was filtered, and the filtrate was washed with Et_2O: mp 172–175 °C dec; mass spectrum, m/e 313 (M^+); $[\alpha]^{26}_D$ +10.4° (c 0.3, EtOH). Anal. ($C_{19}H_{25}ClN_2O_2$) C, H, N, Cl.

(+)-2-Hydroxy-5-[(S)-1-hydroxy-2-[(R)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide Hydrochloride (3a). A solution of 3.0 g (0.0059 mol) of **9a** in 150 mL of EtOH, containing 5.85 mL of 1 N aqueous HCl (0.00585 mol) and 0.25 g of 20% $Pd(OH)_2$ on carbon,[33] was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 2 h. After filtering off the catalyst and removing the solvent in vacuo, we digested the residue with 50 mL of boiling CH_3CN to yield 1.50 g (70%) of **3a**·HCl (GLC 0.6% **2a**): mp 171–172 °C dec; $[\alpha]^{26}_D$ +27.8° (c 1.0, DMF); CD (c 3.0×10^{-4}) $[\theta]_{260}$ 0, $[\theta]_{304}$ +28960 (max), $[\theta]_{340}$ 0. Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

(−)-2-Hydroxy-5-[(R)-1-hydroxy-2-[(S)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide Hydrochloride (3b). This experiment was conducted in the same manner as described for the preparation of **3a** from **9a**, with similar results. Thus, from **9b**, **3b**·HCl (GLC 0.8% **2b**) was obtained: mp 167–168.5 °C dec; $[\alpha]^{26}_D$ −28.4° (c 1.0, DMF); CD (c 2.7×10^{-4}) $[\theta]_{260}$ 0, $[\theta]_{304}$ −30820 (max), $[\theta]_{340}$ 0. Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

Racemic 2 from 2a and 2b. The analytically pure p-TsOH salts of **2a** and **2b** (4.0 mg each) were dissolved in 0.5 mL of hot EtOH. This was cooled in an ice bath, the precipitate was filtered, and the filtrate was dried: mp 185–186 °C; mmp with authentic **2**·p-TsOH (GLC >99.5%) 185.5–186 °C.

Racemic 3 from 3a and 3b. The HCl salts of **3a** and **3b** (5.0 mg each) were dissolved in 1 mL of hot EtOH, which was then evaporated to dryness. The solid residue was triturated with 0.5 mL of cold EtOH and filtered, and the filtrate was dried: mp 212–213 °C dec; mmp with authentic **3**·HCl (GLC >99.5%) 213–214 °C dec.

Hydrogenolysis of a Mixture of 8a and 9a as Free Bases. A 1:1 mixture of **8a** and **9a** (1.0 g, 0.0020 mol) was dissolved in 25 mL of EtOH; 0.1 g of 20% $Pd(OH)_2$ on carbon[33] was added, and it was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 3 h. After filtering off the catalyst and removing the solvent in vacuo, we obtained 0.58 g (88%) of a gummy solid, which corresponded (^1H NMR, TLC) to a 1:1 mixture of **2a** and **3a**, with no evidence for the presence of **11a**.

Hydrogenolysis of a Mixture of 8a and 9a in the Presence of Excess HCl. A 1:1 mixture of **8a** and **9a** (1.0 g, 0.0020 mol) was dissolved in 25 mL of EtOH, containing 0.65 mL (0.00227 mol) of 3.50 N ethereal HCl; 0.1 g of 20% $Pd(OH)_2$ on carbon[33] was added, and it was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 3 h. A TLC analysis of the solution showed the presence of an intense spot corresponding to **2a**/**3a** and a weak spot corresponding to **11a**. The reduction was continued for 5 days; after filtering off the catalyst and removing the solvent in vacuo, we obtained 0.72 g of a gummy solid that corresponded (^1H NMR, TLC) to a 3:1 mixture of **11a**:**2a**/**3a**.

(R,S)-2-Hydroxy-5-[2-[(1-methyl-3-phenylpropyl)-amino]ethyl]benzamide Hydrochloride (11). By Hydrogenolysis of 1. A solution of 20 g (0.055 mol) of **1**·HCl in 420 mL of EtOH was acidified with 80 mL of 3.5 N ethereal HCl ("free" HCl concentrated in resulting solution = 0.56 N), 2.0 g of 20% $Pd(OH)_2$ on carbon[33] was added, and the mixture was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 4 weeks.[34] The gum (homogeneous by TLC R_f = R_f of authentic **11**), obtained after the catalyst was filtered off and the solvent was removed in vacuo, was triturated with boiling CH_3CN and then recrystallized from 2-propanol to afford 9.0 g (47%) of **11**, mp 160–163 °C.

From 11a and 11b. Compounds **11a** and **11b** (5.0 mg each) were dissolved in 1 mL of MeOH, which was then evaporated to dryness. The solid residue was triturated with Et_2O and filtered, and the filtrate was dried, mp 160–163 °C. Compound **11** was identical in all respects (TLC, ^1H NMR, IR, MS, mmp) with an authentic sample, kindly supplied to us by Dr. Geoffrey P. Levy, Allen & Hanburys Research, Ltd., Great Britain.

(34) An experiment under identical conditions, but at 60 °C overnight, resulted in an unidentified mixture of products. No attempt was made to shorten the reaction time by increasing the HCl concentration.

Structure-Activity Relationships of Some Technetium-99m Labeled [(Thioethyl)amino] Carboxylates

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The synthesis, NMR studies, radiochemical labeling with technetium-99m, and tissue-distribution characteristics of some [(thioethyl)amino] carboxylates are described. The ^{99m}Tc agents prepared were eliminated either by the urinary or the hepatobiliary system of mice. The excretion route of the ^{99m}Tc complexes was influenced by the structure and total charge of the ligands.

In the past years, several technetium-99m complexes have been introduced for diagnosis of renal diseases or for kidney function tests. The technetium-99m–iron ascorbate complex introduced by Harper and Lathrop[1] was first suggested for renal studies due to its significant localization in the kidneys. Since then, a great number of ^{99m}Tc complexes have been developed for this purpose. The ligands of these chelates were of various chemical structure, such as ethylenediamine or triamine acetates,[2-4] peptide derivatives,[5] gluconic acids,[6] or thio carboxylates.[7-10]

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Table I. [(Thioethyl)amino] Carboxylates

$R_1SCH_2CH_2N(R_2)CH_2COOH$

compd	R_1	R_2	mp, °C	formula	M_r	proton chem shift, ppm (in Me_2SO-d_6)[b]	
1	H	H	158-160[a]	$C_4H_{10}NSO_2Cl$	171.64	SCH_2	2.90
						CH_2CH_2*N	3.22
						CH_2COO	4.00
						NH	9.84
2	H	CH_2COOH	168-170	$C_6H_{11}NSO_4$	193.22	SCH_2	2.60
						CH_2CH_2*N	2.90
						CH_2COO	3.54
3	H	$CH_2CH_2N(CH_2CH_2SH)CH_2COOH$	135-137 (unhyd)	$C_{10}H_{20}N_2S_2O_4$	296.40	SCH_2	2.50
						$HSCH_2CH_2$*N	2.86
						NCH_2CH_2N	2.77
						CH_2COOH	3.37
4	SCH_2CH_2N-$(CH_2COOH)_2$	CH_2COOH	115-118	$C_{12}H_{20}N_2S_2O_8$	384.42	SCH_2	2.90
						CH_2CH_2*N	2.90
						CH_2COO	3.57
5	CH_2CH_3	CH_2COOH	viscous mass	$C_8H_{15}NSO_4$	221.27	SCH_2	2.65
						CH_2CH_2*N	2.94
						CH_2COO	3.60
						CH_3CH_2S	2.50
						CH_3	1.16
6	$CH_2(CH_2)_2CH_3$	CH_2COOH	109-111	$C_{10}H_{19}NSO_4$	249.33	SCH_2	2.58
						CH_2CH_2*N	2.82
						CH_2COO	3.45
						$(CH_2$*$)_2CH_2S$	1.41
						CH_3	0.87
7	$CH_2C_6H_5$	CH_2COOH	170-171	$C_{13}H_{17}NSO_4$	283.35	SCH_2	2.55
						CH_2CH_2*N	2.92
						CH_2COO	3.73
						$C_6H_5CH_2$*S	3.87
						C_6H_5	3.58
8	$C_{10}H_7$ (β-napthyl)	CH_2COOH	141-143	$C_{16}H_{17}NSO_4$	319.39	SCH_2	2.95
						CH_2CH_2*N	3.18
						CH_2COO	3.47
						$C_{10}H_7$	7.38-8.27

[a] The melting point of compound 1 refers to its hydrochloric salt. [b] An asterisk designates the relevant proton.

Recently, Loberg et al.[11] demonstrated that iminodiacetic acid (IDA), a small-size analogue of EDTA or DTPA, was capable of complexing reduced technetium and easily incorporating into biologically active molecules. They introduced ^{99m}Tc–[(2,6-dimethylacetanilido)imino]diacetate complex (^{99m}Tc–HIDA), which possessed significant hepatobiliary affinity. Based on iminodiacetic acid substitution, numerous ^{99m}Tc hepatobiliary agents were then developed,[12-14] some of them are now being used routinely.

We report here the synthesis and biological distribution in experimental animals of a series of ^{99m}Tc-labeled [(thioethyl)amino] carboxylates. The results were compared to those found for ^{99m}Tc–DMSA (dimercaptosuccinate) and ^{99m}Tc–EHIDA, [(2,6-diethylacetanilido)imino]diacetate, two well-known radiopharmaceuticals for the renal and hepatobiliary system, respectively.

Results and Discussion

The structures of [(thioethyl)amino] carboxylates used in this study, as well as the ^1H NMR data, are presented in Table I. Thin-layer chromatography showed small amounts of free or reduced technetium from the preparations of ^{99m}Tc chelates. The labeling yield was over 90%. ^{99m}Tc complexes 1–4 (Table I) moved along with $^{99m}TcO_4^-$ to the solvent front when acetonitrile/water (1:1) was used as eluent, while reduced technetium stayed at the origin. When 2-propanol was used, reduced and complexed technetium stayed at the origin, while the unbound ^{99m}Tc showed R_f 0.9. The percentage of bound technetium was found by substracting the percentage of free and reduced technetium. ^{99m}Tc derivatives 1–3 were also prepared in acidic pH and showed identical chromatographic behavior. ^{99m}Tc-substituted [(thioethyl)amino]acetates 5–8 were distributed similarly, as described above, on thin-layer chromatograms when acetonitrile/water (3:1) was used. When NaCl (0.9%) was used, complexes 5–8 (Table I) remained near the origin with R_f values depending on the lipophilicity of the sulfur substituent. The radiochemical purity was determined similarly by subtracting bound and unbound technetium.

Table II summarizes the comparative 60-min biodistribution data of ^{99m}Tc–[(thioethyl)amino] carboxylates, ^{99m}Tc–DMSA, and ^{99m}Tc–EHIDA. The values present the percent dose of administered radioactivity in blood, liver, kidney, intestines, urine, and muscles. The renal system was found to be the main elimination path of ^{99m}Tc complexes 1–3. Sixty minutes after the administration, a large portion of activity, 36–50%, accumulated in the urinary bladder. ^{99m}Tc complexes 1–3 show quite low kidney concentration of activity as compared with ^{99m}Tc–DMSA. The latter accumulated activity to the extent of 21.8% of the total dose in kidney (Table II), while complexes 1–3,

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Table II. Percent Injected Radioactivity at 60 min in Tissues Following Intravenous Administration of ^{99m}Tc-[(Thioethyl)amino] Carboxylates, ^{99m}Tc-DMSA, and ^{99m}Tc-EHIDA in Mice[a]

compd	blood	liver + gallbladder	kidneys	intestines	urine	muscle
1[d]	4.176[b] (1.21[c])	5.448 (1.09)	5.318 (0.84)	16.702 (3.00)	36.323 (9.36)	6.988 (0.94)
2	3.593 (0.72)	1.920 (0.32)	5.677 (1.41)	4.743 (1.38)	52.113 (0.99)	7.553 (2.11)
3	3.460 (1.62)	4.300 (1.39)	5.620 (2.29)	16.924 (1.68)	39.570 (7.20)	3.472 (1.81)
4	4.640 (0.79)	3.085 (0.61)	4.240 (2.90)	8.900 (0.99)	50.090 (11.67)	7.973 (2.97)
5	4.550 (0.21)	8.568 (0.90)	3.567 (0.10)	25.533 (1.61)	34.100 (3.81)	3.560 (0.41)
6	2.800 (0.35)	12.700 (1.73)	2.767 (0.96)	52.400 (2.25)	15.967 (3.45)	4.013 (0.60)
7	2.627 (0.87)	8.429 (2.15)	2.083 (0.56)	58.020 (5.68)	11.371 (3.03)	3.320 (0.72)
8	4.406 (1.32)	22.748 (3.48)	1.842 (0.23)	51.300 (4.42)	9.736 (2.46)	5.254 (0.81)
DMSA[e]	18.873 (4.16)	12.617 (0.82)	21.823 (1.09)	5.693 (0.20)	30.040 (5.88)	24.170 (4.57)
EHIDA	0.536 (0.09)	5.938 (3.50)	0.399 (0.02)	57.729 (7.25)	13.865 (9.75)	0.711 (0.29)

[a] Mice were injected in the tail vein. Radioactivity in spleen, heart, or stomach was less than 1% of the injected dose. [b] Represents dose average value of five animals. [c] Represents plus or minus standard deviation. [d] Preparation carried out at pH 7. [e] Prepared from commercial kits.

Table III. Acidity Constants[a]

compd	pK_1	pK_2	pK_3	pK_3'
1	2.6	8.5	11.6	
2	1.9	7.7	10.6	
3	1.1	9.0	11.6	4.6
4	2.3		9.2	
7	2.1		9.1	

[a] 26 °C, 0.02 M in D_2O.

although they possess free sulfhydryl groups like DMSA, were accumulated to the extent of only 5.3–5.6%. Acidic pH, demonstrated previously to be essential[15,16] for kidney localization of thio carboxylates, was unsuccessful with the ^{99m}Tc-[(thioethyl)amino] carboxylates 1–3; ^{99m}Tc derivatives 1–3 prepared in acidic pH and administered in mice showed similar kidney values as when prepared in pH 7. Different intestinal values were obtained when ^{99m}Tc complex 3 was prepared and administered in acidic pH. Sixty minutes postinjection, 7.9% of the total dose was accumulated in the intestinal tract compared to 16.9% when administered at pH 7.

^{99m}Tc chelates 4–8 (Table I) are S-substituted derivatives of 2-[(thioethyl)imino]diacetic acid 2. The substituents are characterized by various degrees of lipophilicity. The substitution of sulfur hydrogen by alkyl or aryl substituents changed the elimination route. Compound 4, although S-substituted, contains four carboxy groups that enhance its hydrophilic character and, thus, is mainly eliminated by the urinary system. The S-substituted compounds 5–8 showed significant hepatobiliary concentration in mice (Table II). The degree of hepatobiliary specificity depended on the molecular weight of the substituent. ^{99m}Tc complex 5, a low-molecular-weight alkyl-substituted derivative, was almost equally excreted via the hepatobiliary and urinary systems. Sixty minutes after administration, 34.1% was found in the urine and 25.5% in the intestinal tract. As the molecular weight of the substituent increased, urinary excretion decreased and hepatobiliary affinity increased. In comparison to ^{99m}Tc-EHIDA, ^{99m}Tc complex 7 showed similar values, while the β-naphthyl derivative, ^{99m}Tc complex 8, showed slower extraction rate from the hepatic cells. Liver values of complex 8 were 22.7% of the total dose, while for ^{99m}Tc complex 7 and ^{99m}Tc-EHIDA they were 8.4 and 5.9%, respectively.

In order to investigate the structural parameters influencing the chelation of ^{99m}Tc with the [(thioethyl)amino]

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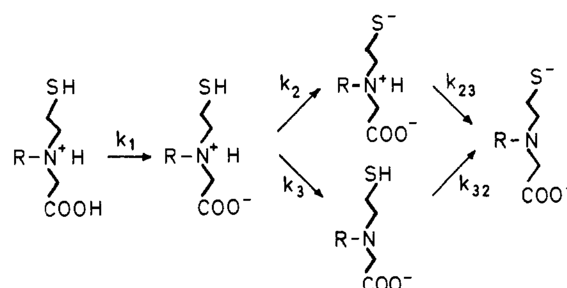


Figure 1. Ionization constants of the [(thioethyl)amino]acetates. K_1, K_2, and K_3 are the macroscopic ionization constants. k_1, k_2, k_3, k_{23}, and k_{32} are the microscopic ionization constants. Assuming that dissociation of the carboxy group is not perturbed by the dissociation of the other groups, $K_1 = k_1$; $K_2 = k_2 + k_3$, and $K_3 = k_{23}k_{32}/(k_{23} + k_{32})$.

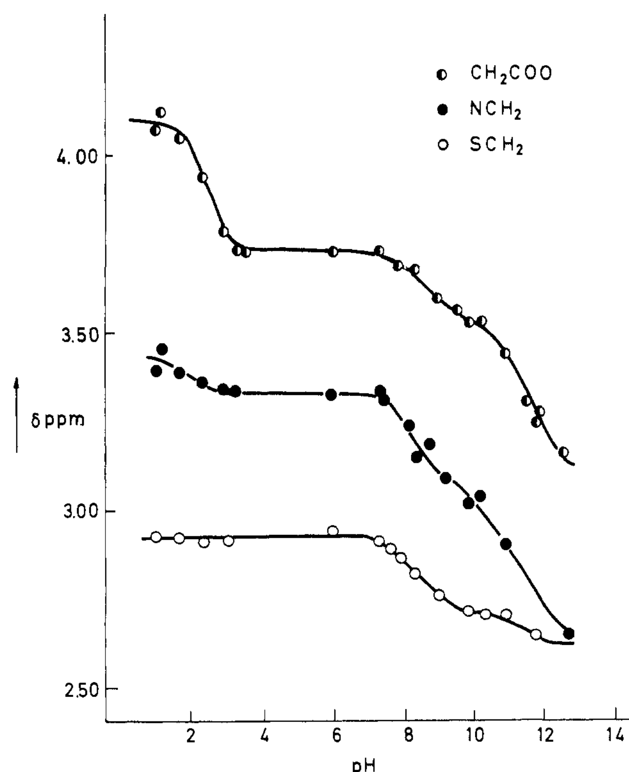


Figure 2. Titration curves of ligand 1. The SCH_2 protons are not sensitive to the ionization of the carboxylic protons. All three groups show separate steps for the dissociation of SH and NH^+.

carboxylates, which might explain the differences observed in the biodistribution of compound 3 (Table II) in acidic or neutral pH, we considered it interesting to study the ionic structures of the ligands at various pH's.

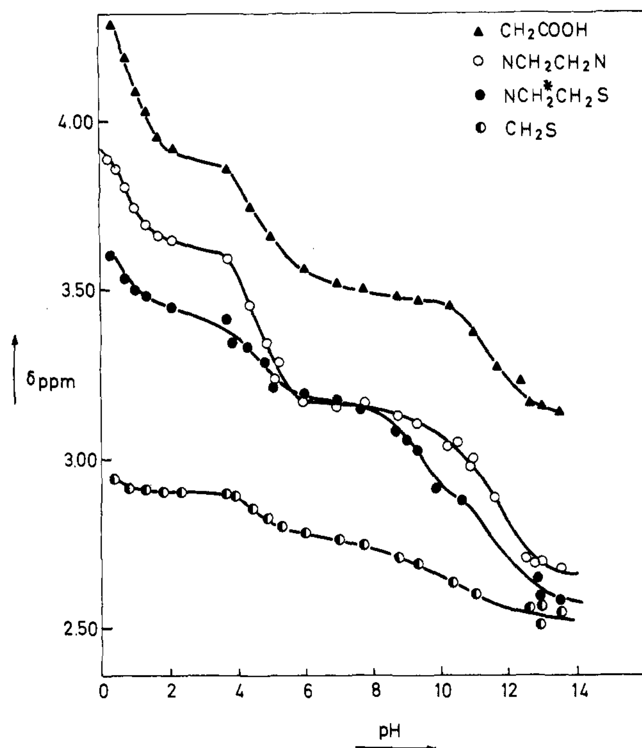
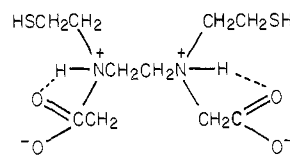


Figure 3. Titration curves of ligand 3. All groups are sensitive to all dissociations. Only $NCH_2{}^*CH_2S$ gives separate steps for the dissociation of SH and the more basic NH^+. Notice the dissociation of the more acidic NH^+ at $pK_3' = 4.6$. All the ligands studied showed a single step for the ionization of the carboxy groups.

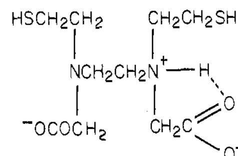
The general scheme for the ionization of the [(thioethyl)amino]acetates is presented in Figure 1. Table III gives the macroscopic acidity constants for the different ionization steps determined by NMR titration experiments in D_2O. Microscopic constants[17] cannot be obtained from these experiments because the COOH, SH^-, and NH^+ dissociations are influencing the chemical shifts of all nonlabile protons (Figure 2). Nevertheless, it should be kept in mind that in the pH region 8 to 12 the species $^-SCH_2CH_2N^+HRCH_2COO^-$ and $HSCH_2CH_2NRCH_2COO^-$ coexist in solution in percentages defined by their microscopic acidity constants. For compounds 1, 2, and 4, only one structure is prevailing at the pH region 3.5 to 7: the carboxylic groups are in the anionic form, while the sulfhydryl group and the immonium group are protonated. The immonium nitrogen cannot coordinate with the metal, having no electrons available, and the SH group is known to be a bad donor for complexation in this type of ligand.[18] Therefore, the coordination throughout this pH region is expected to occur mainly via the carboxy oxygens.

The second nitrogen of ligand 3 has a macroscopic pK of 4.6, which is relatively close to both the carboxylic and sulfhydrylic pK's of the molecule. This increases the complexity of the ionization scheme, so that a variety of ionized forms will be present at a given pH. Grossly, however, we can calculate from the NMR data that at pH 4 85% of ligand 3 is in the zwitterionic form (total charge = 0). One of the resonance forms that can be written is I. The presence of I is confirmed by the characteristic



I

IR absorption for H-bonded COO^- at 1630 cm^{-1} in heavy water solution.[19] Free COO^- and COOH are absent from the IR spectrum. In the remaining 15% of ligand 3, one of the nitrogens is uncharged, as in structure II (total



II

charge = −1), and is therefore a good chelating agent. The presence of II is confirmed from the IR spectrum, which shows both free COO^- at 1585 cm^{-1} and H-bonded COO^- at 1630 cm^{-1}. At pH 7, 100% of ligand 3 has a total charge of −1.

Thus, ligand 3, due to the presence of a nitrogen with a $pK = 4.6$, has two ionic forms with different chelating capacities available for complexation at pH 4, while at pH 7 only one ionic form (II) is present. This may be influencing the biodistribution of the ^{99m}Tc complex. It must be emphasized that solubility products and stability constants will be very important factors in determining the type and structure of the prevailing complex.

In order to correlate the structure of Tc-99m complexes to their biodistribution, one needs to know the actual chelate structure. A structural study under the conditions prevailing in the solution injected to the animals is practically impossible. Not only is the concentration very low (10^{-8} to 10^{-10} M), but also the lifetime of Tc-99m is short and the precise oxidation state of the metal is not defined. Valuable information could result from the study of complexes of the carrier Tc-99. What is more relevant, however, is the structure that the Tc-99m chelates assume in the living organism. In this context, one should be cautioned against any oversimplified comparison of the structure of the free or chelated ligand in vitro to the in vivo state.

In conclusion, knowledge of the various parameters, such as structure of the ligands, ionic forms, pH of preparation, that can influence the chelation and biodistribution of labels is of primary importance in designing ^{99m}Tc-labeled radiopharmaceuticals. By radiolabeling [(thioethyl)amino] carboxylates with technetium-99m, we prepared complexes that are eliminated either through the urinary or the hepatobiliary system. No significant renal fixation of ^{99m}Tc activity was achieved with the molecules containing free sulfhydryl groups. The substitution of the sulfur hydrogen of 2-[(thioethyl)imino]diacetic acid by heavy substituents produced ^{99m}Tc complexes possessing specific hepatobiliary affinity. Further synthetic work on these lines may provide new series of sulfur-containing ^{99m}Tc-labeled hepatobiliary agents.

Experimental Section

A Varian XL-100 spectrometer was used for the NMR measurements. The chemical shifts were measured with respect to sodium 2,2-dimethyl-2-silapentano-5-sulfonate (DSS). The chemical shift of this standard checked against dioxane was found independent of pH in the region of interest. All pH measurements

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were carried out in heavy water (D_2O) and are reported without correction for the deuterium isotope effect. An Orion digital pH meter was used for the pH measurements.

Synthesis of the Ligands. [(Thioethyl)amino]acetates (1-4). The derivatives were prepared according to literature procedures. Thus, 2-[(thioethyl)amino]acetic acid hydrochloride was prepared[20] from ethyl ethyleniminoacetate by the action of H_2S to -75 °C. The reaction product was subjected to acid hydrolysis to form 1. 2-[(Thioethyl)imino]diacetic acid 2 was isolated from [[(benzylthio)ethyl]imino]diacetic acid by the reaction of liquid ammonia and sodium metal.[21] Similarly, *N*,*N'*-bis(2-mercaptoethyl)ethylenediamine-*N*,*N'*-diacetic acid (3) was prepared from *N*,*N'*-bis[2-(benzylthio)ethyl]ethylenediamine-*N*,*N'*-diacetic acid.[22]

Bis[(thioethyl)amino]-*N*,*N*,*N'*,*N'*-tetraacetic acid 4 was isolated by the action of chloroacetic acid to cystamine dihydrochloride.[22]

S-Substituted [(Thioethyl)imino]diacetates (5-8). The various thiols were converted to the corresponding S-substituted thioethylamines via ethylenimine reaction in -15 °C in methanolic solution.[23] The crude thioethylamines were treated with chloroacetic acid in aqueous media and alkaline pH to form the iminodiacetic acid derivatives as described for [[(benzylthio)ethyl]imino]diacetic acid.[21] The derivatives were isolated from the alkaline solution by adjusting the pH to acidic.

Labeling Procedure. ^{99m}Tc Chelates 1-4. To 20 mg of the carrier in water solution was added, with stirring, 0.1 mL of 5 N HCl solution containing $SnCl_2$ (0.2 mg). The pH was then adjusted to 7 using 1 N NaOH. The mixture was filtered with a Millipore filter (0.22 μm), in an evacuated penicillin vial. $^{99m}TcO_4^-$ (30-40 μCi) was then added to the vial to give a final solution volume of 4 mL. The vial was agitated and left for 15 min at room temperature. The solution was then ready for further use.

N,*N'*-Bis(2-thioethyl)ethylenediamine-*N*,*N'*-diacetic acid ^{99m}Tc chelate 3 was prepared using 1 mg of the ligand, 0.4 mg of $SnCl_2$, and 0.7 mg of ascorbic acid as antioxidant. The radiolabeling of the derivatives 1-3 was carried out in a similar manner in acidic pH 3-4.

^{99m}Tc Chelates 5-8. Forty milligrams of the ligands was first dissolved at pH 7-7.5 with 1 N NaOH. $SnCl_2$ solution (0.4 mg in 0.1 mL of 5 N HCl) was added, and the pH of the mixture was adjusted again to 7. Radiolabeling with technetium-99m was then carried out as described above.

Radiochemical Analysis. ^{99m}Tc-[(thioethyl)amino] carboxylates were analyzed for complexed, reduced, or unbound technetium on silicic acid thin-layer strips (ITLC-s.a. Gelman Co.). The solvent systems used were acetonitrile-water (1:1) and 2-propanol for ^{99m}Tc complexes 1-4, while acetonitrile-water (3:1) and NaCl (0.9%) were used for complexes 5-8. The chromatograms were cut into sections of 0.5 cm and counted in a well-type γ-counter. The percent activity was determined, and the R_f values recovered were compared to those found for pertechnetate anion or reduced technetium.

Tissue Distribution Studies. Each radioactive solution (0.2 mL; 2 μCi) was administered intravenously into the tail vein of male swiss albino mice (20-25 g). The animals were put in metabolic cages in order to collect urine. The mice were killed 60 min postinjection with ether vapors, and the liver, kidneys, blood, intestines, stomach, muscles, and urine were dissected out, and the activity was measured. Urination of the animals during death was avoided by ligation of the penis. The organs or tissue samples collected were measured in a γ-counter (ICN-gamma set 500), and the percentage of the injected activity in each organ was calculated. Counts of the tail were subtracted from the total dose to obtain the total injected dose, to correct for any injected dose that infiltrated the tail and did not enter the circulation. Biodistribution studies in mice of ^{99m}Tc-mercaptosuccinate (^{99m}Tc-DMSA, Sorin Biomedica) and ^{99m}Tc-[(2,6-diethylacetanilido)imino]diacetate (^{99m}Tc-EHIDA, Solco Co.) were performed for comparison.

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Notes

Effect of *N*,*N'*-Diethyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines on the 7,12-Dimethylbenz[*a*]anthracene-Induced Mammary Carcinoma of the Rat

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The syntheses and estrogen receptor affinities of *meso*- and (±)-*N*,*N'*-dialkyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (2) are described. They show high binding affinities in both diastereomeric forms but with a preference for the meso isomer, reaching a RBA value of 8.6 (*meso*-**2b**; 17β-estradiol = 100). Both stereoisomers of **2b** exhibit a strong inhibitory effect on the 7,12-dimethylbenz[*a*]anthracene (DMBA) induced hormone-dependent mammary carcinoma of the Sprague-Dawley rat, reducing the tumor area by 74 (*meso*-**2b**) and 24% [(±)-**2b**], respectively, after 4 weeks administration of 6 × 6 (mg/kg)/week. The high uterotrophic potency makes a mode of action likely which corresponds to the effect of high doses of estrogens.

In two previous papers we have reported the affinity of 1,2-bis(2,6-dichlorophenyl)ethylenediamines for the estradiol (E2) receptor[1] and the inhibitory activity of estrophilic 1,2-bis(4-hydroxyphenyl)ethylenediamines against the 7,12-dimethylbenz[*a*]anthracene (DMBA) induced hormone-dependent mammary carcinoma of the Sprague-Dawley rat.[2] It has been shown that the effect of mammary tumor inhibiting compounds correlates with their binding affinity for the estrogen receptor.[3] One way to increase the receptor affinity of the latter ethylenediamines can be the introduction of chlorine atoms into the

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