Indium(III) and Gallium(III) Complexes of Bis(aminoethanethiol) Ligands with Different Denticities: Stabilities, Molecular Modeling, and *in Vivo* Behavior

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Complexes of Ga(III) and In(III) radionuclides are widely used in diagnostic imaging. In this study, the following ligands of denticities 4, 5, and 6 respectively were prepared: *N*,*N*′-bis-(2,2-dimethyl-2-mercaptoethyl)ethylenediamine (4SS), 1-carboxy-*N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine (5SS), and *N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid (6SS). Syntheses of the two new ligands, 5SS and 6SS, are described. Equilibrium constants for their In(III) and Ga(III) complexes were determined by both direct and ligand-competitive potentiometric methods. The formation constant (K_{ML} = [ML]/[M][L]) of In(III)−6SS in 0.100 M KNO_3 at 25.0 °C is $10^{39.8}$, and its pM at physiological pH (7.4 with 100% excess of the ligand) is 30.9. These values are higher than those of any other previous reported ligand for In(III). The stability constants of the complexes of 4SS, 5SS, 6SS, and the analogous ligand EDDASS, *N*,*N*′-bis(2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid, which does not contain *gem*-dimethyl groups, are compared. The thermodynamic stabilities of the In(III) complexes of all ligands except 6SS are greater than those of the corresponding Ga(III) complexes. The presence of the geminal dimethyl groups in 6SS increased the stability of the Ga(III) and In(III) complexes over those of EDDASS. The effects of the *gem*-dimethyl groups on complex stabilities are explained by molecular modeling. The serum stabilities and biodistributions out to 1 h postinjection of $^{67/68}$Ga and ^{111}In chelates of 4SS, 5SS, and 6SS were measured and compared with those of EDDASS. The $^{67/68}$Ga− and ^{111}In−ligand complexes with more donor atoms showed were more stable in serum, both *in vitro* and *in vivo*. The biodistributions of the $^{67/68}$Ga− and ^{111}In−ligand complexes exhibited distinct trends. None of the $^{67/68}$Ga− and ^{111}In−chelates demonstrated significant heart or brain uptake. The majority of uptake for all compounds was in the liver and kidney. The degree of clearance through the liver corresponded to the thermodynamic stability of the complex. Correlations between *in vivo* behavior, molecular modeling data, and thermodynamic stability of the complexes are discussed.

Introduction

The design and synthesis of new chelating agents for effective coordination of Ga(III) and In(III) has long been an important objective of this research group. Table 1 reviews the formation constants of multidentate ligands for the coordination of these metal ions and the corresponding pM values maintained when the ligands are used as metal buffers. These data show that many multidentate ligands containing phenolate or *o*-hydroxypyridyl groups (*i.e.* HBED, TACN-HP and PLED) have high affinity for Ga(III) but do not coordinate In(III) effectively. The polyamino polycarboxylate ligands EDTA and DTPA contain less basic carboxylate donors, form exclusively five-membered chelate rings, and have somewhat higher affinity for In(III) than for the smaller Ga(III) ion. With some exceptions, the cyclic polyamino polycarboxylates show approximately equal performance for either metal ion, except in those cases where the ion-size-fit factor becomes important. Note the conspicuous absence of multidentate ligands containing mercapto groups in Table 1.

Table 1. Formation Constants (log K_{ML}) and pM[a] (−log[M]) of In(III) and Ga(III) Complexes of Multidentate Ligands

ligand	log K_{ML}(In^{3+})	pM (In^{3+})	log K_{ML} (Ga^{3+})	pM (In^{3+})
EDTA[b]	24.9	22.1	21.0	20.0
DTPA[b]	29.0	24.9	24.3	20.2
PLED[b]	26.5	20.2	32.3	25.8
EDDA-HP[c]	28.0	19.7	29.18	20.8
DTTA-HP[c]	28.03	17.4	45.6	34.9
TACN-TX[d]	34.0	14.8	44.2	25.2
N_3O-HP[e]	26.07	15.4	26.81	16.6
HBED[f]	27.9	17.9	38.51	28.6
SHBED[g]	29.37	20.6	37.47	28.3
DOTA[h]	23.9	17.8	21.33	15.2
TRITA[h]	23.0	16.8	19.91	13.7
TETA[h]	21.9	16.2	19.74	14.1
NOTA[i]	26.2	21.6	30.98	26.4
transferrin	18.3,[j,k] 16.4,[l]	18.3	19.8[jk] 18.8[kl]	19.7
N_3O-Ac_3[m]	25.48	21.1	21.3	16.9
N_3O2-Ac_3[m]	23.56	19.9	17.1	13.4
N_3O3-Ac_3[m]			19.2	15.7

[a] 100% excess ligand at pH 7.4. [b] Reference 1. [c] Reference 2. [d] Reference 3. [e] Motekaitis et al., unpublished results. [f] Reference 4. [g] Reference 5. [h] Reference 6. [i] Reference 7. [j] Conditional constant for log K_{ML}. [k] Reference 8. [l] Conditional constant for log K_{M_2L}. [m] Reference 9.

Since the affinity of In^{3+} for OH^- is only a little lower than that of Ga^{3+} (log K_1 (OH^-) for In^{3+} = 10.0 *vs* 11.9 for Ga^{3+}),[1] the difference in stabilities of phenolate complexes is not adequately explained by the difference in basicity. An additional factor favoring higher stabil-

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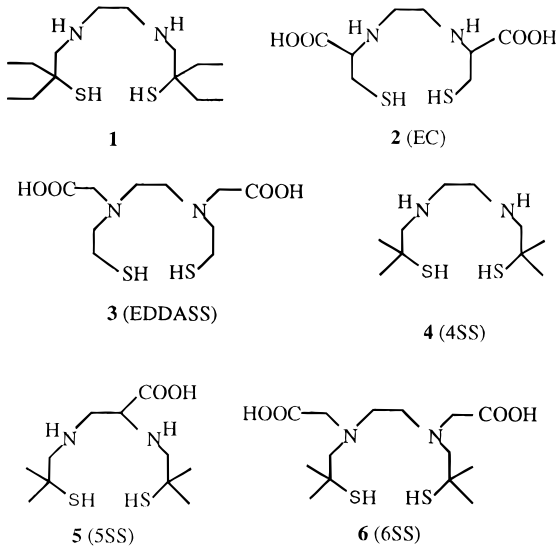
ity of the Ga(III) chelates is the fact that phenolate binding involves the formation of a six-membered chelate ring. It has been shown that six-membered chelate rings form more stable chelates with small metal ions[10,11] and have lower affinity for larger metal ions (the effective ionic radius for octahedral In(III) is 0.80 Å while that of Ga(III) is 0.62 Å[12]). Also, on the hard and soft acids and bases (HSAB) scale,[13,14] a very basic negative oxygen donor has higher affinity for a hard metal ion, such as Ga^{3+}, than for a less hard metal ion, such as In^{3+}. Accordingly, a less basic negative oxygen donor such as a carboxylate group binds more strongly to the In^{3+} ion than to the harder Ga^{3+} ion.

Mercapto (thiolate) groups are not "soft" in the HSAB sense, but may be regarded as intermediate and form complexes of high stability with metal ions such as In^{3+}. It also forms very stable complexes with the smaller and "harder" Ga^{3+} ion, but they seem to be slightly less stable than those of In^{3+}. While the strength of binding of these two metal ions is somewhat similar, the steric effects arising from the relatively large thiolate donor groups leads to different ligand selectivities, based on the fact that the In^{3+} ion is larger than Ga^{3+}.[12] An additional factor favoring coordination to the larger In^{3+} ion is the fact that mercaptoethyl groups form five-membered chelate rings, favoring complexation to larger metal ions.

Over the past 15 years, various kinds of bis(aminoethanethiol) [(BAT) or dithiadiaza (N_2S_2)] ligands were designed for coordination with ^{99m}Tc and were evaluated as radiopharmaceuticals.[15−25] Liu[26] studied the biodistribution of the ^{113m}In complex of *N*,*N*′-bis(2,2-diethyl-2-mercaptoethyl)ethylenediamine, (BAT-TE, **1**) while Kung[27] investigated the ^{68}Ga complex of a cyclohexyl analog of BAT-TE (BAT-TECH). Both ^{113m}In−BAT-TE and ^{68}Ga−BAT-TECH showed some myocardial uptake; however, subsequently, in dog studies the ^{68}Ga−BAT-TECH was shown to dissociate in plasma.[28] According to Liu *et al.*,[26] the ^{113m}In(III)−BAT-TE chelate has desirable biodistribution behavior and shows promise as a possible radiotracer for myocardial perfusion imaging. Cotsyfakis *et al.* prepared 10 different ^{111}In-labeled cationic amino thiol complexes and evaluated them *in vivo*.[29] Subtle changes in the ligand structure produced wide variability in biodistribution results. Although many of the complexes had significant myocardial uptake, the majority of the complexes had considerable liver and kidney uptake at 1 h postinjection.[29] Recently, Anderson *et al.*[30] reported the *in vivo* stability of *N*,*N*′-ethylene-di-L-cysteine, (EC), **2**, with Ga(III) and In(III). Sun *et al.*[31,32] reported that *N*,*N*′-bis(2-mercaptoethyl)-ethylenediamine-*N*,*N*′-diacetic acid (EDDASS), **3**, has unusually high affinity for In(III). This ligand has two mercaptoethyl groups replacing two carboxylates of EDTA.

To explore the potential of this kind of dithiadiaza-containing ligand for use in nuclear medicine, especially for the application of ^{111}In chelates as imaging agents, a series of multidentate ligands, *N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine, (4SS), **4**, 1-carboxy-*N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine, (5SS), **5**, and *N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid (6SS), **6**, were designed so as to contain two amino donors, two mercaptoethyl donors containing *gem*-dimethyl groups,



and a varying number of carboxyl groups. These ligands were synthesized, and the stabilities of their In(III) and Ga(III) complexes were determined. Molecular modeling was applied to the Ga(III) and In(III) complexes of **1**, **3**, **4**, **5**, and **6**, in order to understand their structures and *in vivo* stabilities. In addition molecular mechanics provided an approach to understanding the effects of the *gem*-dimethyl groups found in **4**, **5**, and **6**. The ligands **4**, **5**, and **6** as well as EDDASS were radiolabeled with $^{67/68}$Ga and ^{111}In, and their serum stabilities and biodistributions were determined in normal Sprague−Dawley rats. The biodistributions of ^{67}Ga and ^{111}In complexes of **3** were determined. Correlations between thermodynamic and *in vivo* behavior are discussed.

Experimental Section

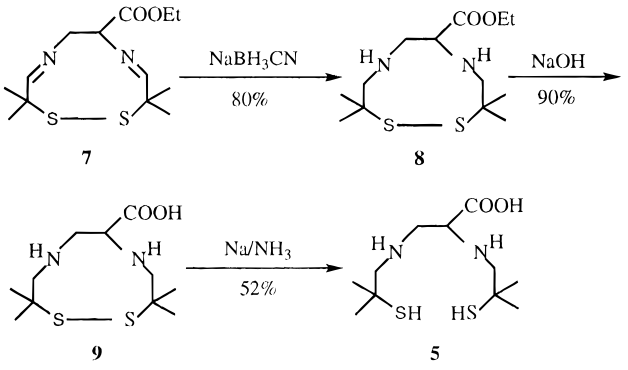
Synthesis and Characterization of the Ligands. Materials and Methods. Bromoacetic acid and sodium cyanoborohydride were obtained from Aldrich Chemical Co. and were used as supplied. Ligand **4**,[33] ethyl 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodeca-4,8-diene-6-carboxylate, **7**,[16] and 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodeca-4,8-diene, **10**,[33] were prepared by previously reported procedures.

The proton and carbon-13 NMR were recorded on a Varian XL-200 spectrometer operating at 200 MHz, and the chemical shifts are reported in ppm relative to tetramethylsilane. The mass spectra were obtained with the Departmental VG analytical 70S high-resolution double-focusing magnetic sector spectrometer with an attached VG analytical 11/250J data system. Measurements were made by the departmental mass spectrometry specialist, Dr. Lloyd W. Sumner. The C, H, N, and Cl analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

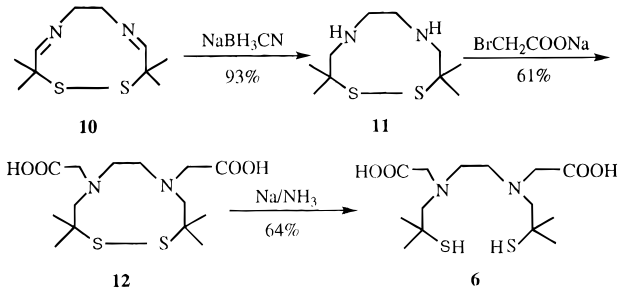
Synthetic Procedure. Outlines of the routes used for the synthesis of 5SS, **5**, and 6SS, **6**, are shown in Schemes 1 and 2.

Ethyl 3,3,10,10-Tetramethyl-1,2-dithia-5,8-diazacyclodecane-6-carboxylate (8). Compound **7** (1.5 g) was dissolved in 30 mL of methanol. Sodium cyanoborohydride ($NaCNBH_3$, 0.42 g) was added portionwise to this solution. The reaction solution was maintained at about pH 6.5 and was stirred at room temperature for 3 h and then acidified with 1 M HCl. The solvents were removed by evaporation. Water (40 mL) and 1:1 concentrated NH_3:H_2O were added to the residue until the pH reached 9.5. This alkaline solution was extracted with CH_2Cl_2. The organic phase was washed with saturated NaCl solution and then dried with anhydrous $MgSO_4$ for 16 h. After removing the drying agent and evaporation of the solvent, 1.06

Scheme 1



Scheme 2



g of colorless oil was obtained. ^{1}H NMR showed it to be the pure cyclic dithiadiaza ethyl ester, **8**. The hydrochloride salt was also made by the addition of 6 M HCl until pH = 1.5; total yield 80%. ^{1}H NMR of compound **8** (in $CDCl_3$): 1.19−1.46 (m, 15H, all CH_3), 2.2−3.2 (m, 6H, all CH_2NH), 2.37 (s, 2H, NH), 3.5−3.7 (m, 2H, CH), 4.12−4.25 (m, 2H, CH_2 of the ethyl). Anal. Calcd for $C_{13}H_{26}N_2S_2O_2 \cdot HCl \cdot H_2O$: C, 43.26; H, 8.10; N, 7.76. Found: C, 43.21; H, 8.21; N, 7.77.

6-Carboxy-3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (9). A solution of 14 mL of 0.5 M KOH in 95% ethanol was added to 1.7 g (0.0056 M) of the dithiadiaza ethyl ester, **8**, and the solution was stirred at room temperature for 1.5 h. To the reaction solution was added 14 mL of water, and the solution was stirred at room temperature for another 0.5 h. A 1.2 M HCl solution was added until pH = 10. The solvent was removed under reduced pressure, and a white residue was obtained. It was dissolved in 1.25 M HCl, and the insoluble material was separated by filtration, and was washed with several milliliters of cold dilute HCl solution. After air drying, 1.58 g of pure product was obtained, yield = 90%. ^{1}H NMR (in D_2O−NaOD, pH = 12.5): 1.01−1.20 (m, 12H, methyl), 2.1−2.4 and 2.6−2.9 (m, 6H, CH_2NH), 3.17−3.3 (m, 1H, CH). Anal. Calcd for $C_{11}H_{22}N_2O_2S_2 \cdot HCl \cdot 1.3H_2O$: C, 38.99; H, 7.63; N, 8.27. Found: C, 39.15; H, 7.42; N, 8.29.

1-Carboxy-*N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine (5). Sodium metal (1.5 g) was added to 60−70 mL of liquid ammonia under argon. Compound **9** (mono-HCl salt, 1.84 g) was added to the deep blue solution portionwise with vigorous stirring over a 30 min period. Solid ammonium chloride was gradually added until the reaction solution became colorless. The liquid ammonia was removed by evaporation. To the white residue was added 50 mL of water, and 2.5 M HCl was used to adjust the pH of the solution to about 1. The solvent was removed by evaporation, and 10 mL of absolute ethanol was added. The insoluble material, NaCl, was filtered off. To the clear ethanol solution was added 2 mL of 6 M HCl, and a colorless crystalline material gradually separated. It was filtered, washed with absolute ethanol and ethyl ether, and vacuum dried over P_2O_5 at 1−2 mmHg for 16 h, and 0.97 g product was obtained; yield 52%. ^{1}H NMR (in D_2O−NaOD): 1.32 and 1.34 (s, 12H, methyl), 2.53 (s, 4H, mercaptoethyl CH_2), 2.76−2.77 (m, 2H, ethylene CH_2), 3.23 (t, 1H, CH). ^{13}C NMR (D_2O−NaOD): 35.8 and 36.3 (methyl), 43.9 and 4.1 (CH_2 of ethylene), 54.8 (CH_2 of mercaptoethyl), 66.6 (CH), 66.4 and 67.8 (HSC), 184.1 (COOH). Anal. Calcd for $C_{11}H_{24}N_2O_2S_2 \cdot NaCl \cdot HCl \cdot 0.5H_2O$: C, 34.37; H, 6.82; N, 7.29. Found: C, 34.61; H, 6.62; N, 7.37.

5,8-Diaza-3,3,10,10-tetramethyl-1,2-dithiacyclodecane (11). The Schiff base **10** (4.6 g, 0.02 mol) was dissolved in 120 mL of methanol at room temperature and was reduced with $NaBH_3CN$ with a procedure similar to that used to prepare **8**. The product was extracted with ethyl ether and dried with anhydrous Na_2SO_4 for 20 h. After the ether was removed by evaporation, 4.6 g of colorless oil was obtained. ^{1}H NMR showed it to be pure diamine;[33] the yield is nearly quantitative. ^{1}H NMR ($CDCl_3$): 1.25 and 1.37 (s, 12H, methyl), 2.16 (s, 2H, NH), 2.55 and 2.61, 2.97 and 3.03 (two d, 4H, CH_2 of the ethyl), 2.02 (s, 4H, ethylene). The dihydrochloride salt was prepared by the addition of concentrated HCl and crystallization from its ethanol−H_2O solution; the yield was 5.7 g (93%).

5,8-Diaza-3,3,10,10-tetramethyl-1,2-dithiacyclodecane-*N*,*N*′-diacetic Acid (12). Compound **11** (2.95 g, 0.0126 mol) was dissolved in 95 mL of absolute ethanol. Bromoacetic acid, 3.85 g (0.0277 mol), was dissolved in 16 mL of ice-cold water. To this solution, cooled by an ice−water bath, was added a solution of 1.11 g (0.0277 mol) NaOH in 16 mL of water until the pH reached 12. The above two solutions were mixed and warmed to 40−42 °C. The pH of the solution was maintained at 11.2−11.5 with 2.5 M NaOH for 6−7 h. The ethanol was removed by evaporation until about 30 mL of solution remained. To this was added 45 mL of water, and the resulting solution was extracted with ethyl ether. The ether phase was discarded. The aqueous phase was acidifed with 6 M HCl to pH 2.5. A large amount of insoluble material separated. It was allowed to stand at 5.0 °C for 3 h, filtered, washed with cold water, and then dried over P_2O_5 at about 5 mmHg for 18 h. The product obtained amounted to 2.2 g. Another 0.04 g of product was obtained from the filtrate. The total yield was 61%. ^{1}H NMR (D_2O−DCl, pH = 0.3): 1.24 and 1.36 (s, 12H, methyl), 3.36 (s, 4H, ethylene), 3.18 and 3.26, 3.39 and 3.47 (two d, 4H, CH_2 of ethyl), 3.88 (s, 4H, CH_2COO^-). Anal. Calcd for $C_{14}H_{26}N_2O_4S_2 \cdot {}^1/_3H_2O$: C, 47.17; H, 7.48; N, 7.86. Found: C, 47.12; H, 7.60; N, 7.85.

***N*,*N*′-Bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-*N*,*N*-diacetic Acid (6).** Compound **12**, 0.7 g (0.002 mol), was added portionwise to a solution of 0.5 g of Na metal in 40 mL of liquid NH_3, and the solution was stirred for 30 min; then the reaction was quenched by the addition of NH_4Cl. The liquid NH_3 was removed by evaporation and then by vacuum evaporation at 10 mmHg at 25.0 °C. Dilute HCl was added until the pH was lowered to 2.5, and the solution was allowed to stand at 5.0 °C for 20 h. A large amount of white precipitate separated. It was filtered, washed with cold water, and vacuum dried over P_2O_5 at room temperature for 16 h. The pure product amounted to 0.45 g (yield 64%). ^{1}H NMR (D_2O−NaOD): 1.30 (s, 12H, methyl), 2.73 (s, 4H, ethylene), 2.83 (s, 4H, CH_2 of ethyl), 3.26 (s, 4H, CH_2COO^-). ^{13}C NMR (D_2O−NaOD): 33.0 (methyl), 42.6 (ethylene), 54.9 (CH_2 of ethyl), 60.5 ($HSCMe_2$), 71.6 (CH_2COO^-), 180.3 (COO^-). FABMS: [M + 1] = 353. Anal. Calcd for $C_{14}H_{28}N_2O_4S_2$: C, 47.70; H, 8.01; N, 7.95. Found: C, 47.46; H, 8.19; N, 7.87.

Other Reagents and Standard Solutions. Metal ion solutions of In(III) and Ga(III) were prepared at about 0.02 M from analytical grade chloride salts with demineralized water and were standardized by complexometric titration with EDTA and by cation exchange (Dowex 50W-X8 cation exchange resin, 20−50 mesh, hydrogen form).

Carbonate-free solution of the titrant, KOH, was prepared by dilution of analytical concentrate Dilut-It (J. T. Baker Chemical Co.) with dimineralized water under a stream of purified argon gas. The solution was standardized with potassium acid phthalate, and the extent of the carbonate accumulation was checked periodically by titration with a standard hydrochloric acid solution.

Potentiometric Equipment and Measurements. A Corning pH/ion analyzer 250 instrument was used together with a Model S-30056-10C Sargent Welch glass electrode and a Fisher 13-639-52 calomel reference electrode. A completely sealed 75 mL glass-jacketed titration cell was used, and the temperature, 25.0 ± 0.1 °C, was controlled with a Fisher Model

Table 2. Protonation Constants[a] and Formation Constants Used in the Ligand−Ligand Competition Experiments

EDTA		PLED[b]	
equilibrium quotient	log *K*	equilibrium quotient	log *K*
[HL]/[H][L]	9.93[c]	[HL]/[H][L]	10.89
$[H_2L]/[HL][H]$	6.05[c]	$[H_2L]/[HL][H]$	10.28
$[H_3L]/[H_2L][H]$	2.68[b]	$[H_3L]/[H_2L][H]$	7.20
$[H_4L]/[H_3L][H]$	2.07[b]	$[H_4L]/[H_3L][H]$	5.73
[InL]/[In][L]	24.90[b]	$[H_4L]/[H_4L][H]$	3.26
[InHL]/[InL][H]	1.5[b]	$[H_6L]/[H_5L][H]$	2.31
		[GaL]/[Ga][L]	32.31
		[GaHL]/[GaL][H]	7.10
		$[GaH_2L]/[GaHL][H]$	6.2

[a] Charges of individual species are omitted. [b] Reference 1. [c] Determined in this work; μ = 0.100 M KCl, t = 25.0 °C.

90 refrigerated bath. Atmospheric CO_2 was excluded from the cell during the titration by passing purified argon through the experimental solution in the reaction cell. The standard base was delivered through a capillary tip just under the surface of the solution by means of a 10-mL capacity Metrohm piston-type buret.[34]

Prior to each potentiometric equilibrium study, a calibration of the pH meter and electrode system was made using standard dilute HCl solutions at ionic strength 0.100 M adjusted with KNO_3 in the thermostated cell at 25.0 °C, so as to read hydrogen ion concentration directly. Thus, the term p[H] in this work is defined as $-\log [H^+]$. The value of $K_W = [H^+][OH^-]$ used in the computations was $10^{-13.78}$.[34] The 1:1 log formation constant of $InCl^{2+}$ is 2.3 at 20 °C (ionic strength 0.7), and that of $InNO_3^{2+}$ is only 0.18 under the same conditions.[1] Therefore in the present study, KNO_3 was used as the ionic medium in all systems at 0.100 M ionic strength.

The potentiometric equilibrium measurements were made on 40−50 mL of ligand solutions initially 5.30×10^{-3} M, first in the absence of metal ions and then in the presence of each metal ion for which $[L]:[M^{3+}]$ ratios were about 1.04:1. The p[H] values were measured after addition of 0.100 mL increments of standard KOH solution. The protonation constants of the ligands and the stability constants of the Ga(III) and In(III) chelates of 4SS and 5SS were obtained directly from the pH titration data. The protonation constants and hydrolysis constants of all metal chelates were calculated from the original p[H] profiles by methods described elsewhere.[34] Whenever the degree of formation of In(III) and Ga(III) complexes, even at low pH, was too high for the determination of stability constants by use of direct potentiometry, the ligand−ligand competition method was also performed. The stability constant of the In(III)−6SS complex was determined by EDTA−6SS competition and that of the Ga(III)−6SS complex by 6SS−PLED competition. The equilibria were confirmed by forward and back titrations. The protonation constants of ligands **4**, **5**, and **6** and the stability constants of In(III) and Ga(III) were obtained from the experimental data with the aid of the BEST program.[34] The species distribution curves were calculated and plotted with SPE and SPEPLOT programs.[34]

The four successive proton dissociation constants for $Ga(III)_{aq}$ ion included in the calculations are $10^{-2.91}$, $10^{-3.70}$, $10^{-4.40}$, and $10^{-5.77}$.[35] The first proton dissociation constant for $In(III)_{aq}$ is $10^{-4.28}$.[35] The protonation constants and formation constants used in the ligand−ligand competition experiments are shown in Table 2.

Preparation of In(4SS)Cl. To 0.0593 g of 4SS dihydrochloride was added 9.43 mL of 0.0191 M $InCl_3$ solution (containing 0.004 M HCl). Then, 0.59 g of KCl was also added to form a clear solution having p[H] 2.44. The p[H] was adjusted to 6.05 through the dropwise addition of 0.0931 M KOH solution. The white insoluble material that formed was isolated by filtration, washed with water, and dried over P_2O_5 under vacuum for 24 h; 0.05 g of white precipitate was obtained. Anal. Calcd for $C_{10}H_{22}ClN_2S_2In \cdot 1.2H_2O$: C, 29.56; H, 6.05; N, 6.90; Cl, 8.73. Found: C, 29.21; H, 5.58; N, 7.00; Cl, 9.14.

Radiochemistry. Materials and Methods. Sodium acetate was purchased from Aldrich Chemical Co. $^{111}InCl_3$ (specific activity, 419 Ci/mg, 50 mCi/mL, pH 1−2) was obtained from Mallinckrodt Medical, Inc., and $^{68}GaCl_3$ was obtained from a 20−35 mCi $^{68}Ge/^{68}Ga$ generator (duPont de Nemours). ^{67}Ga−citrate was from DuPont/NEN. HEPES (*N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid, 0.1 M) was prepared and the pH adjusted to 7.35. Distilled, deionized water (>18 MΩ/cm, Milli-Q, Millipore Corp.) was used throughout. Sprague−Dawley rats were purchased from Sasco (Omaha, NE).

Radio-thin layer chromatography (radio-TLC) was carried out using a BIOSCAN System 200 imaging scanner. Radioactivity counting was accomplished on a Beckman gamma counter containing a NaI crystal. Electrophoretic mobility was measured on Sepharose III cellulose acetate strips (Gelman Sciences) that had been presoaked in 0.1 M HEPES, pH 7.35. The electrophoresis was carried out in 0.1 M HEPES at constant current (1.33 mA/strip) for 1 h. The dianion, ^{111}In−DTPA, was used as a standard. A radiochromatogram scanner was used to determine the migration of the complexes.

Preparation of Radioactive Complexes. In the formation of all the ligand complexes, the acetate complex of the metal ion was first formed. $^{111}In(OAc)_3$ was prepared by diluting 1−10 μL of $^{111}InCl_3$ (0.1−1.0 mCi in 0.1 M HCl) to 500−1000 μL of 0.4 M NaOAc. $^{68}GaCl_3$ was eluted from a $^{68}Ge/^{68}Ga$ generator in 3 mL of 1 M HCl (20−35 mCi) in a robotically controlled remote system as previously described.[36] A 1.0 mL aliquot of the generator eluant was evaporated with heat under nitrogen to dryness, and 0.4 M NaOAc was added to the dried $^{68}GaCl_3$. ^{67}Ga−citrate (0.1−1.0 mCi) was evaporated to dryness with heat under nitrogen. HCl (6 M) was added to the dried ^{67}Ga−citrate, and the solution was extracted three times with ether. The ether was evaporated under nitrogen, and 0.4 M NaOAc was added to the dried $^{67}GaCl_3$.

For complexation with ^{111}In, the ligands 4SS, 5SS, and 6SS (2−3 mg) were dissolved in 250 μL of 0.4 M NaOAc (pH 5.5 for 5SS and 6SS and pH 6.0 for 4SS). $^{111}In(OAc)_3$ (250 μL; 0.4 M NaOAc, pH 5.5 or 6.0) was added to each ligand solution. Complexation occurred at room temperature within 30 min. For complexation with $^{67/68}Ga$, the ligands 4SS, 5SS, and 6SS were dissolved in 0.4 M NaOAc buffer (pH 3.0 for 4SS, pH 4.0 for 5SS, and pH 5.5 for 6SS). The ligand solutions were added to the dry $^{68}GaCl_3$ and incubated for 60 min at 90 °C, whereas $^{67}Ga(OAc)_3$ was added to the ligand solutions and incubated for 60 min at 90 °C. In the preparation of ^{67}Ga− and ^{111}In−EDDASS, the ligand was dissolved in 0.25 mL of 0.4 M NaOAc, pH 5.0 and ^{67}Ga− or ^{111}In−acetate was added to the ligand and incubated for 15 min at room temperature. Quality control was accomplished by radio-TLC using C18-coated plates in 100% methanol or Whatman no. 1 paper in 70:30 of 0.4 M NaOAc pH 5.0:EtOH. ^{111}In-labeled 4SS, 5SS, and 6SS were also analyzed using electrophoresis as described above.

Serum Stability Studies. The ^{111}In and ^{68}Ga complexes were incubated with freshly drawn rat serum in a 37 °C water bath for up to 1 h to determine serum stability. Aliquots of serum were removed at various time points from 2 to 60 min and analyzed by radio-TLC.

In separate experiments, adult, male, Sprague−Dawley rats were injected intravenously with 100 μCi of ^{111}In-labeled 5SS and 6SS and ^{68}Ga-labeled 4SS, 5SS, and 6SS. At various time points out to 1 h, a small volume of blood (250 μL) was withdrawn via cardiac puncture, and the blood was extracted with 250 μL of ethanol and centrifuged. The ethanol extract was analyzed by radio-TLC. A control was performed where the radiolabeled complexes were added directly to rat blood, and extracted with ethanol as previously described. The percent intact complex (as given in Table 6) takes into consideration the percent purity of the injectate, the percent extracted in the control, the percent extracted from the *in vivo* blood sample, and the percent intact in the ethanol extract.

Biodistribution Experiments. All animal studies were performed in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University's Animal Studies Committee. Adult, male rats were injected via the tail vein with 5 μCi of either ^{111}In-labeled 4SS, 5SS, and 6SS and in separate experiments with 50 μCi of

Table 3. Protonation Constants[a] of 4SS, 5SS, 6SS, and EDDASS (t = 25.0 °C, μ = 0.100 M KNO_3)

equilibrium quotient	4SS[b]	5SS[c]	6SS[d]	EDDASS[e]
[HL]/[L][H]	10.99	11.33	11.11	10.79
$[H_2L]/[HL][H]$	9.72	10.3	10.56	9.76
$[H_3L]/[H_2L][H]$	7.79	7.91	8.99	8.19
$[H_4L]/[H_3L][H]$	5.56	4.95	4.24	4.38
$[H_5L]/[H_4L][H]$		1.8	2.7	(1.4)

[a] Charges of individual species are omitted. [b] μ = 0.100M KCl. [c] σ_{fit} = 0.006. [d] σ_{fit} = 0.005. [e] σ_{fit} = 0.010

^{68}Ga-labeled 4SS, 5SS, and 6SS at 2, 5, 15, 30, or 60 min postinjection. The rats were sacrificed by first anesthetizing with Halothane, followed by decapitation, and then dissected. Samples of blood, muscle, liver, and bone were removed along with the entire lung, spleen, kidney, heart, and brain. The tissues were weighed and counted, and the percent injected dose per organ (% ID/organ) and per gram (% ID/g) were determined.

Molecular Modeling. Molecular modeling studies were performed with the commercially available modeling package SYBYL.[37] Force field parameters for bonds between N, O, and S donor atoms with both In(III) and Ga(III) were developed for SYBYL, from structures found in the Cambridge Structural Database.[38] Due to the relatively large size of the In(III) ion the valence force field approach was utilized in order to maintain an octahedral geometry around the ion. These parameters have been used in a study of EC complexes with both Ga(III) and In(III).[30] With both types of metal complex molecular modeling successfully predicted structure and relative stability.

Results

Synthesis of 4SS, 5SS, and 6SS. The synthetic routes for the preparation of 5SS, **5**, and 6SS, **6**, are outlined in Schemes 1 and 2, respectively. The preparation of BAT (bis(aminoethanethiol)) chelating skeleton generally involves the condensation of ethylenediamine or a derivative with 2,2′-dithiobis(2,2-dimethylacetaldehyde) to give the diimine sulfide **7** or **10**. The diimine was then reduced to the diamine with sodium cyanaborohydride, as seen in Schemes 1 and 2, to give **8** and **11**, respectively. The opening of the disulfide bond to the corresponding thiols was accomplished with sodium in liquid ammonia.

Protonation Constants. The protonation constants of 4SS, 5SS, and 6SS are shown in Table 3. The p[H] profiles, representing the original raw data, are given in the supporting information. The protonation constants were determined by direct potentiometric p[H] measurements, since all protonation reactions were observed to take place within the potentiometrically measurable p[H] range. Since the log protonation constants of cysteine are 10.29, 8.16, and 1.91, and for cystine 8.80 and 8.03, it is concluded that the two higher values of the protonation constants of these three ligands correspond to the protonation of the mercapto groups.

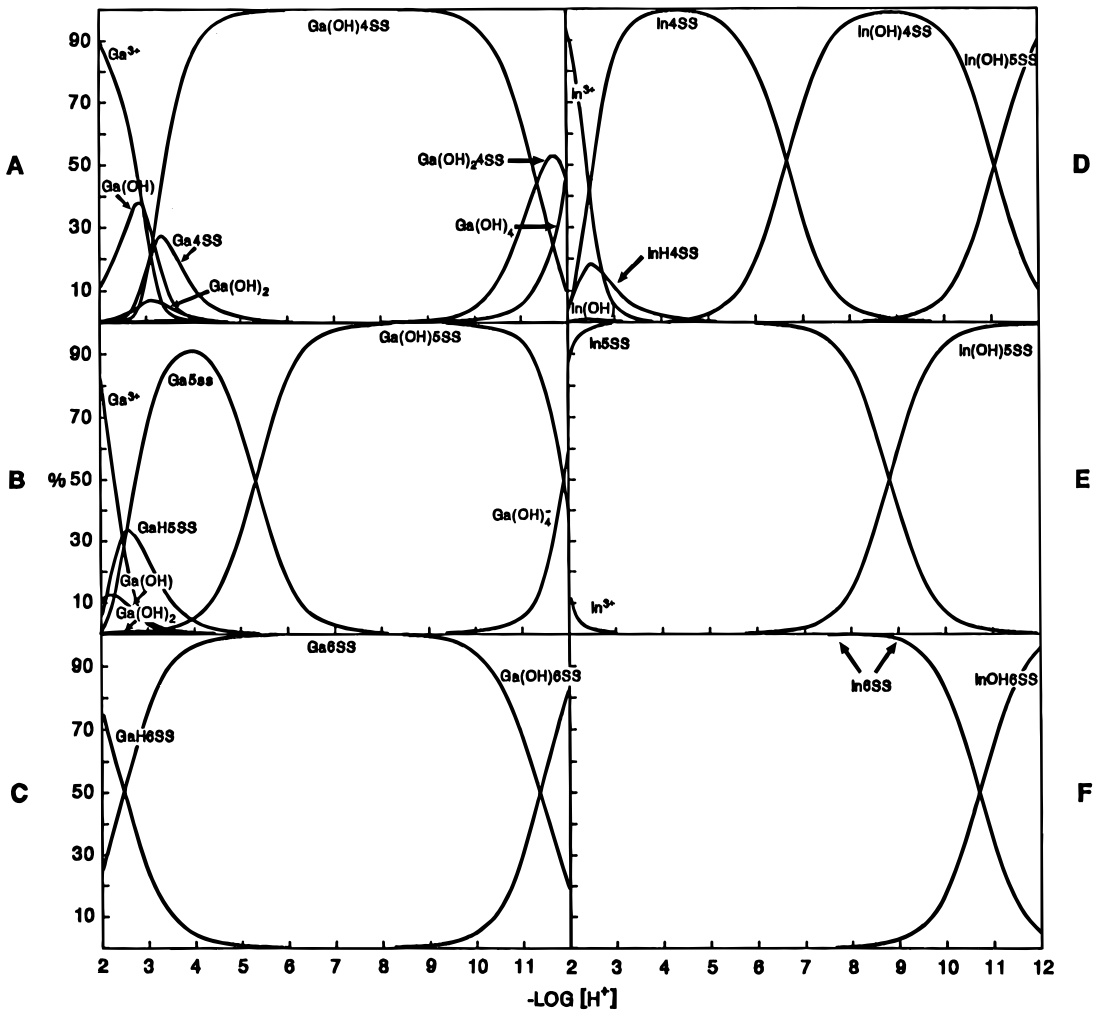


Figure 1. Species distribution curves of Ga(III)− and In(III)−4SS, −5SS, and −6SS. $T_{4SS} = T_{5SS} = T_{6SS} = T_{Ga(III)} = T_{In(III)}$ = 0.00200 M; μ = 0.100 M KNO_3, t = 25.0 °C. % equals percent of species present, with 100% = 0.00200 M.

Table 4. Equilibrium Constants[a] for In(III) and Ga(III) Complexes of 4SS, 5SS, 6SS, and EDDASS (t = 25.0 °C, μ = 0.100 M KNO_3)

equilibrium quotient	4SS	5SS	6SS	EDDASS[k]
	In(III)			
[ML]/[M][L]	27.34[b]	30.9[d]	29.8[g]	37.0
[MHL]/[ML][H]	2.1[b]			
[M(OH)L][H]/[ML]	−6.66[b]	−8.8[e]	−10.7[h]	
$[M(OH)_2L][H]/[M(OH)L]$	−11.1[b]			
pM	21.7	23.7[d]	30.9	30.4
	Ga(III)			
[ML]/[M][L]	24.73[c]	27.37[f]	41.0[i]	35.6
[MHL]/[ML][H]		2.6[f]	2.5[i]	2.4
[M(OH)L][H]/[ML]	−3.09[c]	−5.3[f]	−11.4[j]	−11.1
$[M(OH)_2L][H]/[M(OH)L]$	−11.3[c]			
pM	22.6	22.1	31.6	29.0

[a] Charges of individual species are omitted. [b] σ_{fit} = 0.010. [c] σ_{fit} = 0.020. [d] σ_{fit} = 0.006. [e] Estimated value. [f] σ_{fit} = 0.013. [g] σ_{fit} = 0.024. [h] σ_{fit} = 0.030. [i] σ_{fit} = 0.025. [j] σ_{fit} = 0.019. [k] Reference 31.

Stability Constants. The speciation curves of the 1:1 metal complexes of 4SS, 5SS, and 6SS with In(III) and Ga(III) and their stability constants are shown in Figure 1 and Table 4. The original titration data are given in the supporting information. The stability constants of the 4SS and 5SS of Ga(III) and In(III) were determined from the direct titration data, as described in the Experimental Section. Although 4SS is a tetradentate ligand, its affinity with In(III) at physiological pH is high enough to be comparable to the hexadentate ligand, EDTA (pM 21.7 for 4SS; 22.1 for EDTA). When KCl was used as the ionic medium for In(III), an insoluble material separated out from p[H] 4.5 to 7.5, which was isolated and proved to be the neutral complex, In(4SS)Cl. This information is useful in understanding the biodistributions of the In(III) complexes of this kind of ligand.

To obtain a neutral In(III) complex a carboxylate donor group was added to the ethylene backbone to form a pentadentate ligand, 5SS. A log K_{InL} of 30.9 was found and this neutral complex precipitated at p[H] above 4.5.

The distribution curves in Figure 1 reflect the relative stabilities of the metal chelates of 4SS, 5SS, and 6SS and their hydrolysis products (hydroxo complexes). The Ga(OH)4SS chelate predominates from about p[H] 3.1 to 9.0, while the nonhydrolyzed species become more important as the stability constants and charge of the ligand increases. Thus Ga−5SS predominates from p[H] 3 to 5 and Ga(OH)5SS$^-$ predominates from p[H] 5 to about 11.8. For Ga−6SS, the normal complex, containing no hydroxyl group, predominates over nearly the entire p[H] range (p[H] 2.5−11.2). The distributions of the In(III) chelates reflect the stabilities of In(III) chelates and show an analogous variation, with the nonhydroxylated form of the chelate becoming more predominant as the charge of the ligands and the stabilities of the In(III) chelates increase. The distribution of the In−6SS chelate is also extended at low p[H] by the lack of a protonated form of the chelate.

The 6SS ligand has very high affinity for both Ga(III) and In(III). The log K_{InL} constant was obtained by ligand−ligand competition with EDTA (see Figure 2). This stability constant is 2.8 log units higher than that of EDDASS, its analog without the *gem*-dimethyl groups. The species distribution plot (Figure 1) for the In(III)−6SS system shows 100% formation of the complexes over the range for pH 2−11. With 100% excess of ligand at physiological pH(7.4) the In(III) concentration is $10^{-30.9}$ M which is 6 orders of magnitude lower than that of the In(III)−DTPA complex (pM = 24.5) and is also lower than the value achieved by any other previously reported ligand. The log K_{GaL} of 6SS was determined by 6SS−PLED competition (Figure 2). The speciation curves of the Ga(III)-6SS system (Figure 1) also show 100% formation of the Ga−6SS complex at pH 5−9.

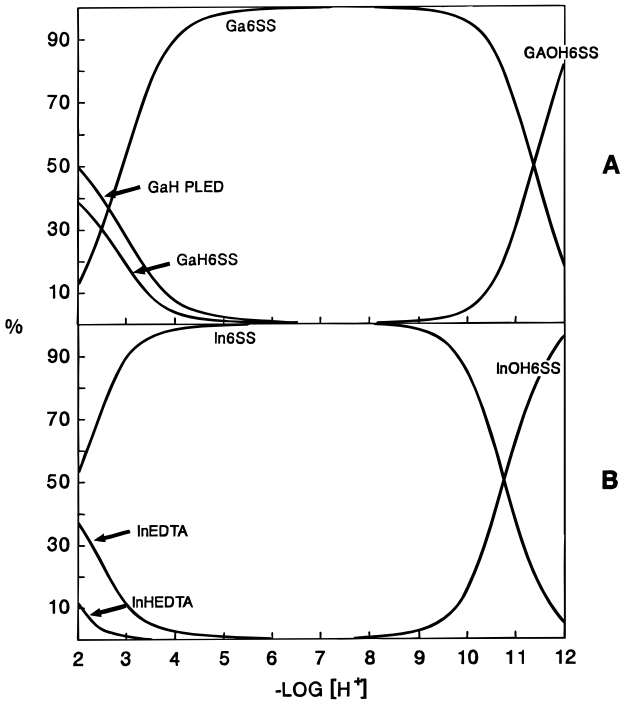


Figure 2. Species distribution curves in the Ga(III)−6SS−PLED and In(III)−6SS−EDTA systems: (**A**) T_{PLED} = 0.00188 M, T_{6SS} = 0.00222 M, $T_{Ga(III)}$ = 0.00205 M, μ = 0.100 M KNO_3, t = 25.0 °C, % equals percent of species present, with 100% = 0.00205 M; (**B**) T_{EDTA} = 0.00212 M, T_{6SS} = 0.00241 M, $T_{In(III)}$ = 0.00223 M, μ = 0.100 M KNO_3, t = 25.0 °C, % equals percent of species present with 100% = 0.00223 M.

Effects of *gem*-Dimethyl Groups in Ligand Design. An approach to understanding the *gem*-dimethyl effect can be taken by performing a molecular mechanics calculation on unsubstituted and *gem*-dimethylmercaptoethylamines (with the methyl substituents adjacent to the thiolate groups) in their extended *trans* forms. The preorganized-for-chelation *cis* rotamer conformations are shown in Figure 3. The result of this calculation shows that the uncomplexed conformers are more stable in their extended *trans* forms for both the unsubstituted model and the *gem*-dimethyl models. Since the *cis* rotamer is the conformation suitable for chelate formation, the calculation implies that in both cases there would be an increase in the strain energy upon chelation. However a comparison of the energy expended in going from *trans* to *cis* rotamers shows that in the *gem*-dimethyl case the net barrier is lower by over 2 kcal/mol. Thus, the ligand with *gem*-dimethyl groups would possess the higher stability constant, all other factors being equal.

Molecular Modeling of Complex Structure. Hancock and co-workers have utilized molecular mechanics to determine the relationship between ligand selectivity and metal ion size.[39−42] The technique utilized in these studies is the calculation of the complex strain energy as a result of the M−L bond length. This is ac-

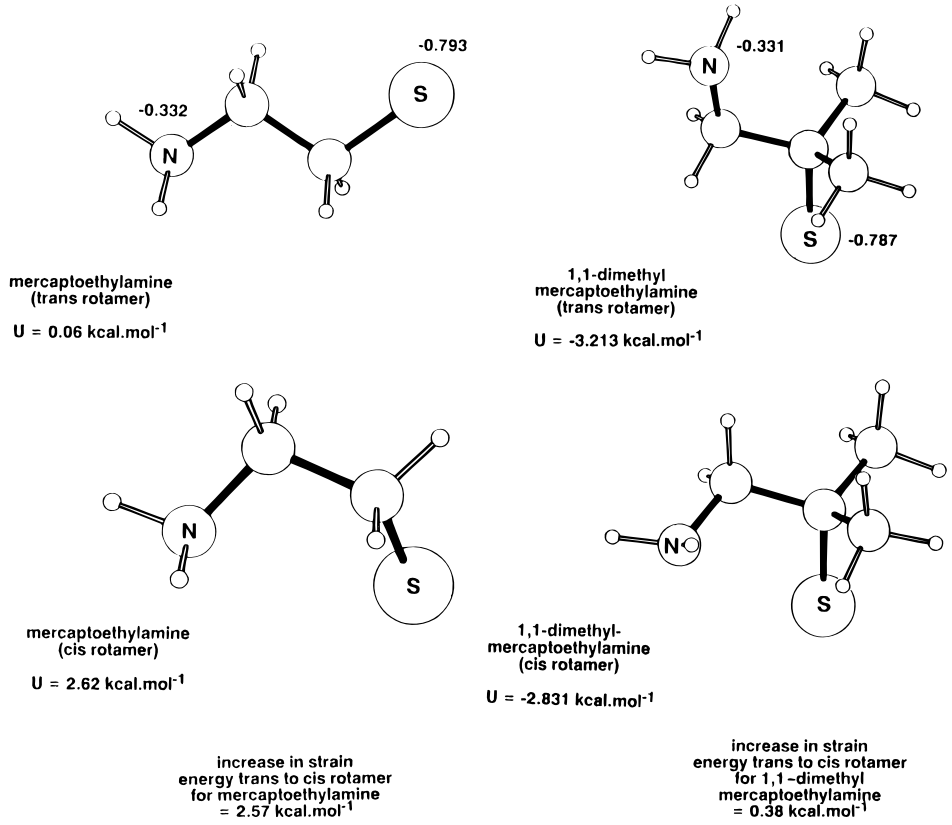


Figure 3. Effect of *gem*-dimethyl groups on conformers of mercaptoethylamine.

complished by modeling the complex with a generic metal while varying its ionic radius. The resultant curves give a minimum energy which corresponds to the best-fitting metal ion radius. In the "coordination scan", similar curves are generated by minimizing complexes with various numbers of water molecules coordinated to the metal ion while changing the M−L bond lengths.[43] The resultant curves are then plotted together and the intersection points, or "crossover points", examined. At the same time the curves also show the ligand selectivity based on ionic size.

Examination of the position of these crossover points in relation to the preferred ionic radius for the metal ion in a given coordination state indicates the preferred coordination number. In order for a given coordination state to be favorable, the ionic radius must be on the correct side of the crossover point. The closer an ionic radius is to a crossover point, the more the other coordination state contributes to the equilibrating system.

Using the coordination scan, each complex was made four, five, six, seven, and eight coordinate by the addition of water molecules to the metal ion. The metal's ionic radius was allowed to range from 0.3 to 1.5 Å. This accounts for the preferred coordination numbers and ionic radii for both gallium and indium.[12] The actual curves from the coordination scans may be found in Figure 4. Figure 5 shows the predicted structures of the gallium complexes of 4SS, 5SS, 6SS, and EDDASS. The crystal structure of Ga(III)−BAT-TECH[44] has been solved and in the solid state possesses a square-pyramidal geometry about the Ga(III) with a chloride filling one of the basal sites. This is in contrast to the predicted structure of Ga−4SS which is octahedral, with two water molecules filling vacant coordination sites. Despite the different coordination numbers, the structures appear quite similar, the only difference being the position of one thiol arm.

For the ligand 4SS the crossover point from four to five coordinate occurs with an ionic radius below 0.3 Å, thus both the Ga(III) and In(III) complexes will be found in a higher coordination state. The next crossover, from five to six coordinate occurs at approximately 0.56 Å. This suggests a coordination number of six for In(III) (radius 0.80 Å) and a coordination number of five (radius 0.55 Å) with a substantial contribution from a six coordinate species (radius 0.62 Å) for Ga(III).

The crossover point from five to six coordinate occurs at 0.75 Å for the ligand 5SS. This indicates that the Ga(III) complex will be five coordinate, while the In-(III) complex will bind a water molecule or some other ligand to form a six coordinate species.

The coordination scan for the complexes formed by 6SS show a crossover from six to seven coordinate at approximately 0.8 Å. This would indicate a six coordinate complex for Ga(III) while In(III) located at the crossover point will likely exist as a mixture of six and seven coordinate species. The scan for the complexes formed with EDDASS shows similar behavior. The crossover from six to seven coordinate occurs at 0.8 Å, again suggesting that the Ga(III) complex will be six coordinate while the In(III) complex will exist as a mixture of both six and seven coordinate species.

Radiochemistry. A summary of the radiochemistry conditions and results is presented in Table 5. The radiochemical purity of ^{111}In- and $^{67/68}$Ga-labeled 4SS, 5SS, and 6SS was routinely >90%. ^{67}Ga- and ^{111}In-labeled EDDASS were routinely >98% radiochemically pure. Using 100% methanol as the eluting solvent and C-18 TLC plates the R_f's of ^{111}In-labeled 4SS, 5SS, and 6SS were 0.59, 0.78, and 0.91, respectively, and that of ^{68}Ga-labeled 6SS was 0.84. In 70:30 ethanol:water and

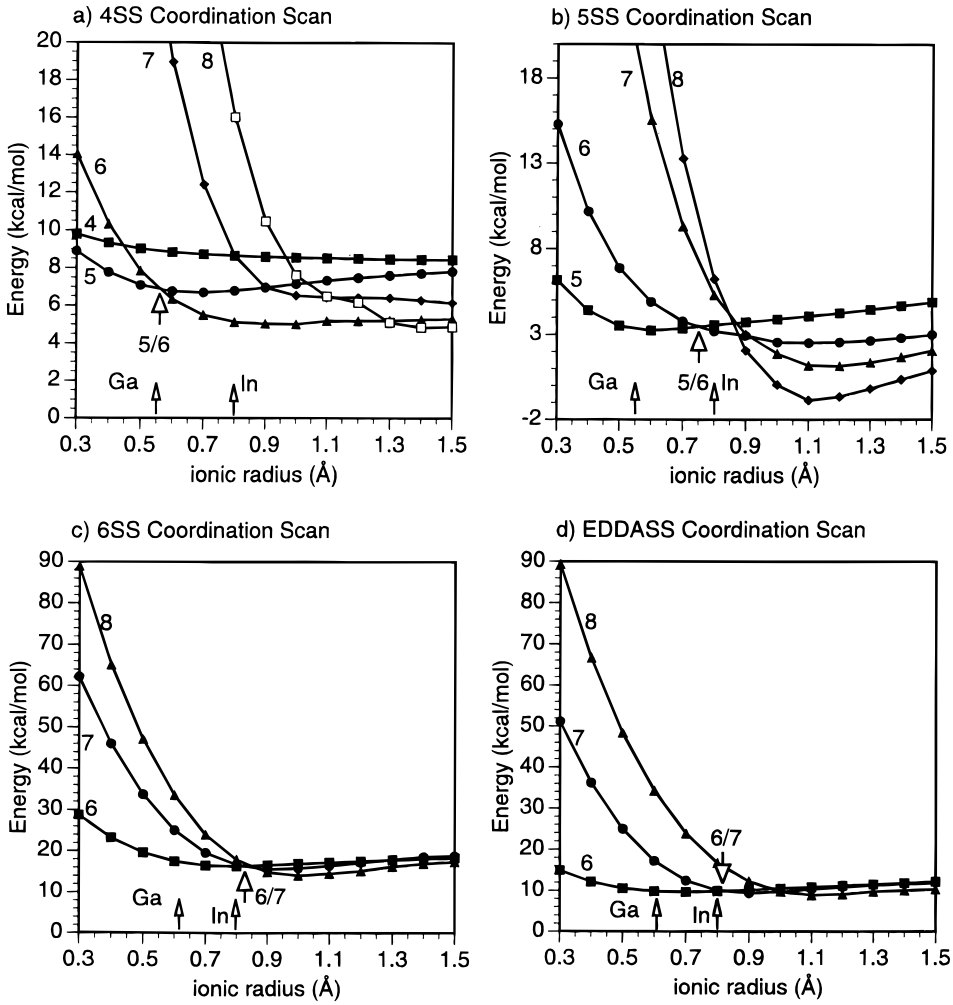


Figure 4. Plots of strain energy as a function of metal ion radius as calculated by molecular mechanics. All coordination numbers greater than that provided by the ligand were generated by coordinating the appropriate number of water molecules to the metal ion. The relevant metal ion radii and coordination crossover points are indicated with arrows on the plots.

Whatman no. 1 paper, $^{67/68}$Ga-labeled 4SS and 5SS had R_f's of 0.78 and 0.70, while ^{67}Ga- and ^{111}In-labeled EDDASS migrated with an R_f of 0.40 and 0.54. In both TLC systems, $^{111}In(OAc)_3$ and $^{67/68}Ga(OAc)_3$ had an R_f of 0. In electrophoresis experiments, ^{111}In-labeled 4SS and 5SS migrated slightly toward the cathode (R_f = 0.07). They are likely neutral complexes, since it has been reported that electroosmotic transport will cause neutral species to migrate toward the cathode.[45] ^{111}In−6SS migrated toward the anode with an R_f of −0.17, which suggests that the complex had a −1 charge; the standard used was the dianion ^{111}In−DTPA which gave an R_f of −0.45.

***In Vitro* and *In Vivo* Serum Stability.** The stabilities of $^{67/68}$Ga- and ^{111}In−labeled 4SS, 5SS, and 6SS were investigated in rat serum *in vitro*, and circulating metabolites were determined by removing blood from rats injected with the radiolabeled ligands. A summary of the results of these studies is presented in Table 6. ^{111}In-labeled 4SS was unstable in rat serum *in vitro* within 2 min after addition, while blood drawn from rats injected with ^{111}In-labeled 4SS showed complete metabolism by 60 min postinjection. ^{67}Ga-labeled 4SS showed moderate *in vitro* stability in rat serum, with about 50% intact complex present out to 30 min. The circulating metabolites of ^{67}Ga−4SS showed about 25−30% of intact complex from 2 to 60 min postinjection. ^{111}In-labeled 5SS was 66% intact in serum after 60 min, and circulating metabolites showed 52% intact complex at 2 min postinjection, decreasing to 19% at 60 min. Circulating metabolites of ^{68}Ga-labeled 5SS showed 60% of intact complex at 2 min, with 19% metabolites present at 60 min. ^{111}In-labeled 6SS was greater than 90% intact both *in vitro* in rat serum and *in vivo*. ^{68}Ga−6SS was 100% stable in serum and *in vivo* at 60 min postadministration.

Biodistribution Studies. Figure 6 shows the blood clearance from 2 to 60 min postinjection of ^{111}In- and ^{67}Ga-labeled 6SS, 5SS, 4SS, and EDDASS. All compounds cleared the blood rapidly, with the exception of ^{111}In−4SS. This is not unexpected as *in vitro* and *in vivo* serum stability data indicated ^{111}In−4SS was >85% uncomplexed 2 min postinjection. Liver clearance of all ^{111}In- and $^{67/68}$Ga-labeled complexes is shown in Figure 7. Both ^{111}In- and ^{68}Ga-labeled 4SS maintained high levels in the liver out to 60 min postinjection (11.3 ± 1.74 and 10.8 ± 0.46% ID/organ at 60 min, respectively). ^{111}In-labeled 5SS cleared less slowly (3.37 ± 1.16% ID/organ at 60 min), whereas ^{67}Ga-labeled 5SS was more rapidly cleared from the liver (1.70 ± 0.13% ID/organ at 60 min). ^{111}In- and ^{68}Ga-labeled 6SS both rapidly cleared the liver (0.79 ± 0.10 and 1.02 ± 0.22% ID/organ, respectively). The liver clearance of the ^{111}In- and ^{67}Ga-labeled six-coordinated EDDASS complexes are compared with those of 4SS, 5SS, and 6SS. ^{67}Ga-labeled EDDASS maintained higher levels of activity

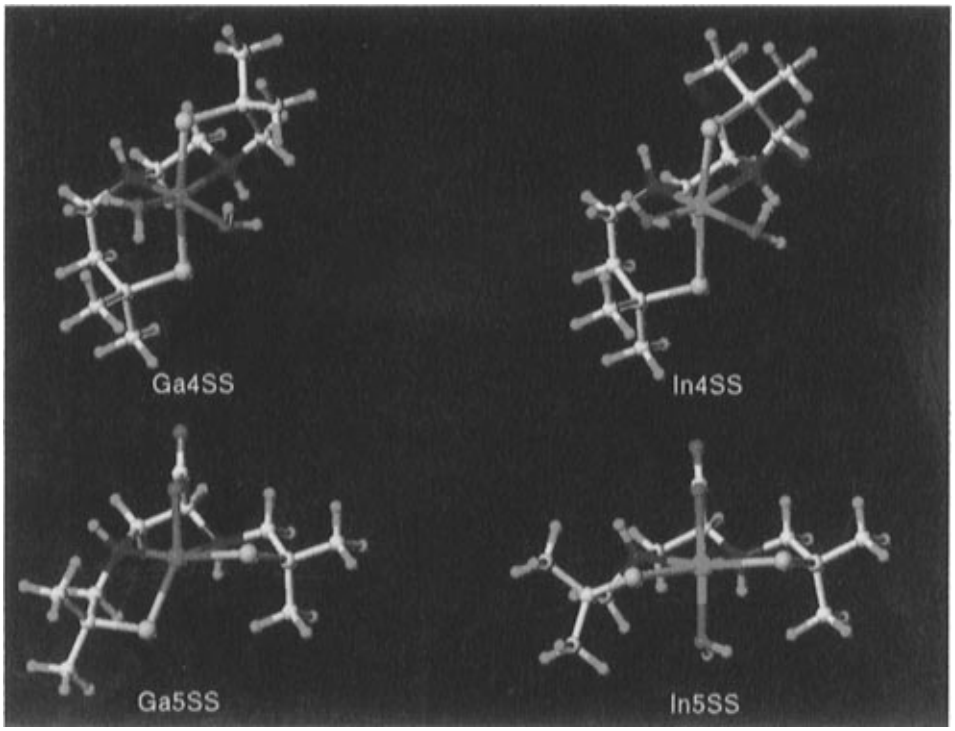


Figure 5. Structures of In(III) and Ga(III) complexes of 4SS and 5SS as predicted by molecular mechanics. Ga(III) is shown in magenta, In(III) in green, O in red, S in yellow, and C in white. Both complexes of 4SS are predicted to be six coordinate and have two water molecules bound to the metal ion. Ga−5SS is predicted to be five coordinate, whereas In−5SS is predicted to be six coordinate with one water molecule bound to the In(III) ion.

Table 5. Labeling and Quality Control Conditions for $^{67/68}$Ga- and ^{111}In-Labeled 4SS, 5SS, 6SS

complex	pH	time, min	temp, °C	TLC system	R_f	purity, %
$^{67/68}$Ga−4SS	3.0	60	90	Whatman no. 1 paper 3:1 EtOH:buffer	0.78	100
$^{67/68}$Ga−5SS	4.0	60	90	Whatman no. 1 paper 3:1 EtOH:buffer	0.70	90
^{68}Ga−6SS	5.0	60	90	C-18; 100% MeOH	0.84	98
^{67}Ga−EDDASS	5.0	15	rt[a]	Whatman no. 1 paper	0.40	100
^{111}In−4SS	6.0	30	rt	C-18; 100% MeOH	0.59	95
^{111}In−5SS	5.5	30	rt	C-18; 100% MeOH	0.78	95
^{111}In−6SS	5.5	30	rt	C-18; 100% MeOH	0.91	100
^{111}In−EDDASS	5.5	15	rt	Whatman no. 1 paper 3:1 EtOH:buffer	0.54	100

[a] rt = room temperature.

Table 6. Serum Stability of ^{111}In- and $^{67/68}$Ga-Labeled 4SS, 5SS, and 6SS *in Vitro* in Rat Serum and from Blood Extracted from Rats Postinjection of the Radiolabeled Ligands[a]

	in vitro serum stability			*in vivo* serum stability		
complex	2 min	15 min	60 min	2 min	15 min	60 min
^{67}Ga−4SS	83	85	0	30	30	25
^{111}In−4SS	4	0	0	13	5	2
^{67}Ga−5SS	98	100	100	60	58	19
^{111}In−5SS	84	74	66	52	22	19
^{67}Ga−6SS	100	100	100	100	100	100
^{111}In−6SS	100	95	92	100	100	100

[a] The data is presented as percent intact complex

in the liver than 5SS (4.27 ± 0.36 *vs* 1.70 ± 0.13% ID/organ at 60 min).

Other biodistribution data (at 15 min post-injection) are shown in Figure 8. None of the complexes exhibited significant brain uptake. Of the eight complexes, ^{111}In−4SS had the highest uptake in the heart and 15 min postinjection (1.69 ± 0.21% ID/g), but this is likely due to blood pool activity of ^{111}In−transferrin, as a result of the instability of ^{111}In−4SS. The blood activity of ^{111}In−4SS was ~6% ID/g. The myocardial uptake of

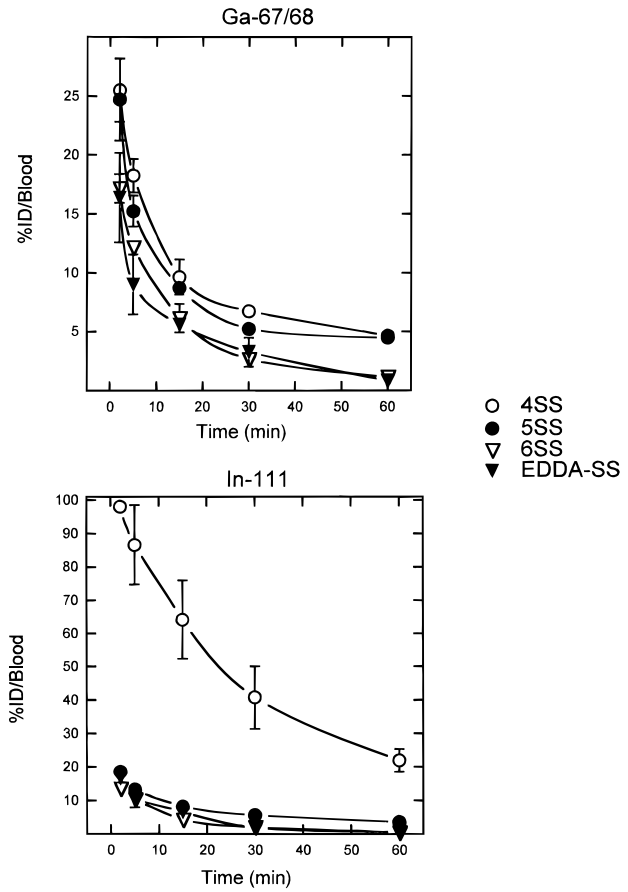


Figure 6. Blood clearance of $^{67/68}$Ga- and ^{111}In-labeled 4SS, 5SS, 6SS, and EDDASS in Sprague−Dawley rats (n = 4 for all time points). The data is presented as % injected dose/organ (mean ± sd).

^{68}Ga−4SS (0.75 ± 0.11% ID/g) was greater than the other complexes (with ^{111}In−4SS an exception), and the

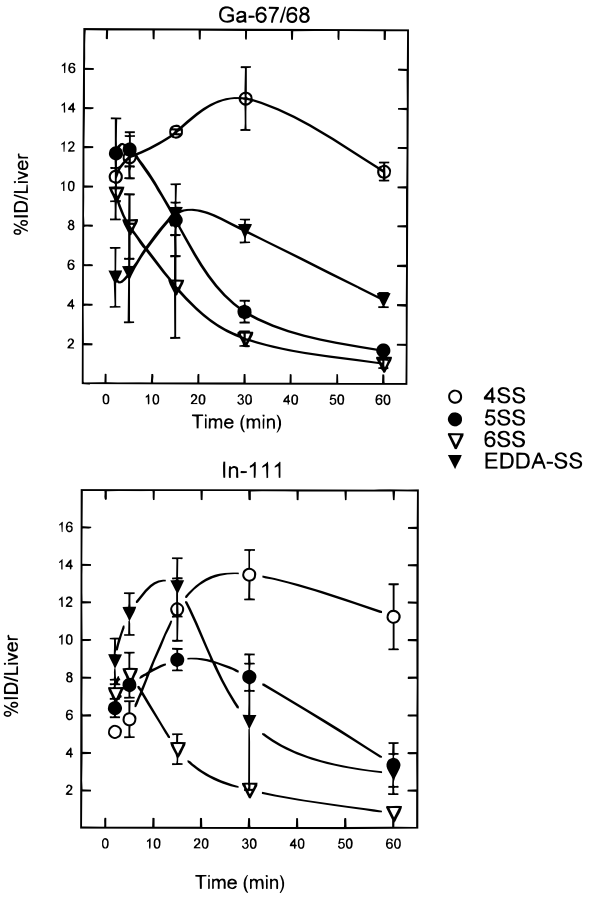


Figure 7. Liver clearance of $^{67/68}$Ga- and ^{111}In-labeled 4SS, 5SS, 6SS, and EDDASS in Sprague−Dawley rats ($n = 4$ for all time points). The data is presented as % injected dose/organ (mean ± sd).

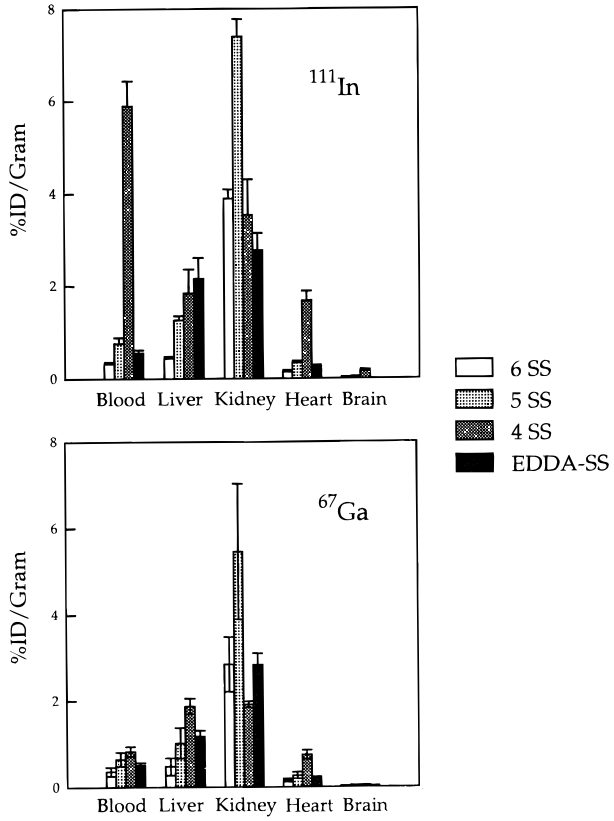
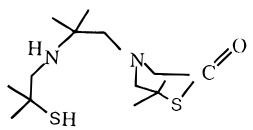


Figure 8. Biodistribution in selected tissues (% ID/g) at 15 min postinjection of $^{67/68}$Ga- and ^{111}In-labeled 4SS, 5SS, 6SS, and EDDASS ($n = 4$). The data is presented as % ID/g (mean ± sd).

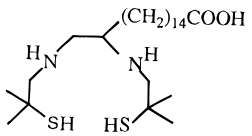
corresponding blood activity was ~0.8% ID/g. All other complexes had myocardial uptake of <0.4% ID/g at 15 min.

Discussion

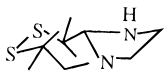
Synthetic Methods. Although more than 50 substituted bis(aminoethanethiol) (BAT) derivatives have been reported as ligands for radiopharmaceutical preparations, only **13**[17] and **14**[18] incorporated a carboxylate group in the tetraalkylated BAT skeleton. Reduction of the diimine formed as a precursor of **13** with sodium borohydride has been shown to result in ring closure to form the dicyclic imidazolidino[1,2-*d*] dithiapine, **15**.[46,47]



13



14



15

Lithium aluminum hydride can be used to reduce the Schiff base to the diamine; however at the same time carboxyl groups are reduced and the disulfide bond is opened. Thus, long and tedious routes were used to synthesize **13** and **14**. In 1992, Apparu *et al.*[33] reported that when $NaBH_4$ was used as the reducing agent and the product was worked up under acidic conditions (by the addition of NH_4Cl), the imine bond is reduced and a pure compound **11** was obtained with nearly quantitative yield. In our synthesis of ligand **5**, the $NaBH_4$−NH_4Cl reaction could not be employed to produce a pure sample of compound **8**. Sodium cyanoborohydride, $NaBH_3CN$, which is a milder and more selective reducing agent than $NaBH_4$ was used, and the Schiff base was smoothly reduced at pH 5−7. The excess reagent can be destroyed at low pH (1−2). Thus strongly basic conditions can be avoided, no bicyclic byproducts are formed, and the disulfide bond and the ester groups remain intact (Scheme 2). Another key step in the synthesis of ligands **5** and **6** is the last step: to open the disulfide bond and still keep the carboxylic acid group intact. For this purpose, sodium in liquid ammonia was found to be a successful reagent.

Protonation Constants. The mean of the first protonation constant in the series 4SS−6SS is 11.14. Since the average absolute deviation from the mean is only 0.12 log units, the addition of remote negative Coulombic charge in the form of carboxylates has little direct influence on the basicity of the first sulfido group. Of course the first protonation constant of EDDASS is 0.32 log units lower than that of 6SS, a direct consequence of basicity differences explained by the presence of *gem*-dimethyl groups (see below).

The range of the second protonation constants in this series is much wider, suggesting differing conformational pathways available upon successive protonations. The mean of 10.19 for the second log protonation constant is almost an order of magnitude less than the

mean of the first log protonation constant. These magnitudes and this difference is consistent with known sulfido protonation constants and remote Coulombic interactions. The mean deviation of 0.32 cannot be rationalized in detail with the information currently available. Because the two sulfur donors are spatially remotely situated, the statistical effect of ~0.6 log unit accounts for a large part (and in the case of 6SS the major part) of the difference.

The third and fourth protonation constants are ascribable to successive protonations on the nitrogen donors. In ethylenediamine derivatives the differences between the first and second log protonation constants are between 3.0 and 4.5 log units. The differences between the third and fourth protonation constants of 4SS, 5SS, and 6SS are approximately what would be expected for ethylenediamine derivatives, resulting from the Coulombic repulsion of one protonated nitrogen for the other. The fact that these protonation constants are considerably lower than those in ethylenediamine is probably due to hydrogen bonding between the sulfhydryl groups and the amino nitrogens, which would increase the first two protonation constants and decrease the third and fourth constants.

Stability Constants. The stability constants determined in this work show trends that are related to the increasing number of donor groups of the ligands. As expected, both In(III) and Ga(III) stability constants increase as the number of donor groups increases and as the negative charge increases in the series 4SS, 5SS, and 6SS. For both metal ions, the C-bound COO^- group is responsible for a ca. 3 log units and 4 log units for Ga(III). The stability constants determined for all complexes are also high when compared to chelates currently used to complex In(III) and Ga(III) (see Table 1). 4SS has a stability constant with In(III) and Ga(III) higher than those with EDTA, one of the more commonly used chelates for medical applications. 6SS has stabilities as high as any observed for ligands with a nonmacrocyclic backbone.

The values shown for 4SS and 5SS favor In(III) over Ga(III) by 2.6 and 3.5 log units, respectively. A similar result had also been noted in EDDASS, where the preference was 1.4 log units. In contrast, the 6SS ligand shows that Ga(III) is somewhat favored over In(III) by 1.2 log units.

It is not unexpected that both In(III) and Ga(III) complexes exhibit at least one hydrolysis constant M(OH)L. The absolute magnitudes of the hydrolysis constants of the normal chelates listed in Table 4 decrease (p*K*'s increase) in the series 4SS, 5SS, and 6SS as expected. Note that the value −8.8 for In−5SS(OH) was estimated from the titration curve in the presence of the insoluble In−5SS. In spite of stability constant differences discussed above, the pM values for corresponding Ga(III) and In(III) complexes are similar in magnitude. For 4SS the pM of Ga(III) is greater than that of In(III) since the contribution of the M(OH)L species outweighs the simple comparison of log K_{ML} values alone. There are no other exceptions in the relative ordering of pM vs log K_{ML}.

Effects of *gem*-Dimethyl Groups in Ligand Design. The increased stabilization of metal chelates caused by *gem*-dimethyl groups described above is supported in a recent report by Lightstone and Bruice.[48] Although *gem*-dimethyl enhancements of ring closure and suppression of ester hydrolysis have been observed in organic chemistry for most of this century, the explanation now offered is based on a similar calculation, but a more elaborate identification of all possible conformers and their energetic correlation with kinetic rate data and chemically logical transition states. Busch *et al.*[49] have introduced pairs of *gem*-dimethyl groups into the cyclidine framework with resulting dramatic increases of O_2 binding to the coordinated iron.

Regular studies of *gem*-dimethyl effects are generally absent in the equilibrium constant realm. However, Hancock and Martell[10] have compiled several examples which show the relationship between restricted rotation and the increase in binding of Ca^{2+}, Cu^{2+}, and Fe^{3+} when the backbone of EDTA and an ethylenediaminepolyol are substituted with alkyl or ring substituents. A search for *gem*-dimethyl-substituted ligands in the NIST database of critical stability constants reveals the 1,1-dimethyl (en) derivative of EDTA to be more strongly bound than the parent EDTA. More dramatically, the 1,1-dimethyl-NTA (acetate) stability constant for Ca^{2+} is 2 log units higher than that of the parent NTA. Although there are not many examples in the literature, it is clear that *gem*-dimethyl groups play an important role in both raising the lipophilicity of the ligand and certainly increasing the stabilities of metal complexes.

Comparison of the 6SS stability constants with the values given for EDDASS in Table 4 shows that the *gem*-dimethyl groups increase the stability of the In(III) chelate by 2.8 log units and increase the stability of the Ga(III) chelate by nearly twice as much (5.4 log units). It seems that the increase in stability caused by *gem*-dimethyl groups has a much greater effect on chelates of small metal ions than the increases observed with the chelates of large metal ions. More data are needed on the stabilities of metal chelates with *gem*-dimethyl groups or other substituents to further explore this interesting effect.

Biodistribution Studies. All $^{67/68}$Ga and ^{111}In complexes cleared the blood rapidly with the exception of ^{111}In−4SS. Although In−4SS has a higher thermodynamic stability than Ga−4SS, In−4SS was less stable in serum. In both *in vitro* and *in vivo* serum studies, ^{111}In−4SS was shown to be very unstable, with <15% intact complex present at 2 min postadministration. The biodistribution of ^{111}In−4SS is indicative of ^{111}In labeled to a protein such as transferrin, which clears the blood slowly, although the stability constant of ^{111}In−4SS would suggest that it may be stable *in vivo* (log K_{In-4SS} = 27.3 vs log K_{In-Tf} = 16.4 or 18.3). *In vivo* the transferrin concentration is much greater than the concentration of the ligand, and at equilibrium the transferrin complex will be favored. The rate of exchange of metal from the low-coordinated complex will be much faster than that of the 6SS and even EDTA where the stability constants are lower. With the exception of In−4SS, the initial biodistributions of the complexes are governed by the lipophilicity of the complex. Only the In−4SS exchanges rapidly with transferrin. Ligands such as 4SS and 5SS can therefore be used to functionalize biomolecules if the biological uptake were rapid. This is important in the design of agents to measure flow and receptor concentration. It is possible that for analogs of 4SS where the approach

of other ligands is hindered, such as Ga−BAT-TECH,[27] the In(III) four-coordinate complex would be more stable *in vivo*.

Molecular modeling studies suggest that stability in the presence of other ligands, as is found *in vivo*, will depend on the complex leading to a favored coordination state; those complexes with a filled coordination should be more stable than those requiring additional coordinating groups. The complex In−4SS wants to fill an additional two coordination sites, while Ga−4SS would require filling only one additional site. This would suggest that In−4SS would be more susceptible to attack by other donor groups than Ga−4SS which is consistent with the results of the serum stability studies found in Table 6 and the biodistribution data (Figure 8).

Ga(III) and In(III) complexes of 5SS, a ligand containing five donor atoms, had intermediate stabilities in serum *in vivo*. Similar to the 4SS complexes, the In(III) complex of 5SS has higher thermodynamic stability than Ga(III)−5SS; however ^{67}Ga−5SS appears kinetically more stable in serum, both *in vitro* and *in vivo*. The complex In−5SS will seek to fill an additional coordination site which should make it less stable than Ga−5SS. The complexes formed by the ligand 6SS require no additional coordination as both the In(III) and Ga(III) complexes are six coordinate. The differences in serum stability likely arise from differences in binding strength between Ga(III) and In(III).

The liver clearance of the $^{67/68}$Ga and ^{111}In complexes indicated that the more stable complexes are cleared rapidly, whereas the less stable complexes were retained in the liver. One possible explanation for this is that in the less stable complexes Ga(III) and In(III) are exchanging with proteins in the liver, whereas the more highly stable complexes can resist such exchange. The relationship between liver clearance and thermodynamic stability of Ga(III) and In(III) complexes has been previously observed by our group. The liver uptake over time of In(III) complexes of a series of phenolic ligands related to HBED were compared and a trend was observed showing the more stable complexes cleared the liver more quickly.[50]

In conclusion, it may be stated that of the chelating agents that were evaluated, those which form the most stable complexes of Ga(III) and In(III), and which in turn are cleared from the liver the most rapidly, would be the best candidates for forming bifunctional ligands which can be covalently linked to biomolecules. Bifunctional chelating agents offering the best chances of success would be derived from 6SS and EDDASS.

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Supporting Information Available: Force field parameters and potentiometric profiles employed for Ga(III) and In(III) complexes are presented (9 pages). Ordering information is given on any current masthead page.

Glossary

DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DTPA	diethylenetrinitrilopentaacetic acid
DTTA-HP	*N*,*N*″-bis(3-hydroxy-6-methyl-2-pyridylmethyl)diethylenetriamine-*N*,*N*′,*N*″-triacetic acid
EDDA-HP	*N*,*N*′-bis(3-hydroxy-6-methyl-2-pyridylmethyl)ethylenediamine-*N*,*N*′-diacetic acid
EDDASS	*N*,*N*′-bis(2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid
EDTA	ethylenedinitrilotetraacetic acid (ethylenediaminetetraacetic acid)
HBED	*N*,*N*′-bis(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid
N_3O-Ac_3	1-oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid
N_3O_2-Ac_3	1,7-dioxa-4,10,13-triazacyclopentadecane-4,10,13-triacetic acid
N_3O_3-Ac_3	1,7,13-trioxa-4,10,16-triazacyclooctadecane-4,10,16-triacetic acid
N_3O-HP	4,7,10-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1-oxa-4,7,10-triazacyclododecane
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid
PLED	*N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetic acid
TACN-HP	1,4,7-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclononane
TACN-TX	1,4,7-tris(3,5-dimethyl-2-hydroxybenzyl)-1,4,7-triazacyclononane
SHBED	*N*,*N*′-bis(2-hydroxy-5-sulfobenzyl)ethylenediamine-*N*,*N*′-diacetic acid
TETA	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
TRITA	1,4,7,10-tetraazacyclotridecane-1,4,7,10-tetraacetic acid
4SS	*N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine
5SS	1-carboxy-*N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine
6SS	*N*,*N*′-bis(2,2-dimethyl-2-mercaptoethyl)ethylenediamine-*N*,*N*′-diacetic acid
p[H]	$-\log[H^+]$ (the negative logarithm of the concentration of the hydrogen ion)
pH	$-\log a_{H^+}$ (the negative logarithm of the activity of the hydrogen ion)
T_χ	total concentration of a component χ in all its forms

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