DALTON
FULL PAPER

New hexadentate ligands composed of 1-hydroxy-2(1*H*)-pyrimidinone, α,ω-diamine and 1,1,1-tris(carboxyethoxymethyl)-ethane or tri(carboxybutyl)isocyanurate. Synthesis and characterization of their iron(III) complexes

Akira Katoh,* Yoko Hida, Jun Kamitani and Junko Ohkanda

Department of Industrial Chemistry, Faculty of Engineering, Seikei University, Musashino, Tokyo 180-8633, Japan

Received 14th September 1998, Accepted 17th September 1998

The successive coupling of 1-benzyloxy-2(1*H*)-pyrimidinone with α,ω-diamines, 1,1,1-tris(carboxyethoxymethyl)-ethane or tri(carboxybutyl)isocyanurate, and final removal of the benzyl protecting group afforded new hexadentate ligands (TEPOH*n* or TCPOH). The UV/VIS spectroscopic analysis in aqueous solution indicated 1 : 1 stoichiometric complexation of the hexadentate ligand with iron(III). The relative stability constants of iron(III) complexes of the hexadentate ligands were estimated to be log *K* 24.4–26.3 by the competitive reaction with EDTA, suggesting that the stability was affected by the methylene chain length. The standard redox potential $E_{1/2}$ of Fe(TEPO2) was measured to be −493 mV at apparent pH 8.0 in 50% aqueous DMF solution. This value was approximately 300 mV higher than that of the iron(III) complex of natural desferrioxamine B (DFB). Further, all synthetic hexadentate ligands effectively removed 3–6 times as much iron(III) from human transferrin as DFB even though the ratio of the synthetic ligand to transferrin was one-fifth that of DFB.

Introduction

A sufficient supply of iron in the human and animal diet is an essential requisite for tissue growth.[1–3] Excess intake of iron induces the iron overload that occurs in widespread genetic diseases like β-thalassemia. Since an excess of iron in the body cannot be entirely removed by normal pathways, iron accumulates at internal organs such as intestines and liver, resulting in organ malfunction and early death. There is a serious need, therefore, to develop efficient and practical iron-chelating agents for treatment of iron overload.[1,3] Microorganisms excrete low-molecular-weight ligands specific for iron(III) ion termed "siderophores" for sequestering iron from the environment and transporting it into a cell through the membrane.

A large number of different siderophores, which have been isolated and identified, fall primarily into two general structural classes: catecholate and hydroxamate.[1,2] A linear trihydroxamate siderophore produced by *Streptomyces pilosus*, DFB, is now the only choice for treatment of iron overload. The stability constant[1,4] of the iron(III) complex was calculated to be log *K* 30.5. However, DFB has some disadvantages, for example, (i) kinetically low efficiency upon iron removal from transferrin, (ii) oral inactiveness, and (iii) a number of side effects such as septicemia. Consequently, many efforts have been devoted to the design and synthesis of novel siderophore analogues.[1–5]

Recently *N*-hydroxyamide-containing heterocyclic monoazines such as 1-hydroxy-2(1*H*)-pyridinone[6] and 3-hydroxy-1,2-dimethyl-4(1*H*)-pyridinone[7] have been paid much attention because of their efficient removal of iron(III) from transferrin, oral activity, and no apparent toxicity.[1,8] Previously we reported that *N*-hydroxyamide-containing diazines such as 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone and 1-hydroxy-5,6-dimethyl-2(1*H*)-pyrazinone showed higher solubility in water and lower pK_a values than those of 1-hydroxy-2(1*H*)-pyridinone by virtue of introduction of the second electron-withdrawing nitrogen atom into the ring, and they formed 3 : 1 iron(III) complexes.[9] Stability constants of the complexes, however, were far below that of DFB. For developing more effective iron(III)-chelating agents, we have investigated the synthesis of hexadentate ligands such as 3-HOPR(X)[10] and 3HOPR*n*CMe[11] possessing 1-hydroxy-2(1*H*)-pyrazinone and 3HOPY*n*[12] bearing 1-hydroxy-2(1*H*)-pyrimidinone as diazine-type ligands. It was revealed that stability constants (log *K*) of these complexes fell into a range from 20.6 to 27.1, but they showed higher iron(III) removal efficiency toward human transferrin than DFB.

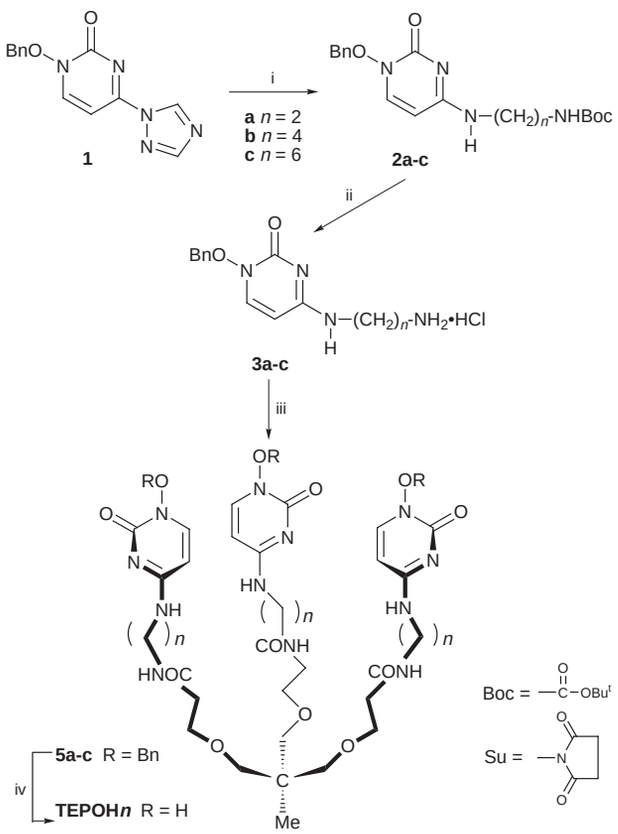
As an extensive study on *N*-hydroxyamide-containing heterocycles, we describe here the synthesis of new hexadentate ligands, TEPOH*n* ($n = 2$, 4 or 6) and TCPOH, bearing 1-hydroxy-2(1*H*)-pyrimidinone as a diazine-type ligand to iron(III), α,ω-diamines as a spacer, and 1,1,1-tris(carboxyethoxymethyl)ethane or *N*,*N*′,*N*″-tri(4-carboxybutyl)isocyanurate as an anchor. Further, characterization of iron(III) complexes of these hexadentate ligands including (i) their iron(III)-chelating properties, (ii) electrochemical behavior and (iii) effect of the methylene chain length and of the shape of the anchor upon the stability constant and iron(III) removal efficiency from human transferrin *in vitro* is discussed.

Results and discussion

Synthesis

The synthetic procedure for THPOH*n* ($n = 2$, 4 or 6) is depicted in Scheme 1. 1-Benzyloxy-4-(1,2,4-triazol-1-yl)-2(1*H*)-pyrimidinone **1**[12] was allowed to react with N^{ω}-*tert*-butoxycarbonyl (Boc)-protected aliphatic diamines to give compounds **2a**–**2c**. The removal of the Boc group of **2a**–**2c** with 4 M HCl in 1,4-dioxane gave the corresponding HCl salts **3a**–**3c**. The coupling of **3a**–**3c** with tris(*O*-succinimide ester) **4**[11] in dry DMF at 38 °C[10–12] gave tripodal compounds **5a**–**5c**. Debenzylation by the catalytic hydrogenation and subsequent purification by gel chromatography on Sephadex LH-20 afforded hexadentate ligands TEPOH*n* ($n = 2$, 4 or 6). The solubility of TEPOH4 and TEPOH6 in water was inferior to that of TEPOH2.

The synthetic procedure for TCPOH is illustrated in Scheme 2. Cyanuric acid was subjected to *N*-alkylation with benzyl 5-bromopentanoate in the presence of NaH to give *N*,*N*′,*N*″-tri(4-benzyloxycarbonylbutyl)isocyanurate **6**. Debenzylation of

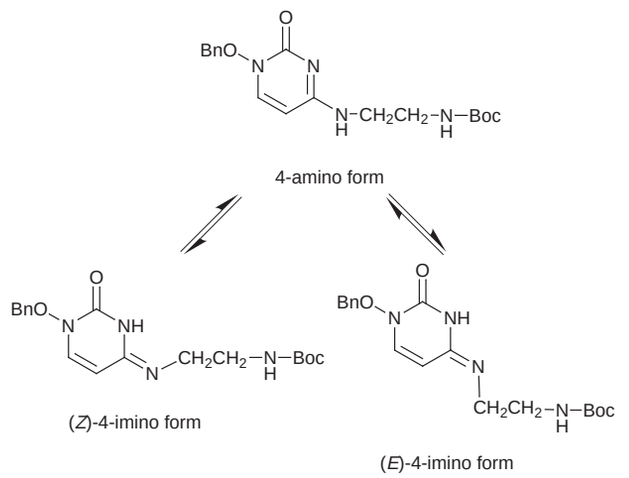


Scheme 1 *Reagents and conditions*: i, $H_2N(CH_2)_nNHBoc$, dry THF, reflux, 9 h; ii, 4 M HCl in 1,4-dioxane, 0 °C, 1 h; iii, $MeC(CH_2O\text{-}CH_2CH_2CO_2Su)_3$ (**4**), DMF, Et_3N, 38 °C, 69 h; iv, H_2, 10% Pd–C, MeOH.

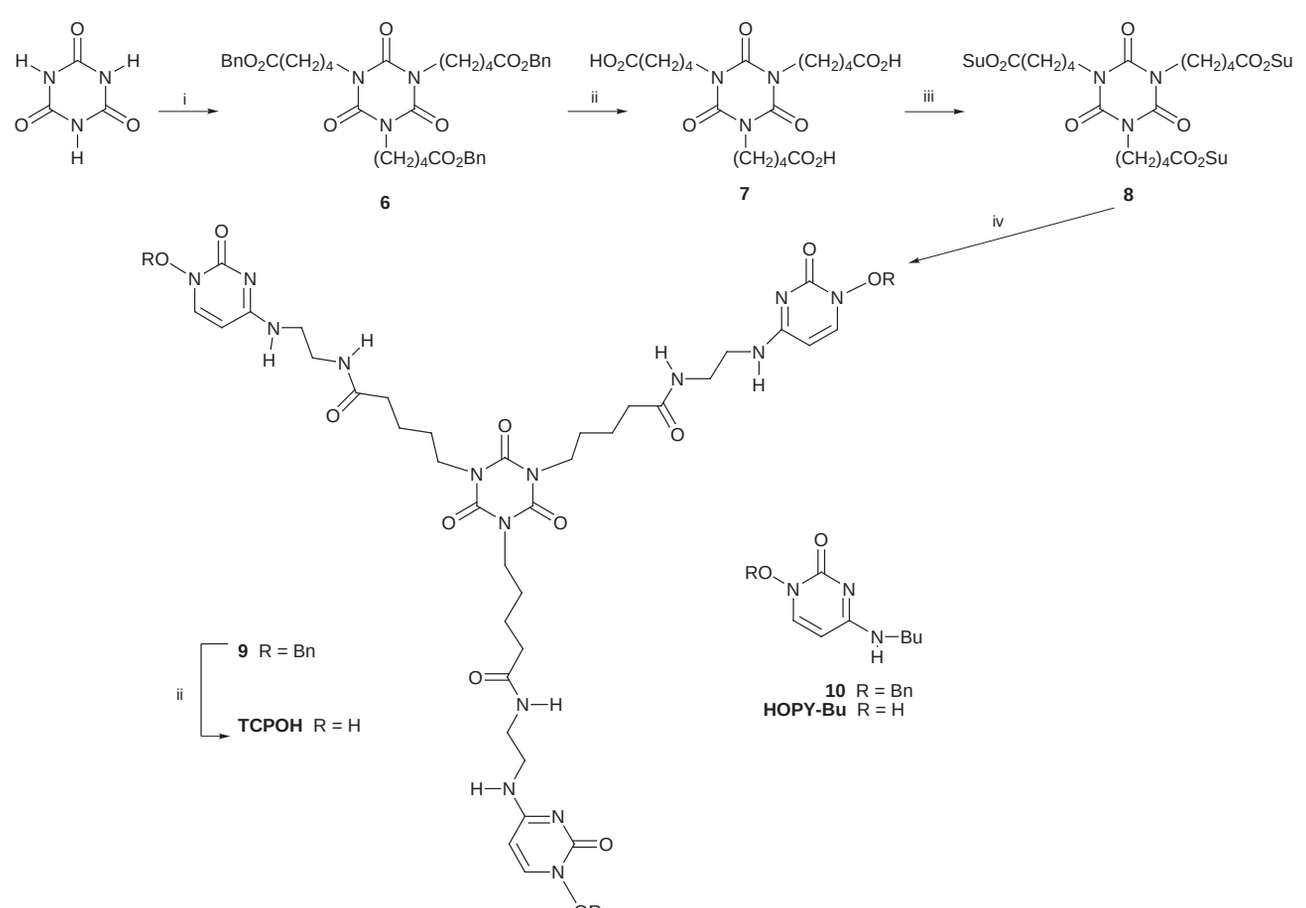
compound **6** by catalytic hydrogenation and subsequent treatment of tris(carboxylic acid) **7** with *N*-hydroxysuccinimide in the presence of WSC·HCl gave the corresponding tris(*O*-succinimide ester) **8**. The coupling of compound **3a** with **8** in a similar fashion to that described above afforded a hexadentate ligand TCPOH.

^{1}H NMR Analysis of tautomers

Compound **2a** showed one set of sharp signals in $(CD_3)_2SO$ solution, *e.g.* the methylene protons adjacent to the nitrogen atom at C-4 position of the pyrimidinone ring as a quartet, indicating that it exclusively existed in the 4-amino form as shown in Scheme 3. In $CDCl_3$ solution, however, **2a** showed



Scheme 3 A possible tautomeric equilibrium of compound **2a** in $CDCl_3$ solution.



Scheme 2 *Reagents and conditions*: i, NaH, NaI, $Br(CH_2)_4CO_2Bn$, dry DMSO, room temperature, 12 h; ii, H_2, 10% Pd–C, MeOH, 12 h; iii, HOSu, WSC·HCl = $EtN{=}C{=}N(CH_2)_3NMe_2$·HCl, DMF–CH_2Cl_2, room temperature, 12 h; iv, **3a**, Et_3N, dry DMF, 38 °C, 40 h.

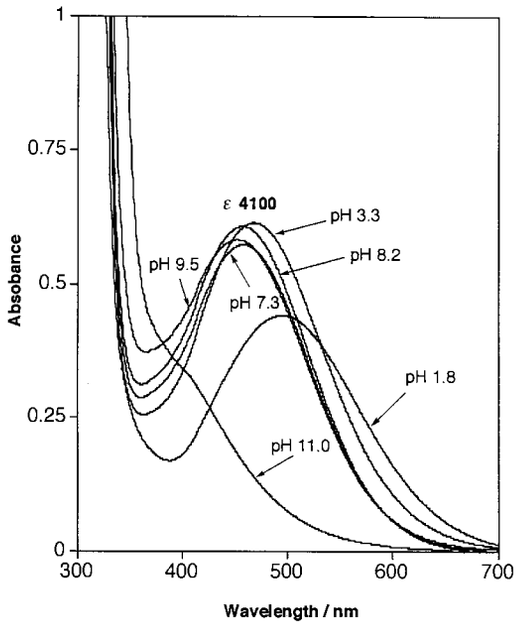


Fig. 1 Spectral change of Fe(TEPO2) in aqueous solution at various pH.

two sets of signals, and a part of the signals of $HNCH_2$ and CH_2NHBoc overlapped each other. For the purpose of obtaining more detailed information, the ^1H NMR spectrum of the simple model, 1-benzyloxy-4-butylamino-2(1*H*)-pyrimidinone **10**[12] was measured. It exhibited almost the same signal pattern as **2a** in $(CD_3)_2SO$ solution, indicating that **10** also existed in the 4-amino form. On the other hand, in $CDCl_3$ solution, two sets of signals at δ 3.15 (broad) and 3.43 (a sharp quartet) (1 : 3 integrated intensity) assignable to $NHCH_2$ protons were observed together with two sets of signals at δ 6.87 (a sharp doublet) and 7.08 (broad) (1 : 3) due to the olefinic proton at C-6. From these spectral data, it seems likely that **2a** exists in a tautomeric equilibrium between 4-amino and two isomers (*E* and *Z*) of 4-imino forms in $CDCl_3$ solution by virtue of internal conversion as shown in Scheme 3.

Iron(III) complex formation

The UV/VIS spectra of a 1 : 1 molar mixture of TEPOH2 and iron(III) in water were measured at various pH. The absorption maximum due to the ligand-to-metal charge transfer (LMCT) of Fe(TEPO2) was observed around 460 nm (ε *ca.* 4100 M^{-1} cm^{-1}) in a wide pH range from 3 to 9 (Fig. 1). The λ_{max} and ε values are comparable to those of a 1 : 1 iron(III) complex of hexadentate ligand 3HOPY5[12] containing 1-hydroxy-2(1*H*)-pyrimidinone (λ_{max} 465 nm and ε 4550 M^{-1} cm^{-1} at pH 5.6), indicating the formation of an intramolecular 1 : 1 complex of iron(III) to TEPOH2. The compounds TEPOH4, TEPOH6 and TCPOH also showed similar behaviors in the acidic to neutral region, but red-brown precipitates except for TCPOH were observed in the neutral to alkaline region. Further characterization of complexes Fe(TEPO2) and Fe(TCPO) was made on the basis of ESIMS (electrospray ionization mass spectrometry) spectra. The sample solution was prepared in aqueous MeOH solution, and the pH adjusted to 6 with 0.1 M NaOH. The ESIMS spectra of the complexes gave *m*/*z* 868.4 and 961.5 assignable to $[Fe(TEPO2) + Na]^+$ and $[Fe(TCPO) + Na]^+$, respectively.

Relative stability of iron(III) complexes

The proton-independent stability constant of the iron(III) complexes with the hexadentate ligands is defined by equilibrium (1). The stability constants of Fe(TEPO*n*) and Fe(TCPO) were

$$Fe^{3+} + L^{3-} \overset{K}{\rightleftharpoons} FeL \quad K = [FeL]/[Fe^{3+}][L^{3-}] \qquad (1)$$

Table 1 The relative stability constants of Fe(TEPO*n*) and Fe(TCPO)

Ligand	K_{eq}[a]	log *K*
TEPOH2	2.55	25.1
TEPOH4	1.80	25.5
TEPOH6	0.69	26.3
TCPOH	0.18	24.4
DFB[b]		30.5

[a] The equilibrium constant. [b] Ref. 14.

estimated by the competitive reaction between the hexadentate ligands and ethylenedinitrilotetraacetate (EDTA).[13] Three pK_a values of the ligand are necessary for calculation of the stability constant. These values, however, were approximated by a pK_a value of the model bidentate ligand, 4-butylamino-1-hydroxy-2(1*H*)-pyrimidinone (HOPY-Bu, pK_a 7.5[12]) owing to the experimental limitation. The competitive reaction was carried out using a 1 : 1 molar mixture of complex and EDTA in 40% aqueous MeOH. Absorbance at 460 nm was monitored in order to determine the equilibrium point. The relative stability constants were calculated from pK_a values of EDTA,[14] pK_a of HOPY-Bu, the equilibrium constant, and the stability constant[15] of Fe(EDTA), and the results are summarized in Table 1. As expected, the relative stability constants (log *K* 25.1–26.3) of Fe(TEPO*n*) were greater than that of Fe(3OPR*n*CMe) (log *K* 20.6–21.7)[11] because the pK_a value of 1-hydroxy-2(1*H*)-pyrimidinone was higher than that of 1-hydroxy-2(1*H*)-pyrazinone. The relative stability constant of Fe(TEPO6) was nearly one order greater than that of Fe(TEPO2), indicating that the stability was apparently affected by the methylene chain length at the spacer moiety. The relationship between the stability constant and the methylene chain length was similar to that in the case of Fe(3OPY*n*).[12] However, these relative stability constants were still below that of the iron(III) complex of natural DFB, because the pK_a value of 1-hydroxy-2(1*H*)-pyrimidinone was about 2 units lower than that of DFB. Further, the relative stability constant of Fe(TCPO) was smaller than of any Fe(TEPO*n*), suggesting that the cyanurate anchor was unfavorable for holding iron(III) by virtue of the divergent arrangement of the ligand.

Electrochemistry of iron(III) complexes

A number of papers have been reported on the electrochemical properties of iron(III) complexes of trihydroxamic acids including DFB. On the contrary, no electrochemical property of an iron(III) complex of a *N*-hydroxyamide-containing heterocycle has been reported, to the best of our knowledge. The electrochemical behaviors of hexadentate TEPOH2 and bidentate HOPY-Bu ligands were examined by means of cyclic voltammetry at apparent pH 8.0 in 50% aqueous DMF. A typical cyclic voltammogram of Fe(TEPO2) is shown in Fig. 2, and the results are summarized in Table 2 together with data for DFB.[16] The standard redox potentials ($E_{1/2}$) of the iron(III) complex of DFB in 50% aqueous DMF are *ca.* 80 mV lower than in an aqueous solution. The electron transfer processes of Fe(TEPO2) and $Fe(OPY\text{-}Bu)_3$ are nearly reversible since the $i_{pc} : i_{pa}$ ratios are close to unity and values of the peak-to-peak separation (ΔE_p) are somewhat larger than 60 mV. It was noteworthy that the $E_{1/2}$ values of the iron(III) complexes of the synthetic ligands are approximately 300 mV higher than that of natural DFB. Further, adsorption[17] of the complexes on the carbon electrode was not observed in the present work.

Kinetics of iron(III) removal from transferrin

The examination of the iron(III) removal efficiency of synthetic hexadentate ligands from human transferrin *in vitro* is one of

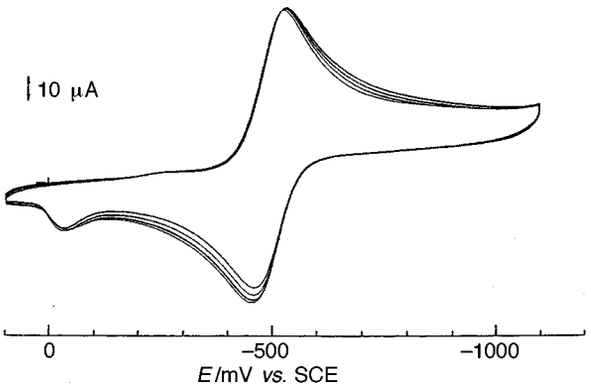


Fig. 2 Cyclic voltammogram of Fe(TEPO2) in 50% aqueous DMF at apparent pH 8.0; scan rate 100 mV s^{-1}.

Table 2 Cyclic voltammetry data for iron(III) complexes in 50% aqueous DMF at apparent pH 8

Iron(III) complex	$E_{1/2}$ *vs.* SCE/mV	ΔE_p/mV	$i_{pc}:i_{pa}$
Fe(TEPO2)	−493	75	1.1
$Fe(OPY\text{-}Bu)_3$	−490	70	1.1
Fe-DFB	−775 (−698)*	60*	1.0*

* In sodium borate–sodium phosphate buffer (pH 8), see ref. 16.

the important requisites for elucidating the potential application to a chemotherapeutic agent for iron overload. After addition of a 20-fold excess of TEPOH2 to a solution of diiron(III) human transferrin ($Tf_{Fe2.0}$)[6a,18–20] in 0.1 M Tris buffer (pH 7.4), the absorbance at 460 nm was measured at appropriate intervals. Plots of log $[(A_\infty - A)/(A_\infty - A_0)]$ *versus* time gave a straight line as shown in Fig. 3, indicating that iron removal from $Tf_{Fe2.0}$ by TEPOH2 proceeded with pseudo-first-order kinetics. The k_{obs} value was calculated from the slope of the line, and the results are summarized in Table 3 together with data for DFB. There was no remarkable difference among the hexadentate ligands (TEPOH*n*) in the kinetic efficiency of iron removal from transferrin. The compounds TEPOH*n* and TCPOH efficiently removed 3–6 times as much iron(III) from transferrin than DFB did even at a smaller concentration ratio ($[L]:[Tf_{Fe2.0}] = 20:1$) at 30 min after the reaction was initiated. Further, it seems that the molecular shape of the anchor moiety affects the kinetic efficiency.

Experimental

Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected. The IR and UV/VIS spectra were recorded on a JASCO FT/IR-230 infrared and a JASCO Ubest V-550 spectrophotometer, respectively, ^{1}H NMR spectra on JEOL GX-270 and JNM-LA400D spectrometers. Chemical shifts are reported in ppm (δ) downfield from internal $SiMe_4$. The ESI mass spectra were taken on a JEOL MS700 instrument (mobile phase MeOH; ring voltage 90 V; desolvation chamber temperature 250 °C). Column chromatography was carried out with Merck Kieselgel 60 (230–400 mesh). Combustion analyses were performed on a Perkin-Elmer Series II CHNS/O Analyzer 2400. Cyclic voltammograms were collected using a HUSO Electro Chemical System (HECS) 315 B Cyclic Voltammograph.

2(1*H*)-Pyrimidinones **1**, **10** and HOPY-Bu were prepared according to the literature method.[12]

General preparation procedure for compounds 2a–2c

A typical example: *N*-(1-benzyloxy-2-oxo-1,2-dihydropyrimidin-4-yl)-*N*′-(*tert*-butoxycarbonyl)ethane-1,2-diamine 2a. A solution of *N*-(2-aminoethyl)carbamic acid *tert*-butyl ester (513 mg, 3.2 mmol) and 2(1*H*)-pyrimidinone **1** (720 mg, 2.7 mmol) in dry THF (20 cm^3) was refluxed for 9 h. After removal of the solvent, water was added to the residue and the aqueous layer extracted with $CHCl_3$ (30 $cm^3 \times 5$). The combined organic phase was washed with 5% citric acid, water, brine, and then dried (Na_2SO_4). Evaporation of the solvent, followed by recrystallization of the residual solid from ethyl acetate, gave compound **2a** (779 mg, 81%) as a yellow solid, mp 136–137 °C (Found: C, 59.9; H, 6.7; N, 15.5. $C_9H_{12}N_2O_2$ requires C, 60.0; H, 6.7; N, 15.55%); $\tilde{\nu}_{max}/cm^{-1}$ 3390, 1706, 1639, 754 and 701; δ_H[270 MHz; $(CD_3)_2SO$] 1.37 (9 H, s, Boc), 3.06 (2 H, q, *J* 6, BocNHCH_2) , 3.26 (2 H, q, *J* 6, NHCH_2), 5.09 (2 H, s, PhCH_2), 5.52 (1 H, d, *J* 7, 5-H), 6.87 (1 H, t, *J* 6, NH), 7.35–7.49 (5 H, m, Ph), 7.58 (1 H, d, *J* 7, 6-H) and 7.75 (1 H, t, *J* 6 Hz, NH).

***N*-(1-Benzyloxy-2-oxo-1,2-dihydropyrimidin-4-yl)-*N*′-(*tert*-butoxycarbonyl)butane-1,4-diamine 2b.** A yellow solid (71%), mp 132–136 °C (Found: C, 61.55; H, 7.1; N, 14.8. C_5H_7NO requires C, 61.8; H, 7.3; N, 14.5%); $\tilde{\nu}_{max}/cm^{-1}$ 3428 1690, 1636, 765 and 703; δ_H(270 MHz; $CDCl_3$) 1.43 (9 H, s, Boc), 1.48–1.68 [4 H, m, $(CH_2)_2$], 3.05–3.24 (2.5 H, m, NHCH_2 and NCH_2), 3.44 (1.5 H, m, NHCH_2), 4.71 (1 H, m, NH), 5.20 (2 H, s, PhCH_2), 5.35 (1 H, d, *J* 8, 5-H), 5.93 (1 H, m, NH), 6.87 (0.75 H, d, *J* 8 Hz, 6-H), 7.09 (0.25 H, m, 6-H) and 7.38 (5 H, s, Ph).

***N*-(1-Benzyloxy-2-oxo-1,2-dihydropyrimidin-4-yl)-*N*′-(*tert*-butoxycarbonyl)hexane-1,6-diamine 2c.** A yellow solid (60%), mp 145–148 °C (Found: C, 63.2; H, 8.0; N, 13.7. $C_{11}H_{16}N_2O_2$ requires C, 63.4; H, 7.7; N, 13.45%); $\tilde{\nu}_{max}/cm^{-1}$ 3308, 1690, 1636, 767 and 703; δ_H(270 MHz; $CDCl_3$) 1.29–1.63 [17 H, m, $(CH_2)_4$, Boc], 3.10 (2.5 H, m, NHCH_2 and NCH_2), 3.44 (1.5 H, m, NHCH_2), 4.58 (1 H, m, NH), 5.22 (2 H, s, PhCH_2), 5.29 (1 H, d, *J* 8, 5-H), 5.57 (1 H, m, NH), 6.89 (0.75 H, d, *J* 8 Hz, 6-H), 7.08 (0.25 H, m, 6-H) and 7.39 (5 H, s, Ph).

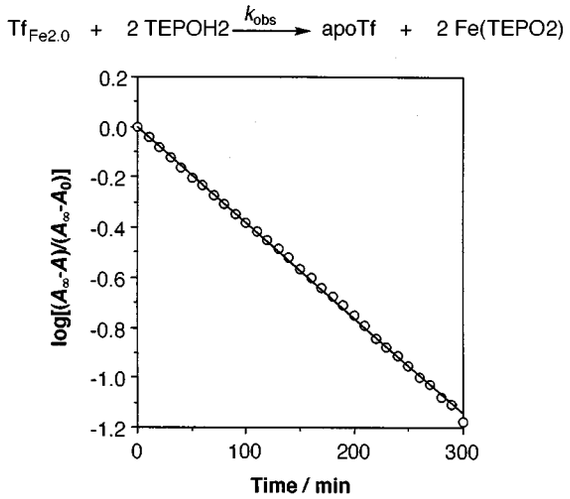


Fig. 3 Plot of log $[(A_\infty - A)/(A_\infty - A_0)]$ *versus* time on iron removal of TEPOH2 from $Tf_{Fe2.0}$.

Table 3 Iron(III) removal from transferrin at pH 7.4

Ligand (L)	$[L]:[Tf_{Fe2.0}]^a$	$k_{obs} \times 10^3/min^{-1}$	% Fe removed[b]
TEPOH2	20	2.69	20
TEPOH4	20	2.62	17
TEPOH6	20	2.30	20
TCPOH	20	4.31	28
DFB	100	0.66	5[c] (5[d])

[a] $[Tf_{Fe2.0}]_0 = 0.02$ mM. [b] At a point 30 min after the reaction was initiated. [c] Ref. 4. [d] The present work.

General procedure for removal of the Boc group of compounds 2a–2c

A typical example: *N*-(1-benzyloxy-2-oxo-1,2-dihydropyrimidin-4-yl)ethane-1,2-diamine hydrochloride 3a. A solution of compound **2a** (701 mg, 1.9 mmol) in 4 M HCl in 1,4-dioxane (12 cm^3) was stirred for 1 h at 0 °C. After removal of the solvent, dry EtOH was added to the residue and then evaporated. Addition and evaporation of dry EtOH were repeated 3 times to give compound **3a** (*ca.* 100%) as a yellow solid which was used for the next reaction without further purification. Similarly compounds **2b** and **2c** were converted into the corresponding HCl salts **3b** and **3c**, respectively.

General preparation procedure for tripodal compounds 5a–5c

A typical example: 1,1,1-tris{2-[2-(1-benzyloxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)ethylaminocarbonyl]ethoxymethyl}-ethane 5a. To a solution of the HCl salt **3a** (580 mg, 1.95 mmol) and Et_3N (482 mg, 4.76 mmol) in DMF (10 cm^3) was added a solution of 1,1,1-tris(2-succinimidooxycarbonylethoxymethyl)ethane **4**[11] (370 mg, 0.59 mmol) in DMF (5 cm^3). The reaction mixture was stirred for 69 h at 38 °C, the solvent was evaporated off under reduced pressure, and the residue dissolved in $CHCl_3$ (400 cm^3). The organic phase was successively washed with water, 5% $NaHCO_3$, 5% citric acid, water, brine, and then dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (8 : 1) and subsequent gel chromatography on Toyopearl HW-40 with MeOH to give the product **5a** (286 mg, 46%) as an amorphous solid (Found: C, 58.6; H, 6.3; N, 15.3. $C_{53}H_{66}N_{12}O_{12}\cdot H_2O$ requires C, 58.9; H, 6.3; N, 15.3%); $\tilde{\nu}_{max}/cm^{-1}$ 3284, 1654, 1637, 764 and 700; δ_H[270 MHz; $(CD_3)_2SO$] 0.76 (3 H, s, CH_3), 2.25 (6 H, m, $COCH_2$), 3.14 (6 H, s, CH_2O), 3.20 (6 H, m, $CONHCH_2$), 3.27 (6 H, m, $NHCH_2$), 3.53 (6 H, m, OCH_2), 5.07 (6 H, s, CH_2Ph), 5.52 (3 H, d, *J* 8, 5-H), 7.42 (15 H, m, Ph), 7.60 (3 H, d, *J* 8 Hz, 6-H), 7.79 (3 H, m, NH) and 7.97 (3 H, m, NH).

1,1,1-Tris{2-[4-(1-benzyloxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)butylaminocarbonyl]ethoxymethyl}ethane 5b. An amorphous solid (27%) (Found: C, 58.6; H, 6.8; N, 14.2. $C_{59}H_{78}N_{12}O_{12}\cdot 3.5H_2O$ requires C, 58.55; H, 7.1; N, 13.9%); $\tilde{\nu}_{max}/cm^{-1}$ 3284, 1636, 756 and 701; δ_H(270 MHz; $CDCl_3$) 0.82 (3 H, s, CH_3), 1.58 [12 H, m, $CH_2(CH_2)_2CH_2$], 2.43 (6 H, m, CH_2CO), 3.21 (12 H, m, CH_2O and $CONHCH_2$), 3.39 (6 H, m, $NHCH_2$), 3.61 (6 H, m, OCH_2), 5.12 (6 H, s, CH_2Ph), 5.62 (3 H, d, *J* 8, 5-H), 6.92 (3 H, d, *J* 8 Hz, 6-H) and 7.30–7.56 (21 H, m, Ph and NH).

1,1,1-Tris{2-[6-(1-benzyloxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)hexylaminocarbonyl]ethoxymethyl}ethane 5c. An amorphous solid (54%) (Found: C, 62.4; H, 7.5; N, 13.2. $C_{65}H_{90}N_{12}O_{12}\cdot H_2O$ requires C, 62.5; H, 7.4; N, 13.45%); $\tilde{\nu}_{max}/cm^{-1}$ 3284, 1650, 754 and 702; δ_H(270 MHz; $CDCl_3$) 0.82 (3 H, s, CH_3), 1.27 [12 H, m, $(CH_2)_2(CH_2)_2(CH_2)_2$], 1.36–1.60 [12 H, m, $CH_2CH_2(CH_2)_2CH_2CH_2$], 2.43 (6 H, m, CH_2CO), 3.06–3.25 (12 H, m, $CONHCH_2$), 3.32 (6 H, m, $NHCH_2$), 3.61 (6 H, m, OCH_2), 5.11 (6 H, s, CH_2Ph), 5.67 (3 H, d, *J* 8, 5-H), 6.91 (3 H, d, *J* 8 Hz, 6-H) and 7.32 (21 H, m, Ph and NH).

General procedure for hexadentate ligands TEPOH*n*

A typical example: 1,1,1-tris{2-[2-(1-hydroxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)ethylaminocarbonyl]ethoxymethyl}-ethane TEPOH2. A suspension of 10% Pd–C (23 mg) in MeOH (10 cm^3) was prehydrogenated with H_2 for 0.5 h. To the suspension was added a solution of compound **5a** (223 mg, 0.2 mmol) in MeOH (100 cm^3). The reaction mixture was stirred for 3 h under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was purified by gel chromatography on Sephadex LH-20 with MeOH to give the product TEPOH2 (88 mg, 53%) as an amorphous solid, hydroxamic acid test positive (Found: C, 47.4; H, 6.4; N, 20.6. $C_{32}H_{48}N_{12}O_{12}\cdot H_2O$ requires C, 47.4; H, 6.2; N, 20.7%); $\tilde{\nu}_{max}/cm^{-1}$ 3284 and 1637; δ_H(400 MHz; CD_3OD) 0.80 (3 H, s, CH_3), 2.40 (6 H, m, CH_2CO), 3.18 (6 H, s, CH_2O), 3.40 (6 H, m, $CONCH_2$), 3.48 (6 H, m, NCH_2), 3.66 (6 H, m, OCH_2), 5.76 (3 H, d, *J* 8, 5-H) and 7.66 (3 H, d, *J* 8 Hz, 6-H).

1,1,1-Tris{2-[4-(1-hydroxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)butylaminocarbonyl]ethoxymethyl}ethane TEPOH4. An amorphous solid (72%), hydroxamic acid test positive (Found: C, 50.5; H, 7.1; N, 18.8. $C_{38}H_{60}N_{12}O_{12}\cdot 1.5H_2O$ requires C, 50.5; H, 7.0; N, 18.6%); $\tilde{\nu}_{max}/cm^{-1}$ 3284 and 1636; δ_H(400 MHz; CD_3OD) 0.82 (3 H, s, CH_3), 1.58 [12 H, m, $CH_2(CH_2)_2CH_2$], 2.40 (6 H, m, CH_2CO), 3.21 (12 H, m, $CONHCH_2$ and CH_2CO), 3.35 (6 H, m, $NHCH_2$), 3.60 (6 H, m, OCH_2), 5.73 (3 H, d, *J* 8, 5-H) and 7.62 (3 H, d, *J* 8 Hz, 6-H).

1,1,1-Tris{2-[6-(1-hydroxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)hexylaminocarbonyl]ethoxymethyl}ethane TEPOH6. An amorphous solid (87%), hydroxamic acid test positive (Found: C, 53.6; H, 7.4; N, 17.1. $C_{44}H_{72}N_{12}O_{12}\cdot 1.5H_2O$ requires C, 53.5; H, 7.65; N, 17.0%); $\tilde{\nu}_{max}/cm^{-1}$ 3284 and 1636; δ_H(400 MHz; CD_3OD) 0.85 (3 H, s, CH_3), 1.37 [12 H, m, $(CH_2)_2(CH_2)_2(CH_2)_2$], 1.48–1.62 [12 H, m, $CH_2CH_2(CH_2)_2CH_2CH_2$], 2.39 (6 H, m, CH_2CO), 3.12–3.38 (18 H, m, $2NHCH_2$ and CH_2CO), 3.63 (6 H, m, OCH_2), 5.71 (3 H, d, *J* 7, 5-H) and 7.62 (3 H, d, *J* 7 Hz, 6-H).

Tris[4-(benzyloxycarbonyl)butyl]isocyanurate 6

Sodium hydride (302 mg, 60% in oil, 7.58 mmol) was washed with hexane and suspended in dry DMSO (10 cm^3). To this suspension was added a solution of cyanuric acid (266 mg, 2.06 mmol) in dry DMSO (6 cm^3) under an argon atmosphere on an ice-bath. The mixture was stirred for 0.5 h at room temperature and again cooled. To this mixture was added NaI (160 mg, 1.1 mmol) and a solution of benzyl 5-bromopentanoate (2 g, 7.38 mmol) in dry DMSO (10 cm^3). After stirring overnight at room temperature, the reaction mixture was taken up in ethyl acetate (80 cm^3). The organic layer was washed with water and then dried ($MgSO_4$). After removal of the solvent, the crude product was purified by column chromatography on silica gel with ethyl acetate–hexane (1 : 3), followed by ethyl acetate–hexane (1 : 1) to give the product **6** (947 mg, 66%) as a colorless oil (Found: C, 66.3; H, 6.3; N, 6.1. $C_{39}H_{45}N_3O_9\cdot 0.5H_2O$ requires C, 66.1; H, 6.5; N, 5.9%); $\tilde{\nu}_{max}/cm^{-1}$ 1732, 1682, 753 and 698; δ_H(270 MHz; $CDCl_3$) 1.67 [12 H, m, $(CH_2)_2$], 2.40 (6 H, m, CH_2CO), 3.87 (6 H, m, NCH_2), 5.10 (6 H, s, CH_2Ph) and 7.33 (15 H, s, Ph).

Tris(4-carboxybutyl)isocyanurate 7

A suspension of 10% Pd–C (30 mg) in THF (10 cm^3) was prehydrogenated with H_2 for 0.5 h. To the suspension was added a solution of compound **6** (188 mg, 0.27 mmol) in THF (10 cm^3). The reaction mixture was stirred for 1 h under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was evaporated to give the product **7** (107 mg, 93%) as a white solid, mp 117–121 °C (Found: C, 50.1; H, 6.4; N, 9.8. $C_6H_9NO_3$ requires C, 50.35; H, 6.3; N, 9.8%); $\tilde{\nu}_{max}/cm^{-1}$ 3310–2480 and 1670; δ_H[270 MHz; $(CD_3)_2SO$] 1.51 [12 H, m, $CH_2(CH_2)_2CH_2$], 2.23 (6 H, m, CH_2CO) and 3.72 (6 H, m, NCH_2).

Tris[4-(succinimidooxycarbonyl)butyl]isocyanurate 8

To a solution of compound **7** (78 mg, 0.18 mmol) and HOSu (89 mg, 0.665 mmol) in dry DMF (2 cm^3) was added a solution of WSC·HCl (140 mg, 0.67 mmol) in dry CH_2Cl_2 (4 cm^3) at −10 °C. The reaction mixture was stirred overnight at room temperature, and then the precipitate was removed by filtration.

The filtrate was evaporated, and the residue dissolved in ethyl acetate (60 cm^3). The organic layer was washed with water, cooled 5% $NaHCO_3$, water, brine, and then dried ($MgSO_4$). Evaporation of the solvent gave the product **8** (130 mg, 100%), which was used for the next reaction without further purication; $\tilde{\nu}_{max}/cm^{-1}$ 1814, 1784, 1739 and 1685; δ_H(270 MHz; $CDCl_3$) 1.77 [12 H, m, $CH_2(CH_2)_2CH_2$], 2.66 (6 H, m, CH_2CO), 2.82 (12 H, s, OSu) and 3.72 (6 H, m, NCH_2).

Tris{4-[2-(1-benzyloxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)-ethylaminocarbonyl]butyl}isocyanurate 9

To a solution of compound **3a** (700 mg, 2.36 mmol) and Et_3N (918 mg) in DMF (25 cm^3) was added a solution of **8** (504 mg, 0.70 mmol) in DMF (10 cm^3). The reaction mixture was stirred for 40 h at 38 °C, the solvent evaporated under reduced pressure, and then the residue dissolved in $CHCl_3$ (400 cm^3). The organic phase was successively washed with water, 5% $NaHCO_3$, 5% citric acid, water, brine, and then dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$, followed by $CHCl_3$–MeOH (8 : 1) to give the product **9** (646 mg, 80%) as an amorphous solid (Found: C, 58.15; H, 6.2; N, 17.4. $C_{57}H_{69}N_{15}O_{12}\cdot H_2O$ requires C, 58.3; H, 6.1; N, 17.9%); $\tilde{\nu}_{max}/cm^{-1}$ 3284, 1654, 1637, 764 and 700; δ_H[270 MHz; $(CD_3)_2SO$] 1.49 [12 H, m, $CH_2(CH_2)_2CH_2$], 2.08 (6 H, m, CH_2CO), 3.16–3.45 (12 H, m, $NHCH_2CH_2NH$), 3.71 (6 H, m, NCH_2), 5.07 (6 H, s, CH_2Ph), 5.52 (3 H, d, *J* 8, 5-H), 7.38–7.46 (15 H, m, Ph), 7.59 (3 H, d, *J* 8 Hz, 6-H), 7.78 (3 H, m, NH) and 7.93 (3 H, m, NH).

Tris{4-[2-(1-hydroxy-2-oxo-1,2-dihydropyrimidin-4-ylamino)-ethylaminocarbonyl]butyl}isocyanurate TCPOH

A suspension of 10% Pd–C (40 mg) in MeOH (5 cm^3) was prehydrogenated with H_2 for 0.5 h. To the suspension was added a solution of compound **9** (200 mg, 0.17 mmol) in MeOH (5 cm^3). The reaction mixture was stirred for 4 h under a hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was evaporated. The residue was purified by gel chromatography on Sephadex LH-20 with MeOH to give the product TCPOH (134 mg, 88%) as an amorphous solid, hydroxamic acid test positive (Found: C, 47.6; H, 6.2. $C_{36}H_{51}N_{15}O_{12}\cdot 1.5H_2O$ requires C, 47.4; H, 6.0%); $\tilde{\nu}_{max}/cm^{-1}$ 3384, 1685 and 1637; δ_H(400 MHz; CD_3OD) 1.61 [12 H, m, $CH_2(CH_2)_2CH_2$], 2.22 (6 H, m, CH_2CO), 3.29–3.38 (12 H, s, CH_2O), 3.85 (6 H, m, $NHCH_2CH_2NH$), 5.74 (3 H, d, *J* 8, 5-H) and 7.65 (3 H, d, *J* 8 Hz, 6-H).

Measurement of UV/VIS spectra of iron(III) complexes

To a solution of each hexadentate ligand (1.19–1.44 mg) in water (5 cm^3) was added a solution (0.45 cm^3) of $Fe(NO_3)_3$ (3.28 mM) in distilled water, and then diluted to 10.0 cm^3 with distilled water (0.15 mM). The pH of the solution was adjusted to an appropriate value with 0.1 or 0.01 M KOH or 0.1 or 0.01 M HNO_3 before spectral measurement.

Measurement of the relative stability constants

Each iron(III) complex solution (0.1–0.3 mM) of hexadentate ligand was prepared by mixing a stock solution of TEPOH*n* or TCPOH (0.3–0.67 mM) in MeOH with an equimolar amount of aqueous $Fe(NO_3)_3$ solution (3.28 mM) and 0.4 M KNO_3 (0.5 cm^3) and then diluting the solution to 5.0 cm^3 with MeOH. At this point, the pH was adjusted to 5–8 with 1 M NaOH. The stock solution of EDTA (0.1–0.3 mM) was prepared by dissolving $Na_2EDTA\cdot 2H_2O$ in water (ionic strength 0.04 M). The iron exchange reaction was initiated by mixing the complex solution (1 cm^3) with EDTA solution (1 cm^3), [complex] = [EDTA] = 0.15 mM, ionic strength 0.04 M, and checked by monitoring the decrease of absorbance at 460 nm. The pH of the solution did not change before and after the reaction. The relative stability constants were calculated by using the stability constant of Fe(EDTA),[15] the pK_a[12] of HOPY-Bu and the equilibrium point at 20 °C.

Cyclic voltammetry of iron(III) complexes

To a solution of each ligand (1.90–3.27 mg) in DMF (2.5 cm^3) was added 0.28 equivalent of aqueous $Fe(NO_3)_3$ solution. The solution was diluted to a volume of 5.0 cm^3 with phosphate buffer (pH 9.0), and then KCl (37 mg) was added. The resulting solution was adjusted to pH 8.0 with 0.05 M $Na_2B_4O_7$, and then filtered to remove insoluble materials. Cyclic voltammetry was carried out by generating triangular waves at ambient temperature. A carbon electrode was used with a saturated calomel electrode as a reference and a platinum wire as an auxiliary electrode. Current–voltage curves were recorded on an X-Y recorder.

Iron(III) removal from transferrin

A commercially available human serum apotransferrin (98%, Sigma) was used; $Tf_{Fe2.0}$ was prepared according to the literature method.[6a,20] The stock solutions of hexadentate ligands (1 cm^3, 0.8 mM) and $Tf_{Fe2.0}$ (1 cm^3, 0.04 mM) in Tris buffer were combined ([ligand] : [$Tf_{Fe2.0}$] = 20 : 1). The absorbance of the solution was monitored at 460 nm. The pseudo-first-order-rate constants (k_{obs}) were obtained from the slopes of plots of $\log[(A_\infty - A)/(A_\infty - A_0)]$ *versus* time.

Acknowledgements

This work was partially supported by Japan Private School Promotion Foundation. The authors are grateful to Mr K. Matuura of JEOL HIGHTECH Co. Ltd. for measuring ESI mass spectra.

References

1 *The Development of Iron Chelators for Clinical Use*, eds. R. J. Bergeron and G. M. Brittenham, CRC Press, Boca Raton, FL, 1992.
2 A. L. Crumbliss, in *Handbook of Microbial Iron Chelates*, ed. G. Winkelmann, CRC Press, Boca Raton, FL, 1991, p. 177.
3 M. T. Ahmet, C. S. Frampton and J. Silver, *J. Chem. Soc.*, *Dalton Trans.*, 1988, 1159.
4 C. J. Carrano and K. N. Raymond, *J. Am. Chem. Soc.*, 1979, **101**, 5401.
5 Z. Hou, D. W. Whisenhunt, jun., J. Xu and K. N. Raymond, *J. Am. Chem. Soc.*, 1994, **116**, 840.
6 (*a*) R. C. Scarrow, D. L. White and K. N. Raymond, *J. Am. Chem. Soc.*, 1985, **107**, 6540; (*b*) R. C. Scarrow, P. E. Riley, K. Abu-Dari, D. L. White and K. N. Raymond, *Inorg. Chem.*, 1985, **24**, 954.
7 M. Streater, P. D. Taylor, R. C. Hider and J. Porter, *J. Med. Chem.*, 1990, **33**, 1749.
8 G. J. Kontoghiorghes, *The Lancet*, 1985, 817; D. M. Taylor and G. J. Kontoghiorghes, *Inorg. Chim. Acta*, 1986, **125**, L38; B. Faller and H. Nick, *J. Am. Chem. Soc.*, 1994, **116**, 3860.
9 J. Ohkanda, T. Tokumitsu, K. Mitsuhashi and A. Katoh, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 841.
10 J. Ohkanda and A. Katoh, *J. Org. Chem.*, 1995, **60**, 1583.
11 J. Ohkanda and A. Katoh, *Tetrahedron*, 1995, **51**, 12995.
12 J. Ohkanda, J. Kamitani, T. Tokumitsu, T. Konakahara and A. Katoh, *J. Org. Chem.*, 1997, **62**, 3618.
13 A. Winston and D. Kirchner, *Macromolecules*, 1978, **11**, 597.
14 G. Anderegg, F. L'Eplattenier and G. Schwarzenbach, *Helv. Chim. Acta*, 1963, **46**, 1400, 1409.
15 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum, New York, 1974, vol. 1.
16 S. R. Cooper, J. V. McArdle and K. N. Raymond, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 3551.
17 K. Shimizu, K. Nakayama and M. Akiyama, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 2421; M. Akiyama, A. Katoh and T. Ogawa, *J. Chem. Soc.*, *Perkin Trans. 1*, 1989, 1213.
18 W. R. Harris, P. K. Bail and M. M. Crowley, *Inorg. Chem.*, 1992, **31**, 2700.
19 G. W. Bates and M. R. Schlabach, *J. Biol. Chem.*, 1973, **248**, 3228.
20 S. A. Nguyen, A. Craig and K. N. Raymond, *J. Am. Chem. Soc.*, 1993, **115**, 6758.

Paper 8/07159D