729-06-2; (±)-(*R**,*R**)-**33**, 103668-26-4; Tc-**33A**, 103639-50-5; Tc-**33B**, 103729-07-3; (±)-(*R**,*S**)-**34**, 103668-27-5; Tc-**34A**, 103729-08-4; Tc-**34B**, 103729-09-5; (*R**,*R**)-**35**, 103729-77-7; Tc-**35**, 103639-51-6; (±)-$NH_2(CH_2)_2CH(NH_2)CO_2H \cdot 2HCl$, 65427-54-5; (±)-$NH_2(CH_2)_2CH(NH_2)C(O)OEt$, 103668-32-2; $ClCH_2C(O)Cl$, 79-04-9; $EtOC(O)CH_2C(O)Cl$, 36239-09-5; $(PhCH_2)_2NH$, 103-49-1; $NH_2CH_2CH(NH_2)CO_2H \cdot HCl$, 6018-55-9; (±)-$NH_2CH_2CH(NH_2)C(O)NHCH_2C(O)OEt$, 103668-28-6; $CH_3CH(N_3)CH(N_3)C(O)OEt$, 103668-29-7; vinylacetic acid, 625-38-7; acrylamide, 79-06-1; (±)-2,3-bis(carbobenzoxyamino)propionic acid, 42548-08-3; (±)-ethyl *N*-[2,3-bis(carbobenzoxyamino)propanoyl]glycinate, 103691-93-6; ethyl crotonate, 10544-63-5; ethyl 2,3-dibromobutanoate, 609-11-0; ethyl 2,3-diaminobutanoate dihydrochloride, 103668-30-0; ethyl 2,3-bis(chloroacetamido)butanoate, 103668-31-1; potassium thiobenzoate, 28170-13-0; sodium thiobenzoate, 51066-54-7; glycine ethyl ester, 459-73-4.