Azaparacyclophanes with Metal-binding Sites. Part 1. Preparation, Characterization, and Molecular Recognition Behaviour of Hexapus Azaparacyclophane and its Iron(III) Complex †

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All the catechol moieties of a hexapus triaza[3.3.3]paracyclophane having three catechol moieties and six hydrocarbon chains (**1**) undergo coordination interactions with the iron(III) ion in a 1:1 molar ratio of **1** to iron(III) above pH 6 in an aqueous medium. Two different iron(III) complexes of **1**, A and B in a 1:1 molar ratio, are in the high spin iron(III) state as characterized by electronic, EPR and Mössbauer spectroscopy: A is a hexacoordinated mononuclear complex with half-protonated catecholato groups, subjected to considerable structural distortion; B is a hexacoordinated mononuclear complex with complete dissociation of all the catechol protons, retaining high structural symmetry. The molecular recognition behaviour of **1** and Fe-complex A toward hydrophobic guest molecules has been investigated in aqueous HEPES and acetate buffers at 30.0 °C and μ 0.10 mol dm^{-3}. The hexapus cyclophane **1** recognizes organic guests through hydrophobic and electrostatic interactions; anionic and non-ionic guests, 7-hydroxy-8-phenylazonaphthalene-1,3-disulfonate (OG) and 2-hydroxy-1-(2-pyridylazo)naphthalene (PAN), respectively, are incorporated into host **1** with binding constants of the order of 10^5 dm^3 mol^{-1}, while a cationic guest, 2-(*p*-dimethylaminostyryl)-1-ethylquinolinium (QR), is not included in the host. The guest-binding behaviour of Fe-complex A toward OG and PAN is much enhanced relative to that of **1** as effected by hydrophobic, electrostatic and coordination interactions under conditions identical to those applied to **1**; the binding constant for OG is nearly pH-independent in a pH range of 3–8, while that for PAN is pH-dependent because the coordination interaction with the host is subject to change by pH.

Biological iron transport is recognized as an essential process for maintaining steady metabolic mechanisms. Siderophores, microbial iron-transporting agents, are excreted by microorganisms for the purpose of solubilizing environmental iron and facilitating its transport into microbial cells. In the case of enteric bacteria, the most effective siderophore is enterobactin which shows an extremely high binding constant (K) toward iron(III) ions; log K = 52.[1]

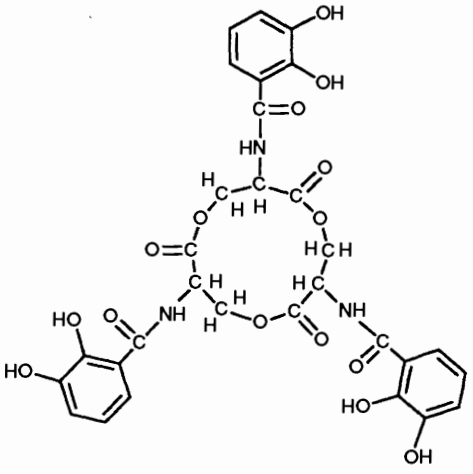
Many studies have been carried out on the binding behaviour of siderophores and synthetic siderophore analogues toward iron(III) ions. In particular, Raymond *et al.* have been actively involved in characterization of enterobactin and its model compounds from the bioinorganic viewpoints.[1–16]

We have prepared octopus azaparacyclophanes, which provide effective hydrophobic sites intramolecularly for binding various hydrophobic guest molecules, exhibiting induced-fit functions originating from the flexible character of their alkyl branches.[17] In relation to the chemistry of 1,5,9-*N*,*N*′,*N*″-tris(2,3-dihydroxybenzoyl)cyclotriazatridecane (CYCAM) which was developed by Raymond *et al.* as an enterobactin model,[2,3] we used in this work a triaza[3.3.3]-paracyclophane derivative having three catechol moieties and six hydrocarbon chains (**1**). The proton-dissociation and iron-coordination behaviour of **1**, as well as its molecular recognition capability toward hydrophobic guest molecules, was investigated in aqueous media. Two different iron(III) complexes of **1** were isolated in a 1:1 molar ratio and characterized by electronic, EPR and Mössbauer spectroscopy. Molecular recognition behaviour of an iron(III) complex of **1**, a hexacoordinated mononuclear complex with half-protonated catecholato groups of **1** (Fe-complex A), toward hydrophobic guests was examined under conditions identical to those applied to **1**. In addition, the guest-binding behaviour of related hosts was also investigated under comparable conditions to demonstrate specific features of the guest-recognition behaviour of Fe-complex A.

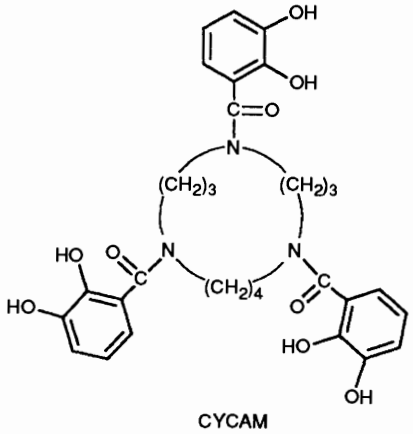
† Preliminary communication, ref. 20.

Experimental

General Analyses and Measurements.—Elemental analyses were performed at the Microanalysis Centre of Kyushu University. A Beckman Φ71 pH meter equipped with a Beckman 39532 combined electrode was used for pH measurements after calibration with a combination of appropriate standard aqueous buffers. Potentiometric titrations were performed with a Kyoto Electronics AT-310 automatic titrator equipped with a C-112 combination electrode. Dynamic light scattering (DLS) measurements were carried out on a Photal (Otsuka Electronics) DLS-700 (He–Ne laser 632.8 nm) spectrometer equipped with a NEC PC-9801 RA2 personal computer in combination with a Photal high speed full correlator unit. IR spectra were taken on a JASCO IR-810 spectrophotometer, while electronic absorption spectra were recorded on a Hitachi 220A, a Hitachi 340 and a Hitachi U-3210 spectrophotometer equipped with a Hitachi SPR-10 temperature controller. EPR spectra were measured with a JEOL JES-FE3XG X-band spectrometer equipped with an Advantest TR-5213 microwave counter and an Echo Electronics EFM-200 NMR field meter, while ^{1}H NMR spectra were taken on a Hitachi R-1500, a Bruker AC-250P and a Bruker AMX-500 spectrometer installed at the Centre of Advanced Instrumental Analysis, Kyushu University. Mössbauer spectroscopy measurements were performed with a constant-acceleration



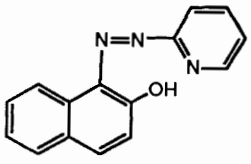
Enterobactin



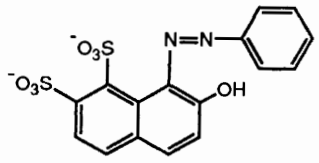
CYCAM

spectrometer of Austin Science Associate, Austin, Texas and data were stored in a 1024-channel pulse height analyser, Type 5200 of Inotech, Inc., Fort Atkinson, Wisconsin.[18] The temperature was monitored with a calibrated copper/constantan thermocouple placed in a variable-temperature cryostat, Type ASAD-4V of Austin Science Associate. A 10 mCi cobalt-57 source diffused into a palladium foil was used for the absorption measurement. The spectra were analysed in terms of Lorentzian curve fitting based on a computer-aided least-squares method at the Computer Centre of Kyushu University. Isomer shifts were evaluated with reference to the signal centroid observed for an iron foil enriched with ^{57}Fe.

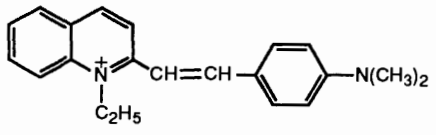
Materials.—The following guest compounds were obtained from commercial sources as guaranteed reagents and used without further purification: 2-hydroxy-1-(2-pyridylazo)naphthalene (PAN; Dojin Chemical Laboratories, Kumamoto, Japan), Orange G (disodium 7-hydroxy-8-phenylazonaphthalene-1,3-disulfonate) [Na_2(OG); Wako Pure Chemical Industries, Osaka, Japan] and Quinaldine Red [2-(*p*-dimethylaminostyryl)-1-ethylquinolinium iodide] [(QR)I; Nacalai Tesque, Kyoto, Japan]. Iron(III) perchlorate and nitrate (Ishizu Seiyaku



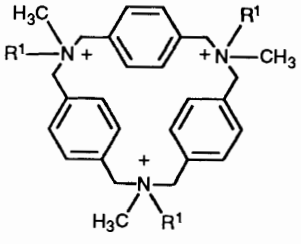
PAN

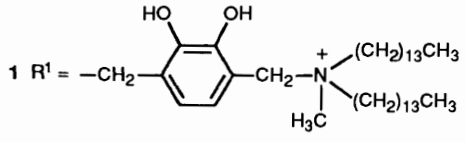


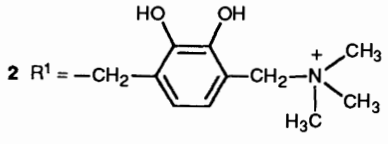
OG

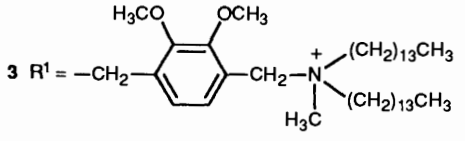


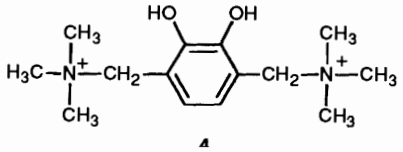
QR



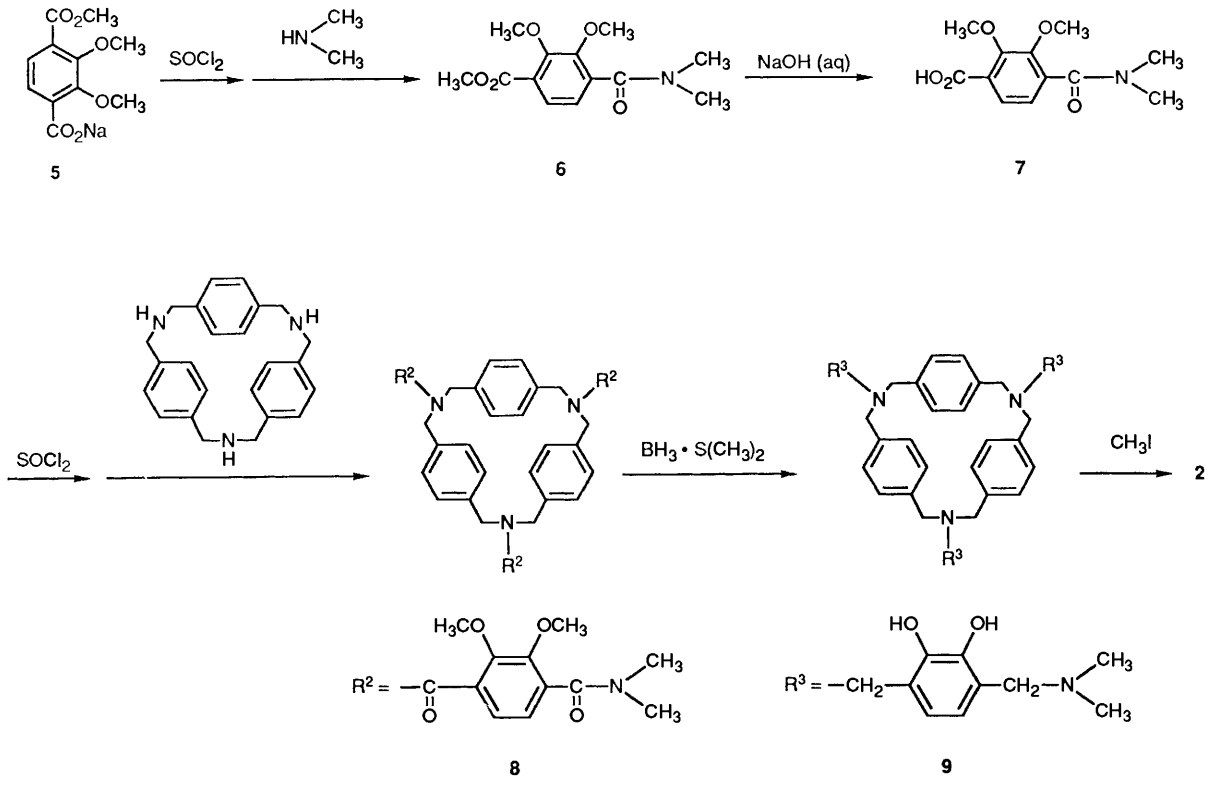




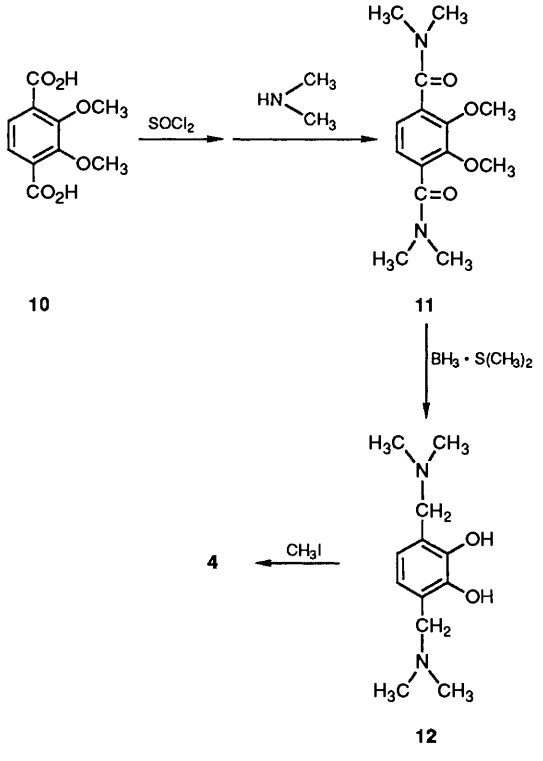




4



Scheme 1



Scheme 2

Co., Osaka, Japan) were dissolved in deionized water and standardized by conventional chelatometric titrations with Chromazurol S (trisodium salt of 3-sulfo-2,6-dichloro-3′,3″-dimethyl-4′-hydroxyfuchsone-5′,5″-dicarboxylic acid; Dojin Chemical Laboratories, Kumamoto, Japan) as an indicator.[19]

A hexapus azaparacyclophane with three catechol segments (**1**) was prepared by a previously reported procedure.[20] Reference compounds **2** and **4** were prepared according to procedures shown in Schemes 1 and 2, and compound **3** was derived from **1** by methylation.

2,3-*Dimethoxy*-4-*methoxycarbonyl*-N,N-*dimethylbenzamide* (**6**). After a mixture of **5**[5] (1.11 g, 4.60 × 10^{-3} mol) and thionyl chloride (30 cm^3, 0.15 mol) was stirred for 24 h at room temperature, excess thionyl chloride was removed under vacuum. A mixture of dimethylamine hydrochloride (750 mg, 9.20 × 10^{-3} mol) and dry triethylamine (1.40 g, 1.39 × 10^{-2} mol) in dry dichloromethane (50 cm^3) was added dropwise over 1 h to the resulting acid chloride dissolved in dry dichloromethane (30 cm^3). The mixture was stirred for 12 h, and washed with aqueous hydrochloric acid (pH 3; 3 × 50 cm^3) and saturated aqueous sodium chloride (2 × 50 cm^3). A viscous brown-red liquid (1.30 g) obtained upon evaporation of the solvent was chromatographed on a column of silica gel (Wako gel C-300) with chloroform as eluent. Evaporation of the solvent under reduced pressure gave a pale yellow viscous oil; yield 1.06 g (86%) (Found: C, 57.85; H, 6.45; N, 5.2. $C_{13}H_{17}NO_5 \cdot 1/6H_2O$ requires C, 57.75; H, 6.45; N, 5.2%); ν_{max}(neat)/cm^{-1} 1735 (ester C=O str) and 1640 (amide C=O str); δ_H(500 MHz; $CDCl_3$) 2.87 and 3.14 [6 H, each s, $N(CH_3)_2$], 3.91 and 3.94 (6 H, each s, OCH_3), 3.93 (3 H, s, CO_2CH_3), 7.01 (1 H, d, phenyl H-6) and 7.54 (1 H, d, phenyl H-5).

4-*Carboxy*-2,3-*dimethoxy*-N,N-*dimethylbenzamide* (**7**). A methanol (50 cm^3) solution containing **6** (784 mg, 2.93 × 10^{-3} mol), sodium hydroxide (235 mg, 5.87 × 10^{-3} mol) and water (2 cm^3) was heated under reflux for 12 h. After addition of water (30 cm^3), methanol was evaporated under reduced pressure. The pH of the resulting solution was adjusted to 2, and the product was extracted with dichloromethane (4 × 50 cm^3). The extract was evaporated to dryness under reduced pressure, and the residue was purified by chromatography on a column of silica gel (Wako gel C-300) with chloroform as eluent. Evaporation of the eluted major fraction under reduced pressure gave a white powder; yield 731 mg (98%) (Found: C, 57.2; H, 6.0; N, 5.55. $C_{12}H_{15}NO_5$ requires C, 56.9; H, 5.95; N, 5.55%); m.p. 103.4–104.2 °C; ν_{max}(KBr disk)/cm^{-1} 1720 (acid

C=O str) and 1610 (amide C=O str); δ_H(500 MHz; $CDCl_3$) 2.89 and 3.15 [6 H, each s, $N(CH_3)_2$], 3.92 and 4.11 (6 H, each s, OCH_3), 7.11 (1 H, d, phenyl 6-H), 7.87 (1 H, d, phenyl 5-H) and 8.64 (1 H, s, CO_2H).

N,N′,N″-*Tris*[2,3-*dimethoxy*-4-(N,N-*dimethylcarbamoyl*)-*benzoyl*]-2,11,20-*triaza*[3.3.3]*paracyclophane* (**8**). A mixture of **7** (337 mg, 1.32 × 10^{-3} mol) and thionyl chloride (20 cm^3, 0.1 mol) was stirred for 24 h at room temperature. Excess thionyl chloride was removed under reduced pressure, and a small amount of dry dichloromethane was added to the residue. The mixture was evaporated under reduced pressure to give the corresponding acid chloride. A dichloromethane solution (20 cm^3) of the acid chloride was added dropwise over 1 h to a solution of 2,11,20-triaza[3.3.3]paracyclophane[21] (119 mg, 3.33 × 10^{-4} mol) and dry triethylamine (150 mg, 1.48 × 10^{-3} mol) in dry dichloromethane (30 cm^3) at room temperature. The resulting mixture was stirred for 12 h at room temperature and evaporated to dryness under reduced pressure. The crude product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluent. Evaporation of the solvent under reduced pressure gave a white powder; yield 316 mg (89%) (Found: C, 63.45; H, 6.35; N, 7.3. $C_{60}H_{66}N_6O_{12}$·$4H_2O$ requires C, 63.5; H, 6.55; N, 7.4%); ν_{max}(KBr disk)/cm^{-1} 1640 (amide C=O str); δ_H(60 MHz; $CDCl_3$) 2.92 and 3.16 [18 H, s, $N(CH_3)_2$], 3.94 and 3.95 (18 H, s, OCH_3), 4.40 and 4.74 (12 H, br s, NCH_2) and 6.68–7.25 (18 H, m, aromatic H).

N,N′,N″-*Tris*[2,3-*dihydroxy*-4-(N,N-*dimethylaminomethyl*)-*benzyl*]-2,11,20-*triaza*[3.3.3]*paracyclophane* (**9**). A dry tetrahydrofuran solution (30 cm^3) of **8** (80 mg, 7.52 × 10^{-5} mol) was deoxygenated by bubbling nitrogen gas through it for 1 h, and borane–dimethyl sulfide complex (2 cm^3, 2.0 × 10^{-2} mol) was added to it. After dimethyl sulfide was removed by distillation, the mixture was heated under reflux for 4 h and evaporated to dryness under reduced pressure. The residue was dissolved in methanol (40 cm^3) saturated with hydrogen chloride and heated under reflux for 1 h. The methanol solution was evaporated to dryness under reduced pressure, and the residue was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluent. Evaporation of the solvent under reduced pressure gave a pale yellow powder; yield 81 mg (96%) (Found: C, 57.45; H, 6.7; N, 7.1. $C_{54}H_{72}Cl_6N_6O_6$·H_2O requires C, 57.3; H, 6.6; N, 7.4%); ν_{max}(KBr disk)/cm^{-1} disappearance of 1640 (amide C=O str); δ_H(60 MHz; CD_3OD) 2.86 [18 H, s, $N(CH_3)_2$], 3.95 [6 H, br s, $CH_2N(CH_3)_2$], 4.35 (18 H, br s, NCH_2) and 7.10 (18 H, br s, aromatic H).

N,N′,N″-*Tris*[2,3-*dihydroxy*-4-(*trimethylammoniomethyl*)-*benzyl*]-2,11,20-*triaza*[3.3.3]*paracyclophane* (**2**). A mixture of **9** (81 mg, 7.27 × 10^{-5} mol), methyl iodide (10 cm^3) and dry *N*,*N*-dimethylformamide (5 cm^3) in a pyrex tube with a magnetic stirring bar inside was deoxygenated by a freeze-pump-and-thaw technique, sealed *in vacuo*, and stirred for 7 days at 30 °C. The mixture was evaporated to dryness under reduced pressure, and the residue was applied on a column of ion-exchange resin (Amberlite IRA-401, Cl-form) with methanol as eluent. The product fraction was evaporated to dryness under reduced pressure and purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluent. Evaporation of the solvent under reduced pressure gave a pale yellow powder; yield 67 mg (78%) (Found: C, 59.85; H, 6.95; N, 6.65. $C_{60}H_{84}Cl_6N_6O_6$·$1/2H_2O$ requires C, 59.7; H, 7.1; N, 6.95%); δ_H(500 MHz; CD_3OD) 3.13 [27 H, s, $N^+(CH_3)_3$], 3.58–3.98 [9 H, m, $(CH_2)_3N^+CH_3$], 4.41 [6 H, br s, $CH_2N^+(CH_3)_3$], 4.72 (18 H, br s, N^+CH_2) and 7.10 (18 H, br s, aromatic H).

N,N′,N″-*Tris*[2,3-*dimethoxy*-4-(*ditetradecylmethylammoniomethyl*)*benzyl*]-2,11,20-*triaza*[3.3.3]*paracyclophane* (**3**). A mixture of **1** (178 mg, 7.77 × 10^{-5} mol), dimethyl sulfate (61 mg, 4.66 × 10^{-4} mol) and potassium carbonate (64 mg, 4.66 × 10^{-4} mol) in acetone (50 cm^3) was heated under reflux for 48 h under argon atmosphere. The insoluble material was separated by filtration from the hot reaction mixture, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluent, and applied on a column of ion-exchange resin (Amberlite IRA-401, Cl-form) with methanol as eluent. Evaporation of the solvent under reduced pressure gave a pale yellow powder; yield 63 mg (34%) (Found: C, 71.0; H, 10.75; N, 3.7. $C_{144}H_{252}Cl_6N_6O_6$·$3H_2O$ requires C, 71.15; H, 10.7; N, 3.45%); ν_{max}(KBr disk)/cm^{-1} 1250 (C–O–C asym str) and 1020 (C–O–C sym str); δ_H(60 MHz; $CDCl_3$) 3.95 (18 H, s, OCH_3).

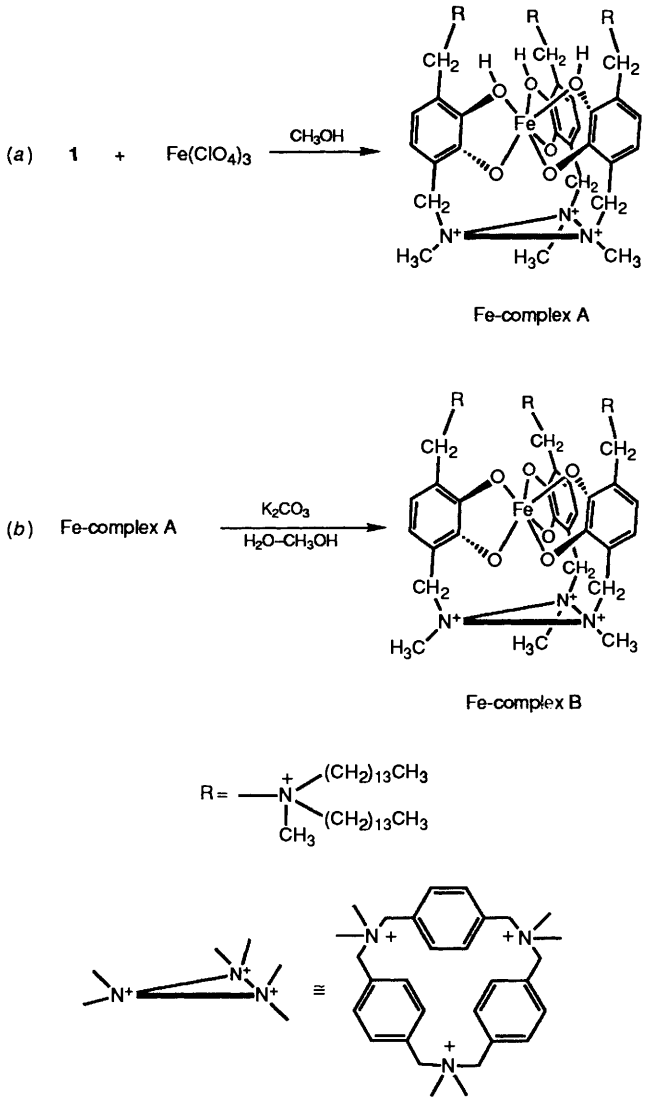
1,4-*Bis*(N,N-*dimethylcarbamoyl*)-2,3-*dimethoxybenzene* (**11**). This compound was prepared from 2,3-dimethoxyterephthalic acid (**10**)[5] (600 mg, 2.65 × 10^{-3} mol), thionyl chloride (30 cm^3) and dimethylamine hydrochloride (432 mg, 5.30 × 10^{-3} mol) in the presence of dry triethylamine (1.2 g, 1.2 × 10^{-2} mol) after the method employed for the preparation of **6**, and isolated as a white powder; yield 530 mg (71%) (Found: C, 59.75; H, 7.25; N, 9.95. $C_{14}H_{20}N_2O_4$ requires C, 60.0; H, 7.2; N, 10.0%); ν_{max}(KBr disk)/cm^{-1} 1640 (amide C=O str); δ_H(60 MHz; $CDCl_3$) 2.87 and 3.12 [12 H, s, $N(CH_3)_2$], 3.88 (6 H, s, OCH_3) and 6.99 (2 H, s, aromatic H).

1,4-*Bis*(N,N-*dimethylaminomethyl*)-2,3-*dihydroxybenzene* (**12**). This compound was prepared by reaction of **11** (200 mg, 7.13 × 10^{-4} mol) with borane–dimethyl sulfide complex (2 cm^3, 2.0 × 10^{-2} mol) after the method employed for the preparation of **9**, and isolated as a white powder; yield 90 mg (43%); ν_{max}(KBr disk)/cm^{-1} disappearance of 1640 (amide C=O str); δ_H(60 MHz; $CDCl_3$) 2.90 [12 H, s, $N(CH_3)_2$], 4.39 (4 H, s, CH_2) and 7.08 (2 H, s, aromatic H).

1,4-*Bis*(*trimethylammoniomethyl*)-2,3-*dihydroxybenzene* (**4**). This compound was prepared by reaction of **12** (90 mg, 3.0 × 10^{-4} mol) with methyl iodide (5 cm^3) in dry *N*,*N*-dimethylformamide (1 cm^3) at 30 °C over 4 days after the method employed for the preparation of **2**, and isolated as a hygroscopic white powder; yield 83 mg (84%) (Found: C, 48.45; H, 8.0; N, 7.95. $C_{14}H_{26}Cl_2N_2O_2$·$5/4H_2O$ requires C, 48.35; H, 8.25; N, 8.05%); ν_{max}(Nujol mull)/cm^{-1} 3400 (indication of H_2O); δ_H(60 MHz; CD_3OD/DCl) 3.17 [18 H, s, $N^+(CH_3)_3$], 4.61 (4 H, s, CH_2) and 7.12 (2 H, s, aromatic H).

Iron(III) *Complexes of* **1**.—Two different iron complexes of **1** were prepared in accordance with reactions shown in Scheme 3. Fe-complex A is a hexacoordinated monomeric iron(III) complex with half-protonated catecholato groups, and Fe-complex B is another hexacoordinated monomeric iron(III) complex obtained by complete dissociation of all the catechol protons. The former complex is soluble in water, while the latter is hardly soluble in water.

(i) *Fe-Complex* A. Solutions of both **1** (2.00 g, 8.73 × 10^{-4} mol) and iron(III) perchlorate (309 mg, 8.73 × 10^{-4} mol) in methanol (200 cm^3 each) were added dropwise simultaneously over 8 h to methanol (1600 cm^3) at an identical rate with vigorous stirring at room temperature, and the mixture was stirred for an additional 72 h and evaporated to dryness under reduced pressure. The residue was applied on a column of ion-exchange resin (Amberlite IRA-401, Cl-form) with methanol as eluent, and the eluted fraction was evaporated to dryness under reduced pressure. The residue was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. Evaporation of the solvent under reduced pressure gave a blue-purple powder; yield 1.59 g (78%) (Found: C, 70.9; H, 9.9; N, 3.75; Fe, 2.25. $C_{138}H_{237}Cl_6FeN_6O_6$ requires C, 70.75; H, 10.2; N, 3.6; Fe, 2.4%); λ_{max}($CHCl_3$)/nm 540. The



Scheme 3

counter ions were replaced with bromide ions by ion-exchange chromatography (Amberlite IRA-401, Br-form; methanol as eluent) (Found: C, 63.5; H, 9.25; N, 3.3. $C_{138}H_{237}Br_6FeN_6O_6$ requires C, 63.6; H, 9.15; N, 3.25%).

(ii) *Fe-Complex* B. A mixture of potassium carbonate (105 mg, 7.59×10^{-4} mol), water (2 cm^3) and methanol (50 cm^3) was added dropwise over 1 h to Fe-complex A (600 mg, 2.55×10^{-4} mol) dissolved in methanol (1000 cm^3) under nitrogen atmosphere. After being stirred for 12 h at room temperature, the reaction mixture was evaporated to half volume under reduced pressure. The resulting precipitates were recovered by filtration and dissolved in chloroform. After undissolved material was removed by filtration, the filtrate was evaporated to dryness. The residue was reprecipitated from dichloromethane upon addition of methanol to afford a reddish-brown powder; yield 370 mg (64%) (Found: C, 74.0; H, 10.4; N, 3.55; Fe, 2.35. $C_{138}H_{234}Cl_3FeN_6O_6$ requires C, 74.15; H, 10.55; N, 3.75; Fe, 2.5%); $\lambda_{max}(CHCl_3)$/nm 510.

Iron(III) *Complex of* **2**.—This complex was prepared from **2** (42 mg, 3.5×10^{-5} mol) and iron(III) perchlorate (18 mg, 3.5×10^{-5} mol) after the method employed for the preparation of Fe-complex A, and isolated as a dark blue-purple powder; yield 20 mg (46%) (Found: C, 56.8; H, 6.2; N, 6.35. $C_{60}H_{81}Cl_6FeN_6O_6 \cdot H_2O$ requires C, 56.8; H, 6.6; N, 6.6%).

Potentiometric Measurements.—Measurements were carried out at 25.0 ± 0.1 °C in a jacketed titration cell (Model MTA-118-5 of Kyoto Electronics), placed on a magnetic stirrer and fitted with inert-gas inlet and outlet tubes, a microburette delivery tube and an electrode. All the titrating solutions, whose ionic strength (μ) was maintained at 0.1 mol dm^{-3} with potassium nitrate, were kept under the carbon dioxide-free argon atmosphere throughout the titrations. Calibration of the automatic titrator was performed by the use of acetic acid buffer as well as standard aqueous hydrochloric acid and potassium hydroxide to measure hydrogen ion concentration directly. To ensure equilibrium attainment, 0.1 mol dm^{-3} aqueous potassium hydroxide was added at the following rates; 2–2.5 mm^3 per hour for **1**, and the same amount for each 10 min for **2** and **4**; approximately 2 days for one potentiometric titration of **1**. Potentiometric data were analysed on the basis of a computer-aided nonlinear least-squares method in a manner as described in the literature.[2,6]

Evaluation of Guest-binding Constants.—The binding behaviour of hexapus cyclophane **1** and related compounds toward various hydrophobic organic guests was examined by electronic absorption spectroscopy according to a method described previously.[22,23] In general, absorbances of the guest molecules were measured in an appropriate aqueous buffer by changing concentrations of the host molecules; absorbances at 480 and 475 nm for OG and PAN, respectively. Binding constants for formation of inclusion complexes at a 1:1 molar ratio of host to guest [K, refer to eqn. (1); H and G stand for a host and a guest, respectively] were calculated on the basis of spectroscopic data obtained at various concentrations of the host molecules.

$$H + G \overset{K}{\rightleftharpoons} H{\cdot}G \quad (1)$$

$$H_6L \overset{K_a(H_6L)}{\rightleftharpoons} H_5L^- + H^+ \quad (2)$$

$$H_5L^- \overset{K_a(H_5L)}{\rightleftharpoons} H_4L^{2-} + H^+ \quad (3)$$

$$H_4L^{2-} \overset{K_a(H_4L)}{\rightleftharpoons} H_3L^{3-} + H^+ \quad (4)$$

Results and Discussion

Potentiometric Titration of Hexapus Cyclophane **1**.—The potentiometric titration of **1**, which has six dissociable protons, was carried out at 25.0 °C and μ 0.10 mol dm^{-3} with KNO_3 by adding 0.1 mol dm^{-3} aqueous KOH under argon atmosphere as shown in Fig. 1A.[20] The acid dissociation constants were evaluated from the titration curve for the first three protons, as defined by eqns. (2)–(4): $pK_a(H_6L)$, 5.18; $pK_a(H_5L)$, 5.96; $pK_a(H_4L)$, 6.54. The corresponding values for an enterobactin model, CYCAM, were reported to be 7.86, 8.65 and 9.26, respectively.[2] The present pK_a values are somewhat smaller than those for CYCAM, probably because of overlapped effects: an electrostatic effect exerted by positive charges placed in the proximity of the catechol moieties, and the formation of strong hydrogen bonds among the catechol moieties as caused by hydrophobic association of the six hydrocarbon chains.

In order to prove this, potentiometric titrations of related

compounds **2** and **4** were carried out under identical conditions. The titration curves for **2** and **4** are shown in Figs. 2 and 3, respectively. The acid dissociation constants evaluated from the titration curves are summarized in Table 1 along with those for CYCAM and 2,3-dihydroxy-*N*,*N*-dimethylbenzamide (DMB). An average of the three constants [$pK_{a(av)}$] for CYCAM is 8.59 and close to the first acid association constant, pK_{a1} = 8.42, for DMB.[1] Similarly, the $pK_{a(av)}$ value for **2** is 7.22 and close to the pK_{a1} value for **4** (a structural fragment of **2**, having a single catechol moiety), 7.49. On the other hand, the $pK_{a(av)}$ value for **1** is 5.89 and much smaller than the corresponding values for **2** and **4**. This must arise from the overlapped effects as mentioned above. In addition, the pK_a values for **2** and **4** are smaller than those for CYCAM and DMB, respectively. These differences arise from an electronegativity effect provided by positive charges placed in proximity to the catechol moieties for **2** and **4**.

The coordination interaction of **1** with iron(III) ions was investigated by potentiometric titration of a solution containing equimolar amounts of **1** and the iron(III) ion under identical conditions [Fig. 1B]. All the catechol moieties are coordinated to the iron(III) ion in a 1:1 molar ratio of **1** to iron(III) above

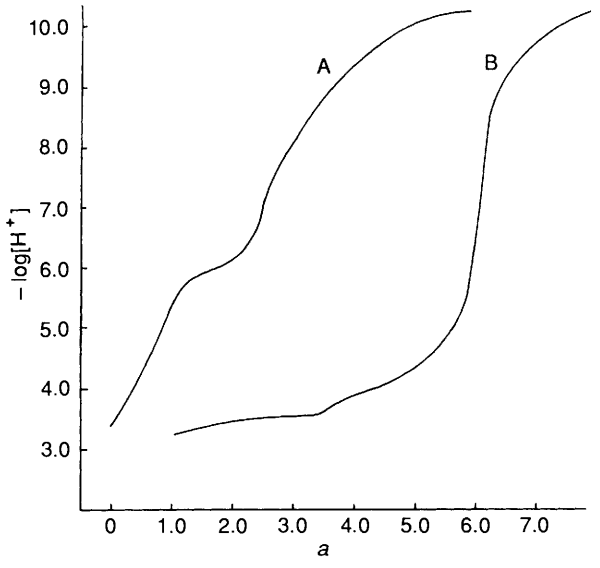


Fig. 1 Potentiometric titration curves of hexapus cyclophane **1** in aqueous solution (μ 0.10 mol dm^{-3} with KNO_3) by adding 0.10 mol dm^{-3} aqueous KOH at 25.0 ± 0.1 °C under argon atmosphere: *a*, moles of base added per mole of ligand; A, **1** alone (1.00 × 10^{-4} mol dm^{-3}); B, **1** + Fe^{3+} (1:1 molar ratio; 1.00 × 10^{-4} mol dm^{-3} each)

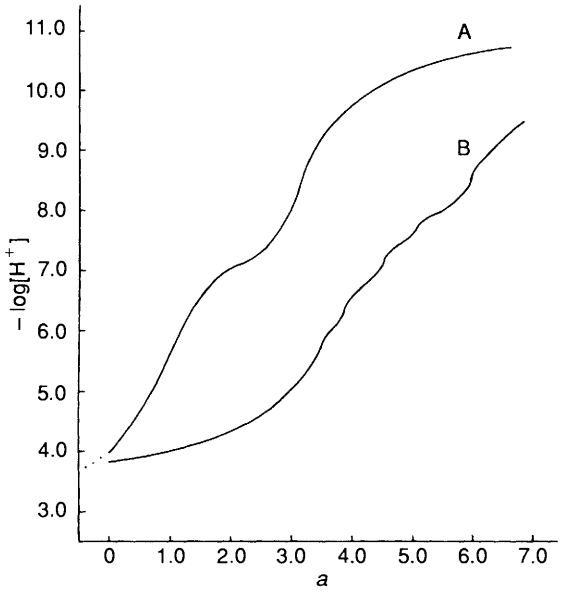


Fig. 2 Potentiometric titration curves of **2** in aqueous solution (μ 0.10 mol dm^{-3} with KNO_3) by adding 0.10 mol dm^{-3} aqueous KOH at 25.0 ± 0.1 °C under argon atmosphere: *a*, moles of base added per mole of ligand; A, **2** (2.00 × 10^{-4} mol dm^{-3}); B, **2** + Fe^{3+} (1:1 molar ratio; 1.00 × 10^{-4} mol dm^{-3} each)

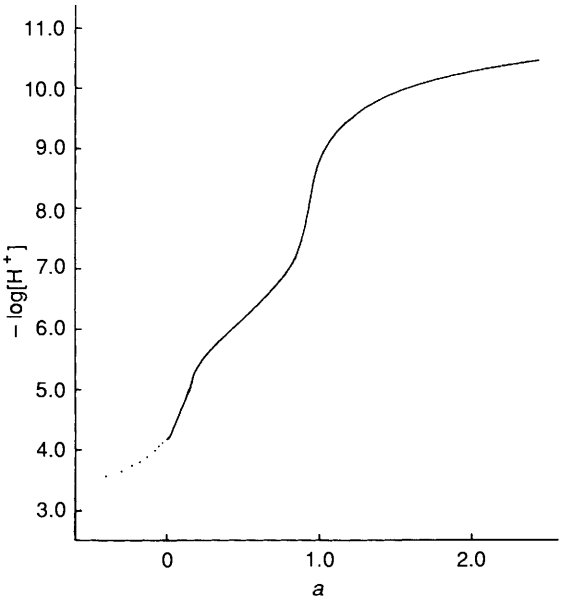
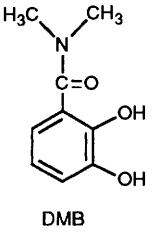


Fig. 3 Potentiometric titration curve of **4** (5.00 × 10^{-4} mol dm^{-3}) in aqueous solution (μ 0.10 mol dm^{-3} with KNO_3) by adding 0.10 mol dm^{-3} aqueous KOH at 25.0 ± 0.1 °C under argon atmosphere; *a*, moles of base added per mole of ligand



DMB

Table 1 Acid dissociation constants (pK_a) for hexapus cyclophane and related compounds at 25.0 ± 0.1 °C and μ 0.10 mol dm^{-3} (KCl)[a]

Compound	$pK_a(H_6L)$[b]	$pK_a(H_5L)$[b]	$pK_a(H_4L)$[b]	$pK_{a(av)}$[c]	pK_{a1}	pK_{a2}
1	5.18	5.96	6.54	5.89		
2	6.79	7.25	7.61	7.22		
4					7.49	11.02
CYCAM[d]	7.86	8.65	9.26	8.59		
DMB[d]					8.42	12.1

[a] Concentrations in mol dm^{-3}: **1**, 1.00 × 10^{-4}; **2**, 2.00 × 10^{-4}; **4**, 5.00 × 10^{-4}. [b] Refer to eqns. (2)–(4). [c] $pK_{a(av)} = 1/3\{pK_a(H_6L) + pK_a(H_5L) + pK_a(H_4L)\}$. [d] Cited from ref. 2.

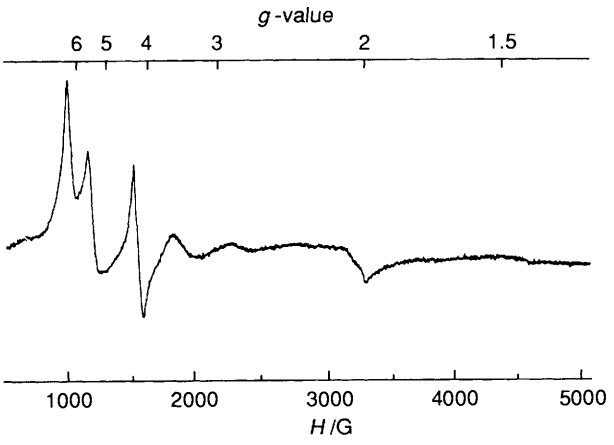


Fig. 4 EPR spectrum of Fe-complex A (2.0×10^{-3} mol dm^{-3}) in chloroform at 123 K

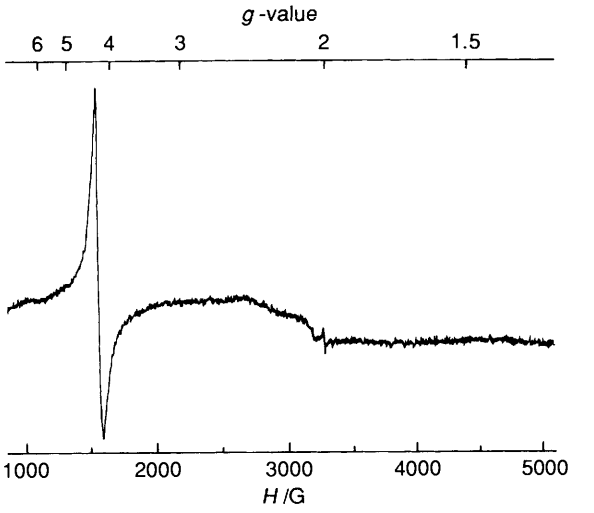


Fig. 5 EPR spectrum of Fe-complex B (2.0×10^{-3} mol dm^{-3}) in chloroform at 123 K

pH 6 upon complete dissociation of all the catechol protons. Reference compound **2** also undergoes a coordination interaction with iron(III) ions as is evident from the titration curve shown in Fig. 2B. However, its affinity for the iron(III) ion is much weaker than that of **1**.

Structures of Iron(III) *Complexes of* **1**.—Two different iron complexes of **1** were prepared as shown in Scheme 3. Fe-complex A (blue-purple) was obtained by the reaction of **1** with iron(III) perchlorate in methanol. Fe-complex A was transformed into Fe-complex B (red-purple) by treatment with an alkaline solution. The structures shown in Scheme 3 were confirmed by electronic, EPR and Mössbauer spectroscopy as well as by elemental analyses.

The iron(III) complexes of both CYCAM and MECAM [1,3,5-*N*,*N*′,*N*″-tris(2,3-dihydroxybenzoyl)triaminomethylbenzene], prepared by Raymond *et al.*, were found to show blue-purple and red colours at pH 4–6 and above pH 7 in water, respectively.[2] The difference in colour reflects the extent of protonation of the catechol moieties in the iron(III) complexes; the former is a hexacoordinated mononuclear iron(III) complex with half-protonated catecholato groups, while the latter is a hexacoordinated mononuclear iron(III) complex with complete dissociation of all the catechol protons. A similar colour change was observed for the iron(III) complex of **1**; absorption maxima for Fe-complexes A and B in chloroform are at 540 and 510 nm, respectively.

EPR spectra of Fe-complexes A and B are shown in Figs. 4 and 5, respectively. The EPR spectrum shown in Fig. 5 is a typical one for the high-spin iron(III) complex with high structural symmetry, and similar to that observed for the enterobactin complex.[8] A Mössbauer spectrum was observed for Fe-complex B even though its intensity was extremely weak in the solid state. Only one absorption (isomer shift $\delta = 0.28$ mm s^{-1}) was observed at 78 K, and the same absorption ($\delta = 0.34$ mm s^{-1}) split into six peaks at 4.2 K. Such Mössbauer behaviour and parameters indicate that Fe-complex **B** is a mononuclear and high-spin iron(III) complex with high structural symmetry. On the other hand, the EPR spectrum given in Fig. 4 shows a complicated splitting pattern in a *g*-range of 4–6. This clearly indicates that Fe-complex A is in the high-spin iron(III) state with structural distortion. A Mössbauer spectrum for Fe-complex A showed doublet peaks of very weak intensity (*ca.* 0.5%) at 78 K ($\delta = 0.62$ mm s^{-1}, quadrupole splitting $\Delta E_Q = 0.998$ mm s^{-1}). The spectral pattern indicates again that Fe-complex A is in the high-spin state with structural distortion. The EPR pattern presumably indicates that there are some stereoisomers for the complex; positional isomers with respect to protonation sites at the catechol moieties. Fe-complexes A and B were separated by HPLC on a column of TSK gel ODS-120T with methanol–chloroform (4:6 v/v) as eluent, but the predicted isomers of Fe-complex A were not separated by the identical HPLC technique.

In summary, both of the iron(III) complexes are in the high-spin state: Fe-complex B, which is a hexacoordinated mononuclear complex with complete dissociation of all the catechol protons, retains high structural symmetry as evidenced by EPR and Mössbauer spectroscopy; Fe-complex A, a hexacoordinated mononuclear complex with half-protonated catecholato groups, is subjected to considerable structural distortion.

Aggregation Behaviour of Hexapus Cyclophane and Fe-Complex A.—Prior to studies on host–guest interactions, we clarified the aggregation behaviour of the hexapus cyclophane and Fe-complex A in aqueous media by means of dynamic light scattering (DLS) measurements. Since cyclophane **1** is quite unstable and readily oxidized to give precipitates under aerobic conditions, we used cyclophane **3** with protected hydroxy groups in place of **1** for DLS measurements. In a concentration range of 1.0×10^{-5} to 1.3×10^{-4} mol dm^{-3} in water at μ 0.10 mol dm^{-3} (KCl), steady and significant light scattering was not observed. This result suggests that hexapus cyclophane **3** does not undergo aggregation as the present DLS instrument is capable of detecting aggregates of hydrodynamic diameters (d_{hy}) as low as 3 nm. At 1.4×10^{-4} mol dm^{-3}, however, the d_{hy} value was evaluated to be 193 nm on the basis of steady light scattering. As a result, the critical aggregate concentration (CAC) value of hexapus cyclophane **1** was estimated to be 1.4×10^{-4} mol dm^{-3}.

As regards Fe-complex A, the DLS measurements were carried out in a concentration range of 5.0×10^{-6} to 1.4×10^{-4} mol dm^{-3} at μ 0.10 mol dm^{-3} (KCl) in water; d_{hy}, 308 nm at 1.3×10^{-4} mol dm^{-3} (CAC).

Molecular Recognition Behaviour of Hexapus Cyclophane **1** *and Its Iron Complex.*—In the light of the above aggregation behaviour, molecular recognition measurements were carried out in the host concentration ranges below their CACs; 2.50×10^{-6}–1.50×10^{-5} and 4.00×10^{-6}–2.00×10^{-5} mol dm^{-3} for **1** and Fe-complex A, respectively. Molecular recognition behaviour of cyclophane **1**, without metal ions, toward hydrophobic guests was investigated in an aqueous 2-[4-(2-hydroxyethyl)piperazinyl]ethanesulfonate (HEPES) buffer (0.01 mol dm^{-3}, pH 8.0, μ 0.10 mol dm^{-3} with KCl) by electronic spectroscopy.[20] Anionic and non-ionic guests (OG and PAN,

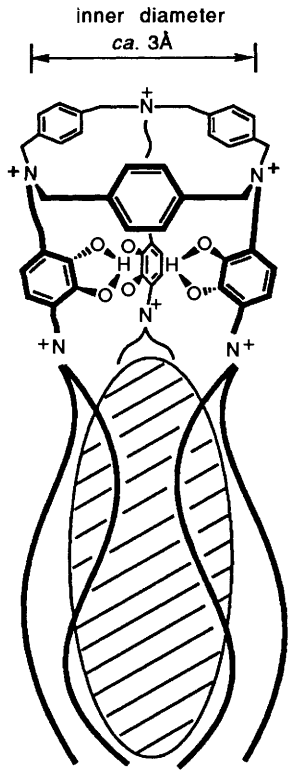


Fig. 6 Schematic representation for the host–guest complex of hexapus cyclophane **1** with a hydrophobic guest molecule

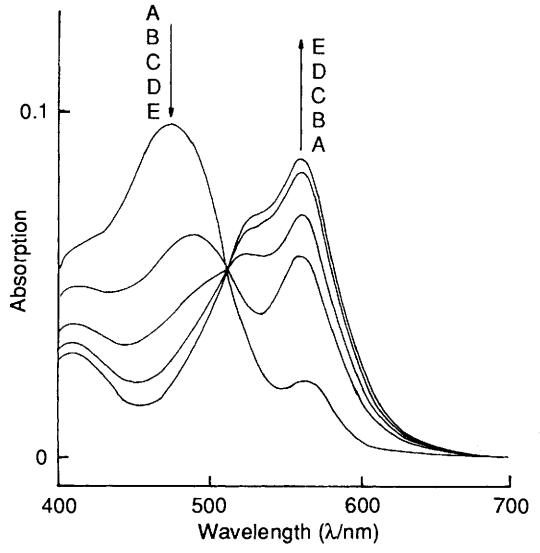


Fig. 7 Electronic spectral changes of PAN (1.0×10^{-5} mol dm^{-3}) upon addition of Fe-complex A in HEPES buffer [0.01 mol dm^{-3}, pH 8.0, μ 0.10 mol dm^{-3} (KCl)] at 30.0 ± 0.1 °C. Concentrations of Fe-complex A: A, 0; B, 4.0×10^{-6}; C, 6.0×10^{-6}; D, 8.0×10^{-6}; E, 1.0×10^{-5} mol dm^{-3}.

respectively), are incorporated into the host with binding constants of 6.7×10^5 and 7.5×10^5 dm^3 mol^{-1}, respectively. However, a cationic guest, QR, is not included in the host. Consequently, the hexapus cyclophane recognizes hydrophobic guests in aqueous media through hydrophobic and electrostatic interactions in a manner as observed with octopus cyclophanes.[24] The CPK model study on **1** suggests that the cavity provided by the [3.3.3]paracyclophane skeleton is narrow (a triangular shape; diameter of the inner free space, *ca.* 3 Å), so that an organic guest molecule is incorporated into the hydrophobic space provided by aggregation of the six hydrocarbon chains as shown in Fig. 6.

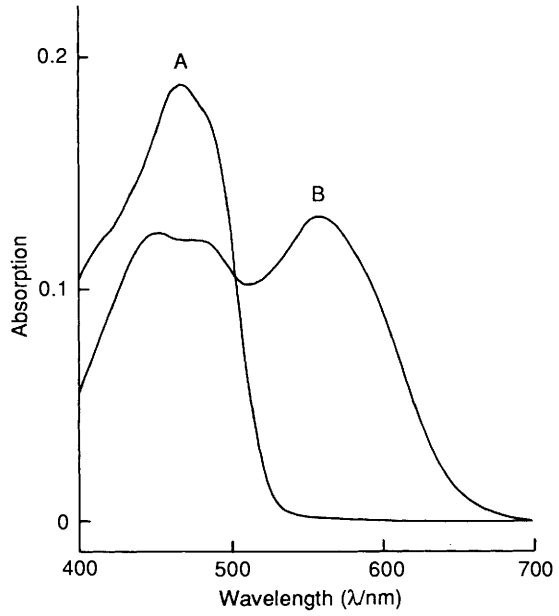


Fig. 8 Electronic spectra of PAN (1.0×10^{-5} mol dm^{-3}) without (A) and with added $Fe(ClO_4)_3$ (1.0×10^{-5} mol dm^{-3}) (B) in chloroform at 30.0 ± 0.1 °C

The guest-binding behaviour of Fe-complex A was examined in water under conditions identical with those applied to **1**.[20] When the catechol segments of the cyclophane are coordinated to the iron(III) ion, binding constants for hydrophobic guests become much larger; 1.7×10^6 and 3.8×10^7 for OG and PAN, respectively. Spectral changes observed upon addition of Fe-complex A to an aqueous solution of PAN indicate formation of the 1:1 complex of PAN and iron(III) as is obvious from the data shown in Fig. 7. The spectral change observed for a combination of iron(III) perchlorate and PAN in chloroform (Fig. 8) is similar to those shown in Fig. 7. Since the absorption band at 557 nm in chloroform is caused by coordination of the iron(III) ion to PAN, the iron(III) ion bound to **1** must undergo a coordination interaction with PAN in the host cage which provides a hydrophobic and apolar microenvironment for the guest. Fe-complex A is transformed into Fe-complex B upon complete dissociation of the catechol protons in aqueous media above pH 6 after a reasonably prolonged period of time as clarified by potentiometric titration (*vide ante*). It must be pointed out here, however, that Fe-complex A in aqueous media does not undergo structural change under present experimental conditions; relatively short reaction periods preclude the possibility of such a transformation. In addition, Fe-complex B is insoluble in aqueous media and no precipitation was detected during the measurements.

In order to make the inclusion behaviour clear, the binding affinity of Fe-complex A for hydrophobic guests in an aqueous buffer was compared with those of reference compounds such as **3** and the iron(III) complex of **2**. The following findings are based on the binding constants evaluated at various pHs for combinations of Fe-complex A and guests (Table 2). (i) The binding constant (K) for the anionic guest molecule (OG) does not depend on the pH in the range 3–8. On the other hand, the binding constant for the non-ionic guest (PAN) depends markedly on the pH. (ii) The K value for PAN at pH 8.0 is larger than that at pH 3.0. Because the pyridyl nitrogen of PAN is protonated in acidic aqueous media (pK_a = 2.9),[25] the guest does not seem to interact effectively with the iron(III) ion of Fe-complex A. The decrease in K in the low pH range must come from an unfavourable electrostatic interaction as well as a weakened coordination interaction between the host and PAN. In fact, the drastic spectral changes shown in Fig. 7, which

Table 2 Binding constants (K) for inclusion of hydrophobic guests in Fe-complex A at 30.0 ± 0.1 °C[a]

	K/dm^3 mol^{-1}			
Guest molecule	pH 3.0[b]	pH 4.5[b]	pH 6.0[b]	pH 8.0[c]
PAN	2.0×10^5	2.4×10^6	5.4×10^6	3.8×10^7
OG	1.6×10^6	1.6×10^6	1.6×10^6	1.7×10^6

[a] Concentrations in mol dm^{-3}: PAN and OG, 1.0×10^{-5}; Fe-complex A, 4.0×10^{-6}–1.4×10^{-5} for PAN and 4.0×10^{-6}–2.0×10^{-5} for OG. [b] In acetate buffer [0.01 mol dm^{-3}, μ 0.10 mol dm^{-3} (KCl)]. [c] In HEPES buffer [0.01 mol dm^{-3}, μ 0.10 mol dm^{-3} (KCl)].

Table 3 Binding constants (K) for inclusion of hydrophobic guests in host **3** at 30.0 ± 0.1 °C[a]

	K/dm^3 mol^{-1}			
Guest molecule	pH 3.0[b]	pH 4.5[b]	pH 6.0[b]	pH 8.0[c]
PAN	3.0×10^5	6.1×10^5	6.3×10^5	7.4×10^5
OG	6.2×10^5	6.1×10^5	6.2×10^5	6.2×10^5

[a] Concentrations in mol dm^{-3}: PAN and OG, 1.0×10^{-5}; **3**, 4.0×10^{-6}–2.6×10^{-5}. [b] In acetate buffer [0.01 mol dm^{-3}, μ 0.10 mol dm^{-3} (KCl)]. [c] In HEPES buffer [0.01 mol dm^{-3}, μ 0.10 mol dm^{-3} (KCl)].

Table 4 Binding constants (K) for inclusion of hydrophobic guests in the iron(III) complex of **2** at 30.0 ± 0.1 °C[a]

	K/dm^3 mol^{-1}		
Guest molecule	pH 4.5[b]	pH 6.0[b]	pH 8.0[c]
PAN	1.0×10^4	1.7×10^4	4.1×10^4
OG	4.0×10^3	4.0×10^3	3.9×10^3

[a] Concentrations in mol dm^{-3}: PAN and OG, 1.0×10^{-5}; the iron(III) complex of **2**, 4.0×10^{-6}–5.0×10^{-5} for PAN and 1.0×10^{-5}–7.0×10^{-4} for OG. [b] In acetate buffer [0.01 mol dm^{-3}, μ 0.10 mol dm^{-3} (KCl)]. [c] In HEPES buffer [0.01 mol dm^{-3}, μ 0.10 mol dm^{-3} (KCl)].

indicate the coordination interaction of PAN with the iron(III) bound to **1**, were not observed at pH 3.0.

For the purpose of estimating the effect of coordination on the inclusion behaviour, the binding affinity of **3** for the hydrophobic guest molecules was examined under identical conditions (Table 3). The results indicate the following aspects. (i) The binding constants for Fe-complex A with OG are about three times greater than those for **3** with OG (without metal ions) in the pH range 3–8. (ii) The K value for Fe-complex A with PAN at pH 8.0 is 50 times larger than that for **3** with PAN. As the pH is lowered, the difference between these binding constants becomes smaller and finally both binding constants are comparable to each other at pH 3. It is now evident that the coordination interaction contributes much to the enhancement of incorporation of a hydrophobic guest molecule into the hexapus cyclophane.

Finally, the hydrophobic effect provided by the six hydrocarbon chains of **1** on the guest-binding behaviour was examined by evaluating binding constants for the iron complex of **2** with hydrophobic guests under identical conditions. The following is evident from the results shown in Table 4. (i) The binding constants for the iron complex of **2** are considerably smaller than those for Fe-complex A. Therefore, the hydrophobic effect provided by the six hydrocarbon chains is important for the efficient inclusion of the guests. (ii) The K value for the iron complex of **2** with OG does not depend on the pH in the range 4.5–8. On the other hand, the K value with PAN does depend on the pH. This may be explained on the basis of the pH-dependent coordination interaction between the host and the guest in a manner as described for the inclusion behaviour of Fe-complex A.

In conclusion, the present metal-bound hexapus cyclophane exhibits marked inclusion capability toward organic guests, as effected by hydrophobic, electrostatic, and coordination interactions. The hexapus cyclophane with three catechol moieties is structurally related to enterobactin and expected to behave as an effective iron(III)-ion carrier. We are now investigating the interaction between the metal-bound hexapus cyclophane and synthetic bilayer membranes[26,27] composed of peptide lipids in aqueous media to mimic metabolic functions of cell surfaces.

Acknowledgements

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