Conformationally Rigid Tricyclic Tripods: Synthesis and Application to Preparation of Enterobactin Analogs

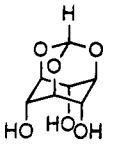
Bruno Tse and Yoshito Kishi*

Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

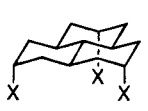
Received June 29, 1994[⊗]

The syntheses of the conformationally rigid triol **2** and triamine **3** are described. One of the key features of these syntheses was that each of the intermediates involved contained a symmetry element, which made their structure elucidation facile and conclusive. The structural similarity between the platform of enterobactin in its metal binding state and triamine **3** was recognized, and the enterobactin analog **14** was synthesized. The K_f value observed for **14** was as high as the K_f value for enterobactin itself, and higher than the K_f value for any enterobactin analog ever reported. In comparison with a number of synthetic enterobactin analogs, it became evident that the conformational rigidity of the enterobactin platform, resulting in good preorganization of the three catechol moieties toward Fe(III)-chelation, was an important factor contributing to its extraordinarily high K_f.

We have been engaged in the synthesis of conformationally rigid, tripodal compounds bearing three axially disposed hydroxyl or amino groups, with the hope that this class of compounds might provide unique opportunities for molecular architecture. Several years ago, we synthesized the mono-orthoformate **1** of *scyllo*-inositol,[1] which has indeed given us insight into the design of enterobactin analogs.[2] We are also interested in conformationally rigid, tripodal compounds, with the three hydroxyl or amino groups being more widely spaced than those in **1**, and noticed that triol **2** and triamine **3** might meet our needs. For example, triamine **3** appeared to provide an ideal platform to prepare an enterobactin analog. Namely, a remarkable structural similarity was recognized between **3** and the triserine lactone platform in the X-ray structure of vanadium(IV) enterobactin. In this paper, we report (1) the syntheses of the conformationally rigid, tripodal triol **2** and triamine **3** and (2) the syntheses and properties of enterobactin analogs derived from these tripodal substrates.



1



2: X = OH
3: X = NH_2

4

Syntheses of Triol 2 and Triamine 3

The triketone **4** appeared to be an ideal precursor for the syntheses of **2** and **3**. Commercially available, inexpensive naphthalic anhydride **5** was chosen as the starting material for the synthesis of **4** because it contained a major portion of the carbon skeleton of **4** (Scheme 1). To introduce oxygen functionalities at the 3 and 6 positions of **5**, a classical phenol synthesis was employed.[3] Thus, upon sulfonation with fuming H_2SO_4, followed by fusion with molten KOH, 3,6-dihydroxynaphthalic anhydride was obtained in 87% yield. Complete reduction of the anhydride moiety to the corresponding diol proved difficult. Therefore, the anhydride was first converted to the corresponding diester; treatment of 3,6-dihydroxynaphthalic anhydride with MeI/KOH in DMF methylated both the anhydride and phenol groups to give compound **6** in 82% yield. Reduction of the dimethyl ester to the diol with DIBAL-H, followed by bromination with PBr_3 gave the dibromide **7** (61% overall yield). To introduce the third ring on **7**, cyclization with acetoacetate was proven to be the most effective. Thus, treatment of **7** with *tert*-butyl acetoacetate and NaH in THF (93% yield), decarboxylation with *p*-TsOH in AcOH (95%), and the addition of a methyl group with MeMgBr (87%) gave compound **8**. The methyl group was added in order to make the subsequent intermediates symmetrical and also to facilitate dehydration in step f.

With the directing effect of the two methoxy groups, Birch reduction of **8**, followed by HCl then NaOH treatments, yielded **9**. Birch reduction of **9**, followed by Jones oxidation, furnished **10**.[4] Dehydration of the tertiary alcohol by conventional methods such as $POCl_3$/pyridine, $SOCl_2$/pyridine, and MsCl/DMAP did not give encouraging results. However, Martin's sulfurane[5] and Burgess reagent[6] smoothly dehydrated **10** to give the tetrasub-

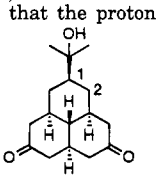
[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1994.

(1) Lee, H. W.; Kishi, Y. *J. Org. Chem.* **1985**, *50*, 4402.

(2) Tse, B.; Kishi, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7892. Biological studies on the enterobactin analogs reported have been performed and will be published elsewhere.

(3) Dziewonski, K.; Majewicz, T.; Schimmer, L. *Bulletin International de l'Academie Polonaise des Sciences* **1936**, *Ser. A*, 43.

(4) The ring systems of **9** and **10** were confirmed to be *trans*-fused at the stage of triketone **4**. The configuration at C-1 of compound **10** was established by ^1H NMR, where the axial proton at C-2 had three large couplings, indicating that the proton at C-1 had to be axial.

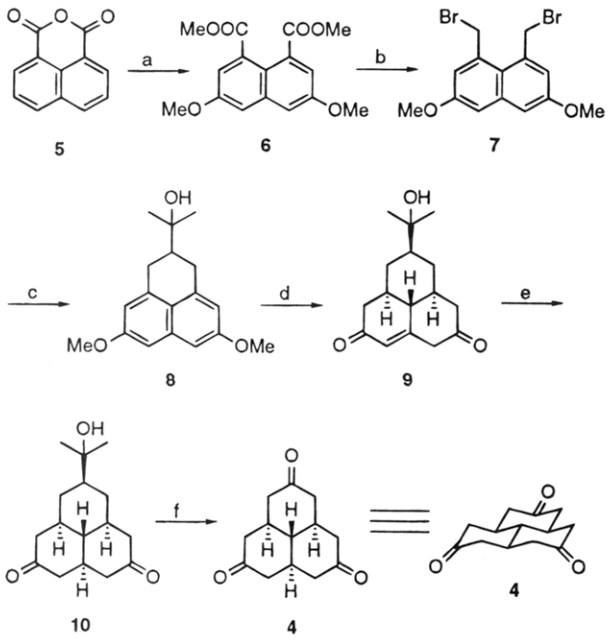


10

(5) Martin, J. C.; Arhart, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 4327.

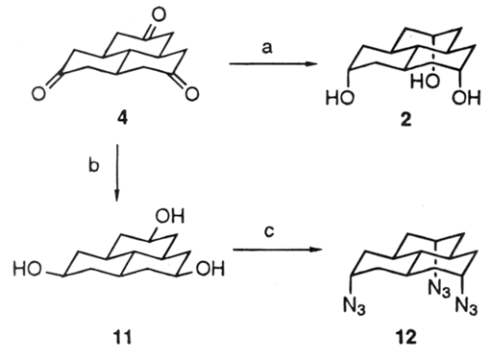
0022-3263/94/1959-7807$04.50/0

Scheme 1[a]



[a] (a) 1. Fuming H_2SO_4/230 °C. 2. KOH/240 °C. 3. MeI/KOH/DMF/rt. (b) 1. DIBAL-H/CH_2Cl_2/0 °C. 2. PBr_3/ether/rt. (c) 1. *tert*-Butyl acetoacetate/NaH/THF/reflux. 2. *p*-TsOH/AcOH/60 °C. 3. MeMgBr/THF/0 °C. (d) 1. Li/NH_3/*t*-BuOH/THF/−33 °C. 2. HCl/rt. 3. NaOH/dioxane/rt. 4. HCl/rt. (e) 1. Li/NH_3/EtOH/THF/−78 °C. 2. Jones reagent/acetone/rt. (f) 1. Martin's sulfurane reagent/CH_2Cl_2/0 °C. 2. O_3/CH_2Cl_2-MeOH/−78 °C, followed by Me_2S workup.

Scheme 2[a]



[a] (a) 1. (*S*)-Alpine hydride/THF/−78 °C. 2. Ac_2O/pyridine/DMAP. 3. Separation. 4. NaOMe/MeOH/rt. (b) 1. Na/NH_3/EtOH/THF/−33 °C. 2. Ac_2O/pyridine/DMAP. 3. Separation. 4. NaOMe/MeOH/rt. (c) 1. MsCl/NEt_3/CH_2Cl_2/rt. 2. *n*-Bu_4NN_3/toluene/80 °C.

stituted olefin which, upon ozonolysis, gave the all-*trans* triketone **4** (22% overall yield from **8**).

Among the various reducing agents studied for the conversion of **4** into the all-axial triol **2**, (*S*)-alpine hydride gave the best selectivity (~3:1) favoring the all-axial substrate over all other stereoisomers (Scheme 2). The triol mixture obtained was acetylated and separated to furnish the all-axial triacetate which could be readily converted to **2** by NaOMe in MeOH (64% overall yield from **4**).

To obtain the all-axial triamine **3**, attempts on reductive amination of **4** were unsuccessful. Thus, the inversion of the all-equatorial triol to the all-axial triazide was pursued. Birch reduction of **4** gave the best selectivity

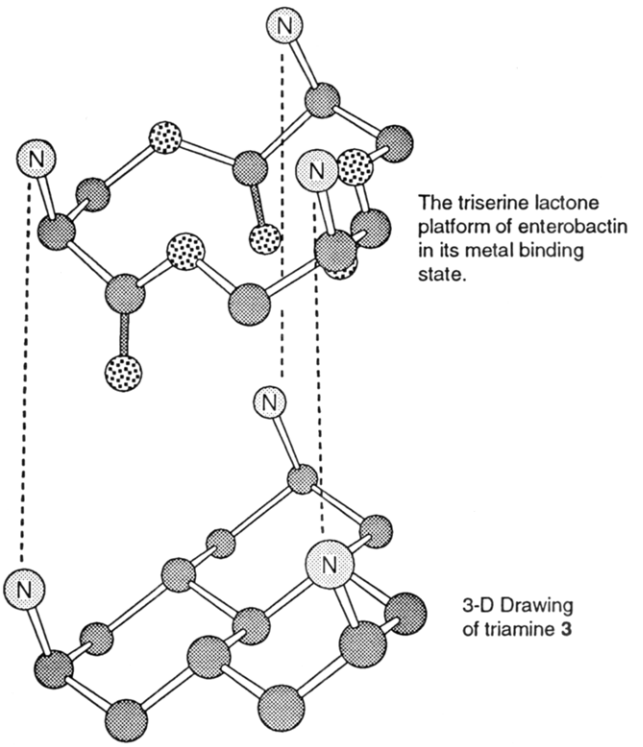


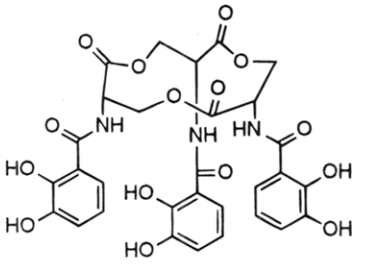
Figure 1. Comparison of the platform of enterobactin in its metal-binding state and the 3-D drawing of triamine **3**.

(~2:1) favoring the all-equatorial triol over all other stereoisomers. The crude triol mixture was acetylated and separated. Cleavage of the all-equatorial triacetate by NaOMe in MeOH gave the all-equatorial triol **11**, which was mesylated and then displaced by *n*-Bu_4NN_3 to give the all-axial triazide **12** (36% overall yield from **4**). Catalytic hydrogenation of **12** furnished the desired triamine **3**, but it was found more convenient to store the triazide **12**.

One of the key features of the syntheses of **2** and **3** was that throughout the syntheses, each of the desired intermediates contained a symmetry element, which made their structure elucidation facile and conclusive.

Synthesis of Enterobactin Analogs

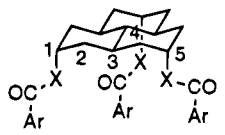
Enterobactin (**13**) is a siderophore produced by enteric bacteria, such as *E. coli* to support the growth of the bacteria,[7] and is known to exhibit unique chemical properties. Enterobactin has an extraordinarily high affinity for ferric ions;[8] the stability constant, K_f, of ferric enterobactin has been estimated to be approximately 10^{49}. It binds Fe(III) with the exclusively right-handed (Δ) configuration at the metal center.[9] The unnatural antipode of enterobactin was found to lack biological activity, suggesting that the chirality at the metal center may play an important role.[10].



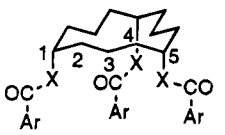
13: enterobactin

(6) Burgess, E. M.; Penton, H. R.; Taylor, E. A. *J. Am. Chem. Soc.* **1970**, *92*, 5224.

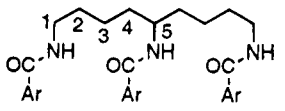
As noted, we hoped that the conformationally rigid, tripodal triol **2** and triamine **3** might provide unique opportunities for molecular architecture. For example, triamine **3** appeared to provide an ideal platform to prepare an enterobactin analog. In this regard, a remarkable similarity was recognized between the structure of **3** and the triserine lactone platform resulting from the removal of the catechol moieties from the X-ray structure of vanadium(IV) enterobactin.[11] This structural similarity suggested that the enterobactin analog **14** derived from **3** has the catechol moieties placed in the ideal positions for Fe(III)-binding. Therefore, the chelation should be entropically favorable. The similar spacing between the amino groups also suggested that the catechol moieties in analog **14**, like those in enterobactin, are able to span an ideal cavity size to chelate Fe(III). Therefore, the chelation should also be enthalpically favorable.



14: X = NH
17: X = O



15: X = NH
18: X = O



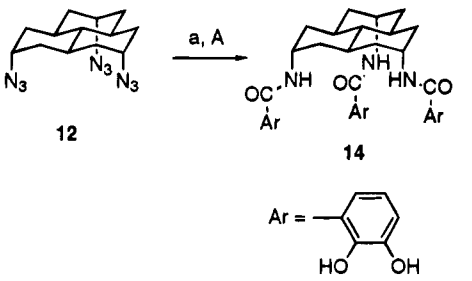
16

We were interested in the effect of the conformational rigidity of the platform on the stability of the Fe(III)-complex formed and chose to use the analogs **15** and **16** as reference compounds. They all had the same number of carbon atoms connecting the catechol moieties. This suggested that all of them could span the suitable space to bind Fe(III), but they differed in the degree of conformational rigidity of the platforms. The platform of **15** was anticipated to be more conformationally flexible than that of **14**, resulting in less preorganization toward Fe(III)-binding. Similarly, the platform in **16** was expected to be even more conformationally flexible, giving even less preorganization toward Fe(III)-binding. Thus, a comparison study of **14** with **15** and **16** should reveal the importance of the conformational rigidity of the platform and the importance of the preorganization of the catechol moieties toward Fe(III)-binding.

The ester analogs **17** and **18** were also synthesized with the hope that these analogs might provide information regarding the advantage of the amide over the ester linkages between the platform and the catechol moieties.

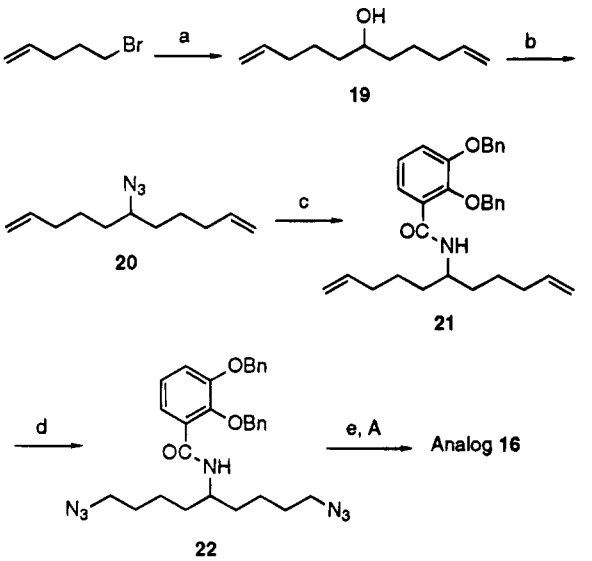
The synthesis of analog **14** from triazide **12** was accomplished in 86% overall yield in 3 steps: (1) catalytic hydrogenation of **12** to **3**, (2) DCC coupling with *O,O*-dibenzyl 2,3-dihydroxybenzoic acid, and (3) catalytic hydrogenolysis (Scheme 3).[12]

Scheme 3[a]



[a] (a) H_2 (1 atm)/$Pd(OH)_2$ on C/MeOH. (A) 1. *O,O*-Dibenzyl-2,3-dihydroxybenzoic acid/DCC/HOBt/CH_2Cl_2–DMF. 2. H_2 (1 atm)/$Pd(OH)_2$ on C/THF–MeOH. Step A will be used again for the synthesis of analogs **15** and **16**.

Scheme 4[a]



[a] (a) 1. Mg/ether/rt. 2. HCOOEt/ether rt. (b) PPh_3/DEAD/DPPA/THF/rt. (c) 1. PPh_3/H_2O/THF/reflux. 2. *O,O*-Dibenzyl-2,3-dihydroxybenzoic acid/DCC/HOBt/CH_2Cl_2/rt. (d) 1. $O_3/CH2Cl_2$–MeOH/−78 °C, followed by Me_2S workup. 2. $NaBH_4/CH_2Cl_2$–MeOH/rt. 3. PPh_3/DEAD/DPPA/THF/rt. (e) PPh_3/H_2O/THF/rt.

Analog **15** was prepared using Corey's procedure with small modifications.[13] Analog **16** was obtained straightforwardly as summarized in Scheme 4.

Analog **17** was synthesized in 90% overall yield from **2** (Scheme 5). It is worthwhile noting that esterification of the sterically hindered alcohols in **2** was most effectively achieved by Yamaguchi's method.[14] Similarly, analog **18** was obtained from *cis,cis*-cyclododecane-1,5,9-triol in 90% yield.

Determination and Comparison of K_f Values

Like enterobactin, analogs **14**–**16** bound ferric ions to give a wine-colored complex (λ_{max} = 495 nm, $\epsilon \sim 4200$ at

(7) (a) Pollack, J. R.; Neilands, J. B. *Biochem. Biophys. Res. Commun.* **1970**, *38*, 989. (b) O'Brien, I. G.; Gibson, F. *Biochim. Biophys. Acta* **1970**, *215*, 393.

(8) Loomis, L. D.; Raymond, K. N. *Inorg. Chem.* **1991**, *30*, 906.

(9) For example, see: (a) Isied, S. S.; Kuo, G.; Raymond, K. N. *J. Am. Chem. Soc.* **1976**, *98*, 1763. (b) McArdle, J. V.; Sofen, S. R.; Cooper, S. R.; Raymond, K. N. *Inorg. Chem.* **1978**, *17*, 3075.

(10) Neilands, J. B.; Erickson, T. J.; Rastetter, W. H. *J. Biol. Chem.* **1981**, *256*, 3831.

(11) Karpishin, T. B.; Raymond, K. N. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 466. The X-ray coordinates for Figure 1 were kindly provided by Professor Raymond.

(12) The steps of DCC coupling of the corresponding amino compound with *O,O*-dibenzyl-2,3-dihydroxybenzoic acid and subsequent catalytic hydrogenolysis are common to the synthesis of analogs **14**–**16** and are denoted as step A collectively.

(13) The procedure described in Corey, E. J.; Hurt, S. D. *Tetrahedron Lett.* **1977**, *45*, 3923 was adopted with the following modifications. To synthesize *cis,cis*-cyclododecane-1,5,9-triol, the method described in Collins, D. J.; Lewis, C.; Swan, J. M. *Aust. J. Chem.* **1974**, *27*, 2593 was followed. In addition, trimesylate, instead of tritosylate, was used, and step A was employed instead of using the acetonide of 2,3-dihydroxybenzoyl chloride.

Scheme 5

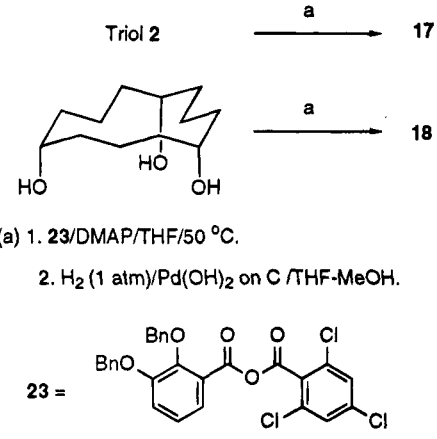


Table 1. Percentage Decrease in Absorbance at λ_{max} at pH 7 upon Addition of Different Concentrations of EDTA Relative to the Absorbance Where No EDTA was Added. λ_{max} for 14_{Fe}, 15_{Fe}, and 16_{Fe} is 495 nm, whereas λ_{max} for 17_{Fe} and 18_{Fe} is 530 nm

concn of EDTA added (mM)	^{14}Fe	^{15}Fe	^{16}Fe	^{17}Fe	^{18}Fe	^{ent}Fe
0.25	6.6	32	81	86	53	
0.5	8.4	42	90	91	65	
2.5	9.2	73	~100	~100	~100	
5	9.9	78	~100	~100	~100	
estimated $\log_{10} K_f$	48.8	47	45	–	–	49 (ref 8)

pH 7). The formation constant of $\mathbf{14_{Fe}}$, determined from UV competition experiments using *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid, was $10^{48.8}$ ($\log_{10} K_f \sim 48.8$), which was remarkably close to the K_f value of ferric enterobactin.[15] This K_f value is the highest among all the synthetic analogs ever reported.[16] Our prediction based on structural similarity, indeed, proved correct.

With the K_f being accurately determined, analog **14** was used as a reference to determine the K_f values of the other analogs. Analogs **14**–**18** were subjected to the competition experiments against EDTA ($K_f \sim 10^{25}$) for ferric ions. Using Raymond's method with small modifications,[17] the K_f values were estimated (Table 1).

The K_f values thus obtained for **14**–**16** clearly shows a trend of how the conformational rigidity of the platform affects the stability of the ferric complexes. The K_f of $\mathbf{15_{Fe}}$ was estimated to be 2 orders of magnitude lower than the K_f of $\mathbf{14_{Fe}}$, and the K_f of $\mathbf{16_{Fe}}$ was, in turn, about 2 orders of magnitude lower than that of $\mathbf{15_{Fe}}$. In other words, changing the rigid platform of **14** to the more flexible platform of **15** costs approximately 2.6 kcal/mol of stability. Roughly the same degree of destabilization was noticed upon changing the platform of **15** to the even more flexible platform of **16**. On the basis of this experiment, it was concluded that the degree of conformational rigidity of the platform, resulting in different extents of preorganization of the catechol moieties for binding Fe(III), is an important factor in determining the stability of the ferric complex. The more conformationally rigid platform provides more preorganization, which makes the chelation of Fe(III) entropically more favorable. The fact that analog **14** and enterobactin have very similar K_f values may suggest that the triserine lactone platform of enterobactin is also conformationally rigid, resulting in good preorganization toward binding.

The ferric complexes of the ester analogs **17** and **18** showed a gradual shift in λ_{max} between pH 7 and pH 10: λ_{max} = 530 nm at pH 7, 520 nm at pH 8, 510 nm at pH 9, and 495 nm at pH 10. The λ_{max} of 495 nm represented chelation with complete deprotonation of the six catecholate protons, whereas the λ_{max} of the higher wavelengths indicated incomplete deprotonation.[18] This showed that, unlike the amide analogs **14**–**16**, pH 7 was not basic enough to deprotonate all the six protons in the catechol moieties of **17** and **18** upon metal chelation. In fact, upon standing at pH 7, a violet precipitate was formed, which corresponded to the neutral Fe(III)-complexes with only three catecholic protons deprotonated. It appeared that the hydrogen bonding between the amide hydrogen and the ortho oxygen on the catechol moiety, as postulated by Raymond,[19] facilitated deprotonation of the catecholate protons. Comparing $\mathbf{17_{Fe}}$ with $\mathbf{14_{Fe}}$ and $\mathbf{18_{Fe}}$ with $\mathbf{15_{Fe}}$ (Table 1), it is noticed that, upon the additions of the same concentrations of EDTA, sharper decreases in absorbance for $\mathbf{17_{Fe}}$ and $\mathbf{18_{Fe}}$ than for $\mathbf{14_{Fe}}$ and $\mathbf{15_{Fe}}$ were observed. Thus, at pH 7, analogs **17** and **18** did not compete with EDTA as well as the amide analogs **14** and **15**.

Conclusion

The syntheses of the conformationally rigid, tripodal triol **2** and triamine **3** were developed. Their unique structural features rendered them useful to applications in molecular architecture. As a demonstration, the enterobactin analog **14** was synthesized from **3**, which exhibited a K_f value as high as enterobactin itself. Comparing analog **14** with the appropriate reference compounds, it was shown that the conformational rigidity of the enterobactin platform, resulting in good preorganization toward binding, is an important factor contributing to the extraordinarily high K_f of its ferric complex.

Experimental Section

General. Reactions sensitive to moisture or air were performed under either argon or nitrogen using anhydrous solvents and reagents. Reagents and solvents were used as supplied otherwise. ^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively. Chemical shifts are reported in parts per million relative to tetramethylsilane. The residual solvent peaks were used as internal references. Coupling constants are reported in hertz. Melting points (hot stage) were uncorrected. IR spectra were measured as films and the wavenumbers are reported in cm^{-1}. For fast atom bombardment (FAB) mass spectra, sodium iodide was added

(14) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.

(15) Using the method described in ref 21, the K_f determination of $\mathbf{14_{Fe}}$ was kindly performed by Dr. Zhiguo Hou in Professor Raymond's laboratories at the University of California, Berkeley.

(16) For example, see: (a) Weitl, F. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2728. (b) Rodgers, S. J.; Lee, C. W.; Ng, C. Y.; Raymond, K. N. *Inorg. Chem.* **1987**, *26*, 1622. (c) Harris, W. R.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6534. (d) Tor, Y.; Libman, J.; Shanzer, A.; Lifson, S. *J. Am. Chem. Soc.* **1987**, *109*, 6517. (e) A recently modified analog was reported in Stack, T. D. P.; Hou, Z.; Raymond, K. N. *J. Am. Chem. Soc.* **1993**, *115*, 6466. The concept of preorganization was used to improve the K_f of a previous analog.

(17) The method used in the estimation of the K_f values is shown in the Experimental Section. The overall pK_a values of the amide analogs are assumed to be the same to allow the approximation of the K_f values. For the ester analogs **17** and **18**, however, the pK_a values are expected to be different from the amide analogs, and estimation for the K_f values is not feasible without knowing the pK_a of the ester analogs.

(18) Cass, M. E.; Garrett, T. M.; Raymond, K. N. *J. Am. Chem. Soc.* **1989**, *111*, 1677.

(19) Garrett, T. M.; Cass, M. E.; Raymond, K. N. *J. Coord. Chem.* **1992**, *25*, 241.

when indicated. Analytical TLC was performed with E. Merck precoated TLC plates, silica gel 60F-254, layer thickness 0.25 mm. Preparative TLC (PTLC) separations were performed on E. Merck precoated TLC plates, silica gel 60F-254, layer thickness 0.50 mm. Flash chromatography was performed with E. Merck Kieselgel 60 (230–400 mesh) silica gel.

Dimethyl Ester 6. KOH (34.3 g, 0.611 mol) was dissolved in 200 mL of water, and the resulting solution was added to 3,6-dihydroxy-1,8-naphthalic anhydride[3] (30.5 g, 0.133 mol). The mixture was stirred at room temperature and 500 mL of DMF was added, followed by the slow addition of methyl iodide (75 mL, 1.20 mol). The mixture was stirred under argon for 4 h and was concentrated *in vacuo*. Aqueous workup (CH_2Cl_2) and silica gel chromatography yielded 33.2 g (82% yield) of **6**, mp (toluene) 133–135 °C. IR: 1725, 1617. ^1H NMR ($CDCl_3$): δ 3.87 (6H, s), 3.89 (6H, s), 7.14 (2H, d, J = 2.6), 7.47 (2H, d, J = 2.6). ^{13}C NMR ($CDCl_3$): δ 52.1, 55.5, 109.6, 118.1, 120.0, 131.2, 137.7, 157.0, 168.8. HRMS (FAB, NaI): calcd for $C_{16}H_{16}O_6Na$ (M + Na) 327.0845; found 327.0860.

Dibromide 7. The dimethyl ester **6** (400 mg, 1.32 mmol) was dissolved in 30 mL of CH_2Cl_2. DIBAL-H (16 mL of 1 M solution in hexanes, 16 mmol) was added under argon at 0 °C. After stirring overnight, the excess DIBAL-H was quenched with MeOH at 0 °C. EtOAc (100 mL) and 50 mL of 3 N HCl were added at 0 °C. The mixture was stirred at room temperature for 2 h. Aqueous workup (EtOAc) gave 400 mg of the corresponding diol as a crude white solid. A small sample was recrystallized from chloroform to give an analytical sample of the diol, mp ($CHCl_3$) 184–186 °C. IR: 3345, 3253, 1616. ^1H NMR (acetone-d_6): δ 3.87 (6H, s), 5.14 (4H, s), 7.14 (2H, d, J = 2.8), 7.16 (2H, d, J = 2.8). ^{13}C NMR (acetone-d_6): δ 55.4, 65.3, 107.3, 119.0, 121.9, 140.2, 141.2, 157.9. HRMS (FAB): calcd for $C_{14}H_{16}O_4$ (M) 248.1048; found 248.1048.

To a mixture of the crude diol (400 mg) and LiBr (200 mg, 2.30 mmol) in 40 mL of anhydrous ether was added PBr_3 (0.40 mL, 4.21 mmol) and the mixture was stirred at room temperature under argon for 18 h. Aqueous workup (CH_2Cl_2) and silica gel chromatography gave 300 mg (61% yield over two steps) of **7**, mp (hexanes) 144–146 °C. IR: 1613. ^1H NMR ($CDCl_3$): δ 3.88 (6H, s), 5.16 (4H, s), 7.06 (2H, d, J = 2.2), 7.11 (2H, d, J = 2.2). ^{13}C NMR ($CDCl_3$): δ 36.6, 55.2, 108.9, 119.5, 122.4, 135.0, 139.4, 156.8. HRMS (FAB): calcd for $C_{14}H_{14}O_2Br_2$ (M) 371.9362; found 371.9366.

Alcohol 8. To a mixture of NaH (44 mg of 60% dispersion, 1.10 mmol) in 40 mL of anhydrous THF under argon was added *tert*-butyl acetoacetate (0.102 mL, 0.615 mmol). The mixture was stirred at room temperature until no more hydrogen was evolved. The dibromide **7** (192 mg, 0.514 mmol) in 20 mL of anhydrous THF was added. The mixture was refluxed at 70 °C for 3 h. Aqueous workup (CH_2Cl_2) and silica gel chromatography gave 176 mg (93% yield) of the cyclized product which was recrystallized to give an analytical sample, mp (5% EtOAc/hexanes) 83–85 °C. IR: 1733, 1712, 1624, 1512. ^1H NMR ($CDCl_3$): δ 1.27 (9H, s), 2.20 (3H, s), 3.40 (2H, d, J = 15.8), 3.45 (2H, d, J = 15.8), 3.85 (6H, s), 6.80 (2H, d, J = 2.4), 6.87 (2H, d, J = 2.4). ^{13}C NMR ($CDCl_3$): δ 26.1, 27.6, 35.9, 55.1, 60.9, 82.2, 104.0, 114.4, 119.9, 134.5, 136.2, 158.1, 170.2, 204.4. HRMS (FAB): calcd for $C_{22}H_{26}O_5$ (M) 370.1780; found 370.1791.

To a solution of this cyclized compound (153 mg, 0.414 mmol) in 50 mL of AcOH was added *p*-TsOH (79 mg, 0.415 mmol). The mixture was stirred under argon at 60 °C overnight. Concentration *in vacuo*, aqueous workup (EtOAc), and silica gel chromatography furnished 106 mg (95% yield) of the corresponding methyl ketone, mp (7% EtOAc/hexanes) 116–117 °C. IR: 1707, 1612. ^1H NMR ($CDCl_3$): δ 2.27 (3H, s), 2.99 (1H, tt, J = 4.0, 10.9), 3.10 (2H, dd, J = 10.9, 15.5), 3.18 (2H, dd, J = 4.0, 15.5), 3.87 (6H, s), 6.78 (2H, br), 6.90 (2H, d, J = 2.4). ^{13}C NMR ($CDCl_3$): δ 28.3, 32.8, 47.5, 55.1, 103.9, 114.2, 120.4, 136.0, 136.4, 158.0, 210.1. HRMS (FAB, NaI): calcd for $C_{17}H_{18}O_3Na$ (M + Na) 293.1154; found 293.1155.

To this methyl ketone (91.6 mg, 0.339 mmol) in 20 mL of anhydrous THF was added MeMgBr (0.22 mL of 3 M solution in ether, 0.66 mmol). The mixture was stirred at 0 °C for 3 h. HCl (50 mL of 0.1 N solution) was slowly added to the mixture at 0 °C. Aqueous workup (CH_2Cl_2) and silica gel chromatography gave 84.2 mg (87% yield) of **8**, mp (20% EtOAc/hexanes) 128–129 °C. IR: 3441, 1622, 1512. ^1H NMR ($CDCl_3$): δ 1.33 (6H, s), 1.99 (1H, tt, J = 3.5, 12.6), 2.84 (2H, dd, J = 12.6, 15.7), 3.11 (2H, dd, J = 3.5, 15.7), 3.87 (6H, s), 6.76 (2H, d, J = 2.1), 6.88 (2H, d, J = 2.1). ^{13}C NMR ($CDCl_3$): δ 27.2, 32.3, 45.5, 55.1, 72.4, 103.5, 113.9, 120.9, 136.3, 138.2, 158.0. HRMS (FAB): calcd for $C_{18}H_{22}O_3$ (M) 286.1569; found 286.1562.

Diketone 10. Birch reduction of **8** was carried out by first condensing NH_3 (500 mL) in a three-necked flask. Compound **8** (0.96 g, 3.36 mmol) in 100 mL of anhydrous THF was added, followed by the additions of 12 mL of *t*-BuOH and lithium metal to maintain a blue color. The reaction was stirred at reflux for 2 h. Solid NH_4Cl was added to quench the excess lithium. The ammonia was evaporated. The bis-enol ether formed was hydrolyzed immediately by adding 3 N HCl to the mixture until acidic. Aqueous workup (CH_2Cl_2) gave 1.21 g of the hydrolyzed bis-enol ether. This crude intermediate (1.21 g) was dissolved in 150 mL of dioxane. NaOH (35 mL of 1 N solution, 35 mmol) was added to the mixture which was stirred at room temperature for 30 min. HCl (60 mL of 1 N solution, 60 mmol) was added, and the reaction mixture was stirred at room temperature for another 3 h. Aqueous workup (EtOAc), concentration *in vacuo*, extraction with 1 N NaOH from ether, acidification, and another aqueous workup (EtOAc) gave 780 mg of the crude vinylogous acid **9**.

Birch reduction of **9** (780 mg) was performed with the same conditions as above, except that EtOH was used instead of *t*-BuOH. Aqueous workup (EtOAc) gave 701 mg of the corresponding triol. CrO_3 (0.5 g, 5 mmol) was dissolved in 1.25 mL of water and 0.5 mL of H_2SO_4. At room temperature, this reagent was added over 2 h to the crude triol (701 mg) in 20 mL of acetone. The excess Jones reagent was quenched by 2-propanol. Aqueous workup (ether), filtration through a plug of silica gel, and concentration *in vacuo* gave 530 mg of the crude diketone **10**, which was used without further purification. A small sample was recrystallized from 20% EtOAc/hexanes, mp (20% EtOAc/hexanes) 141–143 °C. IR: 3500, 1713. ^1H NMR ($CDCl_3$): δ 0.24 (1H, dt, J = 11.6, 11.6), 0.78 (2H, ddd, J = 10.9, 11.8, 13.1), 0.86 (6H, s), 1.17 (1H, tt, J = 5.1, 11.8), 1.21 (1H, dtt, J = 11.6, 3.7, 12.5), 1.48 (2H, dd, J = 12.5, 13.5), 1.50 (2H, dd, J = 12.6, 13.9), 1.64 (2H, ddd, J = 3.7, 5.1, 13.1), 1.69 (2H, ddddd, J = 3.7, 4.2, 10.9, 11.6, 12.6), 2.05 (2H, ddd, J = 2.3, 3.7, 13.5), 2.23 (2H, ddd, J = 2.3, 4.2, 13.9). ^{13}C NMR (C_6D_6): δ 30.3, 34.4, 36.6, 41.4, 41.5, 48.3, 48.9, 49.9, 73.1, 206.6. HRMS (FAB): calcd for $C_{16}H_{25}O_3$ (M + H) 265.1802; found 265.1791.

Triketone 4. The crude **10** (530 mg) obtained above was dissolved in 200 mL of anhydrous CH_2Cl_2. Martin's sulfurane reagent was added at 0 °C until the reaction was complete (TLC). CH_2Cl_2 (50 mL) and MeOH (150 mL) were added to the mixture above. O_3 gas was passed through the mixture at −78 °C until a blue color persisted. N_2 gas was then blown through the mixture to remove the excess O_3. Me_2S (15 mL) was added and the mixture was stirred at −78 °C for 1 h. After concentration *in vacuo* and silica gel chromatography, 163 mg (22% yield from **8**) of the triketone **4** was obtained, mp (20% EtOAc/hexanes) 158–161 °C. IR: 1713. ^1H NMR ($CDCl_3$): δ 1.73 (1H, q, J = 10.7), 1.94 (3H, dtt, J = 10.7, 3.5, 13.4), 2.25 (6H, dd, J = 13.4, 13.9), 2.41 (6H, dd, J = 3.5, 13.8). ^{13}C NMR ($CDCl_3$): δ 40.4, 47.4, 48.0, 206.2. HRMS (FAB): calcd for $C_{13}H_{16}O_3$ (M) 220.1099; found 220.1108.

Triacetate of 2. To a solution of **4** (46.0 mg, 0.209 mmol) in 10 mL of anhydrous THF was added (*S*)-alpine hydride (3.3 mL of 0.5 M solution in THF, 1.65 mmol) at −78 °C. The reaction mixture was stirred at −78 °C for 3 h. AcOH (2 mL) was slowly added. After concentration *in vacuo*, the mixture was applied to a silica gel column which was eluted with 1:1 THF/hexanes and then with 100% THF. The triol mixture obtained was acylated overnight with 7 mL of Ac_2O, 7 mL of pyridine, and 20 mg of DMAP. After concentration *in vacuo* and silica gel chromatography, 46.6 mg (63% yield from **4**) of the all-axial triacetate was obtained, together with 16.7 mg (23%) of undesired isomers, which could be recycled back to the triketone **4** by NaOMe/MeOH, followed by Jones oxidation. The all-axial triacetate was recrystallized, mp (CH_2Cl_2/hexanes) 197–200 °C. IR: 1727. ^1H NMR ($CDCl_3$): δ 0.43 (1H,

q, J = 10.5), 1.16 (6H, ddd, J = 2.7, 13.0, 13.4), 1.71 (3H, dtt, J = 10.5, 2.9, 13.0), 1.78 (6H, ddd, J = 2.9, 3.1, 13.4), 2.09 (9H, s), 5.09 (3H, tt, J = 2.7, 3.1). ^{13}C NMR ($CDCl_3$): δ 21.6, 29.3, 36.9, 51.6, 69.5, 170.6. HRMS (FAB, NaI): calcd for $C_{19}H_{28}O_6Na$ (M + Na) 375.1784; found 375.1783.

Triacetate of 11. The same Birch reduction conditions as before, using sodium, were used. Thus, from 79.8 mg of triketone **4**, 81.4 mg of a crude triol mixture was obtained, which was acylated overnight with 20 mL of Ac_2O, 20 mL of pyridine and 50 mg of DMAP. Concentration *in vacuo* and silica gel chromatography gave 67.5 mg (53% yield from **4**) of the all-equatorial triacetate, and 33.9 mg (27%) of undesired isomers which could be recycled back to the triketone **4** by NaOMe/MeOH, followed by Jones oxidation. The triacetate was recrystallized, mp (CH_2Cl_2/hexanes) 163–165 °C. IR: 1738. ^{1}H NMR ($CDCl_3$): δ 0.30 (1H, q, J = 10.3), 1.11 (6H, ddd, J = 11.2, 11.9, 12.2), 1.24 (3H, dtt, J = 10.3, 3.4, 12.2), 1.94 (6H, ddd, J = 3.4, 4.2, 11.9), 2.00 (9H, s), 4.71 (3H, tt, J = 4.2, 11.2). ^{13}C NMR ($CDCl_3$): δ 21.3, 36.9, 38.4, 50.3, 71.5, 170.4. HRMS (FAB, NaI): calcd for $C_{19}H_{28}O_6Na$ (M + Na) 375.1784; found 375.1776.

Triol 2 and Triol 11. The triacetate of **2** (10.1 mg, 0.029 mmol) was dissolved in 3 mL of 0.01 M NaOMe in MeOH. The reaction was stirred at room temperature overnight. Amberlyst 15 was added until the reaction mixture was neutral. After filtration and concentration *in vacuo*, 6.4 mg (99% yield) of triol **2** was obtained, mp ($CHCl_3$) 245–248 °C. IR: 3192. ^{1}H NMR (CD_3OD): δ 0.29 (1H, q, J = 10.6), 1.09 (6H, ddd, J = 2.7, 12.7, 13.4), 1.58 (6H, ddd, J = 2.2, 3.4, 12.7), 1.91 (3H, dtt, J = 10.6, 3.4, 13.4), 3.96 (3H, tt, J = 2.2, 2.7). ^{13}C NMR (CD_3OD): δ 28.9, 41.1, 54.3, 67.5. HRMS (FAB, NaI): calcd for $C_{13}H_{22}O_3Na$ (M + Na) 249.1467; found 249.1462.

Following the same procedure, 19.0 mg (97% yield) of triol **11** was obtained from 30.5 mg of the triacetate of **11**, mp ($CHCl_3$) 248–250 °C. IR: 3315. ^{1}H NMR (CD_3OD): δ 0.12 (1H, q, J = 10.1), 0.96 (6H, ddd, J = 10.8, 11.4, 12.7), 1.08 (3H, br dt, J = 10.1, 12.7), 1.83 (6H, br dd, J = 4.2, 11.4), 3.52 (3H, tt, J = 4.2, 10.8). ^{13}C NMR (CD_3OD): δ 38.7, 43.6, 52.2, 70.3. No mass spectrum of **11** could be obtained.

Triazide 12. To a mixture of the triol **11** (15.5 mg, 68.6 μmol) in 5 mL of anhydrous THF was added NEt_3 (0.30 mL, 2.15 mmol) and MsCl (0.08 mL, 1.03 mmol). After stirring at room temperature for 1 h, concentration *in vacuo* and aqueous workup (CH_2Cl_2), this crude trimesylate was treated with excess n-Bu_4NN_3 in 5 mL of toluene at 80 °C overnight. After concentration *in vacuo* and silica gel chromatography, 11.7 mg (57% overall yield) of triazide **12** was obtained, mp (CH_2Cl_2/hexanes) 103–105 °C. IR: 2090. ^{1}H NMR (C_6D_6): δ −0.34 (1H, q, J = 10.5), 0.54 (6H, ddd, J = 2.7, 13.0, 13.4), 1.35 (6H, ddd, J = 2.9, 3.1, 13.4), 1.71 (3H, dtt, J = 10.5, 2.9, 13.0), 3.28 (3H, tt, J = 2.7, 3.1). ^{13}C NMR (C_6D_6): δ 29.1, 36.6, 51.7, 57.6. MS (CI): calcd for $C_{13}H_{23}N_{10}$ (M + NH_4) 319.2107; found 319.2098.

***O,O*-Dibenzyl-2,3-Dihydroxybenzoic Acid.** To a mixture of 2,3-dihydroxybenzoic acid (2.0 g, 13.0 mmol) and powdered KOH (8.74 g, 156 mmol) was added 40 mL of DMSO. Benzyl bromide (7.7 mL, 64.8 mmol) was added, and the reaction mixture was stirred for 4 h. Aqueous workup (EtOAc) and recrystallization from CH_2Cl_2/hexanes gave 3.85 g (89% yield) of *O,O*-dibenzyl 2,3-dihydroxybenzoic acid, mp (CH_2Cl_2/hexanes) 125–126 °C. IR: 3063, 2678, 2576, 1692, 1598, 1577. ^{1}H NMR ($CDCl_3$): δ 5.09 (2H, s), 5.16 (2H, s), 7.05–7.65 (13H, m), 11.36 (1H, br). ^{13}C NMR ($CDCl_3$): δ 71.6, 77.1, 119.1, 123.2, 124.5, 124.9, 127.7, 128.5, 128.8, 129.2, 134.8, 135.9, 147.2, 151.4, 165.3. HRMS (FAB, NaI): calcd for $C_{21}H_{18}O_4Na$ (M + Na) 357.1103; found 357.1094.

***cis,cis*-1,5,9-Triazidocyclododecane.** To a mixture of *cis,cis*-cyclododecane-1,5,9-triol[13] (241 mg, 1.12 mmol) in 40 mL of anhydrous CH_2Cl_2 was added NEt_3 (1.4 mL, 10 mmol) followed by MsCl (0.52 mL, 6.72 mmol). The mixture was stirred under argon for 1 h. After aqueous workup (ether), the crude trimesylate obtained was dissolved in 25 mL of DMF. NaN_3 (5.0 g, 0.077 mol) was added and the mixture was stirred at 80 °C for 1 day. The DMF was removed under reduced pressure. Aqueous workup (CH_2Cl_2) and chromatography gave 224 mg of the triazide (69% yield over two steps). IR: 2095. ^{1}H NMR ($CDCl_3$): δ 1.40 (3H, m), 1.49 (3H, m), 1.57 (6H, m), 1.72 (6H, m), 3.52 (3H, m). ^{13}C NMR ($CDCl_3$): δ 18.6, 29.8, 59.9. HRMS (FAB): calcd for $C_{12}H_{22}N_9$ (M + H) 292.1998; found 292.2010.

Perbenzyl-14 and Perbenzyl-15. To a solution of triazide **12** (2.74 mg, 9.1 μmol) in 2 mL of CH_3OH was added 10 mg of Pearlman's catalyst. The mixture was stirred at room temperature under hydrogen (balloon) for 2 h. The mixture was filtered through a piece of tightly packed cotton wool to yield 3.42 mg of crude triamine **3**.

To triamine **3** in 2 mL of anhydrous CH_2Cl_2 and 2 mL of dry DMF was added *O,O*-dibenzyl-2,3-dihydroxybenzoic acid (18.2 mg, 54.5 μmol), HOBt (7.38 mg, 54.6 μmol), and DCC (11.3 mg, 54.8 μmol). The mixture was stirred at room temperature overnight. After concentration *in vacuo*, EtOAc at −78 °C was added to the mixture which was filtered through a sintered glass funnel to remove the urea formed in the reaction. The filtrate was concentrated *in vacuo* and was subjected to purification by PTLC to give 9.8 mg (92% yield) of perbenzyl-**14**. IR: 3385, 3065, 1653, 1576, 1517. ^{1}H NMR (C_6D_6): δ −0.21 (1H, q, J = 10.3), 0.84 (6H, ddd, J = 4.0, 11.8, 13.4), 1.18 (3H, br dt, J = 10.3, 11.8), 1.60 (6H, br d, J = 13.4), 4.43 (3H, br), 4.49 (6H, s), 4.74 (6H, s), 6.53–8.13 (39H, m). ^{13}C NMR ($CDCl_3$): δ 29.9, 37.0, 45.2, 52.5, 71.1, 76.1, 116.6, 123.1, 124.2, 127.4, 128.0, 128.1, 128.4, 128.5, 128.8, 136.2, 136.5, 146.5, 151.6, 165.0. HRMS (FAB, NaI): calcd for $C_{76}H_{73}N_3O_9Na$ (M + Na) 1194.5244; found 1194.5231.

Using the same procedure, 140 mg (71% overall yield) of perbenzyl-**15** was obtained from 49.5 mg of *cis,cis*-1,5,9-triazidocyclododecane. IR: 3375, 1655, 1575, 1520, 1498. ^{1}H NMR ($CDCl_3$): δ 0.95–1.50, (18H, m), 4.08 (3H, m), 5.01 (6H, s), 5.10 (6H, s), 7.07–7.81 (39H, m). ^{13}C NMR ($CDCl_3$): δ 18.5, 30.3, 47.4, 71.3, 76.0, 116.7, 123.5, 124.4, 127.6, 128.2, 128.5, 128.6, 136.4, 136.5, 146.6, 151.6, 163.9. HRMS (FAB, NaI): calcd for $C_{75}H_{75}N_3O_9Na$ (M + Na) 1184.5401; found 1184.5449.

Diene 21. To a dry three-necked flask equipped with a reflux condenser was added 5-bromo-1-pentene (2.5 g, 16.8 mmol) and 30 mL of anhydrous ether. Magnesium turnings (0.679 g, 27.9 mmol) were added. The mixture was heated to initiate reaction and was stirred until no more magnesium dissolved. Ethyl formate (0.64 mL, 7.92 mmol) was added, and the reaction was stirred at room temperature overnight. Aqueous workup (CH_2Cl_2) yielded alcohol **19** (950 mg). To a solution of this crude alcohol **19** (950 mg) in 50 mL of anhydrous THF was added PPh_3 (2.22 g, 8.46 mmol), followed by diethyl azodicarboxylate (1.34 mL, 8.51 mmol) and diphenyl phosphorazidate (1.83 mL, 8.49 mmol). The reaction was stirred at room temperature overnight. After concentration *in vacuo* and silica gel chromatography, 1.32 g of crude azide **20** was yielded. To this crude azide **20** (1.32 g) obtained in 30 mL of THF was added PPh_3 (2.15 g, 8.20 mmol) and water (0.25 mL, 13.9 mmol). The reaction was heated under reflux overnight. The mixture was concentrated and dried *in vacuo* to give the corresponding amine. Following the same procedure as the one used in the synthesis of perbenzyl-**14** from triamine **3**, 2.03 g of **21** was obtained (53% yield over four steps, based on the amount of ethyl formate used). IR: 3372, 3068, 1705, 1656, 1576, 1523, 1498. ^{1}H NMR ($CDCl_3$): δ 1.14–1.44 (8H, m), 1.92–2.04 (4H, m), 4.07 (1H, m), 4.91 (2H, dm, J = 10.3), 4.96 (2H, dm, J = 17.1), 5.10 (2H, s), 5.14 (2H, s), 5.72 (2H, m), 7.12–7.83 (13H, m). ^{13}C NMR ($CDCl_3$): δ 25.2, 33.5, 34.4, 49.1, 71.2, 75.9, 114.5, 116.7, 123.4, 124.3, 127.3, 127.6, 128.2, 128.2, 128.4, 128.5, 128.6, 136.3, 136.4, 138.5, 146.6, 151.6, 164.4. HRMS (FAB, NaI): calcd for $C_{32}H_{37}N_1O_3Na$ (M + Na) 506.2671; found 506.2688.

Diazide 22. Diene **21** (620 mg, 1.28 mmol) was dissolved in 15 mL of CH_2Cl_2 and 30 mL of MeOH. Ozone gas was passed through the reaction mixture at −78 °C until a blue color persisted. The excess O_3 gas was removed by blowing N_2 through the mixture. Me_2S (3 mL) was added, the mixture was stirred at −78 °C for 1 h, and $NaBH_4$ (2.0 g, 54 mmol) was then added. Stirring was continued for 2 h during which the mixture was warmed to room temperature. After aqueous workup (CH_2Cl_2) and silica gel chromatography, 580 mg (92% overall yield) of the corresponding diol was obtained. IR: 3370, 1641, 1575, 1532, 1498. ^{1}H NMR ($CDCl_3$): δ 1.10–1.55 (12H,

m), 1.83 (2H, br), 3.52 (4H, m), 4.04 (1H, m), 5.09 (2H, s), 5.13 (2H, s), 7.10–7.85 (13H, m). ^{13}C NMR ($CDCl_3$): δ 22.1, 32.5, 34.9, 49.1, 62.6, 71.3, 76.0, 116.9, 123.5, 124.4, 127.1, 127.7, 128.3, 128.3, 128.6, 128.7, 128.7, 136.3, 136.5, 146.7, 151.6, 164.9. HRMS (FAB, NaI): calcd for $C_{30}H_{37}N_1O_5Na$ (M + Na) 514.2569; found 514.2585.

To a solution of this diol (460 mg, 0.937 mmol) in 20 mL of anhydrous THF was added PPh_3 (740 mg, 2.82 mmol), followed by diethyl azodicarboxylate (0.45 mL, 2.86 mmol) and diphenyl phosphorazidate (0.61 mL, 2.83 mmol). The reaction was stirred at room temperature overnight. After concentration *in vacuo* and silica gel chromatography, 460 mg (91% yield) of the diazide **22** was obtained. IR: 3371, 2095, 1655, 1576, 1525, 1498. ^{1}H NMR ($CDCl_3$): δ 1.10–1.55 (12H, m), 3.14 (4H, m), 4.02 (1H, m), 5.11 (2H, s), 5.12 (2H, s), 7.10–7.83 (13H, m). ^{13}C NMR ($CDCl_3$): δ 22.9, 28.4, 34.2, 48.6, 51.0, 71.1, 75.7, 116.7, 123.2, 124.1, 126.8, 127.5, 128.0, 128.0, 128.4, 128.4, 128.5, 136.1, 136.4, 146.5, 151.4, 164.4. HRMS (FAB, NaI): calcd for $C_{30}H_{35}N_7O_3Na$ 564.2699; found 564.2715.

Perbenzyl-16. Diazide **22** (92 mg, 0.170 mmol) was dissolved in 10 mL of THF. PPh_3 (134 mg, 0.511 mmol) and water (10 μL, 0.556 mmol) were added and the reaction was stirred at room temperature for 18 h. The mixture was concentrated and dried *in vacuo* to give the crude diamine. Following the same procedure as the one used in the synthesis of perbenzyl-**14** from **3**, 158 mg (83% yield) of perbenzyl-**16** was obtained. IR: 3381, 1655, 1575, 1527, 1498. ^{1}H NMR ($CDCl_3$): δ 1.00–1.30 (12H, m), 3.17 (4H, m), 3.92 (1H, m), 5.04 (4H, s), 5.07 (2H, s), 5.11 (2H, s), 5.12 (4H, s), 7.09–7.90 (39H, m). ^{13}C NMR ($CDCl_3$): δ 23.3, 29.1, 34.6, 39.5, 48.9, 71.2, 71.2, 75.9, 76.3, 116.8, 123.2, 123.4, 124.2, 124.3, 127.5, 127.6, 128.1, 128.2, 128.5, 128.6, 128.7, 136.3, 136.4, 146.7, 151.6, 164.4, 164.9. MS (FAB, NaI): 1144 (M + Na).

Perbenzyl-17 and Perbenzyl-18. *O,O*-Dibenzyl-2,3-dihydroxybenzoic acid (50 mg, 0.150 mmol) was dissolved in 5 mL of anhydrous THF. NEt_3 (24 μL, 0.172 mmol) was added, followed by 2,4,6-trichlorobenzoyl chloride (23 μL, 0.147 mmol). The mixture was stirred at room temperature under argon for 1 h. The solvent was then removed under reduced pressure. The anhydride formed was dissolved in anhydrous ether, and the triethylamine hydrochloride was removed by filtration. The filtrate was concentrated *in vacuo*. To this anhydride in 10 mL of anhydrous THF were added triol **2** (2.14 mg, 9.47 μmol) and DMAP (21.0 mg, 0.172 mmol). The mixture was stirred at 50 °C under argon overnight. Water (10 mL) and NEt_3 (3 mL) were added, and the mixture was stirred at room temperature for 2 h. Aqueous workup (EtOAc) and purification by PTLC gave 10.2 mg (92% yield) of perbenzyl-**17**. IR: 1720, 1580, 1498. ^{1}H NMR ($CDCl_3$): δ 0.56 (1H, q, J = 10.5), 1.25 (6H, br dd, J = 11.7, 13.8), 1.87 (6H, br d, J = 13.8), 1.95 (3H, br dt, J = 10.5, 11.7), 5.04 (6H, s), 5.05 (6H, s), 5.30 (3H, br), 6.82–7.42 (39H, m). ^{13}C NMR ($CDCl_3$): δ 29.9, 37.0, 51.8, 70.2, 71.4, 75.6, 117.9, 122.8, 123.7, 127.5, 127.8, 127.9, 128.0, 128.2, 128.5, 128.6, 136.7, 137.4, 148.5, 152.7, 165.2. HRMS (FAB, NaI): calcd for $C_{76}H_{70}O_{12}Na$ (M + Na) 1197.4765; found 1197.4741.

Using the same procedure, 270 mg (94% yield) of perbenzyl-**18** was obtained from 53.4 mg of *cis,cis*-cyclododecane-1,5,9-triol. IR: 1720, 1579, 1498. ^{1}H NMR ($CDCl_3$): δ 1.27 (3H, m), 1.45 (3H, m), 1.56 (6H, m), 1.75 (6H, m), 5.06 (6H, s), 5.07 (6H, s), 5.18 (3H, m), 6.99–7.40 (39H, m). ^{13}C NMR ($CDCl_3$): δ 18.1, 30.0, 71.4, 73.3, 75.6, 117.8, 122.7, 123.9, 127.5, 127.7, 127.8, 128.1, 128.2, 128.2, 128.6, 136.6, 137.6, 148.2, 152.8, 165.8. HRMS (FAB, NaI): calcd for $C_{75}H_{72}O_{12}Na$ (M + Na) 1187.4921; found 1187.4960.

Analogs 14–18. To a solution of perbenzyl-**14** (7.66 mg, 6.54 μmol) in 1.5 mL of MeOH was added 5 mg of Pearlman's catalyst. The mixture was stirred under hydrogen (balloon) for 2 h and was then filtered through a piece of tightly packed cotton wool. The filtrate was concentrated *in vacuo* to give 3.91 mg (95% yield) of the enterobactin analog **14**. IR: 3378, 1638, 1583, 1532. ^{1}H NMR (acetone-d_6): δ 0.66 (1H, q, J = 10.6), 1.39 (6H, ddd, J = 3.7, 13.0, 13.2), 1.94 (6H, br d, J = 13.2), 2.15 (3H, br dt, J = 10.6, 13.0), 4.35 (3H, br t, J = 3.7), 6.65 (3H, dd, J = 7.8, 7.9), 6.93 (3H, dd, J = 1.2, 7.8), 7.27 (3H, dd, J = 1.2, 7.9). ^{13}C NMR (acetone-d_6): δ 37.6, 46.7, 53.0, 55.2, 116.0, 118.4, 118.9, 119.2, 147.1, 150.4, 170.6. HRMS (FAB): calcd for $C_{34}H_{38}N_3O_9$ (M + H) 632.2608; found 632.2628.

Using the same procedure, 47.6 mg (100% yield) of **15** was obtained from 88.4 mg of perbenzyl-**15**. IR: 3373, 1704, 1638, 1587, 1537. ^{1}H NMR (CD_3OD): δ 1.37 (3H, m), 1.48 (3H, m), 1.61 (6H, m), 1.71 (6H, m), 4.18 (3H, m), 6.60 (3H, dd, J = 7.8, 8.1), 6.81 (3H, dd, J = 1.4, 7.8), 7.17 (3H, dd, J = 1.4, 8.1). ^{13}C NMR (CD_3OD): δ 20.2, 31.6, 49.3, 117.4, 119.1, 119.5, 119.6, 147.2, 149.8, 170.3. HRMS (FAB, NaI): calcd for $C_{33}H_{39}N_3O_9Na$ (M + Na) 644.2584; found 644.2573.

Likewise, 9.9 mg (99% yield) of **16** was obtained from 19.2 mg of perbenzyl-**16**. IR: 3359, 1638, 1591, 1545, 1489. ^{1}H NMR (CD_3OD): δ 1.30–1.60 (12H, m), 3.27 (4H, br t, J = 7.0), 4.02 (1H, m), 6.55–7.15 (9H, m). ^{13}C NMR (CD_3OD): δ 24.6, 30.2, 35.7, 40.3, 50.5, 116.8, 118.6, 118.7, 119.5, 147.3, 150.2, 171.5. HRMS (FAB): calcd for $C_{30}H_{36}N_3O_9$ (M + H) 582.2451; found 582.2455.

Similarly, 5.44 mg (99% yield) of **17** was obtained from 10.2 mg of perbenzyl-**17**. IR: 3436, 1668, 1616. ^{1}H NMR (CD_3OD): δ 0.81 (1H, q, J = 10.4), 1.43 (6H, br dd, J = 11.7, 13.8), 1.93 (6H, br d, J = 13.8), 2.13 (3H, br dt, J = 10.4, 11.7), 5.40 (3H, br), 6.62 (3H, dd, J = 7.9, 8.0), 6.98 (3H, br d, J = 7.9), 7.23 (3H, br d, J = 8.0). ^{13}C NMR (CD_3OD): δ 31.7, 37.6, 52.4, 72.6, 114.6, 120.1, 120.7, 121.7, 147.3, 151.6, 170.8. HRMS (FAB, NaI): calcd for $C_{34}H_{34}O_{12}Na$ (M + Na) 657.1948; found 657.1960.

Finally, 8.65 mg (96% yield) of **18** was obtained from 16.8 mg of perbenzyl-**18**. IR: 3451, 1667. ^{1}H NMR (CD_3OD): δ 1.49 (3H, m), 1.74 (3H, m), 1.87 (6H, m), 1.95 (6H, m), 5.34 (3H, m), 6.70 (3H, dd, J = 7.9, 8.1), 6.97 (3H, dd, J = 1.5, 7.9), 7.29 (3H, dd, J = 1.5, 8.1). ^{13}C NMR (CD_3OD): δ 19.3, 31.2, 75.4, 114.2, 120.0, 121.2, 121.7, 147.2, 151.4, 171.2. HRMS (FAB, NaI): calcd for $C_{33}H_{36}O_{12}Na$ (M + Na) 647.2104; found 647.2078.

Estimations of K_f Values. $FeCl_3$ solution (1 mL of 0.4 mM solution in water), standardized with EDTA according to the procedure described by Welcher,[20] was added to analog L (1 mL of 0.4 mM solution in MeOH), followed by the addition of 2 mL of EDTA solution at particular concentrations in pH 7 buffer. The absorbance was recorded repeatedly until a steady value was observed. The absorbance with no EDTA added was the reference and the decrease in absorbance, where a particular concentration of EDTA was added, was presented in percentage, as shown in Table 1.

The proton-dependent formation constant, K^*, of analog L was estimated using Raymond's method[21] with modifications. K^* can be calculated from eq 1:

$$([\text{Fe-L}^{3-}][\text{H}^+]^6[\text{EDTA}^{4-}])/([\text{Fe-EDTA}^-][\text{H}_6\text{L}]) = K^*/K_{\text{Fe-EDTA}}$$

where $K_{\text{Fe-EDTA}} = 10^{25}$, and $K^* = ([\text{Fe-L}^{3-}][\text{H}^+]^6)/([\text{Fe}^{3+}][\text{H}_6\text{L}])$.

The variables for eq 1 can be obtained from the following set of equations:

$$[\text{Fe}^{3+}]_{\text{total}} = [\text{Fe-L}^{3-}] + [\text{Fe-EDTA}^-]$$

$$[\text{L}]_{\text{total}} = [\text{L}] + [\text{L}^-] + [\text{L}^{2-}] + [\text{Fe-L}^{3-}]$$

$$[\text{EDTA}]_{\text{total}} = \sum[\text{EDTA}^{n-}] + [\text{Fe-EDTA}^-]$$

For simplifications, it was assumed that (1) at pH 7, L only exists as L, L^-, and L^{2-}; (2) Fe-L only exists as Fe-L^{3-} which, for practical purposes, holds for $\mathbf{14_{Fe}}$, $\mathbf{15_{Fe}}$, and $\mathbf{16_{Fe}}$, where λ_{max} = 495 nm. The pK_a values were assumed to be equal to MECAM[8] for the calculations of [L], [L^-], and [L^{2-}]. The differences in orders of magnitude for K^* of $\mathbf{14_{Fe}}$, $\mathbf{15_{Fe}}$, and

(20) Welcher, F. J. *Analytical Uses of Ethylenediaminetetraacetic acid;* D. Van Nostrand: New York, 1958.

(21) Harris, W. R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.; Avdeef, A. E.; McArdle, J. V.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6097.

$\mathbf{16}_{Fe}$ were estimated accordingly. Assuming the overall pK_a values for **14**–**16** are the same, the difference in orders of magnitude of K^* is, therefore, also the difference in orders of magnitude of K_f, where the proton-independent formation constant, $K_f = [Fe\text{-}L^{3-}]/([Fe^{3+}][L^{6-}]) = (K^*[H_6L])/([H^+]^6[L^{6-}])$. Knowing that the K_f for $\mathbf{14}_{Fe}$ is $10^{48.8}$, the K_f values for $\mathbf{15}_{Fe}$ and $\mathbf{16}_{Fe}$ are estimated based on the differences, in orders of magnitude, from the K_f of $\mathbf{14}_{Fe}$ estimated above.

For the ester analogs **17** and **18**, however, the pK_a values are different from the amides. Therefore, estimation of K_f was not possible without knowing the pK_a of the ester analogs.

Acknowledgment. We are indebted to Professor Raymond at the University of California, Berkeley, for providing the X-ray coordinates for Figure 1, and to Dr. Zhiguo Hou of Raymond's group for determining the K_f value of the ferric complex of analog **14**. Financial assistance from the National Institutes of Health (NS-12108) and the National Science Foundation (CHE-9408247) is gratefully acknowledged.

Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra (56 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.