Synthesis, Ligand pK_a, and Fe(III) Complexation Constants for a Series of Bipodal Dihydroxamic Acids

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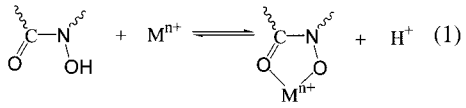
The synthesis of four bipodal dihydroxamic acids containing an apical C atom and amide linkages is described, where **Ia,b** represent "normal" and "retro" hydroxamate isomers: (R)CH[C(═O)NH(CH_2)$_2$NHC(═O)(CH_2)$_n$R′]$_2$ (**Ia**, R = CH_3, R′ = N(OH)(C═O)CH_3, n = 2; **Ib**, R = CH_3, R′ = (C═O)N(OH)CH_3, n = 2; **Ic**, R = CH_3, R′ = (C═O)N(OH)CH_3, n = 3; **Id**, R = C_4H_9, R′ = (C═O)N(OH)CH_3, n = 2.). The pK_{a1} and pK_{a2} values in aqueous solution are reported, and some degree of cooperativity is noted. Complexation equilibria with Fe_{aq}^{3+} are described, and values for stepwise and overall equilibrium constants are reported. log β_{230} values for **Ia**−**d** are 59.22, 59.45, 58.91, and 58.46, slightly lower than for rhodotorulic acid, although the pFe values for the synthetic siderophores are comparable to that for rhodotorulic acid.

Introduction

The chemistry of the siderophores has attracted significant interest in recent years due to their iron acquisition role in microorganisms and plants and their potential application in the treatment of iron overload disease.[1] Among numerous siderophore structures, the hydroxamates are of interest due to their ability to form stable transition metal complexes through the formation of a five-membered chelate ring (eq 1).[2]



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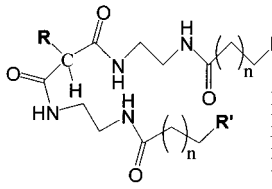
An important feature of their aqueous chemistry is their ability to selectively form stable complexes with Fe_{aq}^{3+} in a milieu of environmental metal ions. This characteristic is utilized by microbes in their synthesis of hydroxamate siderophores as agents to enhance the bioavailability of the essential nutrient iron.[3,4] Hydroxamic acids have been used as therapeutic agents in chelation therapy[5] and as metalloenzyme inhibitors.[6] Other medical applications of the hydroxamates which utilize their affinity for high charge density metal ions include the possible use of their metal complexes as imaging agents.[7]

In this report we describe the synthesis, acidity, and Fe(III) chelation chemistry of a series of four dihydroxamic acids, **Ia**−**d**. These may be viewed as models for the natural dihydroxamate siderophore rhodotorulic acid. Structural features of these hydroxamic acids include a bipodal architecture with an apical C atom, amide linkers, and two geometrical isomers with a "normal" (**Ia**) and "retro" (**Ib**) hydroxamate configuration (relative to rhodotorulic acid).



	R	R′	n
Ia	CH_3	N(OH)C(O)CH_3	1
Ib	CH_3	C(O)N(OH)CH_3	1
Ic	CH_3	C(O)N(OH)CH_3	2
Id	C_4H_9	C(O)N(OH)CH_3	1

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The cytoprotection and iron mobilization of these new dihydroxamate chelators were studied in primary rat hepatocyte cultures, and their efficacy was close to that of ferrioxamine B. The ability of these compounds to scavenge hydroxyl radicals generated from the Fenton reaction was demonstrated, giving the characteristic EPR spectra of stable dinitroxide free radicals.[8]

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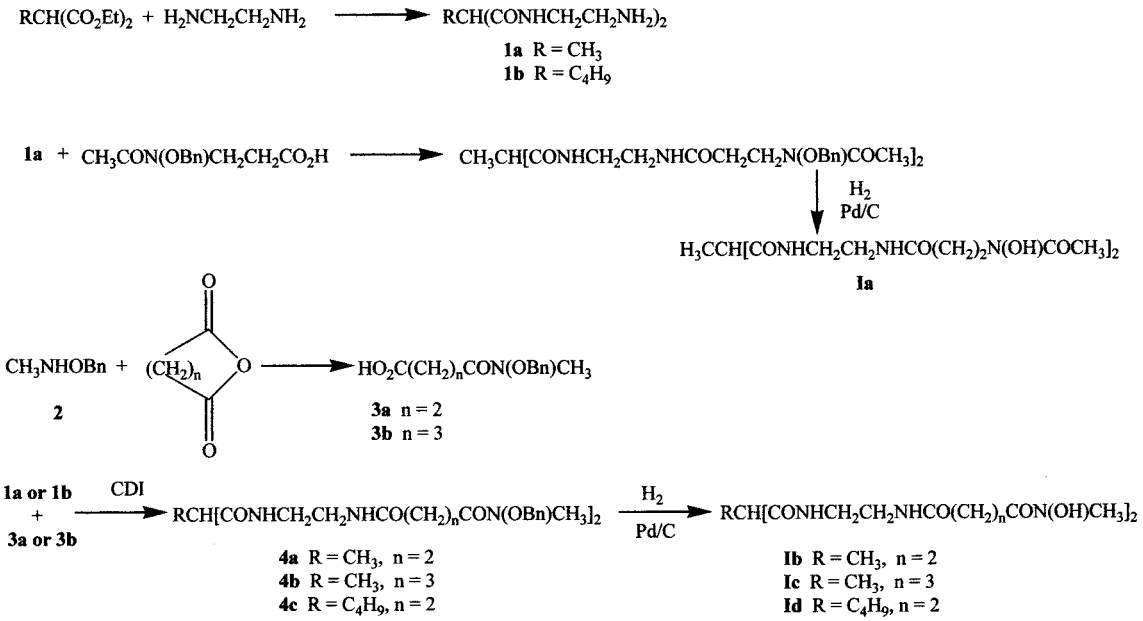
Scheme 1. Synthetic Scheme for the Preparation of Bipodal Dihydroxamic Acids **Ia**−**d**



Some of these compounds demonstrated higher growth promotion of Gram-positive and Gram-negative bacteria than any other synthetic hydroxamate in the literature.[9] These biological data suggest that the Fe(III) chelation characteristics of this series of bipodal dihydroxamic acids are of some significance.

Experimental Section

Dihydroxamic Acid Synthesis. All organic reagents were purchased from Janssen and used without further purification**.** Reagent grade dichloromethane was distilled over phosphorus pentoxide, THF was distilled over sodium−benzophenone ketyl, and ethylenediamine was distilled under Ar. Melting points were determined using a Kofler apparatus. Mass spectra (CI) were recorded using a Ribermag R-10-10 spectrometer at the University of Paris-SUD, Orsay, France. High-resolution mass spectra (FAS) were obtained using a Kratos MS-80 at ICSN spectrometer, CNRS, Gif-sur-Yvette, France. Proton and carbon-13 NMR spectra were obtained using a Bruker AM 250 spectrometer. The chemical shifts are relative to the solvents D_2O and CD_3OD: 4.80 and 7.24 ppm. Infrared spectra were measured using a Bruker IFS 66 spectrometer. Elemental analyses were performed by the CNRS Microanalysis Service at Gif-sur-Yvette, France. All new compounds gave satisfactory analyses.

The synthetic route to all four dihydroxamic acids is illustrated in Scheme 1. Compound numbers in the description below correspond to those in Scheme 1 and the Introduction.

Both the normal (**Ia**) and retro (**Ib**−**d**) dihydroxamic acids were synthesized using the basic scaffold obtained through the synthesis of **1a** or **1b**. 6-Methyl-1,4,8,11-tetraaza-5,7-undecadione (**la)** and 6-butyl-1,4,8,11-tetraaza-5,7-undecadione (**lb**) were synthesized as described by Brik and co-workers.[8] **1a** was obtained as a white hygroscopic solid with a yield of 90%. IR (KBr; ν, cm^{-1}): 3300 (NH), 1660 (CO). ^1H NMR (D_2O; δ, ppm): 1.3 (d, 3H, CH_3); 2.66 (t, 4H, CH_2NH_2); 3.20 (t, 4H, C*H*$_2$NH); 3.33 (q, lH, CH). **1b** was obtained as a white solid with a yield of 85%. IR (KBr; ν, cm^{-1}): 3300 (NH), 1660 (CO). ^1H NMR (D_2O; δ, ppm): 0.81 (m, 3H, CH_3); 1.22 (m, 4H, CH_2CH_2); 1.75 (m, 2H, CH_2); 2.65 (t, 2H, CH_2NH_2); 3.20 (t, 1H, CH).

Synthesis of Dihydroxamic Acid Ia. 1,1-Bis(9-hydroxy-2,5,9-triaza-1,6,10- trioxoundecanyl)ethane (Ia). *N*-Acetyl-*N*-(benzyloxy)-3-aminopropionic acid[10] (1.03 g, 4.34 mmol) and CDI (0.730 g, 4.40 mmol) were dissolved in 30 mL of dry CH_2Cl_2, and the solution was stirred 1 h under Ar at room temperature. A solution of **1a** (0.430 g, 2.17 mmol) in dry CH_2Cl_2 was added and stirred overnight. The mixture was then neutralized with citric acid (0.5 M). The organic layer was dried over Na_2SO_4 and evaporated to give a colorless oil which was crystallized from EtOH/ether in 65% yield (0.650 g). ^1H NMR ($CDCl_3$; δ, ppm): 1.40 (d, 3H, CH_3); 2.10 (s, 3H, $COCH_2$); 2.50 (t, 4H, CH_2); 3.35 (broad s, 12H, CH_2CH_2); 3.95 (m, 6H, CH_2); 4.87 (s, 2H, CH_2); 7.05 (broad s, 1H, NH); 7.40 (s, 10H, H_{arom}). The product (0.650 g, 1 mmol) and 10% Pd/C (23% w/w) were suspended in MeOH, and the reaction mixture was stirred under H_2(g) for 1 h. After filtering of the mixture to remove the catalyst, the solvent was concentrated to provide a viscous colorless oil (94% yield, 0.430 g). ^1H NMR ($CDCl_3$; δ, ppm): 1.21 (d, 3H, CH_3); 1.96 (s, 6H, $COCH_3$); 2.36 (t, 4H, CH_2); 3.10 (m, H, CH); 3.17 (m, 8H, CH_2–CH_2,); 3.74 (t, 4H, CH_2). ^{13}C NMR (D_2O; δ, ppm): 15.03 (CH_3); 20.20 (C_{quat}); 34.17 (CH_2); 39.43−39.74 (2 × CH_2); 45.58 (CH_2); 48.68 (C_α); 174.13−174.92−181.40 (3 × CO). MS-CI (*m*/*e*): M + H^+ = 461. IR (KBr; ν, cm^{-1}): 3360 (NH), 1675 ($COCH_3$); 1640−1660 (CO amide); 1030 (NO).

Synthesis of Dihydroxamic Acids Ib−**d**. *N*-Methyl-*O*-benzylhydroxylamine (**2**), *N*-(benzyloxy)-5-aza-4-oxohexanoic acid (**3a**), and *N*-(benzyloxy)-6-aza-5-oxoheptanoic acid (**3b**) were prepared by literature methods.[11]

1,1-Bis[10-(benzyloxy)-2,5,10-triaza-1,6,9-trioxoundecanyl]-ethane (4a). Compound **4a** was prepared by literature methods[12] as a white solid with 40% yield, mp 110 °C. MS-CI (NH_3; *m*/*e*): M + NH_4^+ = 658. ^1H NMR ($CDCl_3$; δ, ppm): 1.37 (d, 3H, CH_3); 2.42 (t, 4H, CH_2-8); 2.78 (t, 4H, CH_2-7); 3.17 (s, 6H, CH_3N); 3,10 (q, lH, CH); 3.37 (m, 8H, CH_2-3,4); 4.89 (s, 4H, OCH_2); 6.97 (m, 2H, NH); 7.33 (m, 2H, NH); 7.38 (s, 10H, Ar). ^{13}C NMR ($CDCl_3$; δ, ppm): 15.48 (CH_3); 27.68 (CH_2-8); 30.35 (CH_2-7); 33.58 (CH_3N); 38.88 and 39.50 (CH_2-3,4); 47.93 (CH); 76.23 (CH_2O); 128.64−128,90, 129.15 (CH Ar); 134.31 (C−Ar); 171.92 (CO); 173.08 (CO); 174.18 (CC).

1,1-Bis[10-(benzyloxy)-2,5,10-triaza-1,6,9-trioxoundecanyl]pentane (4b). Compound **4b** was prepared by literature methods[12] as a white solid with 40% yield, mp138 °C. MS-CI (*m*/*e*): M + H^+ = 683. ^1H NMR ($CDCl_3$; δ, ppm): 0.8 (t, 3H, CH_3); 1.22 (m, 4H, CH_2–CH_2); 1.80 (m, 2H, CH_2–CH); 2.41 (t, 4H, CH_2-8); 2.78 (m, 4H, CH_2-7); 2.97 (t, lH, CH); 3.16 (s, 6H, CH_3N); 3.36 (m, 8H, CH_2-3,4); 4.88 (s, 4H, OCH_2); 6.92 (m, 2H, NH); 7.38 (s + m, 12H, Ar, NH). ^{13}C NMR

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($CDCl_3$; δ, ppm): 13.83 (CH_3), 22.34 (CH_2); 27.55 (CH_2-8); 29.71 (CH_2); 30.43 (CH_2-7); 31.01 (CH_2); 33.57 (CH_3–N); 39.38−39.98 (CH_2-3,4); 54.51 (CH); 76.35 (CH_2O); 128.67−128.93−129.19 (CH−Ar); 134.36 (C−Ar); 171.47 (CO); 173.08 (CO); 174.10 (CO).

1,1-Bis[11-(benzyloxy)-2,5,11-triaza-1,6,10-trioxododecanyl]-ethane (4c). Compound **4c** was prepared by literature methods[12] with 92% yield as a white solid. ^{1}H NMR ($CDCl_3$; δ, ppm): 1.38 (d, 3H, CH_3); 1.91 (t, 4H, CH_2-8); 2.24 (t, 4H, CH_2-7); 2.39 (t, 4H, CH_2-9); 3.12 (q, lH, CH); 3.16 (s, 6H, CH_3N); 3.46 (m, 8H, CH_2-3 CH_2-4); 4.83 (s, 4H, CH_2–Ar); 7.36 (s, 10H, Ar); 6.75−7.51 (m, 4H, NH). ^{13}C NMR ($CDCl_3$; δ, ppm): 16.09 (CH_3); 22.07 (CH_2-8); 32.28 (CH_2-7); 32.28 (CH_2Ar); 36.26 (CH_2-3); 36.27 (CH_2-4); 39.70 (CH_3N); 40.34 (CH_2-9); 48.89 (CH); 128.64−128.94−129.19 (C−Ar); 173.73−175.27−176 (3CO).

1,1-Bis(10-hydroxy-2,5,10-triaza-1,6,9-trioxoundecanyl)ethane. Dihydroxamic Acid Ib. Compound **4a** (0.768 g, 1.2 mmol) and 12% Pd/C (10%, 0.092 g) were suspended in methanol (20 mL) and stirred under hydrogen gas for l h. The reaction mixture was filtered through microfiber paper. Methanol was evaporated to give a white solid (0.54 g, 98%), mp 94 °C. ^{1}H NMR ($CDCl_3$; δ, ppm): 1.22 (d, 3H, CH_3); 2.33 (t, 4H, CH_2-8); 2.67 (t, 4H, CH_2-7); 3.05 (s, 6H, CH_3N); 3.18 (m, 8H, CH_2-3−4); 3.10 (q, lH, CH). ^{13}C NMR (D_2O; δ, ppm): 14.91 (CH_3); 28.14 and 31.08 (CH_2-7−8); 36.70 (CH_3–N); 39.30 and 39.73 (CH_2-3,4); 48.60 (CH); 174.07 (CO); 174.83 (CO); 176.22 (CO). MS-FAB (*m*/*e*): M + H^+ = 461. IR (KBr; ν, cm^{-1}): 3302 (NH); 3086 (OH); 1666 (CONOH); 1648 (CO amide).

1,1-Bis(11-hydroxy-2,5,11-triaza-1,6,10-trioxododecanyl)-ethane. Dihydroxamic Acid Ic. The hydrogenolysis of **4b** afforded the dihydroxamic acid product **Ic** in 85% yield as a lightly colored solid, mp 175 °C. ^{1}H NMR (CD_3OD; δ, ppm): 1.20 (d, 3H, CH_3); 1.74 (q, 4H, CH_2-8); 2.08 (t, 4H, CH_2-7); 2.34 (t, 4H, CH_2-9); 3.10 (q, lH, CH); 3.13 (s, 6H, CH_3N); 3.15 (m, 8H, CH_2-3,4). ^{13}C NMR (CD_3-OD; δ, ppm): 15.77 (CH_3); 22.11 (CH_2-8); 32.28 (CH_2-7−9); 36.37 (CH_3–N); 39.74 and 40.37 (CH_2-3,4); 49.27 (CH); 173.74 (CO); 175.28 (CO); 175.94 (CO). MS-FAB (*m*/*e*): M + Na^+ = 511, M + H^+ = 489. IR (KBr; ν, cm^{-1}): 3298 (NH); 1666 (CONOH); 1642 (CO amide).

l,l-Bis(l0-hydroxy-2,5,10-triaza-1,6,9-trioxoundecanyl)pentane. Dihydroxamic Acid Id. Dihydroxamic acid **Id** was obtained by the same procedure as **Ib** as a white solid in 93% yield, mp 160 °C. ^{1}H NMR (CD_3OD; δ, ppm): 0.76 (t, 3H, CH_3); 1.16 (m, 4H, CH_2CH_2); 1.65 (q, 2H, CH_2CH); 2.29 (t, 4H, CH_2-8); 2.67 (t, 4H, CH_2-7); 2.93 (t, lH, CH); 3.03 (s, 6H, CH_3N); 3.16 (m, 8H, CH_2-3,4). ^{13}C NMR (CD_3OD; δ, ppm): 14.25 (CH_3); 23.47 (CH_2) 28.62 (CH_2-8); 30.75 and 31.38 (CH_2-7 and 2CH_2); 36.31 (CH_2N); 39.82 and 40.22 (CH_2-3,4); 55.20 (CH); 172.93 (CO); 174.58 (CO); 175.44 (CO). MS−FAB (*m*/*e*): M + H^+ = 503. IR (KBr; ν, cm^{-1}): 3300 (NH); 3085 (OH); 1666 (CONOH); 1649 (CO amide). ^{13}C NMR ($CDCl_3$; δ, ppm): 13.83 (CH_3), 22.34 (CH_2); 27.55 (CH_2-8); 29.71 (CH_2); 30.43 (CH_2-7); 31.01 (CH_2); 33.57 (CH_3–N); 39.38−39.98 (CH_2-3,4); 54.51 (CH); 76.35 (CH_2O); 128.67−128.93−129.19 (CH−Ar); 134.36 (C−Ar); 171.47 (CO); 173.08 (CO); 174.10 (CO).

Determination of Dihydroxamic Acid pK_a and Fe(III) Complex Stability Constants

Materials and Equipment. All experiments were conducted with water that was doubly distilled, first through acidic potassium dichromate and then through basic potassium permanganate. Sodium hydroxide, 0.10 M (Fisher Scientific), was prepared and standardized against potassium hydrogen phthalate to a phenolphthalein end point and stored under N_2. A 2.5 M sodium perchlorate stock solution was prepared from the solid hydrate (Fisher Scientific) that was standardized by passing through a cation-exchange column for hydrogen ion (BIO-RAD, AG 50W-80) and titrated to a phenolphthalein end point. A stock solution of 0.10 M perchloric acid was prepared from 70% perchloric acid (Fisher Scientific) and standardized with standard sodium hydroxide. Each of these two solutions was diluted and combined to make a stock solution of 0.01 M $HClO_4$/0.1 M $NaClO_4$. All solutions were prepared from carbon dioxide free water by boiling doubly distilled water for at least 30 min and allowing it to cool to room temperature under a carbon dioxide free inert-gas blanket before making each solution.

All volumetric glassware and burets were calibrated gravimetrically. All pH and potential measurements were made with a Corning model 250 pH/Ion Analyzer meter and a ROSS combination electrode filled with a 3.0 M KCl reference fill solution.

Acid Dissociation Constants. Potentiometric titrations were conducted in a sealed water-jacketed cell at constant temperature (25 °C). All experiments were conducted under a purified nitrogen atmosphere, in which the nitrogen was passed though a fritted bubbler containing Fiesler's solution and then through a bubbler of carbon dioxide free doubly distilled water. The hydroxamic acid was added to 15 mL of the 0.01 M $HClO_4$/0.1 M $NaClO_4$ solution. This solution was then titrated with standard 0.1 M NaOH, delivered by a 10 mL Scott Gerate mechanical buret, while the potential was recorded in millivolts as well as pH. The electrode was allowed to equilibrate after each ligand titration. A calibration curve was obtained by titrating 10 mL of the perchlorate solution with standard NaOH. Ligand pK_a data were analyzed by two methods in order to determine mixed constants from hydrogen ion activities, $\{H^+\}$, and concentration constants from hydrogen ion concentrations, $[H^+]$.[13] Only the concentration constants used to calculate the Fe(III) complex stability constants are reported. The milivolt data from the strong acid/strong base calibration curves for each experiment were refined using the MAGEC program[13] to obtain the pK_w and E_{zero} for our experimental conditions, using the slope obtained from the calibration of the electrode with buffer solutions. These parameters, along with the millivolt data for the titration of the dihydroxamic acids, were entered into the SUPERQUAD program[14] to convert the $\{H^+\}$ activity read from the pH meter to $[H^+]$ to obtain pK_a values in concentration terms.

Fe(III) Complex Stability Constants. An iron(III) perchlorate stock solution was prepared from recrystallized solid ferric perchlorate hydrate and standardized by two methods: (1) spectrophotometrically in strong acid; (2) titrimetrically by reducing with Sn(II) and titrating against primary standard potassium dichromate. The acid concentration of this solution was determined by passing through a Dowex 50W-X8 strong acid cation-exchange column in H^+ form, titrating the liberated acid, and correcting for ferric ion. An aqueous solution of 4.8 mM EDTA was prepared for competition experiments. All solutions were prepared with carbon dioxide free water. NaOH was delivered to the system by a Scott Gerate T-80 mechanical microburet and a 2.0 mL Gilmont microburet. All UV/vis data were collected using a Hewlet-Packard 8452A diode array spectrophotometer. The temperature of all experiments was held constant at 25 °C.

Fe(III) complex stability constants were determined by pH titration and EDTA competition equilibria, both of which were monitored spectrophotometrically.

Spectrophotometric pH Titration. A spectrophotometric pH titration was used to determine the equilibrium constant for reaction 7, where H_2L represents dihydroxamic acids **Ia**−**d**. The setup consisted of a sealed titration cell linked by polyethylene tubing and a peristaltic circulation pump to a 1 cm glass cuvette in a diode array spectrophotometer. The temperature was held constant at 25 °C by circulating thermostated water through both jacketed cells. All experiments were conducted under N_2 purified by passing though a fritted bubbler containing Fiesler's solution.

The solid ligand and iron stock solution were added to 15 mL of the 0.01 M $HClO_4$/0.1 M $NaClO_4$ solution to give 1.5 and 0.75 mM concentrations, respectively. This solution was then titrated with standard 0.1 M NaOH, delivered by a 10 mL Scott Gerate mechanical microburet (T-80), while the potentials both in mV and pH modes and absorbance spectra were recorded in small pH intervals from 2 to 10. The concentration of iron and ligand at each pH interval, as well as 16 data points from each spectrum, were analyzed using SQUAD.[15]

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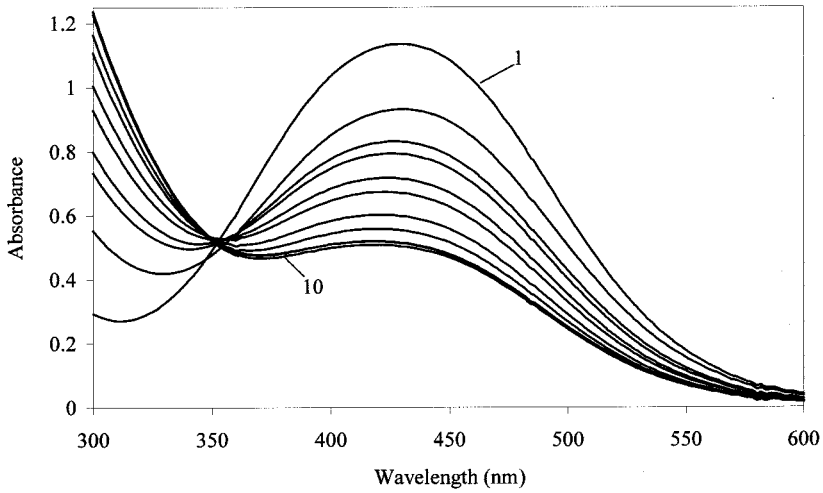


Figure 1. Time-dependent spectra for an EDTA/Fe_2(**Ia**)$_3$ competition experiment at a constant pH of 6.034. Spectrum 1 represents the Fe_2(**Ia**)$_3$ complex solution, and spectrum 10 represents the competition equilibrium spectrum for reaction 2. Conditions: $[Fe(III)]_{tot} = 0.4$ mM; $[\mathbf{Ia}]_{tot} = 0.71$ mM; $[EDTA]_{tot} = 0.48$ mM; $T = 25$ °C; $I = 0.1$ M $HClO_4/NaClO_4$.

Table 1. Bipodal Dihydroxamic Acid Dissociation Constants[a]

hydroxamic acid	pK_{a1}[b]	pK_{a2}[b]	$pK_{a2} - pK_{a1}$
Ia	8.123(0.006)	9.048(0.004)	0.925
Ib	8.067(0.007)	8.955(0.006)	0.888
Ic	8.151(0.007)	9.002(0.006)	0.851
Id	8.145(0.040)	9.156(0.027)	1.011

[a] In 0.100 M $HClO_4/NaClO_4$ at 25 °C. Estimated uncertainties are in parentheses. [b] Concentration constants where $pK_{a1} = -\log [H^+][HL^-]/[H_2L]$ and $pK_{a2} = -\log [H^+][L^{2-}]/[HL^-]$.

EDTA Competition Equilibria. The equilibrium constant for the EDTA competition reaction 2 was determined spectrophotometrically. A 0.2 mM Fe_2L_3 solution was prepared in 0.01 M $HClO_4$/0.1 M $NaClO_4$ with 20% excess ligand. NaOH was added to several 3.0 mL aliquots of this solution in 1 cm plastic cuvettes to prepare a series of solutions with pH values ranging from ca. 5 to 7. The spectrum of each solution was recorded between 300 and 600 nm before adding 150 μL of the concentrated EDTA stock solution. Each solution contained a total of 0.4 mM iron, 0.72 mM ligand, and 0.48 mM EDTA. Spectra were taken over a period of several days until there was no further decrease in the absorbance due to Fe_2L_3, indicating that equilibrium was achieved. At equilibrium, both the final pH and potential in millivolts were measured and the final spectra were recorded. All experiments were thermostated at 25 °C. The total concentration of iron, hydroxamic acid ligand, and EDTA were calculated for each solution. A total of 16 data points and these calculated values, along with the $Fe(edta)^-$ stability constant,[16] were entered into SQUAD[15] for data analysis.

$$Fe_2L_3 + 2H_2edta^{2-} + 2H^+ \rightleftarrows 2Fe(edta)^- + 3H_2L \qquad (2)$$

Results

Dihydroxamic Acid Ligand Synthesis and Acidity. The syntheses of the normal and retro dihydroxamic acids **Ia**−**d** are illustrated in Scheme 1. The acidity of these hydroxamic acids was determined in aqueous 0.1 M $NaClO_4$ at 25 °C. Two readily detectable proton dissociations were observed for all four dihydroxamic acids, where pK_{a1} and pK_{a2} values listed in Table 1 are defined as follows:

$$LH_2 \rightleftarrows H^+ + LH^- \quad pK_{a1} \qquad (3)$$

$$LH^- \rightleftarrows H^+ + L^{2-} \quad pK_{a2} \qquad (4)$$

Iron(III) Complex Equilibria. The dihydroxamic acids **Ia**−**d** act as tetradentate ligands in complexing Fe_{aq}^{3+}. To saturate the six coordination sites on Fe_{aq}^{3+} the diiron species Fe_2L_3 is formed at the appropriate H_2L/Fe^{3+} ratio. Coordination saturation occurs in a stepwise fashion so that the overall formation of Fe_2L_3 shown in reaction 5 occurs in at least two steps as illustrated in reactions 6 and 7. Qualitative evidence for the stoichiometry shown in eqs 6 and 7 comes from equilibrium spectra, since at various $[H^+]$ and H_2L/Fe_{aq}^{3+} ratios the absorption spectra exhibit $\lambda_{max} = 460$ nm, consistent with two hydroxamate groups bound to Fe(III), and $\lambda_{max} = 428$ nm, consistent with three hydroxamate groups bound to Fe(III).[17] The overall equilibrium reaction 5 was investigated by competition experiments with EDTA (reaction 2), conditions where the intermediate species $Fe(L)(OH_2)_2^+$ is not present. The equilibrium reaction 7 was directly monitored by pH titration with spectrophotometric detection. Equilibrium data for reactions 5 and 7 allow us to compute an equilibrium constant for reaction 6.

$$2Fe_{aq}^{3+} + 3H_2L \rightleftarrows Fe_2L_3 + 6H^+ \qquad (5)$$

$$Fe_{aq}^{3+} + H_2L \rightleftarrows Fe(L)(OH_2)_2^+ + 2H^+ \qquad (6)$$

$$2Fe(L)(OH_2)_2^+ + H_2L \rightleftarrows Fe_2L_3 + 2H^+ \qquad (7)$$

Competition equilibrium reactions between Fe_2L_3 and EDTA for **Ia**−**d** over the pH range 4.8−7.4 were carried out (reaction 2). Representative spectra are shown in Figure 1 for dihydroxamic acid ligand **Ia** at pH 6.03. The presence of the isosbestic point indicates there is a distinct equilibrium between the two species $Fe(edta)^-$ and Fe_2L_3, and no intermediates are present. These data were used to calculate β_{230} values summarized in Table 2 that are associated with the following H^+-independent equilibrium:

$$Fe_{aq}^{3+} + 3L^{2-} \rightleftarrows Fe_2L_3 \quad \beta_{230} \qquad (8)$$

The position of equilibrium reaction 7 can be controlled by the solution $[H^+]$. This is illustrated in Figure 2 for the spectrophotometric pH titration of dihydroxamic acid ligand **Ia** in the presence of Fe(III) over the pH range 2−10. The peak at

(16) Martell, A. E., Smith, R. M., Eds. *Critical Stability Constants*; Plenum Press: New York, 1974; Vol. 1, p 207.

(17) Caudle, M. T.; Crumbliss, A. L. *Inorg. Chem.* **1994**, *33*, 4077.

Table 2. Dihydroxamic Acid−Iron(III) Complex Stability Constants[a]

hydroxamic acid	$\log \beta_{110}$[b]	$\log K$[c]	$\log \beta_{230}$[d]	pFe[e]
Ia	19.85	19.39	59.22	21.5
Ib	20.03	19.52	59.45	21.8
Ic	19.69	19.53	58.91	21.4
Id	19.85	18.81	58.46	20.9

[a] In 0.100 M $HClO_4/NaClO_4$ at 25 °C. [b] Equilibrium reaction 10 calculated from the relationship $\beta_{110} = (\beta_{230}/K)^{1/2}$. [c] Equilibrium reaction 9. Results from pH spectrophotometric titrations over the range pH = 2.5−7.1. $[Fe]_{tot}$ = 0.5 mM, and $[H_2L]_{tot}$ = 1.5 mM. [d] Equilibrium reaction 8. Results from EDTA competition experiments. $[Fe]_{tot}$ = 0.4 mM, $[H_2L]_{tot}$ = 0.72 mM, and $[EDTA]_{tot}$ = 0.48 mM. [e] pFe = $-\log [Fe_{aq}^{3+}]$ when $[Fe^{3+}]_{tot} = 10^{-6}$ M, $[H_2L] = 10^{-5}$ M, and pH = 7.4.[24]

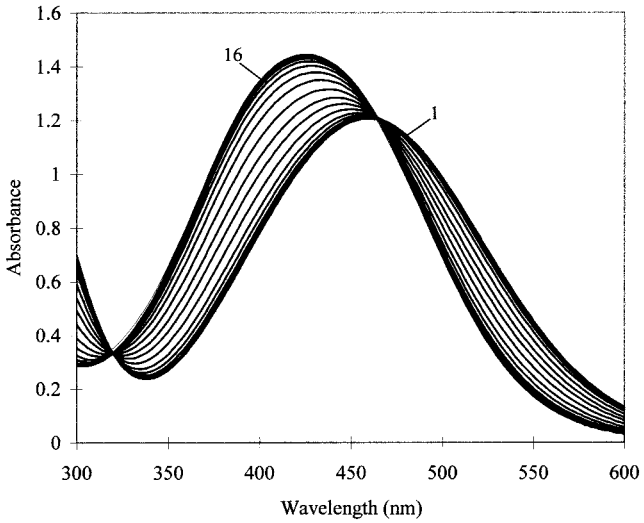


Figure 2. Spectrophotometric pH titration spectra for reaction 7 as a function of pH. Spectrum 1 was obtained at pH 2.518, and spectrum 16 was obtained at pH 7.058. Conditions: $[Fe]_{tot}$ 0.5 mM; $[\mathbf{Ia}]_{tot}$ = 1.46 mM; T = 25° C; I = 0.1 M $HClO_4/NaClO_4$.

460 nm represents the bis coordinated $Fe(L)(OH_2)_2^+$ complex (ϵ = 4050 L/(mol·cm)) formed at pH 2. As the pH increases, the shift in absorbance to 428 nm represents the formation of the tris Fe_2L_3 complex (ϵ = 5100 L/(mol·cm)). The isosbestic point indicates there is a distinct equilibrium with only two light absorbing species present. Data such as these for all of the dihydroxamic acid ligands **Ia**−**d** were used to calculate the H^+-independent equilibrium constants K for reaction 9 that are summarized in Table 2.

$$2Fe(L)(OH_2)_2^+ + L^{2-} \rightleftarrows Fe_2L_3 \quad K \tag{9}$$

The equilibrium constants K and β_{230} can be used to calculate β_{110} for the H^+-independent equilibrium 10 from the relationship $\beta_{110} = (\beta_{230}/K)^{1/2}$. β_{110} values for all of the hydroxamic acid ligands **Ia**−**d** are also summarized in Table 2.

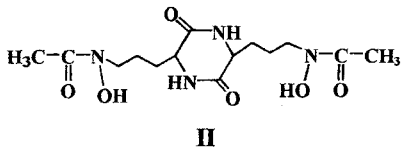
$$Fe_{aq}^{3+} + L^{2-} \rightleftarrows Fe(L)(OH_2)_2^+ \quad \beta_{110} \tag{10}$$

Discussion

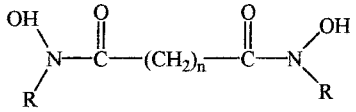
The acid dissociation constants for **Ia**−**d** (Table 1) fall in the normal range (pK_a = 8−10) for hydroxamic acids.[18] Our results may be compared with similar pK_a data in Table 3 for the dihydroxamic acid siderophore rhodotorulic acid (**II**)[19] and a series of alkyl-bridged synthetic analogues (**III**).[20,21] There is an expectation that $pK_{a1} < pK_{a2}$ and that for statistical reasons $pK_{a2} - pK_{a1} \sim 0.6$ for a diprotic acid with two equivalent acid sites.[22] This is generally the case for the data in Table 3, except for rhodotorulic acid (**II**) and **IIIa**, the latter of which appears to be an anomaly. For the dihydroxamic acids studied here $pK_{a2} - pK_{a1} > 0.6$ (Table 1), suggesting some degree of intramolecular interaction between the two hydroxamic acid units. Comparison of our data in Table 1 with literature data in Table 3 suggests that the enhanced difference in pK_{a1} and pK_{a2} is due to a lower value for pK_{a1}. This may arise from a stabilization of the conjugate base HL^-, as a result of the long chain between the hydroxamic acid functional groups that enables them to interact through H-bonding. This is illustrated in eq 11. Such an interaction would decrease pK_{a1} and increase pK_{a2}. This intramolecular H-bonding scheme is also likely to be operative for rhodotorulic acid (**II**) and perhaps for **IIIa**.

Table 3. Dihydroxamic Acid Dissociation Constants

hydroxamic acid	pK_{a1}	pK_{a2}	$pK_{a2} - pK_{a1}$	ref
II	8.49	9.44	0.95	19
IIIa	7.74	10.79	3.05	20
IIIb	9.10	9.79	0.69	20
IIIc	9.16	9.83	0.67	20
IIId	9.21	9.78	0.57	20
IIIe	8.72	9.37	0.65	21
IIIf	8.79	9.37	0.58	21
IIIg	8.86	9.42	0.56	21
IIIh	8.92	9.45	0.52	21
IIIi	8.95	9.47	0.52	21
IIIm	9.05	9.58	0.54	21

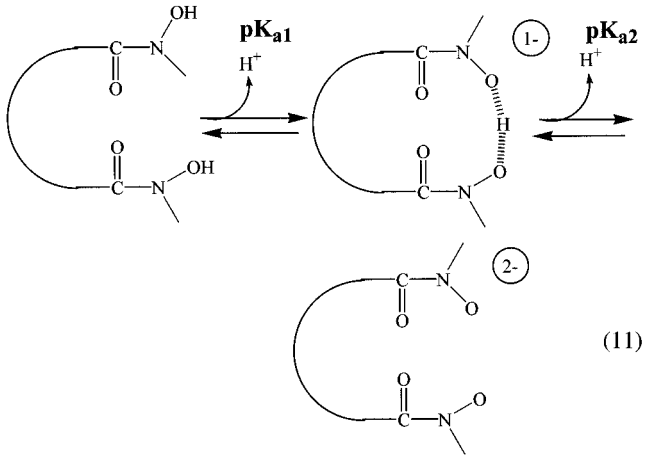


II



R = $CH(CH_3)_2$; n = 3(IIIa), 4(IIIb), 5(IIIc), 6(IIId)
R = CH_3; n = 2(IIIe), 4(IIIf), 6(IIIg), 7(IIIh), 8(IIIi)
R = H; n = 4(IIIj), 6(IIIk), 7(IIIl), 8(IIIm)

III



(11)

The synthetic dihydroxamic acids described here exhibit a high affinity for coordination to Fe(III) as illustrated by the large

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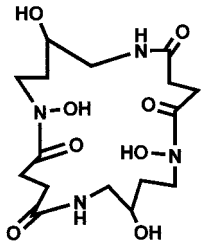
Table 4. Dihydroxamic Acid−Fe(III) Stability Constants

hydroxamic acid	$\log \beta_{110}$	$\log \beta_{230}$	pFe[a]	ref
II	21.55	62.2	21.8	19
IIIa	22.84	62.1		20
IIIb	22.83	62.2		20
IIIc	22.76	62.4		20
IIId	22.63	62.3		20
IIIj	17.60			25
IIIk	18.01			25
IIIl	20.08			25
IIIm	20.30			25
IV	23.5	64.7	23.0	26

[a] pFe = $-\log [Fe_{aq}^{3+}]$ when $[Fe^{3+}]_{tot} = 10^{-6}$ M, $[H_2L] = 10^{-5}$ M, and pH = 7.4.[24]

β and pFe values in Table 2. There is very little variation in stability constants with changes in chain length and alkyl group at the apical C atom or whether the hydroxamate binding group is in the "normal" ($-N(OH)C(O)CH_3$; **Ia**) or "retro" ($-C(O)N(OH)CH_3$; **Ib**−**d**) configuration with respect to rhodotorulic acid (**II**). Martell and co-workers report a 1.7 log unit change in stability for the normal and retro isomers of an apical C atom tripodal trihydroxamic acid chelator.[23] However, in their case the chain length between hydroxamate groups was considerably shorter than in our case and consequently steric effects are more pronounced. The normal isomer is certainly capable of strong Fe(III) binding as illustrated by the high affinity of the siderophore rhodotorulic acid (**II**) for Fe(III) (Table 4).[19] However, the 10 atom distance between hydroxamate groups in rhodotorulic acid is sufficient to relieve steric strain.

The long chain length between hydroxamate binding groups may explain the somewhat lower stability constants for our Fe(III)−dihydroxamate complexes relative to rhodotorulic acid (**II**). (However, the pFe values, which reflect the free uncomplexed Fe_{aq}^{3+} concentration at an arbitrary set of conditions,[24] are comparable for **Ia**−**d** and **II**.) The 19−21 atom distance between hydroxamate groups for **Ia**−**d**, while affording conformational flexibility, may require a heavy entropic price to be paid on proceeding from a highly disordered metal free state to a more ordered Fe(III) chelate. Certainly the interplay between ligand disorder and preorganization plays a role in chelate stability as illustrated by the enhanced stability of the Fe(III) complex of the cyclic dihydroxamic acid alcaligin (**IV**) relative to the linear rhodotorulic acid (**II**) (Table 4)[26,27] and **Ia**−**d** (Table 2). Alcaligin has been shown to be highly preorganized in the metal free state.[26]



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