

New Multidentate Ligands. 20. Isolation, Characterization, and Potentiometric Study of Metal Complexes of Bis[3-(((2-hydroxy-5-methylbenzyl)amino)methyl)-2-hydroxy-5-methylbenzyl]amine-*N,N',N''*-triacetic Acid

ISAO YOSHIDA, RAMUNAS J. MOTEKAITIS, and ARTHUR E. MARTELL*

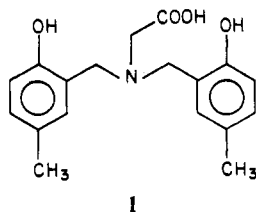
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A new multidentate ligand bis[3-(((2-hydroxy-5-methylbenzyl)amino)methyl)-2-hydroxy-5-methylbenzyl]amine-*N,N',N''*-triacetic acid (TGTC) containing four *p*-cresol units linked by three *N,N*-dimethyleneglycine bridges was isolated from a commercial oligomeric mixture of related compounds and studied for its aqueous coordination with representative divalent and trivalent ions including Cu^{2+} , Ga^{3+} , and Fe^{3+} . TGTC was found to possess two strongly protonated phenolic groups that were too weakly acidic to dissociate appreciably at the highest alkalinity attainable potentiometrically in aqueous solution. The pK 's of these two phenolic groups were obtained spectrophotometrically because of their high values. The remaining ligand dissociation constants, the metal complex formation constants, metal chelate protonation constants, and metal chelate phenolic deprotonation constants were measured potentiometrically. Some interesting and unexpected properties of this complex metal-ligand system are discussed.

Introduction

Ligands possessing a combination of phenolic and amino acetate groups in arrangements favorable toward metal ion complexation have very high stability constants with ions such as Ga(III), In(III), Fe(III), and other highly charged metal ions.¹⁻³ Negative phenolate donor groups are relatively "hard" and, therefore, are good candidates for substitution as structural groups for the coordination of "hard" metal ions in place of the "softer" carboxylate groups of ligands such as EDTA. The purpose of this investigation is to extend the chemistry of aqueous Ga(III) and In(III) to multidentate ligands having "hard" phenolate donor groups, for possible application of the complexes formed as radiopharmaceuticals. Ligands that bind these trivalent metal ions strongly are of interest since they will be relatively resistant to exchange with naturally occurring ligands in biological systems, especially those ligands that serve as natural carriers for the storage and transport of iron(III).

High-resolution NMR spectra had shown that the industrial sequestant "Hamplex" contains a mixture of oligomers in addition to its main constituent, *N,N*-bis(2-hydroxy-5-methylbenzyl)glycine (1). Since CPK models show that the

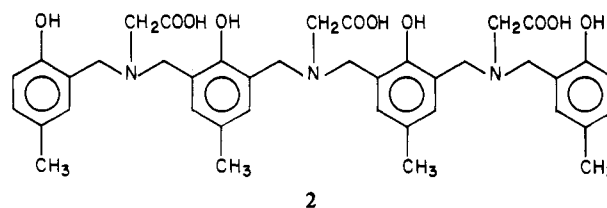


1

donor groups of this ligand, and its higher analogues, are capable of adapting to the coordination sphere of a metal ion, it was decided to isolate and investigate one of these higher molecular weight species, the "tetracresol triglycine" ligand bis[3-(((2-hydroxy-5-methylbenzyl)amino)methyl)-2-hydroxy-5-methylbenzyl]amine-*N,N',N''*-triacetic acid (TGTC) (2).

Experimental Section

Purification of Bis[3-(((2-hydroxy-5-methylbenzyl)amino)methyl)-2-hydroxy-5-methylbenzyl]amine-*N,N',N''*-triacetic Acid (TGTC). A 10.0-g sample of the ligand mixture, Hamplex PCG, was



2

dissolved in 800 mL of water, the pH was adjusted to 3.5 with 1 M HCl, and the mixture was diluted to 1 L and heated to near boiling. The solids remaining (~1 g) were removed, and the filtrate was placed in the refrigerator for 2 days. The solids that separated (~2 g) were removed, the filtrate was made alkaline (pH > 10) with 1 M NaOH, and the volume was reduced under vacuum to 30 mL. This solution was then treated with 1 M HCl to bring the pH down to 3.5, whereby the pure TGTC separated as fine powder after drying at about 60 °C under vacuum; yield 4 g. Anal. Calcd for $\text{C}_{40}\text{H}_{47}\text{N}_3\text{O}_{10} \cdot 3\text{NaCl} \cdot 3\text{H}_2\text{O}$: C, 50.08; H, 5.58; N, 4.38; O, 21.68; Cl, 11.09; Na, 7.19. Found: C, 50.03; H, 5.47; N, 4.37; O, 21.66; Cl, 11.07; Na, 7.48.

Potentiometric Measurements. Because of the extremely high pK 's of the ligand, the values for two of its protonation constants had to be determined from the analysis of UV spectral measurements made as a function of the amount of alkali needed to raise the pH to ≥ 14 . All other procedures and instrumentation have been described previously.⁴

Computations. Although this ligand has seven presumably dissociable protons per molecule (H_7L), it was decided to treat it as an H_3L ligand and express complex formation reactions involving dissociation of phenolic protons as proton displacement reactions. Even though the primary purpose of this is to circumvent the publication of formation constants that may be less accurate (measurement of the high pK 's involves a high degree of uncertainty), a secondary purpose was achieved: it avoided computer overflows in handling the computations. In addition, the division of the protonation steps is a natural one, since there are three basic nitrogens and four phenolic groups in the ligand. All formation constants were calculated from potentiometric data with the program BEST.⁵

Results

Ligand. The isolation of the ligand was difficult since the presence of many substances possessing similar chemical and physical properties precluded the development of a facile separation procedure. While, in retrospect, the final procedure described in the Experimental Section appears simple, it is a

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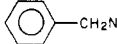
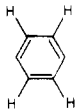
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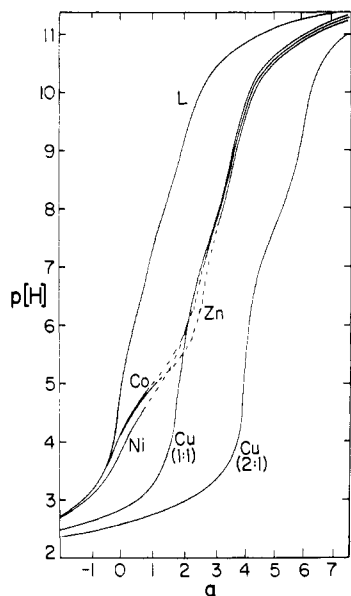
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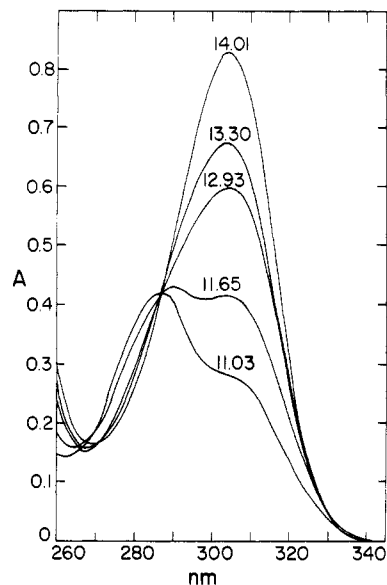
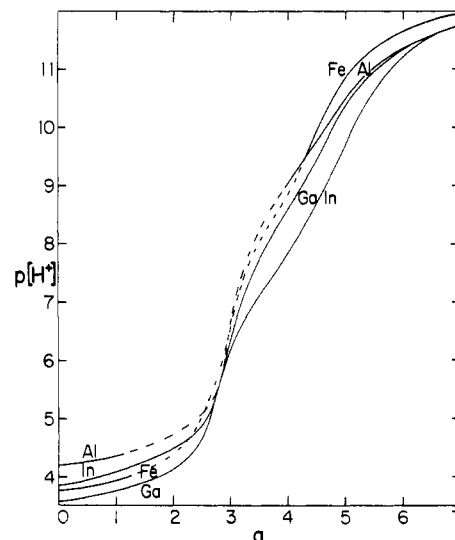
Table I. NMR Spectral Data (90 MHz) of Aqueous TGTC in Acidic and Alkaline Solutions vs. an Internal Me₄Si Capillary

group	δ	
	0.50 M DCl	0.50 M NaOD
CH ₃	2.35, 2.42 (12)	2.30, 2.36 (12)
CH ₂ COO	4.55, 4.66 (7.6)	3.37 (6)
	4.88-4.96 (12)	3.83 (11)
	6.87, 6.97, 7.16, 7.40 (10)	6.70, 6.78, 7.06, 7.14, 7.27 (10)

**Figure 1.** Potentiometric equilibrium measurement of $-\log [H^+]$ in solutions containing a 1:1 molar ratio of TGTC to Co(II), Ni(II), Zn(II), and Cu(II) and a 1:2 ratio of TGTC to Cu(II) at $t = 25.0$ °C, $\mu = 0.100$ M, and $T_L = 1.32 \times 10^{-3}$ M; $a = \text{mol of base/mol of ligand}$.

result of an extensive series of separation attempts that included many solvent combinations as well as chromatographic separations involving silica gel, ion exchange, and gel permeation. In general, these methods resulted in the isolation of products with unacceptable levels of impurities. Since the empirical formula was found to contain three NaCl and three H₂O molecules, it is reasonable, at this stage, to conclude that this formulation results in a sparingly soluble precipitate, which is the reason for its success. The identity of the compound was further confirmed by its high- and low-pH NMR spectra illustrated in Table I. The analyses of the NMR spectra obtained as well as the chemical shifts relative to known structures give chemical shift comparisons⁶ as a function of pH that are consistent with structure 2.

Protonation Constants of TGTC. The potentiometric curve for the ligand TGTC shown in Figure 1 features a steeply sloping buffer region from $a = 0$ to $a = 3$, indicating three almost nonoverlapping protonation equilibria. Above $a = 3$ the curve enters the highly alkaline region wherein the computer was able to sort out two addition dissociation constants with reasonable accuracy. Also, not immediately apparent is a low protonation constant below $a = 0$. Above $a = 5$, the data were analyzed by considering the variability of absorbance at 305 nm with pH. Typical data shown in Figure 2 show the variation of absorbance with dissociation of successive phenolic

**Figure 2.** Ultraviolet absorbance spectra of 5.0×10^{-5} M TGTC as a function of $-\log [H^+]$ at 305 nm, $t = 25.0$ °C, and $\mu = 0.100$ M.**Figure 3.** Potentiometric equilibrium curves of TGTC in the presence of equimolar concentrations of Ga(III), Al(III), Fe(III), and In(III) at $t = 25.0$ °C, $\mu = 0.100$ M, and $T_L = 1.32 \times 10^{-3}$ M; $a = \text{mol of base/mol of ligand}$.**Table II.** Protonation Constants and ϵ_n (L mol⁻¹ cm⁻¹) of TGTC^a

n	$\log k^n_H$	ϵ_n	n	$\log k^n_H$	ϵ_n
0		20500	5	9.33	1400
1	14.17	18800	6	7.50	1260
2	13.76	12100	7	5.10	1020
3	11.48	5300	8	1.96	1000
4	11.08	3060			

^a 350 nm; $\mu = 0.100$ M; $t = 25.0$ °C.

groups. From the appropriate simultaneous equations for mass balance and total absorbance

$$T_L = [L](1 + \beta_1[H] + \beta_2[H]^2 + \dots + \beta_7[H]^7) \quad (1)$$

$$A = [L](\epsilon_0 + \epsilon_1\beta_1[H] + \epsilon_2\beta_2[H]^2 + \dots + \epsilon_7\beta_7[H]^7) \quad (2)$$

the elimination of $[L]$ gives

$$A = T_L \frac{\epsilon_0 + \epsilon_1\beta_1[H] + \epsilon_2\beta_2[H]^2 + \dots + \epsilon_7\beta_7[H]^7}{1 + \beta_1[H] + \beta_2[H]^2 + \dots + \beta_7[H]^7} \quad (3)$$

On the basis of these relationships, β_1 , β_2 , ϵ_0 , ϵ_1 , ϵ_2 , ϵ_3 , ϵ_4 , ϵ_5 , ϵ_6 , and ϵ_7 were varied systematically until the sums of the

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Table III. Stability Constants of Mononuclear Complexes^{a,b}

equilibrium quotient	Cu(II)	Ni(II)	Co(II)	Zn(II)	Al(III)	Fe(III)	Ga(III)	In(III)
[ML]/[M][L]	23.27	10.30	9.80	10.41	15.29	25.00	19.20	16.65
[MHL]/[ML][H ⁺]	6.17	6.07	5.86	5.39	3.05	1.70		3.21
[MH ₂ L]/[MHL][H ⁺]		4.40	4.39	4.55				
[MH ₃ L]/[MH ₂ L][H ⁺]	5.02							
[MH ₋₁ L][H ⁺]/[ML]	-8.08	-8.02	-7.95	-7.72	-6.19	-4.54	-5.01	-5.73
[MH ₋₂ L][H ⁺]/[MH ₋₁ L]	-10.73	-10.35	-10.16	-9.91	-8.23	-7.54	-7.48	-7.17
[MH ₋₃ L][H ⁺]/[MH ₋₂ L]		-11.83	-11.73	-11.47	-10.02	-9.87	-9.60	-9.44
[MH ₋₄ L][H ⁺]/[MH ₋₃ L]					-11.43	-11.05		-10.99

^a $\mu = 0.100$ M; $t = 25.0$ °C. ^b $\log K$ values for binuclear complexes: [Cu₂L]/[Cu][CuL] 15.04; [Cu₂H₋₁L][H⁺]/[Cu₂L], -3.07; [Cu₂H₋₂L][H⁺]/[Cu₂H₋₁L], -6.60; [Cu₂H₋₃L][H⁺]/[Cu₂H₋₂L], -7.97; [Cu₂H₋₄L][H⁺]/[Cu₂H₋₃L], -10.13; [Cu₂HL]/[Cu₂L][H⁺], 2.47; [Cu₂H₂L]/[Cu₂HL][H⁺], 2.74.

squares of the deviations between observed and calculated values of A at all pH values measured were minimized. As expected, the values of ϵ_5 , ϵ_6 , and ϵ_7 were almost identical with the directly computed values at low pH.

The combined results from both potentiometry and spectroscopic analysis for the protonation constants for this ligand are listed in Table II.

From a microscopic point of view, the values of the protonation constants for the cases of $n = 5-7$ should correspond mainly to nitrogen protonation, the case where $n = 8$ is the protonation of any of the three glycine carboxylate moieties to give a monoprotonated ion, while the cases where $n = 1-4$ are essentially the phenolic protonation constants. It appears that addition of base corresponding to pH 14.2 was just sufficient to displace half of the final phenolic-bound proton from the hexanegative ligand.

Nevertheless, the variation of the increase in ϵ that occurs in the conversion of one species to the next less protonated form is an indication of the participation of microspecies involving functional groups other than phenolate donors. Unfortunately there are no suitable reference compounds possessing analogous (*o*-hydroxybenzyl)amine groups. A well-known bis(*o*-hydroxybenzyl) ligand, HBED, has been reported to have absorptions at $\lambda = 294$ with the following extinction coefficients for the three highest pH species: $\epsilon_{1+} = 8300$, $\epsilon_{HL^-} = 4000$, $\epsilon_{H_2L^{2-}} = 650$. Since the pK_a 's of HBED are somewhat more separated than those of the ligand reported here, the former is estimated to have an increase in ϵ of about 3400 for the first phenol deprotonation, with an overall cumulative effect of an increase of 4300 per deprotonation. Similarly, the UV spectrum of ((*o*-hydroxybenzyl)imino)diacetic acid also shows $\epsilon_0 = 4028$, $\epsilon_1 = 292.7$. Precise intensity comparisons cannot be made since TGTC possesses *p*-methyl substituents and bis(methylene)glycine bridges, which would be sufficiently interactive to affect the intensities of the individual phenolate extinction parameters. The overall increase in ϵ_0 of about 20 500 is about right, however. A full analysis of the relative concentrations of microspecies cannot be carried out at this stage without detailed spectroscopic data, which are currently not available. Therefore, further elaboration of the microspecies formed as a function of pH for this complex ligand will not be attempted at this time.

Complexes of Divalent Metal Ions. The potentiometric data for the 1:1 molar ratio of metal ion to ligand are shown as continuous curves in Figure 1 for Cu(II), Ni(II), Co(II), and Zn(II). The features of each curve are similar, except that of copper(II), which shows unusually high stability even below $a = 0$. However, in each case the species present are also of similar stoichiometries. Thus, the formation region involves the presence of MH_2L^+ and MHL^0 species, which subsequently dissociates to ML^- , which then undergoes further deprotonation until the monoprotonated complex MH_3L^{4-} is

formed. The remaining phenolic group does not dissociate, even at high pH in the presence of divalent metal ions. The values for the various stability constants thus contained are listed in Table III. Although the major trends follow the expected spectrochemical order, the unusually large value for $K_{ML}(Cu^{2+})$ relative to those of Ni(II), Co(II), and Zn(II) reveals a difference in coordinate bonding that cannot be ignored. Examination of space-filling models shows that the ligand may easily adapt to a configuration whereby two (or three) phenols as well as an amino nitrogen and an acetate group form a favorable coordination pocket. Such a complex, "CuL", would still possess two protonated phenol groups as well as two protonated nitrogens. Putting aside any doubt that this is indeed the case, attention is called to the analogy that $\log K_{ML}(Cu^{2+})$ for glycine is only 8.13⁸ while that of HBED¹ is 21.38. In the latter case it had been shown that two phenolate oxygens and two basic amino groups participate in coordinate bonding to Cu(II) and that as the pH is lowered, stepwise displacement of Cu(II) from coordinate phenolate groups through protonation has been observed.

It should be pointed out that Ni(II), Co(II), and Zn(II) are also involved in phenolate coordination, although to a lesser degree. The logarithm of the stability constants of the glycine chelates of these metal ions are in the range of $\sim 5-5.7$, so it seems that at least one phenoxide coordination is involved in the formation of the complex ML with these metal ions. This interpretation is supported by the fact that the $\log K_{MLH}^{M}$ of NiHBED of 15.36, with one phenolic group bound, is 5 log units larger than that of the Ni(II)-TGTC chelate. Harris et al.⁷ have shown that the participation of a phenoxide donor increases the stability by about 5.6-5.9 log units relative to coordination by the donor groups of iminodiacetic acid alone. Another rough estimate for the $\log K$ for participation of an additional phenolic group in Ni(II) coordination is ~ 5.5 ($\log K(NiHBED) = 19.3$, $-\log K(\text{nickel } ((o\text{-hydroxybenzyl})\text{imino})\text{diacetate}) = 13.8$). If the phenoxide term is additive, then it follows that $4.8[\log K(NiL(N,N\text{-dimethylglycine}))] + 5.6[\text{phenoxide participation}] = 10.4$, a value which is numerically almost equal to the observed TGTC interaction listed in Table III. Similar estimates may be made for coordination of Co(II) and Zn(II). It is also significant that, even in these complexes, the pK_a for the dissociation constant of the last proton of the coordinated ligand was found to be too high to be measured potentiometrically. This is not entirely surprising in view of the low charge of the metal ion, the large negative ligand charge, and the decadentate nature of the ligand.

The stability constant for the addition of a second Cu(II) ion to the 1:1 Cu(II) complex is $10^{15.04}$, which is about 8 orders of magnitude lower than that of the mononuclear complex. Reasoning similar to that employed above leads to the interpretation that perhaps one additional phenolate is involved in Cu(II) coordination, together with participation by the co-

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ordination of the second metal ion by other donor groups present in the ligand. Binuclear coordination is probably also associated with redistribution of some of the donor groups associated with Cu(II) in the mononuclear complex, thus contributing to the lower formation constant for the second step. The 2:1 Cu(II) complex also undergoes two protonation reactions ($\log K^H_{M_2HL} = 2.47$, $\log K^H_{M_2H_2L} = 2.74$) and a series of four deprotonation reactions ($\log K^{-H}_{M_2HL} = -3.07$, $\log K^{-H}_{M_2LH_2} = -6.60$, $\log K^{-H}_{M_2LH_3} = -7.97$, $\log K^{-H}_{M_2LH_4} = -10.13$). The first three deprotonations probably involve the displacement of N-bound protons, in view of the implications made above that each Cu(II) in the binuclear complex is bound to one or two phenolate donors.

Tripositive Metal Ions. From the equilibrium constants shown in Table III, it is apparent that the trivalent ions form a group of their own and are therefore discussed together. The Fe(III) chelate is seen to have the highest stability constant, closely followed by Ga(III), In(III), and Al(III). Since the $\log K_{ML}$ of Fe(III) (25.0) is much lower than that of HBED (39.8), it follows that the coordinate bonding of Fe(III) in the TGTC chelate may be similar to that of Cu(II) with two phenolate groups bound. However, there must be some structural differences, since the Fe(III) and Ga(III) chelates do not undergo protonation as readily as is the case with

Cu(II). Besides a weak one for Fe(III), a protonated chelate MHL was found for Al(III) and In(III) as well as for all the chelates of the divalent metal ions. At higher pH values, it seems that Fe(III) has greater affinity for phenolate donor groups, as is evidenced by the lower proton affinity of FeL ($\log K = -4.54$) as compared to those of Ga(III), In(III), and Al(III). Since subsequent dissociation reactions show little discrimination between the trivalent metal ions, these reactions must also take place at remote sites such as the amino donor atoms.

It is unfortunate that stability constant information for metal complexes of other chelating ligands having a single phenolic group are almost nonexistent. Such data would be useful in sorting out the coordination possibilities with more complex ligands such as TGTC. In future studies by this group, the reactions of trivalent metal ions with sexadentate or highly polydentate ligands having only one phenolic donor group will be investigated.

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Contribution from the Mass Spectrometry Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts 02115

Synthesis and Characterization of Hexakis(alkyl isocyanide) and Hexakis(aryl isocyanide) Complexes of Technetium(I)

MICHAEL J. ABRAMS, ALAN DAVISON,* ALUN G. JONES, CATHERINE E. COSTELLO, and HENRIANNA PANG

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The complexes $[Tc(CNR)_6]PF_6$ (where R = *tert*-butyl, methyl, cyclohexyl, and phenyl) have been prepared by reduction of the pertechnetate ion with aqueous $Na_2S_2O_4$ in the presence of the isocyanide ligands. These complexes have been characterized by elemental analysis, optical, infrared, and 1H NMR spectroscopy, conductance, cyclic voltammetry, and field desorption mass spectrometry.

Introduction

The widespread use of the metastable isotope of technetium (^{99m}Tc) in diagnostic nuclear medicine procedures is well documented.^{1,2} This, in part, is due to the favorable nuclear properties of this isotope ($\gamma = 140$ keV, $t_{1/2} = 6$ h) that allow γ camera images of high resolution to be obtained with a low-radiation dose to the patient. During the last 7 years, the use of macroscopic amounts of the readily available long-lived radionuclide ^{99}Tc , a β -particle emitter ($t_{1/2} = 2.12 \times 10^5$ years), has enabled substantial progress to be made in the basic chemistry of this element. Much of this work has been directed toward the investigation of kinetically inert complexes formed in oxidation states that are readily accessible in aqueous media by the reduction of the pertechnetate ion. These studies have demonstrated that it is possible to prepare classes of stable complexes in both the V and the III oxidation states with the appropriate choice of ligands.^{3,4}

Recently, we reported the preparation of hexakis(*tert*-butyl isocyanide)technetium(I) hexafluorophosphate from the reaction of hexakis(thiourea-S)technetium(III) chloride with *tert*-butyl isocyanide in refluxing methanol.⁵ We now report

the synthesis and characterization of this and related complexes by direct reduction of the pertechnetate ion in aqueous ethanol with sodium dithionite ($Na_2S_2O_4$) in the presence of the isocyanide ligands.

This in turn has enabled us (a) to synthesize this class of air- and water-stable complexes at tracer concentrations (ca. 10^{-8} – 10^{-9} M) with metastable ^{99m}Tc , (b) to begin to evaluate the biological distributions of these complexes in animals, and (c) to establish structure-function correlations on a potentially large class of well-characterized complexes. We have demonstrated that certain members of this class have potential use in diagnostic nuclear medicine for lung perfusion studies, imaging of normal myocardial tissue, the detection of vascular

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* To whom correspondence should be addressed at the Department of Chemistry, Massachusetts Institute of Technology.