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β-Cyclodextrin Bound Retrohydroxamate Ferrioxamines. Chiral Iron(III) Coordination and Biological Activity of Synthetic Siderophores

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Two β-cyclodextrin bound synthetic siderophores that mimic linear and cyclic desferrioxamines are prepared. These synthetic siderophores form stable 1:1 iron(III) complexes with a Δ-selective coordination and show the growth promotion activity when tested with *Aureobacterium flavescens*.

Microorganisms produce iron-chelating agents called siderophores and take up iron(III) via siderophore-mediated iron transport systems.[1-3] Siderophore-mediated iron uptake involves molecular level recognition of iron binding and receptor matching, thus providing an area of intense current research, and much effort has been devoted to the design and synthesis of artificial siderophores.[4] Of great interest in the design are such analogs that have distinctive molecular features in shape and size.

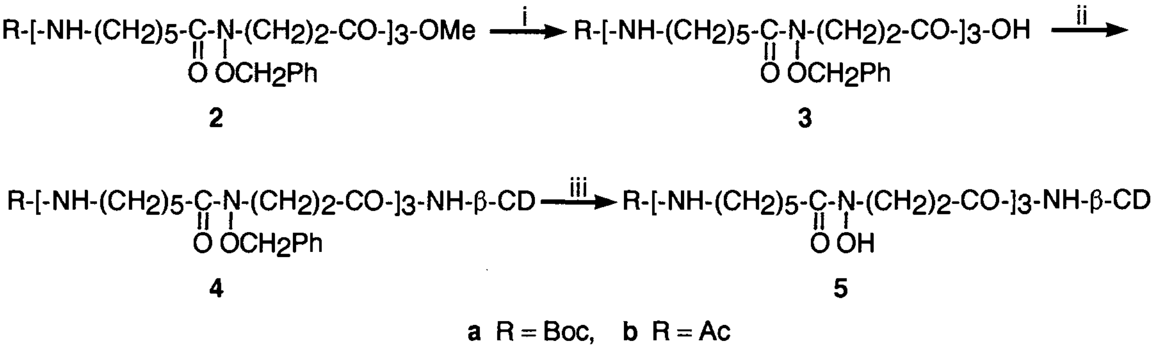
A cyclodextrin is a large chiral molecule holding a cavity capable of accommodating a variety of compounds.[5] Synthetic siderophores appended with a β-cyclodextrin (β-CD) unit should serve as excellent probes in iron(III) coordination and biological activity. Among a great number of biomimetic analogs of cyclodextrins there have been reported a few metal-ligating derivatives to date,[6] yet examples of chiral coordination and microbial activity are unprecedented in β-CD metal ion complexes.

Ferrioxamines are iron(III) complexes of trihydroxamate siderophores.[7] Previously we have synthesized cyclic and linear retrohydroxamate ferrioxamines E and G (**RFE** and **RFG**) by use of suitably protected 6-aminohexanoyl-3-(N-hydroxy)aminopropanoyl units.[8] We report here that retrohydroxamate ferrioxamines show several interesting features when bonded to mono-6-amino-6-deoxy-β-cyclodextrin (**1**) by an amide linkage.

The synthesis is outlined in Scheme 1. A previous procedure provided a useful starting material **2a**, and the N-terminal acetyl compound **2b** was obtained from **2a**.[9] The carboxylic acid derivatives **3a** and **3b** were condensed with the amino-β-cyclodextrin **1** (NH_2-β-CD) via N-hydroxysuccinimide (HOSu) esters to give β-CD derivatives **4a** and **4b**. Hydrogenation of **4** afforded the β-CD bound retrohydroxamate desferrioxamines **5a** and **5b** after purification by gel chromatography.[10]

By virtue of attachment to β-CD, they have considerable water solubility (250 mg / mL), which is one of desirable proprties for synthetic siderophores.[3]

The 1:1 iron(III) complexes (**5a**-Fe and **5b**-Fe), β-CD bound retrohydroxamate ferrioxamines, were obtained by combining **5a** and **5b** with an equimolar amount of aqueous $Fe(NO_3)_3$ solution at pH 3. Molecular modeling indicates that an octahedral iron complex moiety stays outside the β-CD cavity. The absorption spectra exhibit maxima at 428 nm (ε 2800) for **5a**-Fe and at 420 nm (ε 2950) for **5b**-Fe at pH 7, characteristic of the 1:3 complex of iron(III) with the hydroxamate group.[11)] This was confirmed by mole ratio plots, and absorbance vs pH plots showed the presence of these complexes over a wide pH range (pH 3-10) (not shown here).



$$R\text{-}[\text{-}NH\text{-}(CH_2)_5\text{-}\underset{\underset{O}{\|}}{C}\text{-}\underset{\underset{OCH_2Ph}{|}}{N}\text{-}(CH_2)_2\text{-}CO\text{-}]_3\text{-}OMe \xrightarrow{i} R\text{-}[\text{-}NH\text{-}(CH_2)_5\text{-}\underset{\underset{O}{\|}}{C}\text{-}\underset{\underset{OCH_2Ph}{|}}{N}\text{-}(CH_2)_2\text{-}CO\text{-}]_3\text{-}OH \xrightarrow{ii}$$

2 **3**

$$R\text{-}[\text{-}NH\text{-}(CH_2)_5\text{-}\underset{\underset{O}{\|}}{C}\text{-}\underset{\underset{OCH_2Ph}{|}}{N}\text{-}(CH_2)_2\text{-}CO\text{-}]_3\text{-}NH\text{-}\beta\text{-}CD \xrightarrow{iii} R\text{-}[\text{-}NH\text{-}(CH_2)_5\text{-}\underset{\underset{O}{\|}}{C}\text{-}\underset{\underset{OH}{|}}{N}\text{-}(CH_2)_2\text{-}CO\text{-}]_3\text{-}NH\text{-}\beta\text{-}CD$$

4 **5**

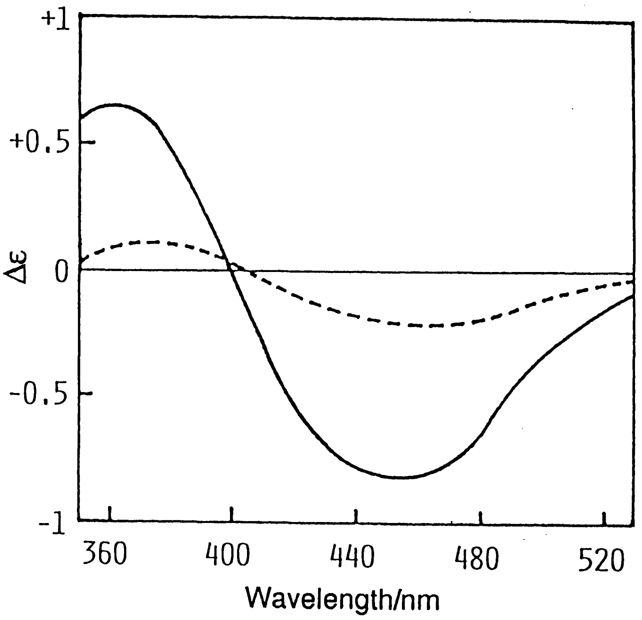
a R = Boc, **b** R = Ac

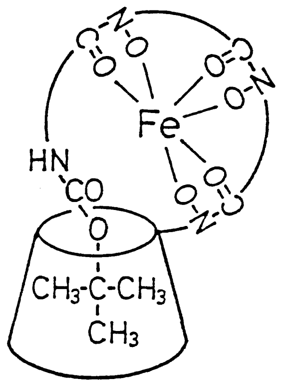
Scheme 1. Reagents and conditions: i) 1 M NaOH (2 equiv.) in MeOH at r. t.; ii) HOSu - 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.HCl in DMF-CH_2Cl_2, then with **1** in pyridine at 38 °C for 2 days; iii) H_2/10% Pd-C in MeOH for 15-20 h.

Interestingly, the cyclodextrin unit exerts its chiral influence on iron(III) coordination of the achiral ligand. **5a**-Fe and **5b**-Fe show circular dichroism (CD) spectra with different magnitudes of intensity (Fig. 1). The pattern and magnitudes of the negative (at 455 nm) and positive (at 370 nm) bands suggest that both **5a**-Fe and **5b**-Fe have a Δ-selective coordination, though not exclusive, around the metal ion.[12)] The different intensity implies that the two complexes behave differently under these conditions. By the addition of a good binding substrate, 2-adamantanecarboxylate (50 molar excess), at pH 9 the CD magnitude of **5a**-Fe was decreased by a factor of 4 approaching to that of **5b**-Fe after 3 days, whereas **5b**-Fe was unaffected. Thus the Boc group of **5a**-Fe apparently enters the β-CD cavity to form a pseudo cyclic structure, thereby the metal coordination being fixed more efficiently. ^{1}H-NMR NOE measurements lend some support for the cyclic structure **(A)**.[13)] The unaffected complex **5b**-Fe remains acyclic.

Iron transfer experiments indicate that acyclic (**5b**-Fe) and pseudo-cyclic (**5a**-Fe) structures are predominant species in solution. Iron(III) was transferred from complexes (0.32 mM) to excess EDTA (8.3 mM) with pseudo-first-order rate constants 2.6×10^{-5} (**5a**-Fe) and 1.0×10^{-4} (**5b**-Fe) s^{-1} in acetate buffer (pH 5.3) at 25 °C. Relative to a value (7.4×10^{-5} s^{-1}) for **RFG**,[8)] a lower rate for **5a**-Fe is consistent with strong holding, as was the case of cyclic analog **RFE**,[8)] whereas a high rate suggests a destabilization of **5b**-Fe by β-CD.

Equilibrium determinations under these similar conditions gave approximate stability constants of 10^{32} and 10^{30} for **5a**-Fe and **5b**-Fe,[14] which correspond to those of cyclic and linear ferrioxamines, respectively.[11]





Structure (A)

Fig. 1. Circular dichroism spectra for β-CD bound retrohydroxamate ferrioxamines in water at pH 7.0: **5a-Fe** (—); **5b-Fe**(----). The same spectra were obtained at pH 9.0.

Finally, we examined the growth promoting activity by the literature procedure[1] using *Aureobacterium flavescens*,[15] an auxotroph for hydroxamate siderophores. Both **5a**-Fe and **5b**-Fe showed a weak but significant halo of exhibition of growth despite the presence of the β-CD unit. The activity is estimated from the diameter to be 10% (23 mm) to that (35 mm) of potent ferrioxamine B. This phenomenon is interesting, since the β-CD unit is not considered to pass into the cell membrane.

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9) **2a** corresponds to **5** in Ref. 8. Treatment of **2a** with HCl-dioxane /Ac_2O gave **2b**.
10) Both obtained as ninhydrin negative products after Sephadex G-15 gel chromatography with water: **5a**, 41% yield from **2a**, mp 183 °C dec., $[\alpha]_D^{27}$ 93° (*c* 0.13, H_2O); ^1H NMR (200 MHz in DMSO-d_6 at 50 °C) δ 1.15-1.55 (18H, m, 3xβ,γ,δ-$(CH_2)_3$-), 1.37 (9H, s, t-Bu), 2.25-2.40 (12H, m, 6xCH_2-CO), 2.89 (2H, q, J=6.1 Hz, Boc-NH-$\underline{CH_2}$-), 3.02 (4H, q, J=5.9 Hz, 2x$\underline{CH_2}$NHCO), 3.20-3.42 (16H, m, β-CD 14H (C-2 and C-4 and β-CD-$\underline{CH_2}$-NH), 3.50-3.75 (32H, m, 3x-$\underline{CH_2}$N(OH)CO and β-CD 26H (C-3, C-5 and C-6)), 4.34 (6H, br s, β-CD-OH (C-6)), 4.83 (7H, d, J=2.7 Hz, β-CD C-1-H), 5.59 (14H, br s, β-CD-OH (C-2 and C-3)), 6.60 (1H, t, J=6.1 Hz, Boc-NH), 7.68 (2H, t, J=5.9 Hz, β-CD-NHCO), 7.78 (2H, t, J=5.9 Hz, 2x-NHCO), and 9.57 (3H, br s, 3xN-OH). Anal. Found: C, 47.96; H, 7.02; N, 5.29%. Calcd for $C_{74}H_{127}N_7O_{45}.H_2O$: C, 47.97; H, 7.02; N, 5.29%.

    5b, 45% yield from **2b**, mp 179 C dec. A similar ^1H NMR spectrum was obtained. Anal. Found: C, 47.23; H, 7.26; N, 5.89%. Calcd for $C_{71}H_{121}N_7O_{44}.H_2O$: C, 47.52; H, 6.91; N, 5.46%.
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13) Upon irradiation of the Boc methyl protons in DMSO-d_6 at 50 °C, signal intensity at δ 3.64 enhanced by 2% for **5a** and 1% for **5a**-Ga(III).
14) Stability constants for **5a**-Fe and **5b**-Fe were calculated from the EDTA-Fe stability constant and values for the following equilibrium ([Fe-EDTA][**5a**])/([**5a**-Fe][EDTA])=0.60, ([Fe-EDTA][**5b**])/([**5b**-Fe][EDTA])=17.5 at 25 °C (pH 5.3) using pK_1=8.70, pK_2=9.46, and pK_3=10.05 for desferri-**RFG.**
15) ATCC 25091, formerly registered as *Arthrobacter flavescens* Jg-9; B. F. Burnham and J. B. Neilands, *J. Biol. Chem.*, **236**, 554 (1961).

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