Artificial Siderophores as a Model for Ferrichrome. Control of the Δ- or Λ-Configuration of Iron(III) Complexes of Tripodal Hydroxamates by Linking to the C- or N-Terminus of the Same L-Alanyl-L-alanyl-β-(N-hydroxy)alanine Unit

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New L-alanyl-L-alanyl-β-(N-hydroxy)alanine-based tripodal hydroxamic acids have been prepared. They form stable chiral complexes with iron(III). The chirality of these complexes can be controlled by a tripodal molecule that links to the C-terminus or N-terminus of the same N-hydroxy peptide unit to give the Λ-*cis* or Δ-*cis* configuration about the metal ion, respectively.

Iron is essential in living systems. Microorganisms produce iron(III)-chelating agents called siderophores in order to acquire iron from the environment. Siderophores usually contain hydroxamate or catecholate groups to form stable six-coordinate octahedral complexes with iron(III).[1,2] Ferrichrome [Fe(III)-**1**] is a representative tripodal iron(III) hydroxamate complex for fungi, while enterobactin is a typical triscatecholate siderophore excreted from bacteria, such as *Escherichia coli.*[1,2] Iron(III)-siderophore complexes are actively transported into the cells after recognition by membrane receptor proteins. There are factors crucial for the recognition, including the chirality as well as the shape and size of iron(III)-siderophores.[1-4] Among them, the importance of the chirality has been demonstrated for growth of test organisms; neither enantiomeric ferrichrome[5] nor enantio-enterobactin[6] is effective, because of the opposite chirality, while *Rhodotorula pilimane* was less acceptable for a Λ-Cr(III) isomer than for Δ-Cr(III) isomers, derived from rhodotorulic acid.[7]

In our approach by way of N-hydroxy peptide derivatives[8] to the design and synthesis of artificial ferrichromes,[9,10] we report here that Ala-Ala-β(HO)Ala[11]-based tripodal hydroxamic acids (**2**- **4**) produce chiral iron(III) complexes (Figure 1) and that the

$\text{N[CH}_2\text{CO-Ala-Ala-}\beta\text{(HO)Ala-OMe]}_3$ **2**

$\text{[Ac-Ala-Ala-}\beta\text{(HO)Ala-Ala-NHCH}_2\text{CH}_2\text{]}_3\text{N}$ **3**

$\text{[Boc-Ala-Ala-}\beta\text{(HO)Ala-NHCH}_2\text{CH}_2\text{]}_3\text{N}$ **4**

chirality of these complexes can be controlled by the choice of a tripodal molecule, nitrilotriacetic acid (nta) or tris-(2-aminoethyl)-amine (tren), respectively. Chiral control of iron(III) complexes that have the same optical components has not been realized yet.

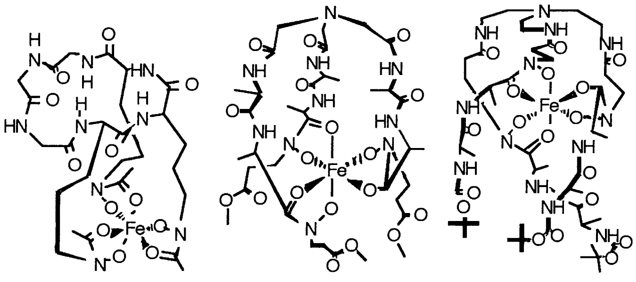
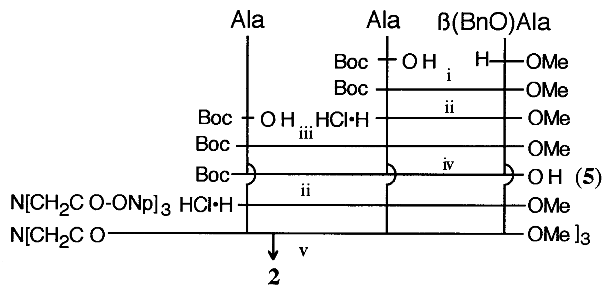

Fe(III)-**1**, Λ-*cis* Fe(III)-**2**, Λ-*cis* Fe(III)-**4**, Δ-*cis*

Figure 1. Schematic representation of Fe(III)-**1**, Fe(III)-**2** and Fe(III)-**4**. See text for the assignment.



Scheme 1. *Reagents:* i ClCO_2Bu^i / Et_3N, -15 °C; ii 6 M HCl in dioxane, 0 °C; iii EDC / HOBt; iv 1 M NaOH; v H_2, Pd-C in MeOH.

The synthesis of tripodal ligands (**2** - **4**) was carried out via condensations of suitably protected $\text{Ala}^1\text{-Ala}^2\text{-}\beta\text{(HO)Ala}$ units with nta[10a] or tren, followed by hydrogenation to remove benzyl protective groups. A typical example is shown in Scheme 1. We also used tris(Ala-NHCH_2CH_2)N in place of tren for the synthesis of ligand **3**, since coupling of peptide **5** with tren gave ligand **4** only in poor yield, although ligand **4** compares to ligand **3** in iron(III)-holding capacity. These final products (**2** - **4**) were characterized by HPLC, IR, ^{1}H NMR and elemental analysis.[12] The presence of hydroxamic acid groups was confirmed by observing a full intensity for the Ala α-protons adjacent to the -CON(OH)- group in ^{1}H NMR spectra determined in DMSO-d_6.

The iron(III) complexes of ligands (**2** - **4**) were produced by mixing a slight excess (1.03 equivalent) of each ligand with an aq $\text{Fe(NO}_3\text{)}_3$ solution and by the subsequent gradual neutralization with 0.1 M KOH. These 1:3 iron(III) complexes showed their molar absorptivity of λ_{max} 425 nm and ε ca. 2800 M^{-1} cm^{-1} (Table 1), characteristic of a typical 1:3 iron(III) complex.[1,2,13]

The stability of Fe(III)-**2** and Fe(III)-**3** against attack of H^+ or OH^- ions was estimated by plotting an absorption at 425 nm vs pH. These complexes showed a range of constant absorption regions over pH 4.0 - 9.0 (data not shown). A similar trend was

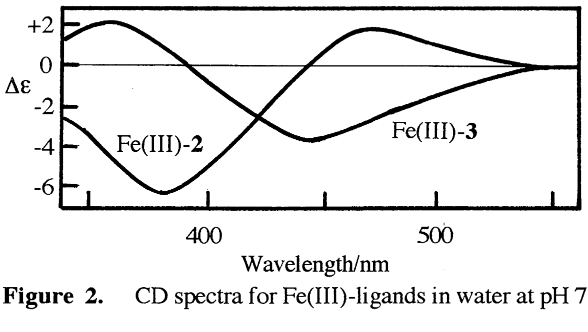


Figure 2. CD spectra for Fe(III)-ligands in water at pH 7.0.

Table 1. VIS and CD Spectra, and Iron Exchange Rates[a]

Complex	VIS λ_{max}/nm (ε)	Type	CD λ/nm ($\Delta\varepsilon$)	Exchange rate k_{obs}/s^{-1}
Fe(III)-**1**	425 (2900)[b]	Λ	465, 360 (+2.4, -3.7)[b]	6.1×10^{-4} [c]
Fe(III)-**2**	424 (2700)	Λ	465, 380 (+2.1, -6.2)	2.3×10^{-3}
Fe(III)-**3**	425 (2800)	Δ	445, 365 (-4.0, +2.3)	2.7×10^{-2}
Fe(III)-**4**	430 (2900)	Δ	455, 375 (-5.3, +3.0)	1.1×10^{-2}

[a] VIS and CD spectra were taken in water at pH 7.0. The ε values are in $M^{-1} cm^{-1}$. Ligand **1** stands for deferriferrichrome. The iron exchange reaction was carried out, as described in the text. [b] Ref. 2. [c] Ref. 14.

observed for Fe(III)-**1**.[14] Fe(III)-**3**, for example, gave an isosbestic point at 447 nm in its absorption spectra over pH 2.6 - 3.6 (data not shown), which indicated the presence of a protonation equilibrium between the 1:3 complex and its protonated species, providing evidence for the 1:3 complex formation.[2,13]

$$\text{Fe(III)-L} + H^+ = \text{Fe(III)-HL}^+ \qquad (1)$$

The chirality of these iron(III) complexes was determined by their circular dichroism (CD) spectra, as shown in Figure 2. Two pairs of different negative and positive bands for Fe(III)-**2** and Fe(III)-**3** are assigned to predominant Λ and Δ configurations about the metal ion, respectively, with reference to the literature determination.[15] Table 1 contains these results. It is notable that a pair of opposite configurations reflect the inverse directionality of the hydroxamato group in the same chiral sequence, although the inverse directionality itself has been shown to affect little on biological activity.[16] The CD intensity of these complexes is comparable to ferrichrome which has a well-defined geometry in the crystals and in solution.[15] A comparison of Fe(III)-**4** and Fe(III)-**3** reveals that an Ala unit added to tren has no effect on the chiral tendency. CPK molecular models show that the iron(III) complex of these tripodal ligands must have the *cis* orientation of all of the three hydroxamato-chelation rings, therefore, the configuration assigned here is either the Λ-*cis* or Δ-*cis*.

The stability constants of Fe(III)-**2** and Fe(III)-**3** were obtained from the following equimolar, iron(III), ligand-EDTA exchange reaction in water at 25 °C, pH 5.4, and ionic strength 0.1, using

$$\text{Fe(III)-L} + H_2EDTA^{2-} + H^+ = \text{Fe(III)-EDTA}^{1-} + H_3L \quad (2)$$

1.0×10^{-4} M of each iron(III) complex. The resultant proton dependent equilibrium constants, $K^* = [\text{Fe(III)-EDTA}^{1-}][H_3L] / \{[\text{Fe(III)-L}][H_2EDTA^{2-}][H^+]\}$, were determined by observing the amount of iron(III) complexes unexchanged [Fe(III)-**2**, 18 % and Fe(III)-**3**, 10 %], and converted into the stability constants by use of the protonation constants of the ligand and EDTA.[13]

The protonation constants for ligand **3** were obtained by potentiometric titration in water at 25 °C and ionic strength 0.01; the values being pK_1 9.69, pK_2 9.03, and pK_3 8.26. This data and the Fe(III)-EDTA stability constant (10^{25})[13] allowed us to calculate a stability constant for Fe(III)-**3** to be 10^{28}. For Fe(III)-**2** a value of 10^{29} was tentatively obtained when the same pK values were used for the calculation, assuming that a similar molecular environment is provided for ligand **2**. Each value is close or equal to the ferrichome value of 10^{29}.[13]

The iron(III)-holding capacity of the complexes were examined further in terms of a ligand exchange reaction, as eq 2, in aq acetate buffer at pH 5.4, ionic strength of 0.1, and 25 °C, using 3.8×10^{-4} M of each complex and excess molar EDTA (20 equivalent) as a competing ligand. A slightly higher, iron(III)-preserving capacity for ligand **1** is noted. These results are also included in Table 1.

The present finding of the chiral control shows a possibility of affording tripodal iron(III) complexes of the Δ or Λ configuration from a range of N-hydroxy peptide units of the same optical series.

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11 Abbreviations: Ala, L-alanyl; β(BnO)- or β(HO)-Ala, β-(N-benzyloxy)- or β-(N-hydroxy)-alanyl; (HO)Gly, (N-hydroxy)glycyl; Boc, *t*-butoxycarbonyl; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; ONp, *p*-nitrophenoxy; TFA, trifluoroacetic acid; HOBt, 1-hydroxybenzotriazole: 1 M = 1 mol dm^{-3}.

12 Ligand **2**: $[\alpha]_D^{25}$ -62 (c 1.1, MeOH); Ala^2 α-H 4.78 (3H, quint. J=7.2 Hz); C, H, N ± 0.3% as $C_{36}H_{60}N_{10}O_{18}\cdot H_2O$. Ligand **3**: $[\alpha]_D^{25}$ -83 (c 0.3, H_2O); Ala^2 α-H 4.79 (3H, quint. J=7.0 Hz); C, H, N ± 0.3% as $C_{48}H_{84}N_{16}O_{18}\cdot 4H_2O$.

Ligand **4**: $[\alpha]_D^{25}$ -39 (c 1.1, MeOH); Ala^2 α-H 4.79 (3H, quint. J=7.0 Hz); C, H, N ± 0.3% as $C_{48}H_{87}N_{13}O_{18}H_2O$.

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