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Absolute Configurations of Iron(III) Complexes of an Enterobactin Model, a Hexapus Cyclophane with Catechol Segments

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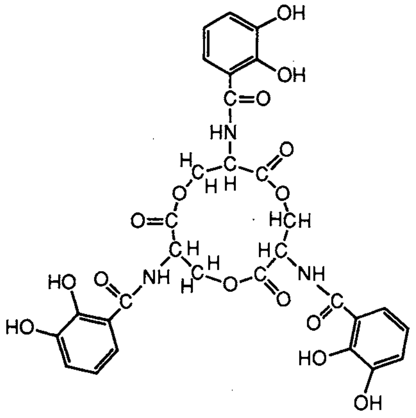
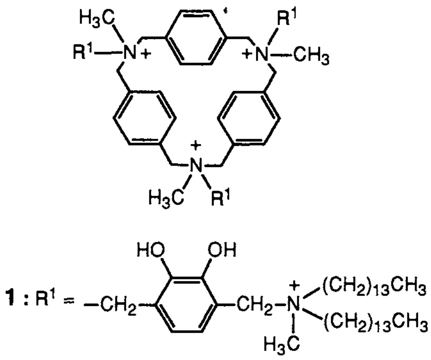
The absolute configuration of an iron(III) complex of *N,N',N''*-tris[*N*-(4-ditetradecylcarbamoyl-2,3-dihydroxybenzoyl)alanyl]-2,11,20-triaza[3.3.3]-paracyclophane, an enterobactin model, was subjected to change by the chirality of the alanine residues; the L-alanine residue prefers to give out the Λ-configuration, while the D-residue stabilizes the Δ-configuration.

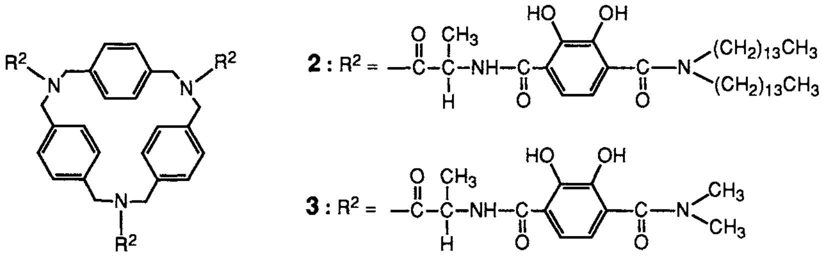
Siderophores, microbial iron-transporting agents, are excreted by microorganisms for the purpose of solubilizing environmental iron and facilitating its incorporation into the microbial cell. The most effective siderophore involved in enteric bacteria is enterobactin, which shows a stability constant (K) for the iron(III) ion as large as $\log K = 52$.[1] We have previously reported the preparation of hexapus cyclophane **1** and its physicochemical properties as an enterobactin model.[2] Because its iron(III) complex is hardly soluble in water, its stability constant was not determined in aqueous media under ordinary conditions. Raymond et al. proposed that the amide portions of enterobactin, which are interposed between the catechol groups and the cyclic triserine skeleton, are indispensable components for efficient recognition of the enterobactin complex by its receptor on the cell surface.[3] In this regard, we now prepared a novel hexapus cyclophane with alanine (Ala) residues, *N,N',N''*-tris[*N*-(4-ditetradecylcarbamoyl-2,3-dihydroxybenzoyl)alanyl]-2,11,20-triaza[3.3.3]paracyclophane (**2**), and the complexation and protonation equilibria for its iron(III) complex as well as the absolute configurations of the complexes with enantiomeric Ala residues have been investigated. Compound **3** with L-Ala residues [**3**(L-Ala)], lacking long hydrocarbon segments, was also prepared as a reference to characterize specific features of **2** with L-Ala residues.

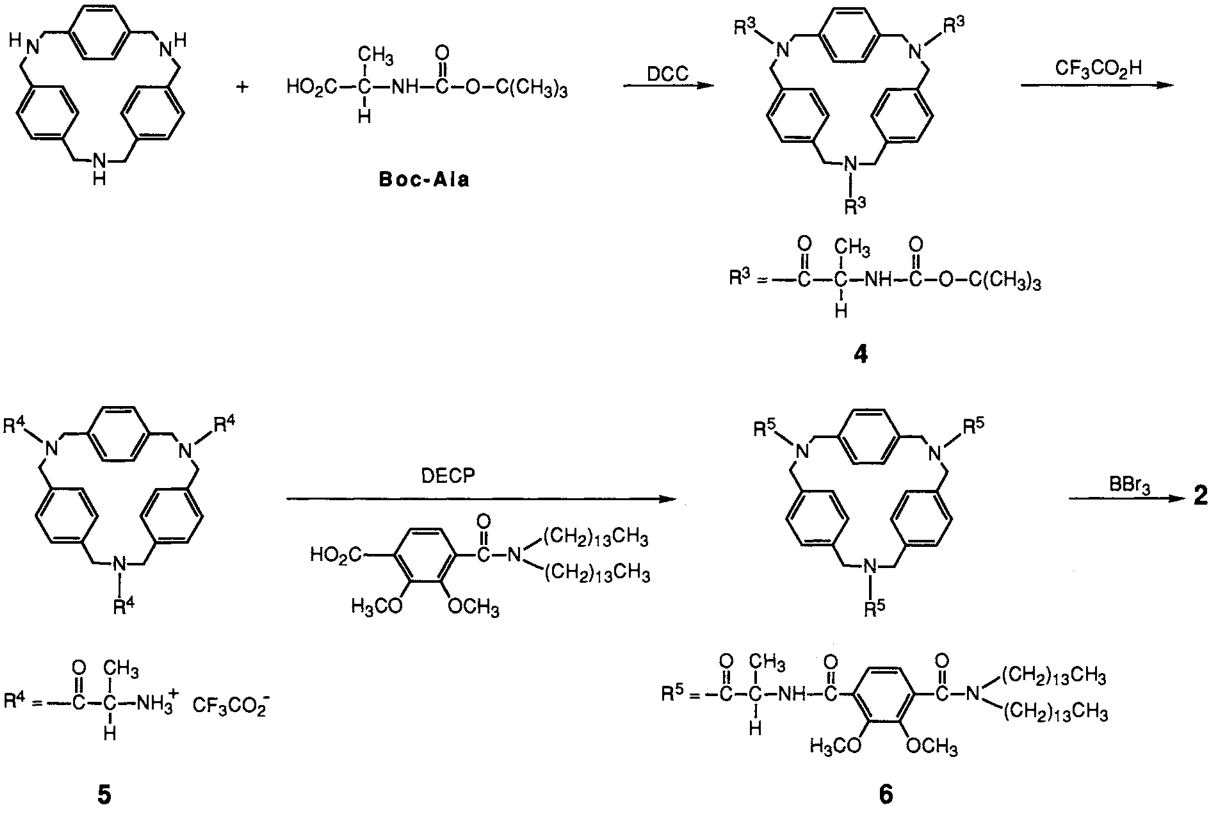
Hexapus cyclophane **2** with L-Ala residues [**2**(L-Ala)] was prepared by following the reaction steps similar to those applied to the preparation of **1**,[2] as shown in Scheme 1. The reaction of **6** (500 mg, 2.11×10^{-4} mol) with boron tribromide (529 mg, 2.11×10^{-3} mol) was carried out in dry dichloromethane under argon atmosphere, and the product was purified by gel-filtration chromatography [Sephadex LH-20, methanol–chloroform (1:1 v/v)] to give **2**(L-Ala); yield 427 mg (89%). Found: C, 73.53; H, 9.89; N, 5.43%. Calcd for $C_{141}H_{225}N_9O_{15}\cdot H_2O$: C, 73.49; H, 9.92; N, 5.47%. Compound **2** with D-Ala residues [**2**(D-Ala)] was prepared by the same procedure in a comparable yield.



enterobactin







Scheme 1.

Complexation equilibria for the formation of the iron(III) complexes with **2**(L-Ala) and **3**(L-Ala) and protonation equilibria for these complexes were determined by electronic spectroscopy. Electronic spectra of the iron(III) complexes (2.0 x 10^{-5} mol dm^{-3}) were measured at 25.0 °C and μ 0.10 (KNO_3) as a function of pH [pH 5–12 for **3**(L-Ala), pH 6–12 for **2**(L-Ala)]. The protonation constants (K_{MHL}, K_{MH_2L}, and K_{MH_3L}; refer to Eqs. 2–4) for the iron(III) complexes were determined after the Schwarzenbach's method:[4] pK_{MHL} = 7.24 for the iron(III) complex of **2**(L-Ala), and pK_{MHL} = 8.88, pK_{MH_2L} = 7.58, and pK_{MH_3L} = 5.99 for the iron(III) complex of **3**(L-Ala).

The overall formation constants (K_{ML}; refer to Eq. 1) for the iron(III) complexes of **2** and **3** were not determined directly because the iron binding is so strong that the complexes are not appreciably dissociated into the free ligand and the free metal ion. Therefore, proton-dependent stability constants were determined spectrophotometrically at pH 9.0 in competition with EDTA after the method of Raymond et al.[5] Since the acid dissociation constants for **2** were not evaluated exactly, these values were estimated from those for a related compound, a single catechol fragment of **2** without long hydrocarbon chains and the macrocycle. The complexation constants (K_{ML}) are $>10^{41}$ and 10^{38} mol dm^{-3} for **2**(L-Ala) and **3**(L-Ala), respectively.

It was reported that the iron(III) complex of enterobactin assumes Δ-configuration and the specific chirality recognition may be operating on the cell surfaces.[3] In this regard, the absolute configurations of the iron(III) complexes of hexapus cyclophanes with L-Ala and D-Ala

$$L^{6-} + M^{n+} \xrightleftharpoons{K_{ML}} ML^{n-6} \qquad (1)$$

$$ML^{n-6} + H^{+} \xrightleftharpoons{K_{MHL}} MHL^{n-5} \qquad (2)$$

$$MHL^{n-5} + H^{+} \xrightleftharpoons{K_{MH_2L}} MH_2L^{n-4} \qquad (3)$$

$$MH_2L^{n-4} + H^{+} \xrightleftharpoons{K_{MH_3L}} MH_3L^{n-3} \qquad (4)$$

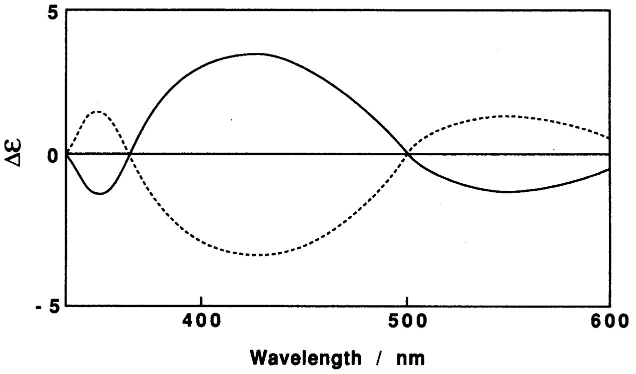


Fig. 1. CD spectra of iron(III) complexes of **2**(L-Ala) (—) and **2**(D-Ala) (---) in Tris buffer (pH 9.0) at 30.0 °C.

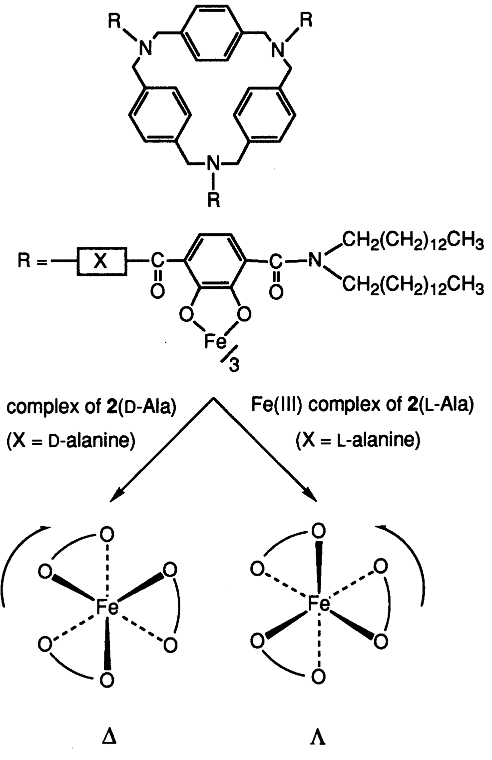


Fig. 2. Absolute configurations of iron(III) complexes of **2**(L-Ala) and **2**(D-Ala).

residues were examined by circular dichroism (CD) spectroscopy as shown in Fig. 1. The assignments of absolute configurations, Δ and Λ, to these complexes were based on the CD spectral patterns for tris(catecholato)chromate(III) and the chromium(III) complex of enterobactin.6) The CD spectra indicate that the iron(III) complexes of **2**(L-Ala) and **2**(D-Ala) prefer to assume the Λ- and Δ-configurations, respectively, as summarized in Fig. 2.

The iron(III) complex of **3**(L-Ala) without long hydrocarbon chains showed a CD spectral pattern similar to that for **2**(L-Ala). Low energy configurations for the iron(III) complex of **3**(L-Ala) in the gas phase were examined on the basis of molecular mechanics (BIOGRAPH, MM2, and MMP2)7) and molecular dynamics (BIOGRAPH, AMBER, and CHARMM)8) calculations as well as by Monte Carlo9) conformational search on an IRIS-4D/220GTX workstation (Silicon Graphics). The total molecular energy (E_{total}) is expressed as an energy sum of bonded and non-bonded interactions, and an enantiomer complex with a lower energy value is regarded to be more stable relative to the other (see Eq. 5). The bonded interactions consist of bond stretching (E_b), bond angle bending (E_θ), dihedral angle torsion (E_φ), and inversion (E_i) terms, while the non-bonded interactions are composed of van der Waals (E_{vdw}), electrostatic (E_{el}), and hydrogen bond (E_{hb}) terms. The E_{total} values for the low energy conformations of the iron(III) complex of **3**(L-Ala) are 1434.63 and 1275.67 kJ mol^{-1} for Δ- and Λ-configurations, respectively. This comes from a difference in the energy of bond angle bending term (E_θ = 429.11 and 237.10 kJ mol^{-1} for Δ- and Λ-configurations, respectively). This result is consistent with the absolute configuration of Λ clarified by the CD spectroscopy for the iron(III) complex of **2**(L-Ala).

$$E_{\text{total}} = \underbrace{E_b + E_\theta + E_\varphi + E_i}_{\text{bonded}} + \underbrace{E_{vdw} + E_{el} + E_{hb}}_{\text{non-bonded}} \qquad (5)$$

In conclusion, the absolute configuration of the iron(III) complex is controlled by the chirality of the alanine residues introduced into the hexapus cyclophane. We are now investigating interactions between the hexapus cyclophane and synthetic bilayer membranes composed of peptide lipids in aqueous media in order to establish a cell surface model.

References

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