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New Multidentate Ligands. 29. Stabilities of Iron(III) and Gallium(III) Complexes of a Tris(hydroxamate) Cryptand and Two Open-Chain Analogues

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Two open-chain tris(hydroxamic acid) analogues of a tris(hydroxamic acid) cryptand have been synthesized. The stability constants of these two ligands and of the cryptand with Fe(III) and Ga(III) are reported. The stability constants of the tris(hydroxamate) chelates are found to be lower than those of the Fe(III) and Ga(III) cryptates.

Introduction

This research group has been actively involved in the design, synthesis, and characterization of synthetic hydroxamate-containing siderophores, especially macrocycles containing endocyclic hydroxamate groups.[1-3] The first tris(hydroxamate) cryptand was recently synthesized in this laboratory.[4,5] For further exploration of structure–stability relationships and the macrocyclic and cryptate effects of hydroxamate ligands, two open-chain analogues of the tris(hydroxamate) cryptand were synthesized, one with terminal acetyl groups and the other with terminal methylhydroxylamino functions. The stability constants of the Fe(III) and Ga(III) chelates of these synthetic ligands have been measured and are compared with those of desferriferrioxamine B and other related ligands.

Experimental Section

N-Methylhydroxylamine hydrochloride salt, benzyl bromide, and silica gel 60 were purchased from Aldrich Chemical Co. Formic acid (88%) and chloroform were obtained from Fisher Scientific; methyl alcohol and ethyl ether were from Mallinckrodt and were used without further purification. Acetyl chloride, benzoyl chloride, and pyridine were purified by conventional methods.[6] Benzene was dried with activated 4A molecular sieves. *N*-Methyl-*O*-benzylhydroxylamine was prepared by the method of Moore et al.[7] Palladium black catalyst was freshly prepared by the method of Greenstein and Winitz.[8]

The ^1H and ^{13}C NMR spectra were recorded with a Varian XL-200E NMR spectrometer operating at 200 MHz, and the chemical shifts are reported in ppm relative to tetramethylsilane. The mass spectra were obtained with a VG analytical 70S high-resolution double-focusing magnetic sector spectrometer, with an attached VG analytical 11/250J data system of the Texas A&M Mass Spectrometry Center. The C,H,N elemental analyses were performed by Galbraith Labora ories, Inc.

Potentiometric Equilibrium Measurements. Equipment. A Corning Model 150 pH meter was attached to an extension reference and high-pH glass electrodes mounted in an air-protected, sealed, thermostated jacketed cell maintained at 25.0 ± 0.05 °C, equipped with a stirrer and a 10.00-mL-capacity Metrohm piston buret. The pH meter–electrode system was calibrated to read $-\log [H^+]$ (designated as p[H]) directly by means of known concentrations of HCl solutions.

Procedure. The details are described in ref 10. The protonation constants were calculated by means of the computer program BEST with the use of data obtained on 50.0-mL (initial volume) solutions containing $\sim 1 \times 10^{-3}$ M ligands and 0.100 M KCl as supporting electrolyte. Similar solutions containing $\sim 1 \times 10^{-3}$ M gallium(III) were used for the direct determination of potentiometric equilibrium data for gallium complexes. The iron(III) stability constants were determined spectrophotometrically at very low pH values on solutions, $\sim 1 \times 10^{-4}$ M in each component. The p[H] range was 0.5–1.5 and was determined from the amount of added HCl. The required KCl was added to maintain ionic strength numerically at 0.10 M; however, it is realized that significant deviations from ideality become increasingly important as the HCl concentration increases. The gallium(III) cryptand formation constant was determined from potentiometry at high p[H] where the formation of gallate anion, $Ga(OH)_4^-$, competes with the cryptate. It was necessary to carry out this determination because of the insolubility of the Ga(III) cryptate in neutral and acid solutions.

1,1,1-Tris((2-((*N*-methyl-*O*-benzylhydroxamino)carbonyl)ethoxy)methyl)ethane (2). Approximately 0.0080 mol of 1,1,1-tris((2-(chlorocarbonyl)ethoxy)methyl)ethane (**1**), which was prepared from 2.69 (0.0080 mol) of 1,1,1-tris((2-carboxyethoxy)methyl)ethane[5] was dissolved in 50 mL of dry benzene. This solution was added dropwise to a benzene solution of 3.4 g (0.025 mol) of *N*-methyl-*O*-benzylhydroxylamine, 9.5 g (0.12 mol) of dry pyridine, and 150 mL of benzene within a period of 40 min at room temperature under dry Ar. It was then allowed to stand at room temperature for 4 h. The pyridinium chloride salt was removed by filtration, and excess pyridine and solvent were removed by distillation under reduced pressure; 6.0 g of pale yellow oil was obtained. This crude product was dissolved in 100 mL of dichloromethane and washed with 2 and 0.1 M HCl. The organic phase was separated and dried with anhydrous $MgSO_4$ for 12 h. The 5.5 g of pale yellow oil obtained was purified by flash chromatography on a silica gel 60 column, with $CHCl_3$ as an eluent. The eluent spots, which gave an $R_f = 0.58$ ($CHCl_3$:MeOH = 95:5), were pooled. Solvent removal gave 4.3 g of colorless oil; yield 78%. ^1H NMR ($CDCL_3$): 7.39 (s, 15 H, aromatic), 4.86 (s, 6 H, $-CH_2Ph$), 3.66 (t, 6 H, $-OCH_2CH_2CON-$), 3.26 (s, 6 H, $-C(CH_2O-)_3$), 3.19 (s, 9 H, $-CONCH_3-$), 2.66 (t, 6 H, $-OCH_2CH_2CON-$), 0.89 (s, 3 H, $CH_3C(CH_2O-)_3$). ^{13}C NMR ($CDCl_3$): 173.1 (–CON–), 134.7 (C-1 CH_2Ph), 129.4–128.8 (C-2,3,4 CH_2Ph), 76.6 ($-CH_2Ph$), 73.9 ($-CCH_2O-)_3$), 67.3 ($-OCH_2CH_2-$), 41.0 ($CH_3C(CH_2O-)_3$), 33.7 ($-OCH_2CH_2CON-$), 32.7 ($-CONCH_3$), 17.5 ($CH_3C(CH_2-)_3$). FAB MS: $(M + H)^+ = 694$.

1,1,1-Tris((2-((*N*-methylhydroxyamino)carbonyl)ethoxy)methyl)ethane (3). A 0.5-g amount of the tribenzyl ester **2** was dissolved in 25 mL of 4.4% HCOOH–CH_3OH solution. This solution was added to a flask containing about 0.6 g of freshly prepared palladium black catalyst in 25 mL of 4.4% HCOOH in CH_3OH. This reaction mixture was stirred at room temperature under argon for 48 h. After removal of the catalyst and solvent, the crude product was dissolved in methanol and purified by flash chromatography with silica gel 60. The product was eluted with $CHCl_3$:CH_3OH = 95:5, $R_f = 0.35$ ($CHCl_3$:CH_3OH = 9:1). After solvent removal, the product was vacuum-dried at 0.1 mmHg and 35.0 °C for 4 h; 0.27 g of colorless oil was obtained; yield 90%. Anal. Calcd for $C_{17}H_{33}N_3O_9$: C, 48.32; H, 7.80; N, 9.93. Found: C, 48.42; H, 7.77; N, 9.58. ^1H NMR (in CD_3OD): 4.9 (b, 3 H, *H*ON–), 3.70 (t, 6 H, $-OCH_2CH_2-$), 3.32 (s, 9 H, $>NCH_3$), 3.25 (s, 6 H, $>CH_2O$), 2.77 (t, 6 H, $-OCH_2CH_2-$), 0.91 (s, 3 H, $CH_3C<$). FAB MS: $(M + H)^+ = 424$; $(M + Na)^+ = 446$; $(M + K)^+ = 462$.

1,1,1-Tris((3-(benzyloxy(methylcarbonyl)amino)propoxy)methyl)ethane (5). 1,1,1-Tris((3-(*O*-benzylhydroxyamino)propoxy)methyl)ethane,[5] 2.9 g, was dissolved in 80 mL of dry ethyl ether containing 7.7 mL of pyridine. This solution was added dropwise within 40 min to a solution of 120 mL of dry ethyl ether containing 1.5 g of acetyl chloride cooled in an ice water bath. The reaction mixture was stirred at this temperature for another 4 h and then at room temperature for 2 h. To the reaction mixture, 40 mL of water and 40 mL of the 2 N HCl were added, and the mixture was shaken vigorously. The ether phase was separated and washed with dilute HCl and 2% Na_2CO_3 and then with saturated NaCl solution. The ether solution obtained was dried with anhydrous sodium sulfate for 16 h. After removal of the solvent and

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Scheme I. Syntheses of Tris(hydroxamate) Ligands Analogous to Tris(hydroxamate) Cryptand

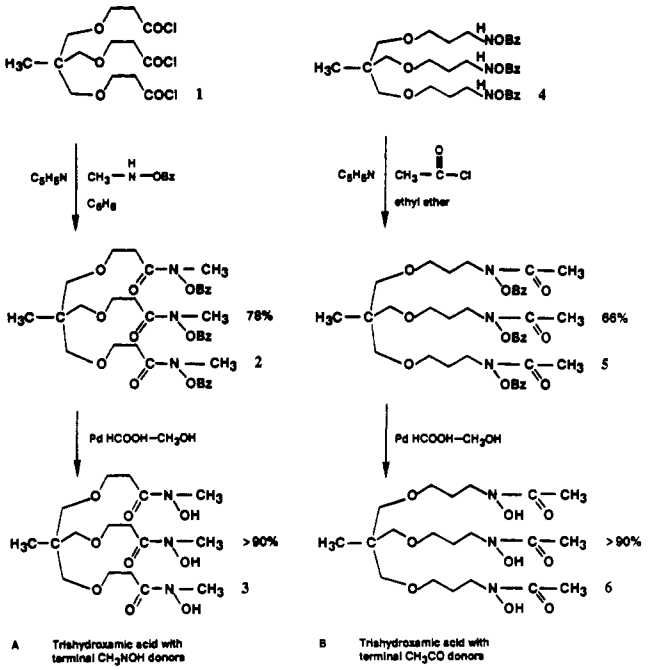

Table I. Protonation Constants for the Tris(hydroxamic acids) **3**, **6**, and **7** at 25.0 °C and μ = 0.100 M (KCl) in Aqueous Solution[a]

	3, H_3L	6, H_3L	7, H_3L
log K_1^H = $[HL^{2-}]/[L^{3-}][H^+]$	9.75	9.69	10.67
log K_2^H = $[H_2L^-]/[HL^{2-}][H^+]$	8.99	9.02	9.56
log K_3^H = $[H_3L]/[H_2L^-][H^+]$	8.26	8.39	8.93

[a] The standard deviation of $\sigma_{fit} = [\sum w_i[(pH_{obs} - pH_{calc})_i]^2/\sum w_i]^{1/2}$ where $w_i = 1/(pH_{i+1} - pH_{i-1})^2$; σ_{fit}: **3**, 0.002; **6**, 0.001; **7**, 0.01.

drying agent, the colorless oil was purified with silica gel 60 by gradient flash chromatography; 2.3 g of pure product was obtained; yield 66%. R_f = 0.53 ($CHCl_3$:CH_3OH = 95:5).

^1H NMR ($CDCl_3$): 7.40 (s, 15 H, aromatic), 4.83 (s, 6 H, $-CH_2Ph$), 3.73 (t, 6 H, $-CH_2NOBz-$); 3.42 (t, 6 H, $-OCH_2CH_2CH_2-$), 3.26 (s, 6 H, $-C(CH_2O-)_3$), 2.09 (s, 9 H, $-COCH_3$), 1.90 (quintet, 6 H, $-OCH_2CH_2CH_2-$), 0.95 (s, 3 H, $CH_3C(CH_2-)_3$). ^{13}C NMR ($CDCl_3$): 172.5 ($-NOBzCO-$), 134.9 (C-1 CH_2Ph), 128.9–129.5 (C-2,3,4 CH_2Ph), 76.5 ($-CH_2-$, CH_2Ph), 73.9 ($-C(CH_2O-)_3$), 69.0 ($-OCH_2CH_2CH_2-$), 43.3 ($-CH_2CH_2CH_2NOBz-$), 41.2 ($-C(CH_2O-)_3$), 27.6 ($-CH_2CH_2CH_2-$), 20.9 ($-COCH_3$), 17.8 ($CH_3C(CH_2O-)_3$). FAB MS: $(M + H)^+$ = 736.

1,1,1-Tris((3-(hydroxy(methylcarbonyl)amino)propoxy)methyl)ethane (6). 0.50 g of the tribenzyl ester was treated in the same way as described in the procedure for the preparation of compound **3**. The product was eluted from a silica gel column with $CHCl_3$:CH_3OH (95:5), 0.28 g of colorless oil was obtained after vacuum drying; yield 90%. R_f = 0.28 ($CHCl_3$:CH_3OH = 9:1). Anal. Calcd for $C_{20}H_{39}N_3O_9 \cdot {}^1/_2H_2O$: C, 50.63; H, 8.44; N, 8.86. Found: C, 50.91; H, 8.53; N, 8.25. ^1H NMR (in CD_3OD): 4.88 (s, 4 H, >NOH), 3.67 (t, 4 H, $-OCH_2CH_2CH_2N<$), 3.42 (t, 6 H, $-OCH_2CH_2CH_2-$), 3.27 (s, 6 H, $>CCH_2O-$), 3.21 (t, 2 H, $-OCH_2CH_2CH_2N<$), 2.08 and 1.91 (s, 9 H, $-C(=O)CH_3$), 1.81 and 1.71 (t, 6 H, $-CH_2CH_2CH_2-$), 0.91 (s, 3 H, $CH_3C<$). FAB MS: $(M + H)^+$ = 466; $(M + Na)^+$ = 488; $(M + K)^+$ = 504.

Results

Synthesis. The synthetic routes for the preparation of the two open-chain analogues are shown in Scheme I. Two acylation reactions were selected to form the benzyl-protected tris(hydroxamic acid) derivatives, which are relatively easily purified at that stage. The benzyl groups were then removed by catalytic transfer hydrogenation with formic acid[9] under relatively mild conditions.

In Table I are listed the protonation constants of the three compounds studied in this investigation: the shorter open-chain tris(hydroxamate), **3**; the larger open-chain tris(hydroxamate), **6**; and the previously reported[4] but newly measured values for the tris(endocyclic hydroxamate) cryptand, **7**.

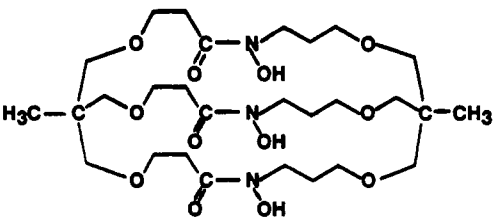

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Table II. Comparison of Fe(III) and Ga(III) Ion Affinities of the Tris(hydroxamate) Cryptand with Open-Chain Tris(hydroxamate) Chelating Ligands

ligand	equilibrium quotient	log K	pM[a] at pH 7.4
tris(hydroxamic acid) cryptand, H_3L, **7**	$[HL]/[L][H]$	10.67[b]	
	$[H_2L]/[HL][H]$	9.56	
	$[H_3L]/[H_2L][H]$	8.93	
	$[FeL]/[Fe][L]$	29.12 (8)	22.1
	$[FeHL]/[FeL][H]$	4.05 (1)	
	$[GaL]/[Ga][L]$	27.48[c]	20.5
	$[GaHL]/[GaL][H]$ (estd)	~6	
open-chain chelating ligand A (terminal CH_3NOH) H_3L, **3**	$[HL]/[L][H]$	9.75[b]	
	$[H_2L]/[HL][H]$	8.99	
	$[H_3L]/[H_2L][H]$	8.26	
	$[FeL]/[Fe][L]$	28.11 (5)	23.3
	$[FeHL]/[FeL][H]$	3.72 (1)	
	$[GaL]/[Ga][L]$	25.58[d]	20.7
	$[GaHL]/[GaL][H]$	2.56 (1)	
	$[GaH_2L]/[GaHL][H]$	0.6 (1)	
open-chain chelating ligand B (terminal $COCH_3$) H_3L, **6**	$[HL]/[L][H]$	9.69[b]	
	$[H_2L]/[HL][H]$	9.02	
	$[H_3L]/[H_2L][H]$	8.39	
	$[FeL]/[Fe][L]$	26.42 (7)	21.5
	$[FeHL]/[FeL][H]$	3.74 (1)	
	$[GaL]/[Ga][L]$	27.29[e]	22.4
	$[GaHL]/[GaL][H]$	2.32 (1)	
	$[GaH_2L]/[GaHL][H]$	1.6 (1)	

[a] Calculated with 100% excess ligand. [b] Ligand uncertainties indicated in footnote a of Table I. [c] σ_{fit} = 0.004. [d] σ_{fit} = 0.004. [e] σ_{fit} = 0.010.

Metal Ion Stability Constants. Although iron(III) stability constants cannot be determined by direct potentiometric p[H] measurements, all systems studied potentiometrically clearly showed the presence of MHL^+ species in addition to the expected complex, ML. Potentiometry also showed evidence for the existence of GaH_2L^{2+} for the open-chain ligands **3** and **6**. Whether such species exist for the cryptand **7** could not be determined due to insolubility of the complex in acid solution. The absence of the FeH_2L complex was inferred from the ability to do successful calculations assuming the formation of FeHL as the only protonated Fe(III) chelate in the acid region. The numerical values for the constants determined in this study are presented in Table II. During the course of spectral investigations it was determined that the Fe(III) complex of ligand **3**, FeL, has a λ_{max} at 420 nm, ϵ_{ML} = 2760. Upon protonation, the complex $FeHL^+$ that forms has an ϵ_{MHL} = 2210 at 466 nm. Similarly for ligand **6** FeL, ϵ_{ML}^{425} = 2880 and ϵ_{MHL}^{475} = 2030.

Discussion

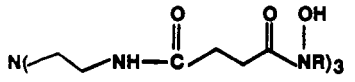
Protonation Constants. The most notable aspect of the data in Table I is that the basicity (as measured by protonation constants) of the cryptand exceeds those of both open-chain ligands for all protonation steps. Thus the first proton is bound more strongly by ~1 log unit than the open-chain analogues, a probable consequence of the steric restrictions imposed by the macrobicyclic ring structure, which effectively holds the hydroxamate donors close to each other. The protonation constants for the two open-chain tris(hydroxamates) indicate that the pendant arms are nearly independent of each other, since the difference in successive protonation constants of ~0.6 log unit are close to the statistical value (0.48). Thus the protonation of one hydroxamate has little influence on the protonation of the next, indicating a minimal

Table III. Protonation Constants, Fe(III) and Ga(III) Stability Constants, of Hydroxamic Acid Ligands

ligand	equilibrium quotient	log K^a	pM at pH 7.4[b]
acetohydroxamic acid, HL	[HL]/[H][L]	9.36	
	[FeL]/[Fe][L]	11.42	17.9
	$[FeL_2]/[Fe][L]^2$	21.10	
	$[FeL_3]/[Fe][L]^3$	28.33	
desferriferrioxamine, H_4L^+	[HL]/[L][H]	10.79	
	$[H_2L]/[HL][H]$	9.55	
	$[H_3L]/[H_2L][H]$	8.96	
	$[H_4L]/[H_3L][H]$	8.32	
	[FeHL]/[Fe][HL]	30.99	25.9
	[FeHL]/[FeL][H]	10.40	
	[GaL]/[Ga][HL]	28.65	23.5
	[GaHL]/[GaL][H]	10.31	

[a] Reference 14. [b] 100% excess ligand.

degree of preorganization in these ligands. A similar situation is found for desferriferrioxamine B and Trendrox (**8**).[11]



8 (Trendrox), R = *p*-tolyl

"Trendrox" is an analogous tris(hydroxamic acid) based on tren tris amide as the central organizing unit with slightly longer pendant arms containing bidentate secondary hydroxamate donors. The bridgehead tertiary nitrogen becomes protonated with a log K_4^H below those of the hydroxamate group. The previously reported tris(hydroxamate) BAMTPH[12] also shows the same type of statistical protonation sequence.

Stability Constants. The Fe(III) complexes of the two open-chain tris(hydroxamate) ligands **3** and **6** have similar UV–vis spectra, as expected. Taken together with the $\epsilon_{ML} \simeq 2750$ at λ_{max} = 430 nm of previously published cryptand complexes, there does not seem to be sufficient differences in the electronic spectra to be useful in distinguishing between these complexes. In other words, the origins of the absorbance spectra, the charge-transfer interactions between metal ion and hydroxamate donors, are quite similar for a given metal ion for all hydroxamates, whether with open chains or with closed macrobicyclic ring structures.

The log stability constants themselves are also quite similar in magnitude. If one considers ferric tris(acetohydroxamate) as the reference complex in which any bonds between ligands are absent, then its 28.33 overall stability constant can be considered as a basis for evaluating stability enhancement for the other hydroxamic acid ligands, in which there are possible preorganizational stability factors, countered by steric constraints. Even for the natural siderophore DFB (desferriferrioxame B), the ferric complex has a log K_{ML} of 30.99, barely 1.66 units above that of the tris(acetohydroxamate). Both open-chain ligands in this study form less stable complexes, yet the log K of cryptand **7** exceeds that of tris(acetohydroxamate) by barely 0.8 log units. Therefore there is no evidence here for a large cryptate effect.

The computed pM values of Tables II and III tell quite a different story. The higher the pM, the more effective is the metal binding at physiological pH. Acetohydroxamic acid is by far the weakest ligand in this respect, while DFB is still the most effective ligand when measured by pM values, by over 3 log units, compared to the cryptand. The surprise is that the open-chain tris(hydroxamate) **3** is more effective than the cryptand by 1.2 pM units.

While it is generally considered that Ga(III) stability constants with a given ligand are usually lower than those of Fe(III), it is interesting to note that the open-chain ligand **6** with the longer pendant arms binds Ga(III) more tightly than Fe(III) by nearly a full pM unit.

A survey of ref 13 reveals the fact that ligands having higher affinities for the Ga(III) ion than for the Fe(III) ion are not uncommon. For the compilations of data through 1985[13] 15 such ligands were found. In addition, there are 20 ligands for which the Fe(III) stability constants are from 0 to 1 log K unit higher than those of Ga(II). There are also 27 ligands, mainly polyamino polycarboxylic acids such as EDTA, for which the Fe(III) constants are more than 1 log unit higher than those of Ga(III). The fact that the effective ionic radius of six-coordinate Ga(III) is about 0.02 Å lower than that of Fe(III)[14] seems to be in accord with the small differences in the reported stability constants. While the differences may be due in part to incorrect measurements, they may also be due to subtle factors associated with the coordinate bond strengths of Ga(III) and Fe(III) complexes and the conformations of the ligands to which they are bound.

While the lack of metal-binding enhancement of hexadentate tris(hydroxamates) relative to bidentate tris(acetohydroxamic acid) complexes has been observed previously for BAMTPH and elsewhere, Trendrox (**8**) seems to be exceptional with a K_{ML} value of 32.9, making it even more effective than DFB by several log units. It appears then that increasing the length of the short bridges of cryptand **7** may well provide a more effective ligand than Trendrox.

While cryptand **7** has proved to be less effective for Ga(III) and Fe(III) than some open-chain tris(hydroxamate) ligands, preliminary molecular mechanics calculations[15] show that the fit of ligand donor groups around these metal ions is less than perfect. Therefore, in view of its superior preorganization, a similar cryptand ligand with more closely adjusted ring sizes should provide more effective binding for Fe(III) and Ga(III) than any of the other ligands considered in this paper.

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Registry No. **1**, 123074-24-8; **2**, 132234-46-9; **3**, 132259-53-1; **4**, 123074-27-1; **5**, 132234-47-0; **6**, 132234-45-8; **7**, 123074-29-3; *N*-methyl-*O*-benzylhydroxyamine, 22513-22-0; 1,1,1-tris((2-carboethoxy)-methyl)ethane, 64020-01-5.

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