ligand trans to CO in the ferrous form[10] or trans to thiolate or phosphine in the ferric form,[14] the results observed in this study of phosphine binding, together with the previous findings with CO and thiolate, strongly support the conclusion that the heme iron of chloroperoxidase has an endogenous thiolate axial ligand. Given the well-established presence of an endogenous thiolate ligand to the heme iron of P-450, this conclusion is further strengthened by the close similarities observed between the UV-visible absorption, MCD, and, for ferric forms, EPR spectra of the phosphine adducts of chloroperoxidase and P-450. The spectroscopic evidence for thiolate ligation to chloroperoxidase stands in contrast to the chemical evidence against such a ligand.[2]

Acknowledgment. This work was supported by Grants GM-26730 (J.H.D.) and GM-07768 (L.P.H.) from the U.S. Public Health Service. The electromagnet for the circular dichroism spectrophotometer was purchased through a grant from the Research Corp. We wish to thank Dr. Kim Smith Eble and Cheryl Shigaki for purification of cytochrome P-450-CAM, John A. Alberta for electrophoretic analysis of the enzymes, and Joseph V. Nardo and Edmund W. Svastits for assembling the computer-based spectroscopic data acquisition and manipulation system. J.H.D. is the recipient of a Camille and Henry Dreyfus Teacher/Scholar Award (1982-1987), an Alfred P. Sloan Foundation Research Fellowship (1983-1987), and a National Institutes of Health Research Career Development Award (1983-1988; AM-01123).

Registry No. Cytochrome P-450, 9035-51-2; bis(hydroxymethyl)-methylphosphine, 5958-52-1; dimethylphenylphosphine, 672-66-2; chloroperoxidase, 9055-20-3; heme, 14875-96-8; iron, 7439-89-6; cysteine, 52-90-4.

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New Multidentate Ligands. 27. Synthesis and Evaluation of Metal Ion Affinities of New Endocyclic Hydroxamate Macrocycles

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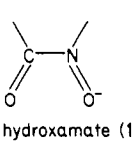
Two synthetic pathways for the synthesis of endocyclic polyhydroxamate macrocycles are explored: peroxomolybdate oxidation of endocyclic amides and the cyclization of benzyl-protected hydroxamic acid intermediates. Because of the apparent sensitivity of the peroxomolybdate oxidation step to steric effects, the benzylhydroxamate route was found to be superior, with the smallest stepwise yield being the ring closure (~26%) and with good yields of other reaction steps up to the 80-90% range, for an overall yield of 2% based on the triethylene glycol starting material. The two endocyclic dihydroxamate macrocycles synthesized, the sexidentate 5,14-dihydroxy-4,15-dioxo-1,5,14,18-tetraaza-8,11,21,24-tetraoxacyclohexacosane and its octadentate 1,18-diacetic acid derivative, were characterized by potentiometric measurement of their proton, Ni(II), and Fe(III) affinities. The results show stability enhancement over analogous complexes having exocyclic hydroxamate donor groups.

Introduction

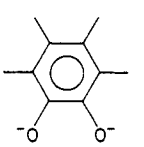
There is considerable interest in the development of new selective ligands for the formation of iron(III), gallium(III), and indium(III) complexes of high stability, particularly for the treatment of iron overload disease (Cooley's anemia)[1-4] and for the imaging of tumors and organs in the human body.[5] Considerable attention has also been given to the siderophores,[6] which are microbial hydroxamate and catecholate ligands having high affinity for iron(III) and other trivalent metal ions. Synthetic ligands containing three bidentate catecholate donor groups have been synthesized as models of the siderophores and have been found to have high affinities for iron(III).[4] Thus far no synthetic hydroxamate ligands having iron(III) affinities comparable to those of the natural trihydroxamate siderophores have been reported.

The structures of the natural siderophores vary considerably with respect to the placement of the bidentate hydroxamate or catecholate donor groups. The hydroxamate siderophores contain functional groups in acyclic, exocyclic, and endocyclic arrangements.[6] Of these, the endocyclic ligands, such as deferriferrioxamine E, show the highest affinities for iron(III), because of the more effective operation of the macrocyclic effect in this type of structure. Thus far no natural endocyclic catecholate siderophores have been identified. Enterobactin, which has the highest iron(III) affinity of any ligand yet measured,[4] has three exocyclic catecholamide donor groups. Reports are now appearing, however, on the design and synthesis of endocyclic catecholate ligands modeled after the siderophores.[2,7-9] On the other hand, synthetic endocyclic trihydroxamates have thus far not been reported.

In addition to the macrocyclic effect, the synthesis and study of endocyclic hydroxamates and catecholates are of interest because of the specificities that can be built into the structure by variation of ring size. The structures of the hydroxamate and catecholate donor groups, indicated by **1** and **2**, place stringent



hydroxamate (1)



catecholate (2)

demands on the substituent polyatomic groups that link them together in such a manner as to place six negative oxygens symmetrically around an octahedral metal ion. The trigonal nature of these functional groups demands that the bridging groups approach from the back side for hydroxamate and from the back side or at the 3,6-positions for the catecholates. The use of molecular models and comparison with natural siderophores indicate that connecting chains of eight atoms or more are needed

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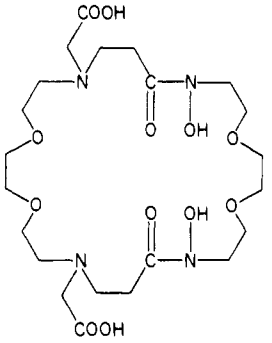
for the hydroxamates. It is the purpose of this paper to report the synthesis and study of a new sexidentate macrocycle containing six donor groups, four of which are negative oxygens derived from endocyclic hydroxamate groups.

Ligand Design

In working out the design of a macrocyclic ligand containing endocyclic hydroxamate groups, it was decided to prepare a ligand with two hydroxamate groups and two bridging amino groups. The latter would be useful in themselves as possible donors. If the groups were secondary amines, they would serve as the positions for attaching additional donor functions. In addition to these considerations, it was considered necessary to have connecting groups containing sufficient numbers of atoms to utilize the hydroxamate groups fully as bidentate donors. Furthermore, it was necessary to allow for the sensitivity of the hydroxamate hydroxy group to many of the rather reactive reagents used in the synthesis of macrocycles.

In the selection of **3** as the first example of this type of macrocycle, placement of the amino groups in adjacent cis positions rather than alternating trans positions was in this case a matter of convenience in synthesis. Two synthetic routes were investigated, the synthesis of the corresponding endocyclic diamide as an intermediate, followed by oxidation to the hydroxamate (Scheme I), and protection of the preformed hydroxamic acid functions by benzylation, as indicated in Scheme II. Both routes require high-dilution steps involving an acid chloride intermediate.



5,14-dihydroxy-4,15-dioxo-1,5,14,18-tetraaza-8,11,21,24-tetraoxahexacosane-1,18-diacetic acid (**3**)

This paper describes the synthesis of intermediates for both synthetic routes leading to **3**, a critical comparison of the two routes as practical methods of making endocyclic hydroxamates, and a physicochemical evaluation of **3** and its immediate precursor macrocyclic intermediate for complexing divalent and trivalent transition-metal ions, with Ni(II) and Fe(III) chelates as examples.

Experimental Section

Melting points were taken on a Fisher-Johns apparatus in open capillaries and are uncorrected. ^{1}H NMR spectra were recorded with an EM-390 spectrometer and ^{13}C NMR spectra with an FT-80 spectrometer. Chemical shifts are given in ppm, with the following abbreviations: s = singlet; d = doublet; t = triplet; qa = quadruplet; m = multiplet; br = broad. The mass spectra were obtained by a californium-252 plasma desorption mass spectrometer through the courtesy of Drs. R. Macfarlane and C. McNeil of this department. Thin-layer chromatography (TLC) was performed with commercially prepared silica gel 60 F_{254} sheets purchased from Merck (layer thickness 0.2 mm).

Potentiometric equilibrium measurements were made in a scrubbed-N_2 protected 75 mL capacity vessel thermostated at 25 °C and equipped with high-pH glass and calomel reference extension electrodes, first calibrated with standard acid at μ = 0.1000 M (KNO_3) to read −log [H^+] directly. Similarly, at high −log [H^+] standard base was used at μ = 0.100 M (KNO_3) to calibrate the system. K_w = [H^+][OH^-] = $10^{-13.79}$ was used for calibration and computation.

Although exact quantities were taken, in rounded terms 0.0500 mmol of ligand in 30.0 mL of μ_0 = 0.100 M KNO_3 was equilibrated at 25 °C with successive additions of 0.1 M KOH and −log [H^+] was recorded on a Corning 145 meter. The data were worked up with use of the program BEST[10] on a PDP 11/40 computer. The main purpose of working up the data immediately was to ascertain the precise molecular weight so as not to add excess metal ion. The solution was made acidic with standard 0.10 M HNO_3, and 0.05 mmol of standard Ni^{2+} was added. A slight adjustment for this dilution in ionic strength was made by addition of KNO_3, and then the solution was equilibrated forward and backward in the case of ligand **3**. The data from back-titration of **3** was used, as it was noted that the forward equilibrium for that ligand was too slow. Iron(III) titrations were done similarly, but their equilibrations were more rapid. The data were worked up with use of the program BEST.[11]

1,8-Diphthalimido-3,6-dioxaoctane (5) and 1,8-Diamino-3,6-dioxaoctane (6). The procedures of Bogatskii et al.[9] have been modified as follows:

To a suspension of 44.4 g (0.24 M) of potassium phthalimide in 110 mL of DMF, heated to oil bath temperature 100 °C, was added 18.7 g (0.1 M, 16.1 mL of 97% reagent from Aldrich) of 1,8-dichloro-3,6-dioxaoctane in 10 mL of DMF at a rate such that the temperature did not rise above 105 °C. The mixture was stirred and kept at 95–100 °C (oil bath) for 22 h. It was cooled and then poured onto 400 mL of crushed ice and allowed to stand for 1 h until precipitation was complete. The precipitate was filtered off, washed with water until no Cl^- remained in the filtrate, and then dried at 60 °C for 16 h. The yield of 38.5 g was 94% of the theoretical amount; mp 187–188 °C (lit.[9] mp 185–186 °C, yield 72%). ^{1}H NMR ($CDCl_3$): 3.6 (s, 4 H, $-OCH_2CH_2O-$); 3.6–3.9 (m, 8 H, $-CH_2CH_2NPh$); 7.5–7.8 (m, 8 H, arom H).

To a suspension of 79 g (0.19 M) of 1,8-diphthalimido-3,6-dioxaoctane in 400 mL of absolute ethyl alcohol was added 14.59 g (0.456 M, 15 mL) of 97% hydrazine, and the mixture was refluxed for 12 h. After the mixture was cooled, 6 M HCl was added to bring the pH to 1. The mixture was refluxed for 1 h and allowed to stand overnight.

The precipitate (phthaloyl hydrazide) was filtered off and washed with 5 × 100 mL of water to pH 5 and vacuum evaporated to remove the EtOH and water. The filtrate was evaporated nearly to dryness. A 35-g amount of KOH pellets was slowly added to the dihydrochloride, and the paste was extracted with 4 × 100 mL of CH_2Cl_2. The CH_2Cl_2 phase was taken out, filtered, and dried with anhydrous $MgSO_4$ for 30 min. The clear CH_2Cl_2–diamine solution was vacuum evaporated to remove CH_2Cl_2. The crude product (75% yield) was vacuum distilled, and the fraction with bp 76–78 °C at 1 mmHg was collected (38% yield of distilled product, lit.[9] distilled yield 54%). ^{1}H NMR ($CDCl_3$): 2.4 (s, br, 4 H, $-NH_2$); 2.8 (t, 4 H, $-CH_2N$); 3.5 (t, 4 H, $-OCH_2CH_2-$); 3.6 (s, 4 H, $-OCH_2CH_2O-$).

***N,N'*-Bis(*p*-tolylsulfonyl)-1,8-diamino-3,6-dioxaoctane (7).**[11] A 12-g (0.085 M) amount of 1,8-diamino-3,6-dioxaoctane was dissolved in 150 mL of water, and 6.3 g (0.16 M) of NaOH (in 20 mL of water) was added. A 4.0-g (0.21 M) sample of *p*-toluenesulfonyl chloride was dissolved in 80 mL of diethyl ether, and the solution was washed twice with 10% NaOH (2 × 90 mL). The two solutions were mixed and shaken for 1 h. The pale yellow oil that separated was dissolved by the addition of 3 g of NaOH (in a small amount of water). The reaction mixture was shaken for another 3 h and allowed to stand overnight. NaOH (1 g) was added to the mixture to dissolve the oil that separated. The clear ether solution (no TsCl odor) was removed and washed with 120 mL of 1 M NaOH. The combined aqueous phase and washings was acidified with concentrated HCl to pH 3 (5–6 mL of HCl used). The separated pale yellow oil was extracted with CH_2Cl_2, washed twice with saturated NaCl solution, and then dried over $MgSO_4$. The solvent was removed under reduced pressure and dried at 60 °C for 20 h. A total of 34.2 g of white solid was obtained: yield 88%; mp 104–106 °C. ^{1}H NMR ($CDCl_3$): 2.4 (s, 6 H, 2 CH_3); 3.15 (qa, 4 H, 2 CH_2N); 3.5 (m, 8 H, $-CH_2OCH_2CH_2OCH_2-$); 5.5 (t, 2 H, −HNTs); 7.25, 7.75 (m, 8 H, arom H). ^{13}C NMR ($CDCl_3$): 143.0, 136.7, 129.4, 126.7 (arom C); 69.8, 69.1 ($-OCH_2CH_2OCH_2-$); 42.5 ($-CH_2NHTs$); 21.2 (CH_3).

Dimethyl 4,13-Bis(*p*-tolylsulfonyl)-4,13-diaza-7,10-dioxahexadecane-1,16-dioate (8).[12] A mixture of *N,N'*-bis(*p*-tolylsulfonyl)-1,8-diamino-3,6-dioxaoctane (8.7 g, 0.019 M), methyl acrylate (11.2 mL, 10.63 g, 0.124 M), K_2CO_3 (5.25 g, 0.038 M), and 14 mL of DMF was stirred and heated to 95–100 °C under reflux for 22 h. After it was cooled, the mixture was vacuum evaporated to remove the excess methyl acrylate and DMF (1 mmHg). A 70-mL amount of water was added and then 70 mL of $CHCl_3$ to extract the product. The separated aqueous phase was extracted with 30 mL of $CHCl_3$. The combined $CHCl_3$ solutions were washed with 100 mL of saturated NaCl solution and allowed to stand until two clear phases separated. The $CHCl_3$ phase was removed and dried with anhydrous $MgSO_4$. The $CHCl_3$ was removed, and the product was dried under vacuum (1 mmHg); 9.9 g of crude product was obtained

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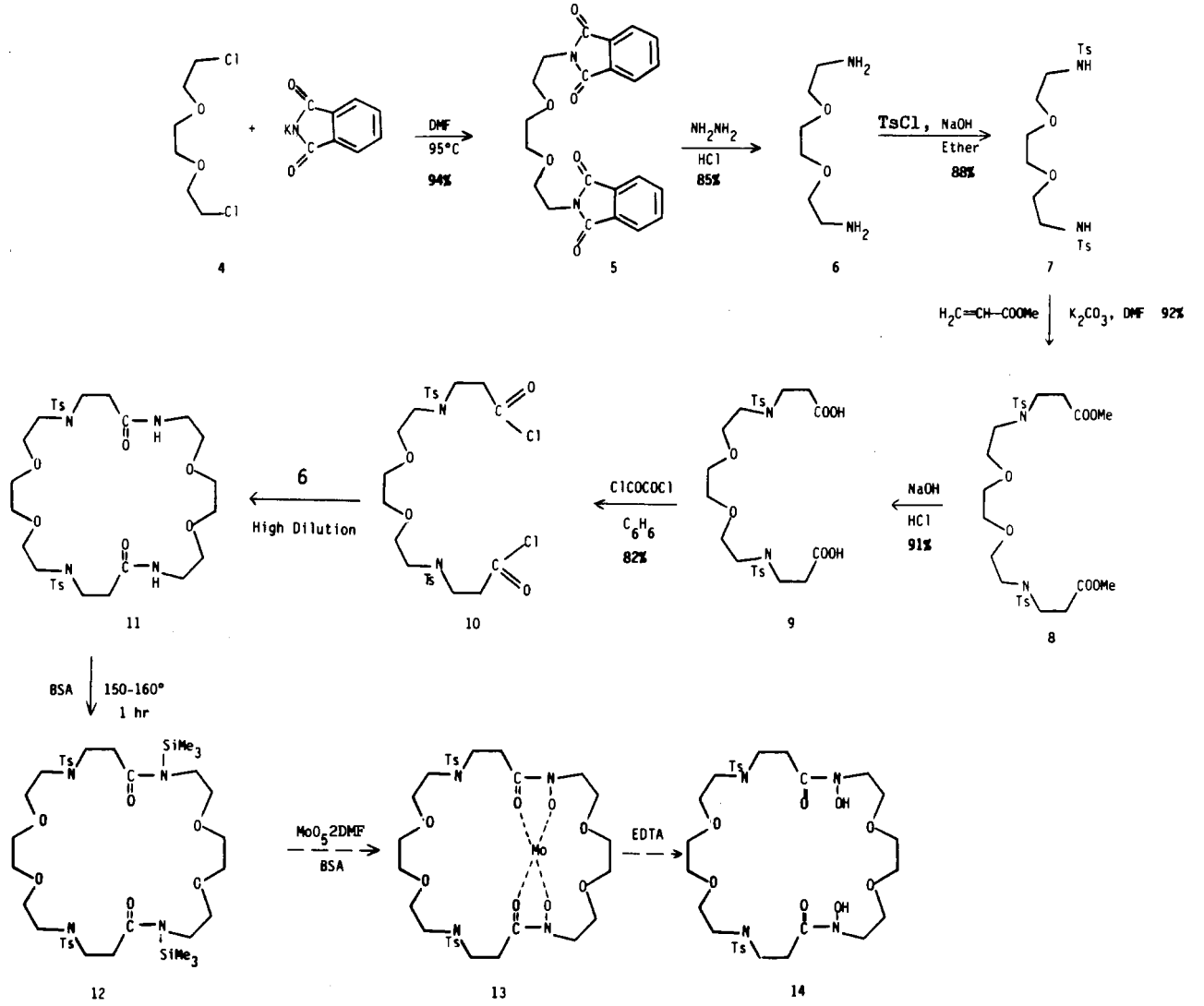
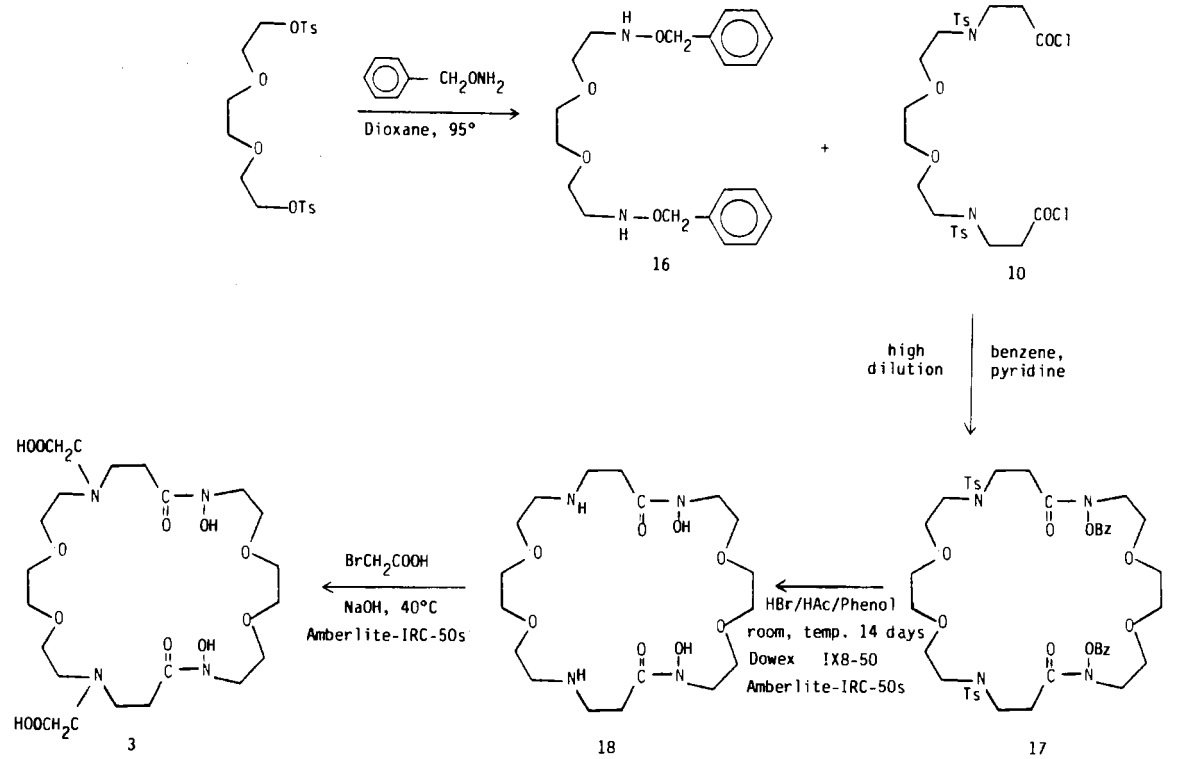
Scheme I



Scheme II



(yield 92%). This crude product was used without further purification for the following step.

For further purification, the 9.9 g of crude product was dissolved in 10 mL of $CHCl_3$, 15 g of silica gel was added to form the loaded phase, and the mixture was air-dried. The loaded silica gel was added to the top of a dry silica gel column (70 g of untreated silica gel 40, 70–230 mesh ASTM Ex Reagent from MC/B; Manufacturing Chemists, Inc., column size 42 × 80 mm). The column was washed with $CHCl_3$–MeOH (95:5), and the first pale yellow band was collected. About 150 mL of solution was obtained (500 mL of eluent was used). The solvent was removed with vacuum; 5.2 g of pure product was obtained (yield 53%). ^1H NMR ($CDCl_3$): 2.4 (s, 6 H, 2 CH_3); 2.7 (t, 4 H, $-CH_2COOR$); 3.2–3.8 (m, 22 H, $-CH_2OCH_2-$, $-CH_2NTsCH_2-$, $-COOCH_3$); 7.3, 7.7 (d, 8 H, arom H). ^{13}C NMR ($CDCl_3$): 171.2 (–COOR); 142.9, 136.0, 129.3, 126.6 (arom C); 69.7 ($-CH_2OCH_2-$); 51.0 (CH_3 ester); 48.3, 44.9 (CH_2NTsCH_2); 33.7 (CH_2COOR); 20.8 (tolyl CH_3).

4,13-Bis(*p*-tolylsulfonyl)-4,13-diaza-7,10-dioxahexadecane-1,16-dioic Acid (9).[13] An 8.25-g amount of NaOH was dissolved in 160 mL of water. This 5% NaOH solution was added to 52 g of the diester and stirred at room temperature for 20 h until the oily diester gradually disappeared. Some insoluble colloidal material was filtered out. The product was vacuum evaporated to remove part of the CH_3OH until the volume of the solution was reduced to half of the original. Two volumes of water were added to dilute the reaction mixture. Some white precipitate separated and was filtered out. The clear filtrate (pH 8–9) was treated with 20 mL of concentrated HCl. The white gummy diacid separated and was extracted with 4 × 100 mL of $CHCl_3$. The $CHCl_3$ solution was washed with 3 × 150 mL of saturated NaCl solution. The $CHCl_3$ solution was filtered and dried with anhydrous $MgSO_4$ overnight. After removal of the solvent and vacuum drying, 45 g of product was obtained; yield 91%. ^1H NMR ($CDCl_3$): 2.4 (s, 6 H, 2–CH_3); 2.7 (qa, 4 H, 2 CH_2COO^-) 3.2–3.7 (m, 16 H, $-CH_2OCH_2-$, $-CH_2NTsCH_2-$); 7.3, 7.8 (d, 8 H, arom H); 10.6 (s, 2 H, –COOH). ^{13}C NMR ($CDCl_3$): 142.9, 135.4, 129.1, 126.4 (arom C); 69.5 ($-CH_2O$); 48.2, 44.6 ($-CH_2NTsCH_2-$); 33.5 ($-CH_2COOH$); 20.7 ($-CH_3$); 175.6 (–COOH).

4,13-Bis(*p*-tolylsulfonyl)-4,13-diaza-7,10-dioxahexadecane-1,16-dioic Acid Chloride (10).[14] A 26.3-g (0.0438 M) amount of the diacid was dissolved in 56 mL of dry benzene, and the solution was added to 12 mL of oxalyl chloride, with 3 drops of pyridine as catalyst. The reaction mixture was protected from moisture and was stirred at room temperature for 20 h. The reaction mixture was filtered very quickly, and the solvent and excess reactant were removed from the filtrate by vacuum evaporation. Two volumes of 70 mL of dry benzene were added to evaporate off excess oxalyl chloride. The yellow solid residue was vacuum-dried for 30 min. A 26.8-g amount of crude product was obtained (96%). From ^{13}C NMR the only impurity in the yellow crude product was benzene, so that precipitation from ether was not necessary for the following step.

To this material was added 100 mL of dry ether, and the suspension was stirred at room temperature for 30 min. The yellow impurities dissolved in the ether solution (yellow), which was decanted or filtered under nitrogen. The white precipitate was washed with 50 mL of dry ether. A 21.5-g amount of pure acid chloride (white) was obtained; yield 80%. ^1H NMR ($CDCl_3$): 2.5 (2, 6 H, 2–CH_3); 3.2–3.7 (m, 20 H, $-CH_2OCH_2$, $-CH_2NTsCH_2-$); 7.2, 7.6 (m, 8 H, arom H). ^{13}C NMR ($CDCl_3$): 143.5, 135.5, 129.6, 126.8 (arom C); 171.8 (–COCl), 70.3, 69.9 ($-CH_2OCH_2$); 49.3, 46.9, 45.0 ($-CH_2NTsCH_2CH_2-$); 21.2 ($-CH_3$).

4,15-Dioxo-1,18-bis(*p*-tolylsulfonyl)-1,5,14,18-tetraaza-8,11,21,24-tetraoxacyclohexacosane (11) (High-Dilution Method).[14] A 19.6-g (0.0307 M) amount of the diacyl chloride was dissolved in 500 mL of dry benzene. A 9.6-g (0.0614 M) amount of the diamine was dissolved in 500 mL of dry benzene. (The benzene was dried by reflux with Na for 24 h and then distilled). The solutions were added to 1.2 L of dry benzene (dried by activated molecular sieves) through two separation funnels at about 1 drop/s. The addition was accomplished in 7 h. During the addition, the reaction mixture was vigorously stirred. The reaction mixture was allowed to stand overnight and was then filtered. The flask was thoroughly washed with three volumes of 200 mL of dry benzene. The combined filtrate and washings were vacuum evaporated, and about 17 g of crude product was obtained. Its TLC spectrum showed one main product and a small amount of polar impurities. A 13-g amount of the crude product was dissolved in 100 mL of $CHCl_3$, which was then loaded on 170 g of alumina (neutral, 80–200 mesh, Fisher Scientific A-950, Brockman activity 1). This loaded alumina was put on a 30–40 mm diameter column and was eluted with $CHCl_3$ (MC/B, CX 1055-9, Reagent ACS) until no spot of R_f 0.3 was detected on a TLC plate (developed by 95:5 $CHCl_3$–MeOH). The 500 mL of eluate was collected and vacuum evaporated to remove the solvent, and the residue was crystallized from a solvent mixture of 100 mL of benzene and 50 mL of petroleum ether (30–60 °C). The total yield of crystalline product (mp 142–143 °C) was 12.1 g, 56%. ^1H NMR ($CDCl_3$): 2.4 (2, 6 H, $-CH_3$); 2.6 (t, 4 H, $-CH_2CONH-$); 3.2–3.75 (m, 28 H, $-CH_2OCH_2-$, $-CH_2NTsCH_2-$); 6.6 (t, 2 H, –CONH–); 7.3, 7.7 (d, 8 H, arom H). ^{13}C NMR ($CDCl_3$): 170.3 (–CONH–); 143.2, 135.4, 129.5, 126.9 (arom C); 69.9, 69.3 ($-CH_2OCH_2$); 49.2, 46.8 ($-CH_2NTsCH_2-$); 39.0 ($-CONH-CH_2-$); 36.0 ($-CH_2CONH$); 21.1 ($-CH_3$(Ts)). Mass spectrum: m^+/e = 713 (100); m^-/e = 711 (100); fw = 712.

5,14-Dihydroxy-4,15-dioxo-1,18-bis(*p*-tolylsulfonyl)-1,5,14,18-tetraaza-8,11,21,14-tetraoxacyclohexacosane (14).[15] To 0.712 g (0.002 M) of the diamide ring **11** was added 5 mL (0.02 M) of bis(trimethylsilyl)acetamide (BSA). This mixture was refluxed at 150–160 °C under dry N_2 for 1 h and then was vacuum distilled under 1 mmHg in 0, 30, and 70–80 °C baths to remove the excess BSA and $CH_3CONHSiMe_3$. A pale yellow liquid was obtained. ^1H NMR showed it to be the silylated diamide ring **12**. To this was added 2 mL of CH_2Cl_2. After all the silylated compound was dissolved, 0.99 g of MoO_5.2DMF was added and the reaction mixture was stirred at room temperature for 5.5 days. After the solvent was removed, 10 mL of 1 M Na_4EDTA (pH 9.7) was used to decompose the molybdenum complex **13**. This solution was warmed to about 60–70 °C for 20 min and then 1:1 HCl was used to adjust the pH of the solution to 7.5. It was then extracted with 75–100 mL of $CHCl_3$. The chloroform solution obtained was washed with saturated NaCl solution and dried with $MgSO_4$ overnight.

The dried $CHCl_3$ solution was concentrated to about 10 mL and loaded onto 17 g of neutral alumina (A-950, Fisher Co.). Washing with $CHCl_3$ gave 300–400 mL of eluate that contained the unreacted diamide ring. The column was then washed with $CHCl_3$–MeOH (8:2), and about 30 mL of eluate was collected, which contained a component with R_f 0 (developed by 95:5 $CHCl_3$–MeOH). After removal of the solvent and vacuum drying, 44 mg of pale yellow oil, **14**, was obtained; yield 6%.

This compound reacts with Fe^{3+} at pH 1 to form a wine red compound, which showed an absorption of 510 nm (in MeOH). This Fe^{3+} complex was dissolved in $CHCl_3$ and washed 8–10 times with 0.6 M HCl until no Fe^{3+} could be detected by KCNS. The organic phase was dried with saturated NaCl and $MgSO_4$, the solvent was removed, and from ^1H NMR the original dihydroxamic acid ring was obtained. ^1H NMR ($CDCl_3$): 2.4 (s, 6 H, CH_3); 2.6 (t, 4 H, CH_2CON-); 3.1–3.8 (m, 28 H, $-CH_2OCH_2-$, CH_2NTsCH_2-, $-N(OH)CH_2$); 7.3, 7.7 (d, 8 H, arom H).

1,18-Bis((benzyloxy)amino)-3,6-dioxaoctane (16).[16] (Benzyloxy)amine was prepared from its hydrochloride (from Aldrich) by slowly adding 5.6 g of KOH dissolved in 10–20 mL of water to a suspension of 15.9 g of (benzyloxy)amine hydrochloride in 30–50 mL of water until the aqueous solution was alkaline to phenolphthaline indicator. The free base was extracted with CH_2Cl_2 twice (50 and 25 mL) and then dried with anhydrous $MgSO_4$ for 2 h. The solution was rotavaporated at 25–30 °C to remove the solvent; 12.3 g of colorless oil was obtained.

A 12.3-g amount of (benzyloxy)amine was dissolved in about 15 mL of dry *p*-dioxane. A 7.64-g portion of 1,8-bis(((*p*-tolylsulfonyl)oxy)-3,6-dioxaoctane[16] (**15**) was dissolved in 45 mL of *p*-dioxane. The latter was added slowly to (benzyloxy)amine at room temperature. The mixture was kept at 95–100 °C under N_2 for 3 days. When the solution was cooled to room temperature, the white crystalline salt of (benzyloxy)-amine and *p*-toluenesulfonic acid that separated out was filtered off and washed with dry ether (3 × 20 mL). To the filtrate (combined with the ether washings) was added 2.5 mL of concentrated HCl, and the HCl salt of the unreacted (benzyloxy)amine was precipitated. This was also filtered out and washed with 2 × 25 mL of dry ether. The dioxane and ether filtrate was rotavaporated under vacuum (about 1 mmHg) to near-dryness. To the residue was added 25 mL of CH_2Cl_2 and 40 mL of 2.5 N HCl, and the mixture was shaken, giving three layers. The bottom layer was removed and washed with 2 × 20 mL of 2.5 N HCl. The aqueous washing was added to the other two layers.

NaOH (10 g) dissolved in about 20 mL of water was slowly added to the reaction mixture until the pH reached 11–12. The pale yellow oil that separated was extracted with 5 × 25 mL of CH_2Cl_2 until no spot was produced on a TLC plate (developed by 95:5 $CHCl_3$–MeOH). The CH_2Cl_2 solution was dried with Na_2SO_4 for 2 h. The solvent was removed and the residue was vacuum-dried at 50–60 °C for 3 h (a small amount of residual (benzyloxy)amine with bp 40 °C at 1 mmHg was removed in this way.). The 4.9 g of product obtained amounted to

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(15) Matlin, S. A.; Sammes, P. G.; Upton, R. M. *J. Chem. Soc., Perkin Trans.* **1979**, 2481.
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approximately 100% of the theoretical yield. ^{1}H NMR ($CDCl_3$): 3.1 (t, 4 H, CH_2N–); 3.5–3.65 (m, 8 H, CH_2OCH_2); 4.7 (s, 4 H, CH_2 of Bzl); 5.4 (s, br, 2 H, HN): 7.3 (s, 10 H, arom H). ^{13}C NMR ($CDCl_3$): 137.7, 126–129 (arom C); 75.3 (CH_2 of Bzl); 69.8, 67.0 (CH_2OCH_2); 51 (CH_2NH).

5,14-Bis(benzyloxy)-4,15-dioxo-1,18-bis(*p*-tolylsulfonyl)-1,5,14,18-tetraaza-8,11,21,24-tetraoxacyclohexacosane (17) (High-Dilution Method). A 3.19-g amount of the diacyl chloride **10** was dissolved in 160 mL of dry benzene. 1.8-g amount of the bis(benzyloxy)amine **16** was dissolved in 160 mL of dry benzene (the benzene was previously refluxed with Na for 24 h and distilled). These two solutions were added to 1.2 L of dry benzene to which 8 mL (7.9 g, 0.1 M) of dry pyridine had been added. The addition was accomplished in 6 h with vigorous stirring. After the addition the reaction mixture was allowed to stand for 16 h. The pyridine salt was filtered out, and the benzene solvent was removed by rotavaporation at 40–50 °C. The residue was dissolved in 80 mL of CH_2Cl_2, which was then washed with 0.6 N HCl (75, 75, 50 mL), washed with saturated NaCl solution, and dried with $NaSO_4$.

After removal of the CH_2Cl_2, the yellow oil was dissolved in 15 mL of $CHCl_3$. This solution was added to a column containing 100 g of neutral alumina (A-950, Fisher Co.) and washed with dry benzene until no impurities of R_f 0.9 could be detected in the eluate by TLC developed with 95:5 $CHCl_3$–MeOH. The column was then eluted with $CHCl_3$ to give a product of R_f 0.5 (developed with 95:5 $CHCl_3$–MeOH). After removal of the solvent and vacuum drying, 0.51 g of product was obtained.

A 2.5-g portion of the impure material (about 70%) from the above was air-dried, loaded on 40 g of neutral alumina, and washed with $CHCl_3$. An additional 0.47 g of pure product was obtained. (1.4 g of impure product was also collected).

The 1.4 g of impure product from the above column treatment was dissolved in 15 mL of $CHCl_3$, was loaded on 12 g of Al_2O_3, and put on 120 g of Al_2O_3 in a 20 × 300 mm column, and the column was washed with $CHCl_3$; 0.70 g of pure product was obtained (yield 1.21 g (26%)). ^{1}H NMR ($CDCl_3$): 2.4 (s, 6 H, CH_3); 2.8 (t, 4 H, CH_2CO–); 3.2–3.7 (m, 28 H, CH_2OCH_2, CH_2NTsCH_2, CH_2NOBzl); 4.8 (s, 4 H, CH_2 of Bzl); 7.2, 7.3, 7.6 (s, d, 18 H, arom H). ^{13}C NMR ($CDCl_3$): 173.5 (–CO–); 143.5, 136.9, 135.1, 130.9–126 (arom C); 79.4 (CH_2 of Bzl); 70.5 (CH_2OCH_2); 67.2 (NCH_2); 49.2, 45.9 (CH_2NTsCH_2); 33.2 (CH_2CO–); 21.8 (CH_3). Mass spectrum: m^+/e, 926 (100); m^-/e, 923 (100); fw = 924.

5,14-Dihydroxy-4,15-dioxo-1,5,14,18-tetraaza-8,11,21,24-tetraoxacyclohexacosane (18).[17] A 33-mL amount of a 33% solution of HBr in acetic acid and 1.78 g of phenol were added to 2.84 g of the ditosyl dibenzyl macrocycle (which was vacuum-dried at 1 mmHg for 2 h). The reaction mixture was kept at room temperature (about 25 °C), with exclusion of moisture, for 2 weeks. The bottle was then chilled in ice, and the mixture was poured into ca. 200 mL of cold dry ether (cooled at 2–3 °C in an ice–water bath). The mixture was stirred at this temperature for 1–2 h until the solution became completely clear. The red oily product adhered on the wall of the flask. The ether solution was decanted, and the product was washed with 2 × 50 mL of cold dry ether. The hygroscopic product was taken up by 40 mL of water. A small amount of charcoal was used to obtain a colorless aqueous solution. The aqueous solution was passed over a 20 × 300 mm bed of Dowex 1/8-50 (Cl form). The column was washed with water, and the fractions giving a positive Fe^{3+} test were pooled and vacuum evaporated under 50 °C bath to near-dryness. The excess free acid was removed by evaporation with water several times. A total of 0.8 g of crude product was obtained. This product was dissolved in a small amount of water and washed with ether two to three times. Dilute NaOH aqueous solution was added to the above aqueous phase to obtain pH 5–5.6. This solution was loaded on 20 mL of Amberlite-IRC-50s (a 25-mL buret was used as the column), and the column was washed with water and with pH 2 HCl aqueous solution. The fractions showing a positive Fe^{3+} test were combined. After the solvent was removed, and the product was evaporated with water two times, 0.44 g of pure material was obtained; yield 25%. ^{1}H NMR (D_2O): 2.9 (t, 4 H, CH_2CO–); 3.3 (m, 12 H, CH_2NHCH_2, $CON(OH)CH_2$); 3.8 (m, 16 H, CH_2OCH_2).

1,18-Bis(carboxymethyl)-5,14-dihydroxy-4,15-dioxo-1,5,4,18-tetraaza-8,11,21,14-tetraoxacyclohexacosane (3).[18] A 0.18-g (0.3-mmol) amount of 5,14-dihydroxy-4,15-dioxo-1,5,14,18-tetraaza-8,11,21,24-tetraoxacyclohexacosane HCl salt was dissolved in 4–5 mL of water and was neutralized with 2.5 M NaOH to pH 7–9. A 0.095-g amount of bromoacetic acid was dissolved in 2 mL of water and was neutralized with 2.5 M NaOH to pH 6–10. The above two solutions were mixed. The pH of the reaction mixture gradually dropped to lower than 8. A 2.5 M NaOH solution was added dropwise, and the temperature of the solution was kept at 40–45 °C. The pH of the solution was measured by a Beckman Research pH meter with a combination glass electrode. Whenever the pH of the solution was dropped to lower than 9, another drop of 2.5 M NaOH was added, until the stoichiometric amount of NaOH was added, at which time the pH of the solution reached 9.5. The reaction mixture was allowed to stand overnight. This solution was loaded on a 9 × 300 mm Amberlite-IRC-50S (H^+ form) column and eluted with water. After about 40 mL of eluate with HBr and HCl was collected, the portion that gave a positive Fe^{3+} test appeared. After removal of the solvent and vacuum drying at 60 °C for 2 h, 0.055 g of product was obtained; yield 28%. ^{1}H NMR (D_2O): 2.8 (t, 4 H, CH_2CO–); 3.6 (s, 4 H, CH_2COO–); 3.5, 3.8 (m, 28 H, CH_2OCH_2, CH_2NCH_2, $CH_2N(OH)$).

Results and Discussion

Synthesis. The synthetic route outlined in Scheme I was first selected as the most direct and efficient method of preparing the desired macrocyclic ligand **3**. All of the reaction steps **4**–**10** prior to ring closure were successfully accomplished in high yield (82–94%). The high-dilution ring closure **10** → **11** to form the macrocyclic diamide **11** was also accomplished in very good yield for that type of reaction (56%). The main problem encountered in this synthesis is the difficulty of oxidizing the macrocyclic diamide to the corresponding endocyclic dihydroxamic acid **12** → **14**. A review of the literature indicated that the best method of converting secondary amides to secondary hydroxamic acids is oxidation with diperoxooxomolybdenum(VI), MoO_5. Although several reaction conditions were tried that in our hands gave good yields of hydroxamic acid from acyclic secondary amides, the yield of dihydroxamic acid **14** obtained from **12**, the trimethylsilyl derivative of the endocyclic diamide **11**, was consistently low, about 6% of the theoretical yield. The unsatisfactorily low yield in the system under investigation could be due to steric problems, resulting from the inability of the molybdenum center to span the two amide groups of **11** to give an intermediate structure such as **13**. This problem could be due to steric crowding resulting from trimethylsilylation of the amide nitrogens, a problem that would not be a factor in the oxidation of a single open-chain secondary amide.

In view of the low yield obtained through molybdenum(VI) oxidation, it was decided to attempt a completely different route to the desired macrocyclic hydroxamic acid, indicated in Scheme II. In this case the yields of the required reaction steps prior to macrocyclic ring closure were found to be highly satisfactory (80–90%). In this sequence the only poor yield involved the high-dilution ring closure, which, at 26%, still provided a reasonable route to the desired macrocycle.

Complexes of the Diamino Bis(hydroxamate) Macrocycle 18. Potentiometric equilibrium curves of the endocyclic dihydroxamic acid with two coordinating amino groups **18** in the absence of and in the presence of equimolar concentrations of Ni(II) or Fe(III) are presented in Figure 1. The ligand is formally an H_2L type ligand, which may be protonated at the amino groups to form species such as H_3L^+ and H_4L^{2+}. Since the two amino nitrogens are adjacent to ether oxygens and carbonyl groups spaced two carbons away, their basicities would be expected to be considerably lower than that of a normal aliphatic amino group. The potentiometric equilibrium curve in Figure 1 for the ligand alone shows dissociation of only one protonation site, indicating considerable coulombic interaction. The first deprotonation is assigned to one of the amino groups. The three overlapping deprotonation reactions from a = 1 to a = 4 are assigned to successive amino group deprotonation followed by dissociation of the hydroxamic acid groups. The values obtained for the last two are 9.21 and 9.86, the relative magnitudes of which imply electronic isolation (the values are close to statistical). The magntidues of the protonation constant of these hydroxamic acid groups are close to that of acetohydroxamic acid itself (log K^H_1 = 9.36), further suggesting independence of these groups from electronic interactions with other functional groups.

The ligand protonation constants and the stability constants of the Ni(II) and Fe(III) chelates are listed in Table I. The equimolar Ni–L potentiometric curve indicates some binding at

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(18) Haskell, B. E.; Bowles, S. B. *J. Org. Chem.* **1976**, *41*, 159.

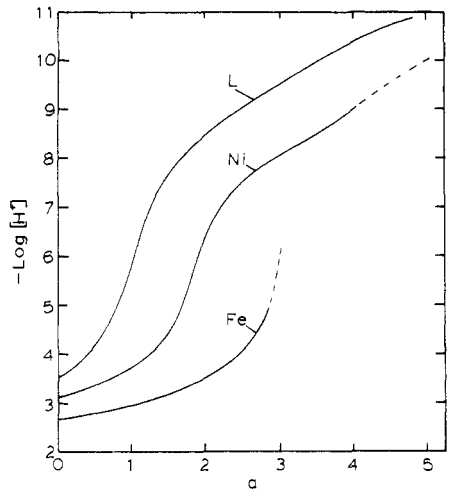

Figure 1. Potentiometric equilibrium curves for **18** in the presence and absence of Ni, Fe^{3+}, and H^+ ions (T_L ($=T_M$) = 1.2×10^{-3} M, μ = 0.100 M (KNO_3), t = 25.0 °C) as a function of −log [H^+].

Table I. Diamino Bis(hydroxamic acid) (H_2L) **18** Protonation Constants and Ni(II) and Fe(III) Stability Constants (μ = 0.100 M KNO_3, t = 25.0 °C in Aqueous Solution)

quotient Q	symbol	log Q
$[HL^-]/[H^+][L^{2-}]$	K^{H}_{1}	9.86
$[H_2L]/[H^+][HL^-]$	K^{H}_{2}	9.21
$[H_3L^+]/[H^+][H_2L]$	K^{H}_{3}	7.70
$[H_4L^{2+}]/[H^+][H_3L^+]$	K^{H}_{4}	4.14
$[NiL]/[Ni^{2+}][L^{2-}]$	K_{NiL}	10.82
$[NiHL^+]/[NiL][H^+]$	K^{H}_{NiHL}	8.10
$[NiH_2L^{2+}]/[NiHL^+][H^+]$	$K^{H}_{NiH_2L}$	7.95
$[NiH_3L^{3+}]/[NiH_2L^{2+}][H^+]$	$K^{H}_{NiH_3L}$	4.11
$[FeL^+]/[Fe^{3+}][L^{2-}]$	K_{FeL}	
$[FeHL^{2+}]/[Fe^{3+}][HL^-]$	K^{HL}_{FeHL}	15.78
$[FeH_2L^{3+}]/[Fe^{3+}][H_2L]$	$K^{H_2L}_{FeH_2L}$	10.58

ca. pH 3 involving the displacement of at least two protons from H_2L^{2+} as indicated by the initial rise in p[H] and inflection at a = 2. Beyond a = 2 there is evidence for formation of the species $NiHL^+$ and NiL in the buffer region prior to precipitation of $Ni(OH)_2$. Additional mathematical analysis of the potentiometric curve indicates the formation of the triprotonated species NiH_3L^{3+} as the initial complex.

It is helpful to rearrange the constants listed in Table I to correspond to the nickel(II) complex formation reactions shown as eq 1–4 in order to make comparisons based on the ligand donor groups involved.

$$Ni^{2+} + L^{2-} \rightleftharpoons NiL \qquad \log K_{NiL} = 10.82 \qquad (1)$$

$$Ni^{2+} + HL^- \rightleftharpoons NiHL^+ \qquad \log K^{HL}_{NiHL} = 9.06 \qquad (2)$$

$$Ni^{2+} + H_2L \rightleftharpoons NiH_2L^{2+} \qquad \log K^{H_2L}_{NiH_2L} = 7.80 \qquad (3)$$

$$Ni^{2+} + H_3L^+ \rightleftharpoons NiH_3L^{3+} \qquad \log K^{H_3L}_{NiH_3L} = 4.21 \qquad (4)$$

Acetohydroxamic acid serves as a convenient reference ligand for comparison with these binding constants. The stability constants of 5.3 log units for the 1:1 Ni–acetohydroxamic acid complex and 4.0 log units for the 1:2 complex give a log β value of 9.3 for the combination of Ni(II) with two hydroxamate groups. Since log K_{NiL} (eq 1) is greater than 9.3, the Ni(II) ion must be bound to two hydroxamic acid groups in NiL, with a macrocyclic stabilization effect of 1.5 log units. Because the experimental solution, after the potential break at a = 2, is yellow, the coordination sphere around Ni(II) must be planar. This conclusion applies to the three complexes NiL, $NiHL^+$, and NiH_2L^{2+}, with the last two involving protonation of the amino groups. The species NiH_3L^{3+} (eq 4) must therefore involve coordination by a single hydroxamate group. The value for its formation constant (log $K^{H_3L}_{NiH_3L}$ = 4.21) is lower than that of acetohydroxamic acid (5), probably because of the unfavorable electronic effects of adjacent polar groups in the ring.

The possibility of simultaneous coordination of Ni(II) by the two amino groups and the hydroxamate nitrogens should be pointed out. Such a complex might be expected to have low stability and thus be a minor species in solution because of the unusual bonding mode of the hydroxamate group and the formation of a 6-membered chelate ring. On the other hand, the presence of such a species could account for the pale yellow color observed in the reaction mixture. Thus there seems to be a definite possibility that more than one microspecies of the Ni(II) chelate exist simultaneously in solution. All of these complexes are relatively weak, and nickel(II) hydroxide precipitates from alkaline solution, as is indicated by the broken section of the equilibrium curve in Figure 1.

From the equilibrium curves in Figure 1, it is apparent that the Fe(III) ion behaves in a manner different from that of Ni(II). The initial potential jump at a = 3 (Figure 1) indicates a coordination process involving the displacement of three protons. Since $Fe(OH)_3$ separates as a solid phase, it is impossible to draw any conclusions for the data beyond a = 3. However, two complex equilibria perfectly explain the curve from a = 0 to a = 3. Probably because of the higher charge of Fe^{3+} relative to Ni^{2+}, the data showed no evidence for the triprotonated complex species FeH_3L^{4+}. However, the less protonated complexes FeH_2L^{3+} and $FeHL^{2+}$ were found to be present in this p[H] range. The presence of solid $Fe(OH)_3$ prevented computation of log K_{FeL^+}; hence the results are expressed in terms of eq 5 and 6. Here also, the binding

$$Fe^{3+} + HL^- \rightleftharpoons FeHL^{2+} \qquad \log K^{HL}_{FeHL} = 15.78 \qquad (5)$$

$$Fe^{3+} + H_2L \rightleftharpoons FeH_2L^{3+} \qquad \log K^{H_2L}_{FeH_2L} = 10.58 \qquad (6)$$

affinity of acetohydroxamic acid to Fe^{3+} provides a useful analogy to infer the type of coordination occurring in the macrocycle. The logarithms of stability constants of the 1:1 and 1:2 Fe^{3+}–acetohydroxamic acid complexes are 11.42 and 9.68. The value obtained with the monoprotonated endocyclic dihydroxamic macrocycle is clearly much larger (15.78 vs. 11.42) than that of the monoacetohydroxamic acid, and binding of two endocyclic hydroxamic groups per Fe^{3+} ion is indicated. The stability constant is not as large, however, as the overall constant of the 2:1 acetohydroxamate chelate (11.42 + 9.68) probably because of coulombic effects of the positive protonated nitrogens in the ligand, and perhaps also to some extent to the head-to-head arrangement of the hydroxamates in the macrocycle, for which there are no analogous simpler complexes. It is interesting to note that the protonation constant for eq 7 is found to be 3.70 log units, a value

$$FeHL^{2+} + H^+ \rightleftharpoons FeH_2L^{3+} \qquad \log K^{H}_{FeH_2L} = 3.70 \qquad (7)$$

that is only 0.44 unit lower than the fourth protonation constant of the metal-free ligand. This shows that protonation of the aliphatic amino groups is only slightly affected by Fe(III) coordination and that, therefore, these amino groups are not involved in the coordination of the Fe^{3+} ion.

Spectrophotometric measurement of the iron(III) chelate gave a typical hydroxamate absorption maximum at 475 cm^{-1} but a relatively weak absorptivity of 240. Since the p[H^+] of this measurement is 2.46, within 2% accuracy the only Fe(III) complex species present is FeH_2L and the spectrum must therefore correspond to that species. According to Neilands,[19] monohydroxamates of Fe(III) absorb at 510 nm, while trihydroxamates absorb at 440 nm. Since our complex absorbs at the intermediate value of 475, the visible spectrum is compatible with the interpretation that two hydroxamate groups are bound per Fe(III) in the macrocycle. The intensity of this band is somewhat less than would be expected for coordination by two hydroxamate groups. It is noted that the amino groups probably do not coordinate Fe(III), since they are not sterically favorably positioned for participation of octahedral coordination of a metal ion, when the

(19) Neilands, J. B. *Struct. Bonding (Berlin)* **1966**, *1*, 59.

Table II. Diamino Bis(acetato) Bis(hydroxamic acid) H_4L, **3**, Constants and Ni(II) and Fe(III) Stability Constants (μ = 0.100 M KNO_3, t = 25.0 °C in Aqueous Solution)

quotient Q	symbol	log Q
$[HL^{3-}]/[H^+][L^{4-}]$	K^H_1	11.91
$[H_2L^{2-}]/[H^+][HL^{3-}]$	K^H_2	9.79
$[H_3L^-]/[H^+][H_2L^{2-}]$	K^H_3	8.11
$[H_4L]/[H^+][H_3L^-]$	K^H_4	4.25
$[H_5L^+]/[H^+][H_4L]$	K^H_5	2.70
$[H_6L^{2+}]/[H^+][H_5L^+]$	K^H_6	2.18
$[NiL^{2-}]/[Ni^{2+}][L^{4-}]$	K_{NiL}	13.64
$[NiHL^-]/[NiL^{2-}][H^+]$	K^H_{NiHL}	9.50
$[NiH_2L]/[NiHL^-][H^+]$	$K^H_{NiH_2L}$	6.37
$[NiH_3L^+]/[NiH_2L][H^+]$	$K^H_{NiH_3L}$	3.87
$[FeL^-]/[Fe^{3+}][L^{4-}]$	K_{FeL}	24.45
$[FeHL]/[FeL^-][H^+]$	K^H_{FeHL}	4.73
$[FeH_2L^+]/[FeHL][H^+]$	$K^H_{FeH_2L}$	3.67
$[FeH_3L^{2+}]/[FeH_2L^+][H^+]$	$K^H_{FeH_3L}$	2.70

two bidentate endocyclic hydroxamate groups are coordinated to the metal ion. It is possible that such steric effects also influence the strength of the binding of the hydroxamate groups. This steric problem was verified by the use of space-filling models and acounts for the relatively low stability of the Ni(II) and Fe(III) complexes. In addition the hydroxamate groups are arranged head to head, and it is difficult to asses the consequence of this arrangement in terms of the molar absorbance since such models are not currently available.

Metal Chelates of the Diacetic Acid Ligand 3. The substitution of exocyclic acetate groups on the secondary amino group should improve the ability of the ligand to conform to an octahedral structural coordination sphere, since the acetate groups can provide two additional donor oxygens that can share octahedral sites along with the four hydroxamate oxygens.

The two additional negative charges of the acetic acid substituents make a marked difference in the magnitudes of the protonation constants (Table II) of the macrocycle. The order of protonation reaction sites of the most basic form of the ligand is probably the same as that of the unsubstituted macrocycle: the two hydroxamates, followed by the two amino groups and finally by acetate oxygens. The first protonation constant is raised by about 2 log units, reflecting the added charge (total of 4–) and possible hydrogen binding of the first proton. The second protonation constant is only slightly higher than that of the amino analogue. The numerical difference between the third and fourth protonation constants is 3.86 and compares well with the corresponding values (3.57, Table I) for the amino macrocycle. The magnitudes of the hydroxamate protonation constants are also comparable with those of **3** being slightly higher than those of **18**. The acetate protonation constants 2.70 and 2.18 are in agreement with the values expected for α-amino acids.

The nickel stability constant log K_{NiL} (Table II) is almost 3 log units higher as the result of acetate substitution. There are also three chelate protonation constants as in the case of the amino macrocycle, but their magnitudes are different, reflecting the participation of the acetate groups in the coordination and stabilization of the complex. When the corresponding equilibria are expressed as formation reactions (eq 8–11, comparisons with the

$$Ni^{2+} + L^{4-} \rightleftharpoons NiL^{2-} \qquad \log K_{NiL} = 13.64 \qquad (8)$$

$$Ni^{2+} + HL^{3-} \rightleftharpoons NiHL^- \qquad \log K^{HL}_{NiHL} = 11.27 \qquad (9)$$

$$Ni^{2+} + H_2L^{2-} \rightleftharpoons NiH_2L \qquad \log K^{H_2L}_{NiH_2L} = 7.81 \qquad (10)$$

$$Ni^{2+} + H_3L^- \rightleftharpoons NiH_3L^+ \qquad \log K^{H_3L}_{NiH_3L} = 3.57 \qquad (11)$$

formation constants for eq 1–4 may be made directly. The first two formation constants of **3** are significantly larger for the direct reactions involving hydroxamate groups. However, surprisingly the final two formation constants are not higher than those of the amino macrocycles in spite of the more favorable charges of the ligand species involved. It is possible that eq 10 and 11 involve some coordination between Ni^{2+} and the aminoacetate groups, **3**, with the hydroxamate groups participating in protonation.

Even though the numerical value of the normal stability constant is quite high, the strong basicity of the ligand made it possible to compute log K_{FeL} as well as log K^H_{FeHL}, log $K^H_{FeH_2L}$, and log $K^H_{FeH_3L}$ by direct potentiometry with a very favorable standard deviation in calculated vs. observed pH values. When the p[H] value is elevated to the point where all four ligand protons are neutralized, iron hydroxide precipitates, and it is not possible to carry the potentiometric measurements further. Nevertheless, the ligand shows tight wrapping around the metal ion, pushing the K^H_{FeHL}, $K^H_{FeH_2L}$, and $K^H_{FeH_3L}$ values down relative to those observed for nickel(II). In this case further information concerning the complexing behavior can be obtained by the rearrangement of the Fe^{3+} data in Table II according to eq 12–15 The value

$$Fe^{3+} + L^{4-} \rightleftharpoons FeL^- \qquad \log K_{FeL} = 24.45 \qquad (12)$$

$$Fe^{3+} + HL^{3-} \rightleftharpoons FeHL \qquad \log K^{HL}_{FeHL} = 17.28 \qquad (13)$$

$$Fe^{3+} + H_2L^{2-} \rightleftharpoons FeH_2L^+ \qquad \log K^{H_2L}_{FeH_2L} = 11.16 \qquad (14)$$

$$Fe^{3+} + H_3L^- \rightleftharpoons FeH_3L^{2+} \qquad \log K^{H_3L}_{FeH_3L} = 5.74 \qquad (15)$$

of log K_{FeL} cannot be compared to that of the amino macrocycle; however, other comparisons are possible. It is seen that log K^{HL}_{FeHL} is only 1.5 log units higher for **3** than for **18** and that eq 14 is barely favored for **13**, further indicating that the acetates exert most of their activity through new coulombic attraction. Computations support the formation of FeH_3L^{2+} as the most protonated species present at low pH value. The visible spectra show a broad shoulder at 460–480 nm on a generally upward sloping absorbance curve, but since various protonated species are in equilibrium in the p[H] range of the measurements, it is not possible to assign a precise molar absorbance to any single species. However, taking into account the degree of formation of the Fe(III) complexes and assuming that protonation at remote sites has little influence, the approximate value of ϵ_{475} is 120 cm^{-1} M^{-1}.

All of the natural endo trihydroxamic acids have a head-to-tail arrangement of hydroxamate groups. Because of the synthetic route chosen, the new ligand described in this work has a head-to-head arrangement. While the implications of this arrangement over that of the natural compounds are in need of more synthetic model ligands for comparison, it is clear that the macrocycle **18** without acetates shows quite a stability enhancement over what one would expect without the benefit of encompassing the groups in a cycle. This incremental increase in stability for several hydroxamic acids was noted by Schwarzenbach[20] some time ago for the natural hydroxamate siderophores.

The presence of additional amino and acetate donor groups in the macrocycle helped increase metal ion affinity somewhat but not sufficiently to prevent the precipitation of $Fe(OH)_3$ at higher pH values. The efficacy of a given ligand is conveniently measured in terms of p[Fe^{3+}] at p[H] 7.2 for 10^{-3} M total iron in the presence of 10% excess ligand at 25 °C. The values found are 13.0 and 15.2 for ligands **18** and **3**. In order to design more effective ligands, this result means that aminoacetate donor groups are not particularly helpful in augmenting stabilization already established by the endocyclic hydroxamate groups. Apparently the way to increase the value of pM of this type of ligand is to incorporate an additional endocyclic hydroxamate group as in the natural trihydroxamic acids. However, it should be possible to maximize the stability of the two endocyclic hydroxamic acid groups by adjustment of the ring size to optimize the size of the cavity for the containment of the Fe^{3+} ion. Since neither the available amino nitrogens nor added acetates are particularly good donors for ferric ions, their presence for the most part is not particularly useful and therefore should be considered as potentially disposable. Another possible variation in structure would be to add the third endocyclic hydroxamate group as part of a macrobicyclic (cryptand) cagelike ligand, as was suggested earlier.[2]

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Substitution and Rearrangement Reactions of Nickel(III) Peptide Complexes in Acid

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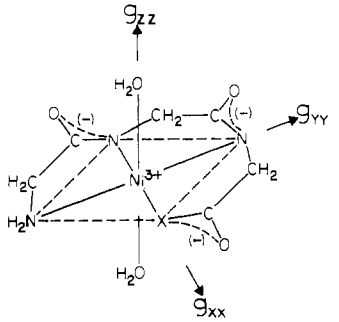
The terminal N(peptide) bonds to nickel in complexes of tetraglycine, $Ni^{III}(H_{-3}G_4)(H_2O)_2^-$, and tetraglycinamide, $Ni^{III}(H_{-3}G_4a)(H_2O)_2$, break rapidly in acid with first-order rate constants that range from 0.1 to 15 s^{-1} as the hydrogen ion concentration increases (0.004–1.0 M). However, the other three equatorial Ni(III)–N bonds are relatively inert to substitution. EPR spectra at room temperature and in frozen aqueous glasses help to characterize several protonated species for these complexes and for nickel(III) tripeptide complexes. The substitution reactions of the terminal peptide nitrogen in the Ni(III) complexes of G_4 and G_4a are reversible in dilute acid, and the nitrogen coordinates to the metal once again in the process of freezing the aqueous solutions for EPR measurements. The ternary complexes $Ni^{III}(H_{-2}peptide)(phen)(H_2O)$ and $Ni^{III}(H_{-2}peptide)(terpy)$ form readily in dilute acid with phen chelated to an axial and an equatorial site and with terpy coordinated to two axial sites and one equatorial site of nickel(III).

Introduction

Chemical or electrochemical oxidation of nickel(II) peptides yields nickel(III) complexes that have been characterized by electron paramagnetic resonance (EPR) and ultraviolet–visible spectroscopy as well as by cyclic voltammetry.[1-4] The UV–vis spectra of these Ni(III) species have two ligand-to-metal charge-transfer bands, one between 325 and 360 nm ($\epsilon \sim 5000$ M^{-1} cm^{-1}) and the other between 230 and 260 nm ($\epsilon \sim 11\,000$ M^{-1} cm^{-1}). The reduction potentials of the Ni(III,II) peptide couples fall in the range of 0.79–0.96 V (vs. NHE)[2] and vary less with the nature of the coordinated equatorial donors than is the case for the Cu(III,II) peptides.[5] Temperature-dependent electrode potential studies indicate that two coordinated water molecules are released when Ni(III) peptides are reduced to Ni(II) peptides.[4] EPR spectra (−150 °C) of frozen aqueous glasses of Ni(III) peptide solutions show that the metal is located in a tetragonally distorted octahedral environment and that the unpaired electron resides in a molecular orbital that has a large amount of d_{z^2} character.[3,6] Structures IA–C give the proposed



IA, $Ni^{III}(H_{-2}G_3)$, X = O
B, $Ni^{III}(H_{-3}G_4)^-$, X = NCH_2COO^-
C, $Ni^{III}(H_{-3}G_4a)$, X = NCH_2CONH_2

coordination geometry for $Ni^{III}(H_{-2}G_3)(H_2O)_2$, $Ni^{III}(H_{-3}G_4)(H_2O)_2^-$, and $Ni^{III}(H_{-3}G_4a)(H_2O)_2$, where G is the glycyl residue, a is an amide, and H_{-n} indicates n deprotonated peptide nitrogens. The x and y axes have different combinations of donor groups that contribute to a broad $g_\perp$ EPR signal and can be resolved into x and y components (g_{xx} and g_{yy}). In previous work[3] it has been shown that the magnitude of the equatorial g value increases as the strength of the equatorial donor increases in the order N^-(peptide) ≈ N^-(amide) > $-NH_2$ > imidazole ≈ CO_2^-.

In this work, the reactions of Ni(III) peptides with acid are examined in regard to EPR and UV–vis evidence for structural changes and for the kinetics of these changes. It also is shown that Ni(III) peptides readily form ternary complexes with terpy (2,2′:6′,2″-terpyridine) or phen (1,10-phenanthroline).

Experimental Section

Reagents. Triglycine (G_3), tri-L-alanine (A_3), L-alanylglycylglycine (AG_2), glycyl-L-alanylglycine (GAG), triglycinamide (G_3a), tetraglycine (G_4), and tetraglycinamide (G_4a) were obtained from Biosynthetika or Vega Fox. The purity of the peptides was confirmed by chromatographic analysis and by elemental analysis. Nickel perchlorate, prepared from nickel carbonate and perchloric acid, was recrystallized, and a stock solution was standardized by EDTA titration to a murexide end point. Sodium perchlorate solution, prepared from sodium carbonate and perchloric acid, was boiled to remove carbon dioxide and was standardized gravimetrically. Oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ from E. I. du Pont de Nemours & Co.) was used for rapid chemical oxidation to give nickel(III) complexes. Buffer solutions (0.050 ± 0.003 M) with an ionic strength of 0.1, adjusted with $NaClO_4$, were prepared from $ClCH_2CO$-OH for pH 2.4–3.6, CH_3COONa for pH 3.9–5.1, and NaH_2PO_4 above pH 6.3.

Nickel(II) peptide complexes were prepared in solution by the reaction of nickel perchlorate with excess peptide. (The excess was 2% for G_4a, 5% for G_4, 20% for G_3a, and 30% for the tripeptides.) This prevented the precipitation of $Ni(OH)_2$ when the pH of the solutions was adjusted to 10.5 by slow addition of NaOH solution, controlled by a Radiometer Model ABU 13 pH stat.

Equipment and Procedures. Nickel(III) peptide samples were prepared electrochemically by passing the corresponding Ni(II) peptide solution through a graphite powder working electrode that was packed in a porous-glass column and wrapped with a platinum-wire auxilary electrode.[7] Generally, the fully deprotonated form of the Ni(II) peptide complex (pH 10.5) was oxidized at a potential 200 mV more positive than the $E°$ value for the Ni(III,II) couple with flow rates below 1.5 mL min^{-1}. The accompanying electrolysis of water lowered the pH and the collected solution was adjusted immediately to the desired pH with buffers or standard acid solution, because the nickel(III) peptides un-

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