Chiral Analogs of Enterobactin with Hydrophilic or Lipophilic Properties

Bruno Tse and Yoshito Kishi*

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

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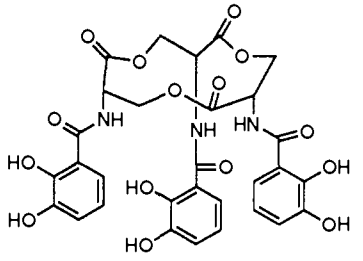
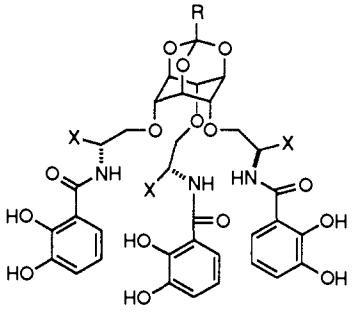
Enterobactin (**1**) is a siderophore produced by enteric bacteria to trap ferric ions under iron-deficient conditions.[1] It exhibits unique chemical properties, including (1) extraordinarily high affinity for ferric ions (stability constant K_f of ferric enterobactin $\approx 10^{49}$)[2] and (2) chirality at the metal center with the exclusively right-handed (Δ) configuration.[3] The unnatural antipode of enterobactin was shown to lack biological activities, suggesting that the chirality at the metal center plays an important role.[4] There have been substantial efforts at synthesizing lipophilic enterobactin analogs; as increasing the lipophilicity may affect the tissue distribution of the metal complex, such analogs might have potential medical applications.[5] In this communication, we report the synthesis of chiral enterobactin analogs which exhibit not only excellent affinities for ferric ions but also hydrophilic or lipophilic properties.



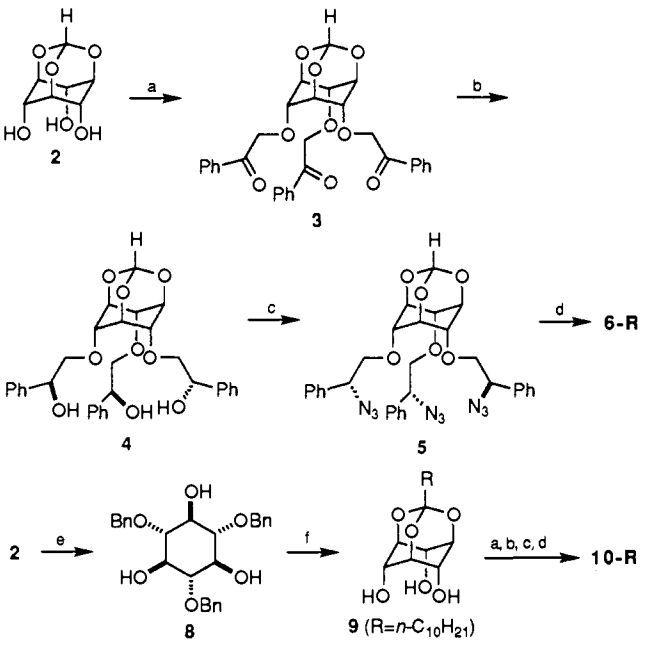
1 : enterobactin



6-R : R=H, X=Ph
6-S : the enantiomer of **6-R**
7 : R=H, X=H
10-R : R=*n*-$C_{10}H_{21}$, X=Ph

Several years ago, we reported the monoorthoformate **2** of *scyllo*-inositol.[6] We were particularly intrigued by the three hydroxyl groups axially disposed at the 1, 3, and 5 positions of the cyclohexane ring and felt that this class of compounds might provide unique opportunities for molecular architecture. For example, **2** has appealing structural features for the design of enterobactin analogs: (1) a chiral ligand can be attached to each of the three hydroxyl groups in **2**, and the resulting compound may form a complex with chirality at the metal center and (2) the orthoformate group in **2** can be replaced by other ortho esters, which may allow tuning of the polarity of the metal complexes.

Scheme I[a]



[a] Reagents and reaction conditions. (a) 1. $Ph(CH_2{=})CCH_2Br/NaH/THF–DMF$/reflux. 2. O_3/CH_2Cl_2–MeOH/–78 °C, followed by Me_2S workup. (b) (*S*)-CBS reagent/catechol borane/toluene/–78 °C. (c) 1. $MsCl/NEt_3/CH_2Cl_2$/rt. 2. *n*-Bu_4NN_3/toluene/80 °C. (d) 1. H_2(1 atm)/$Pd(OH)_2$ on C/THF–MeOH/rt. 2. *O*-Dibenzyl 2,3-dihydroxybenzoic acid/HOBt/DCC/THF. 3. H_2(1 atm)/$Pd(OH)_2$ on C/THF–MeOH/rt. (e) 1. BnBr/NaH/THF/reflux. 2. *p*-TsOH/MeOH/rt. (f) *n*-$C_{10}H_{21}C(OMe)_3$/*p*-TsOH/toluene/reflux. 2. H_2(1 atm)/$Pd(OH)_2$ on C/THF-MeOH/rt.

Thus, treatment of *scyllo*-inositol monoorthoformate **2** with 3-bromo-2-phenyl-1-propene[7] in the presence of NaH, followed by ozonolysis, gave the triketone **3** (77% overall yield) (Scheme I). Asymmetric reduction of **3** with Corey's (*S*)-oxazaborolidine ((*S*)-CBS) reagent[8] furnished the all-(*S*) triol **4** ($[\alpha]_D$ +129°) with excellent stereoselectivity.[9] Mesylation of **4**, followed by treatment with tetrabutylammonium azide, yielded the all-(*R*) triazide **5** ($[\alpha]_D$ –54°) in 64% overall yield from **3**. Catalytic hydrogenation of **5**, coupling with *O*-dibenzyl 2,3-dihydroxybenzoic acid, and catalytic hydrogenolysis furnished the chiral enterobactin analog **6-*R*** ($[\alpha]_D$ –40°) in 68% overall yield from **5**. Its enantiomer **6-*S*** ($[\alpha]_D$ +40°) was obtained by following the same procedure using Corey's (*R*)-CBS reagent.

Both **6-*R*** and **6-*S*** bind ferric ions to give deep red complexes (λ_{max} 495 nm, $\epsilon \approx 3600$ at pH 7). As expected, the CD spectra (Figure 1) of their ferric complexes **6-R_{Fe}** and **6-S_{Fe}** were found to be mirror images. Like the ferric complex of enterobactin, **6-R_{Fe}** and **6-S_{Fe}** showed CD bands in the ligand-to-metal charge-transfer region (~540 nm), indicating that the ligand chirality of **6-*S*** and **6-*R*** induced a preference for the right-handed (Δ) metal complex over the left-handed one (Λ) or *vice versa*.

(1) Pollack, J. R.; Neilands, J. B. *Biochem. Biophys. Res. Commun.* **1970**, *38*, 989.
(2) Loomis, L. D.; Raymond, K. N. *Inorg. Chem.* **1991**, *30*, 906.
(3) For example, see: (a) Isied, S. S.; Kuo, G.; Raymond, K. N. *J. Am. Chem. Soc.* **1976**, *98*, 1763. (b) Stack, T. D. P.; Karpishin, T. B.; Raymond, K. N. *J. Am. Chem. Soc.* **1992**, *114*, 1512 (c) Karpishin, T. B.; Raymond, K. N. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 466.
(4) Neilands, J. B.; Erickson, T. J.; Rastetter, W. H. *J. Biol. Chem.* **1981**, *256*, 3831.
(5) For example, see: (a) Weitl, F. L.; Raymond, K. N. *J. Org. Chem.* **1981**, *46*, 5234. (b) Kappel, M. J.; Pecoraro, V. L.; Raymond, K. N. *Inorg. Chem.* **1985**, *24*, 2447. (c) Miller, M. J.; Malouin, F. *Acc. Chem. Res.* **1993**, *26*, 241.
(6) Lee, H. W.; Kishi, Y. *J. Org. Chem.* **1985**, *50*, 4402.
(7) Hatch, L. F.; Patton, T. L. *J. Am. Chem. Soc.* **1954**, *76*, 2705.
(8) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, *109*, 5551.
(9) Up to 5% of the undesired isomers of triol **4** may be present. Vigorous purification was done at the stage of triazide **5**. Both ^1H and ^{13}C NMR spectra indicated the presence of a symmetry element in **4**. The assignment of the absolute configuration of **4** was based on literature precedent.[8]

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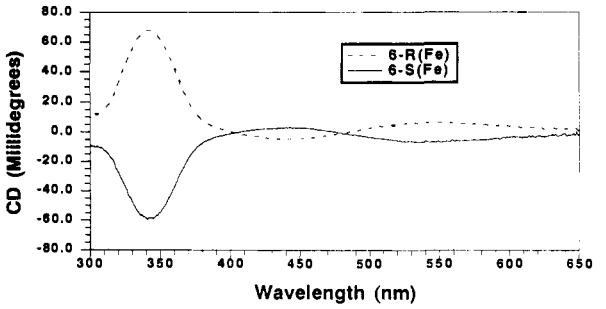


Figure 1. CD spectra of ferric complexes of **6**-*R* and **6**-*S* recorded at 10^{-4} M concentration in a 1:1 mixture of MeOH and 0.01 M phosphate buffer (pH 7.0).

Comparing the signs of the CD bands of **6**-R_{Fe} and **6**-S_{Fe} at ~540 nm with the literature data allowed the assignment of Δ-cis to **6**-S_{Fe} and Λ-cis to **6**-R_{Fe}.[3b] However, the ratio of Δ:Λ of the complexes cannot be deduced from CD spectroscopy alone. Proton NMR spectra of the diamagnetic Ga(III) complexes of enterobactin and its analogs were shown to be useful for determining the ratio of Δ:Λ.[3b] Thus, the Ga(III) complexes of **6**-*R* and **6**-*S* were prepared and subjected to variable-temperature NMR studies. Over a wide range of temperatures (230 to 333 K), ^{1}H NMR of the Ga(III) complexes showed only one set of well-defined signals, showing that the complexes were exclusively Δ-cis in the case of **6**-S_{Ga} and exclusively Λ-cis in the case of **6**-R_{Ga}. With the similarities of the coordination chemistry of Fe(III) and Ga(III),[5] the same assignment can be made for the ferric complexes of **6**-*R* and **6**-*S*.

It is worthwhile to mention the stability constants K_f observed for **6**-S_{Fe} and **6**-R_{Fe}. For the purpose of comparison, the achiral analog **7**, which differs from **6** only in lacking the phenyl groups, was synthesized.[10] As expected, the chemical behavior of **7** toward ferric ions was parallel to that of **6**, yet, the stability constant K_f for **7** was found to be at least 2 orders of magnitude[11] lower than that of **6**. We attribute this difference primarily to an entropic factor; with the small *scyllo*-inositol as the platform, the relatively bulky phenyl groups point outward to minimize steric congestion, and the three catechol groups are preorganized in such a way that the entropy lost upon binding with Fe(III) is less for **6** than for the relatively flexible **7**. It is intriguing to note that the stability constant K_f for **6** was estimated to be only 1 order of magnitude lower than K_f for enterobactin itself.

Using an undecyl group as an example, the feasibility of structural modification at the ortho ester moiety was demonstrated. Hydrolysis of 1,3,5-tri-*O*-benzyl *scyllo*-inositol monoorthoformate readily gave 1,3,5-tri-*O*-benzyl *scyllo*-inositol (**8**). Treatment of **8** with trimethyl orthoundecanoate[12] in the presence of a catalytic amount of *p*-TsOH, followed by catalytic hydrogenation, furnished the orthoundecanoate **9** (73% overall yield). Using the same procedure as before, **9** was successfully converted to the chiral analog **10**-*R* ($[\alpha]_D$ −8.1°). As anticipated, the analog **10**-*R* was found to be much more lipophilic than the corresponding **6**. Like **6**-*R*, **10**-*R* was found to chelate ferric ions and give a deep-red complex (λ_{max} 495 nm, $\epsilon \approx 4000$ at pH 7), with a K_f similar to that of **6**-R_{Fe}.[11] The CD spectrum of **10**-R_{Fe} was virtually identical to that of **6**-R_{Fe}. The ^{1}H NMR spectrum of **10**-R_{Ga} exhibited only one set of well-defined signals, suggesting that **10**-*R* also bound Ga(III), and thus Fe(III), exclusively in the Λ-fashion. The most exciting observation was that **10**-R_{Fe} showed excellent solubility in organic solvents; upon partitioning between water and EtOAc or $CHCl_3$, **10**-R_{Fe} appeared only in the organic layer, whereas **6**-R_{Fe} appeared only in the aqueous layer.

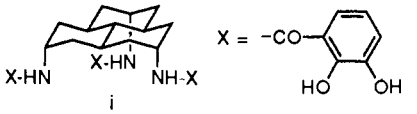
Further investigations on the design and synthesis of metal ligands using monoorthoformate **2** and related substances, the modification of chemical properties of metal complexes by tuning the orthoester moiety, and applications of these ligands are in progress in our laboratories.

Acknowledgment. We are indebted to Professor Kemp at the Massachusetts Institute of Technology for the use of his CD spectrometer. Financial assistance from the National Institute of Health (NS-12108) and the National Science Foundation (CHE 89-09762) is gratefully acknowledged.

Supplementary Material Available: ^{1}H NMR spectra of the compounds shown in Scheme I (14 pages). Ordering information is given on any current masthead page.

(10) The achiral analog **7** was synthesized using the same synthetic reactions shown in Scheme I, except that allyl bromide was used instead of 3-bromo-2-phenyl-1-propene in step a, and LAH was used instead of (*S*)-CBS reagent in step b.

(11) With a small modification of the method established by Raymond (*J. Am. Chem. Soc.* **1979**, *101*, 6097), the K_f values were estimated as follows. We synthesized the enterobactin analog **i**, the ferric complex of which was kindly determined to have $K_f = 10^{48.8}$ by Professor Raymond and co-workers (details to be published elsewhere).



i

We then performed EDTA competition experiments on both **6** and **i**. Assuming[2] the overall pK_a of the six catecholic protons to be 58.5 and measuring the decrease in ϵ of the ferric complex at 495 nm in the presence of 5×10^{-4} to 10^{-2} M EDTA, the K_f value of $\mathbf{6}_{Fe}$ was estimated to be approximately 1 order of magnitude lower than that of the ferric complex of **i**. The K_f values for other analogs were estimated likewise.

(12) McElvain, S. M.; Kent, R. E.; Stevens, C. L. *J. Am. Chem. Soc.* **1946**, *68*, 1922.