N-Hydroxy Amides. Part 9.[1] Synthesis and Iron(III) Complexes of Tripodal Hydroxamic Acids derived from ω-(*N*-Hydroxyamino)alkanoic Acids and Tris-(2-aminoethyl)amine

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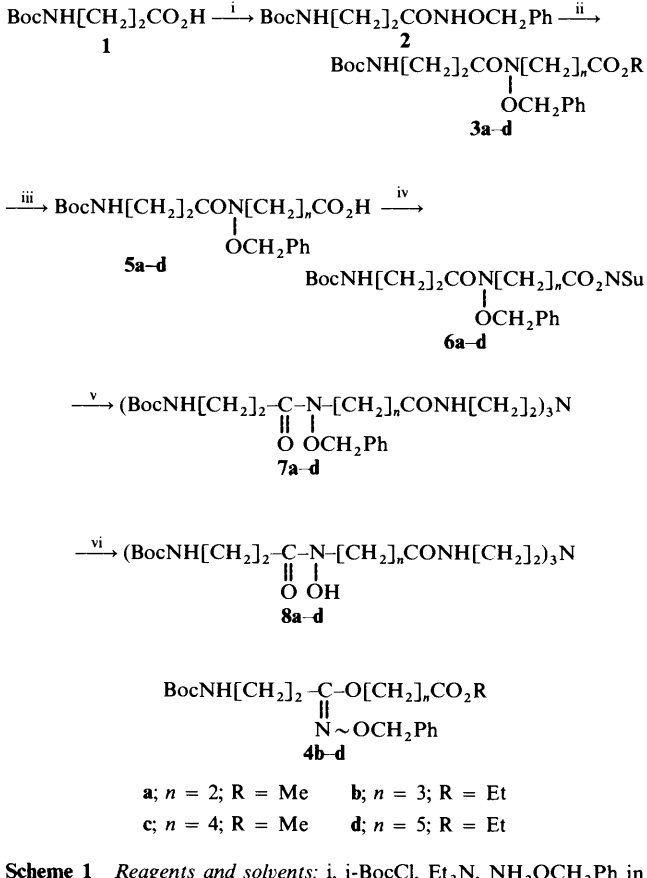
Tripodal oligoamide hydroxamic acids with different chain lengths (compounds **8a–d**) are prepared *via* condensation of *N*-hydroxysuccinimide esters of *N*-acyl-*N*-benzyloxyaminoalkanoic acids with tris-(2-aminoethyl)amine. These synthetic trihydroxamic acids form iron(III) complexes in aqueous DMF solution. The behaviour of these iron(III) complexes is investigated in terms of absorption *vs* pH and of iron(III) exchange reactions with EDTA. A biological assay performed with *Aureobacterium flavescens* reveals that the trihydroxamic acids have substantial growth-promoting activity although weak relative to that of natural desferrioxamine B.

Naturally occurring hydroxamic acids exhibit unique biological activity, acting as antibiotics and growth factors,[2–6] or effecting sensitization of tumour cells to macrophage-mediated cytolysis.[7] Microorganisms excrete iron-transporting agents called siderophores, which in general possess three groups of the hydroxamate or catecholate type, in order to sequester iron(III) and to transport it into the cell.[3,4,6,8,9] In mimicking natural siderophores, a number of artificial hydroxamate ligands have been synthesized to date,[1,8,10–13] and some of them have been demonstrated to serve as biological probes for microbial iron(III)-uptake.[8,14,15] Iron(III)-holding properties and biological activities of synthetic trihydroxamic acids are of considerable interest in relation to naturally occurring compounds. Several synthetic trihydroxamic acids conveniently utilize tris-(2-aminoethyl)amine as a basic building block for making trifunctional molecules.[13,15] Previously we reported on the synthesis of several oligoamide trihydroxamic acids which contain *N*-hydroxy-α- or -β-amino acid units as the functional moieties.[16] In the course of our studies on *N*-hydroxy amides, we were interested in testing tripodal oligoamide hydroxamic acids. This paper describes the synthesis of novel trihydroxamic acids composed of ω-(*N*-hydroxyamino)alkanoic acids and tris-(2-aminoethyl)amine as important building blocks, together with their functional properties, including iron(III) complex-forming tendency and microbial growth-promoting activity.

Results and Discussion

Synthesis.—There seems to exist an optimal spacing between hydroxamic acid units in order for them to make stable iron(III) complexes. In natural trihydroxamate siderophores, a 10-atom spacing is seen for ferrichromes and fusarinines, and an 8- or 9-atom spacing for ferrioxamines. Spacing between the hydroxamic acid units in tripodal molecules is adjustable by changing the alkyl chain length of ω-(*N*-hydroxyamino)alkanoic acids. The synthetic procedure for tris-(2-aminoethyl)amine-based trihydroxamic acids **8a–d** is depicted in Scheme 1. 3-(Butoxycarbonylamino)propanoic acid **1** was coupled with *O*-benzylhydroxylamine by the mixed anhydride (MA) method to give the benzyloxyamide **2** in 89% yield. The amide **2** was allowed to react with ω-bromoalkanoic acid esters in dry *N*,*N*-dimethylformamide (DMF) in the presence of sodium hydride to afford the *N*-alkylation **3a–d** and *O*-alkylation products **4b–d**,[17] both of which were easily separated by column chromatography. Acids **5a–d** obtained by hydrolysis of compounds **3a–d** were converted into *N*-hydroxysuccinimide (HONSu) esters **6a–d** by the use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl) in high yield. The desired products **8a–d** were obtained by coupling of the active esters **6a–d** with tris-(2-aminoethyl)amine, followed by hydrogenation to remove the *O*-protecting benzyl group from compounds **7a–d**.



Scheme 1 *Reagents and solvents:* i, i-BocCl, Et_3N, NH_2OCH_2Ph in THF; ii, NaH, $Br[CH_2]_nCO_2R$ in DMF; iii, 1 mol dm^{-3} NaOH; iv, HONSu, EDC·HCl in DMF–CH_2Cl_2 (1:2); v, $(NH_2CH_2CH_2)_3N$ in DMF; vi, H_2/10% Pd–C in MeOH

Iron(III) *Complex Formation.*—The 1:1 molar mixture of iron(III) and a trihydroxamic acid **8a–d** produced iron(III) complexes in 50% aq. DMF solution. Visible spectra of these complexes were measured at different pH_{app} (apparent pH).

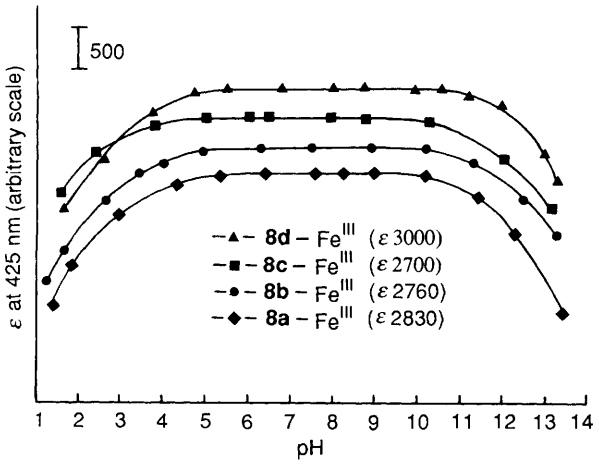


Fig. 1 Molar absorption coefficient (ε) *vs* pH for the 1 : 1 molar mixture of iron(III) and trihydroxamic acids **8a–d** in 50% aq. DMF. Each curve is shown arbitrarily with the scale given to indicate the onset of curvature. The ε value for each complex is shown in parentheses.

Table 1 Iron(III)-exchange reactions with EDTA[a]

Ligand (L)	k_{tr}/s^{-1}	Relative rate
8a	8.9×10^{-4}	6.8
8b	1.3×10^{-4}	1
8c	1.5×10^{-4}	1.1
8d	5.1×10^{-4}	4
DFB[b]	6.5×10^{-6}	

[a] An exchange reaction rate (k_{tr}) was measured under pseudo-first-order conditions; $[Fe\text{–}L]_0$ 1×10^{-4} mol dm^{-3}, $[EDTA]_0$ 9.7×10^{-4} mol dm^{-3} at pH_{app} 6.9 in 50% DMF at 25 °C. [b] $[Fe\text{–}DFB]_0$ 3.2×10^{-4} mol dm^{-3}, $[EDTA]_0$ 8.3×10^{-3} mol dm^{-3} under the similar conditions.

Table 2 Growth-promotion activity of tripodal hydroxamic acid **8a–d**–iron(III) complexes for *Aureobacterium flavescens*[a]

Ferric complex with	**8a**	**8b**	**8c**	**8d**	DFB	DW
Diameter (mm)	25	22	22	28	48	0

[a] An aliquot (15 mm^3) from iron(III) complex stock solutions (230 μmol dm^{-3}) was absorbed onto a 6 mm filter paper disc. DFB–Fe^{III} in a 23 μmol dm^{-3} stock solution was used as a reference, and distilled water (DW) as a blank. The diameter of the halo of growth was measured.

Values of λ_{max} (425 nm) and the molar absorption coefficient ε (2800–3000) for the complexes at neutral pH correspond to those for the 1 : 3 complex of iron(III) with the hydroxamic acid unit.[18] Plots of ε for the complexes *vs* pH are shown in Fig. 1. A constant-absorbance region is seen for every iron(III) complex over the ranges pH 5–10, where iron(III) is completely entrapped by a hexadentate trihydroxamic acid. In both acidic (pH < 5) and basic (pH > 10) regions, λ_{max} shifted to a longer wavelength and the absorbance at 425 nm decreased, which indicated transformation of the 1 : 3 complexes into 1 : 2 and then into 1 : 1 complexes by attack of H^+ or OH^- ions.[2a] The pH stability of these complexes is estimated roughly to be in the order **8c**–Fe^{III} > **8b**–Fe^{III} > **8d**–Fe^{III} > **8a**–Fe^{III} from the span of the plateau region of the curves. Examination of molecular models reveals that **8a**–Fe^{III} is a tight, small molecule, while **8d**–Fe^{III} has a rather expanded shape.

Relative Stability of Iron(III) *Complexes.*—The pseudo-first-order rate constants (k_{tr}) of iron(III) exchange reactions [equation (1)] between these complexes and ethylenediaminetetraacetic acid (EDTA) were measured by following the decrease in absorbance at 425 nm in the presence of a large excess of EDTA.

$$\text{Fe–L} + \text{EDTA} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{L} + \text{Fe–EDTA} \qquad (1)$$

$$\text{where } k_{tr} = k_f + k_r \fallingdotseq k_f$$

The results are summarized in Table 1. Although the exchange reactions are reversible, we can estimate the relative kinetic stability (k_f) for these complexes from the initial rates (k_{tr}) or iron(III) transfer from Fe–L to EDTA under the present conditions.[19] From Table 1, compounds **8b** and **8c** hold iron(III) more tightly than do compounds **8a** and **8d**. The relative stability of the complexes falls in the order of ligand **8b** ≒ **8c** > **8d** > **8a**, indicating that the spacing between the hydroxamic acid functions affects iron(III)-holding capacity. Under similar conditions desferrioxamine B(DFB)–Fe^{III}, a typical natural trihydroxamic acid complex, showed a much slower rate in spite of a more forcing concentration. An iron(III) stability constant of 10^{30} was obtained in water for DFB, and synthetic analogues of DFB showed a trend similar to that of DFB in 50% aq. DMF;[16b] DFB is considered to have an optimal 9-atom spacing between the hydroxamic acid groups.[18] Spacing atoms for the present ligands vary from 13 for **8a** to 19 for **8d**. The iron(III)-exchange reaction showed that favourable atom numbers for complex formation are 15 and 17 for **8b** and **8c**, comparable with the pH-stability order obtained from the absorbance *vs* pH plot. The fact that optimal spacing-atom numbers of the present ligands are different from that of DFB indicates a different complex-forming tendency between tripodal and linear molecules. The data suggest that under the present conditions the tripodal ligands produce less stable iron(III) complexes relative to linear ligands.

Biological Activity.—The growth-promotion activity of iron(III) complexes was examined by using *Aureobacterium flavescens** which is a mutant auxotroph for hydroxamate siderophores and does not synthesize such siderophores. The strain, therefore, is useful for diagnosis whether a synthetic compound acts as an artificial siderophore or not. The assay was performed according to the standard procedure.[2c] The activity was evaluated by measuring the diameter of the halo of exhibition of growth. All iron(III) complexes of synthetic ligands **8a–d** showed the halo of growth of similar magnitude, irrespective of iron(III)-exchange stability and the alkyl chain length, *i.e.* molecular shape. The potent natural siderophore DFB exhibited almost twice the halo of growth even at one-tenth concentration, indicating that the activity of these synthetic compounds is significant, but far less than that of DFB. These data are given in Table 2.

Experimental

M.p.s were measured on a silicon bath and are uncorrected. IR spectra were recorded on JASCO Model A-302 and FT/IR-5M Fourier-transform IR spectrometers. ^{1}H NMR spectra were taken with a JEOL JNM-FX 200 spectrometer with $SiMe_4$ as the internal standard in $CDCl_3$ or $(CD_3)_2SO([^2H_6]DMSO)$; *J*-values are given in Hz. UV spectra were recorded on a Hitachi 320A spectrometer. The pH of solutions was measured with a TOA Model HM-20B digital pH meter. Gel chromatography was performed using Sephadex LH-20 with methanol as the eluent, and Wako gel C-300 was used for column chromato-

* ATCC 25091, which was formerly registered as *Arthrobacter flavescens* Jg-9. Biological activity: ref. 20.

graphy. DMF was purified with both BaO and ninhydrin before use.

Benzyl 3-(t-Butoxycarbonylamino)propanohydroxamate **2**.—To a cooled mixture of 3-(t-butoxycarbonylamino)propanoic acid **1** (4.08 g, 21.6 mmol) and triethylamine (2.3 g, 22.7 mmol) in tetrahydrofuran (THF) (65 cm^3) were added a solution of isobutyl chloroformate (3.1 g, 22.7 mmol) in THF (20 cm^3) at −15 °C, and *O*-benzylhydroxylamine (2.34 g, 19 mmol), at −17 °C, after 15 min. The mixture was kept at −15 °C for 3 h and at room temperature for 20 h, and was then filtered. The filtrate was evaporated off; the residue was dissolved in AcOEt (150 cm^3), and the organic phase was washed successively with 5% aq. $NaHCO_3$, 5% aq. citric acid, and water, and was then dried (Na_2SO_4). Evaporation of the solvent gave a solid product **2**, which was recrystallized from Et_2O–hexane to give the *product* **2** (4.97 g, 89%), m.p. 103–104 °C (Found: C, 61.3; H, 7.5; N, 9.7. $C_{15}H_{22}N_2O_4$ requires C, 61.2; H, 7.5; N, 9.5%); ν_{max}(KBr)/cm^{-1} 1685, 1640, 750 and 700; δ($CDCl_3$) 1.42 (9 H, s), 2.25 (2 H, m), 3.34 (2 H, q, *J* 6.1), 4.85 (2 H, s), 5.18 (1 H, br s), 7.2–7.4 (5 H, m) and 9.3 (1 H, br s).

General Procedure for Compounds **3a–d**: *A Typical Example, Methyl 3-[N-Benzyloxy-3-(t-butoxycarbonylamino)propanamido]-propanoate* **3a**.—Compound **2** (2.35 g, 8 mmol) and NaH (60% in oil; 0.38 g, 9.45 mmol) were stirred in DMF (20 cm^3) for 1 h at room temperature, and then a solution of methyl 3-bromopropanoate (1.2 g, 9.25 mmol) in DMF (10 cm^3) was added dropwise to the mixture at 5 °C. The mixture was heated for 3 h at 100 °C, and was then poured onto ice. The resulting solution was extracted with AcOEt (50 cm^3 × 3). The organic phase was washed with water (80 cm^3 × 5), dried (Na_2SO_4) and evaporated. The product was purified by column chromatography on silica gel with AcOEt–hexane (1:1) to afford *compound* **3a** as an oil (2.2 g, 80%) (Found: C, 59.9; H, 7.5; N, 7.35. $C_{19}H_{28}N_2O_6$ requires C, 60.0; H, 7.4; N, 7.35%); ν_{max}(neat)/cm^{-1} 3360, 1735, 1705, 1655, 750 and 700; δ($CDCl_3$) 1.43 (9 H, s), 2.59 (4 H, t, *J* 6.8), 3.37 (2 H, q, *J* 5.9), 3.62 (3 H, s), 3.95 (2 H, t, *J* 6.8), 4.80 (2 H, s), 5.17 (1 H, br s) and 7.37 (5 H, s).

Ethyl 4-[N-benzyloxy-3-(t-butoxycarbonylamino)propanamido]butanoate **3b** was obtained as an *oil* in 47% yield (Found: C, 61.55; H, 8.0; N, 6.75. $C_{21}H_{32}N_2O_6$ requires C, 61.75; H, 7.9; N, 6.85%); ν_{max}(neat)/cm^{-1} 3350, 1720, 1705, 1655, 745 and 700; δ($CDCl_3$) 1.23 (3 H, t, *J* 7.1), 1.43 (9 H, s), 1.95 (2 H, quint, *J* 6.8), 2.32 (2 H, t, *J* 6.8), 2.62 (2 H, t, *J* 5.8), 3.38 (2 H, q, *J* 5.8), 3.71 (2 H, t, *J* 6.8), 4.11 (2 H, q, *J* 7.1), 4.79 (2 H, s), 5.25 (1 H, br s) and 7.4 (5 H, s).

Methyl 5-[N-benzyloxy-3-(t-butoxycarbonylamino)propan amido]pentanoate **3c** was obtained as an *oil* in 35% yield (Found: C, 61.5; H, 7.8; N, 6.95. $C_{21}H_{32}N_2O_6$ requires C, 61.75; H, 7.9; N, 6.85%); ν_{max}(neat)/cm^{-1} 3300, 1720, 1695, 1650, 740 and 700; δ($CDCl_3$) 1.41 (9 H, s), 1.4–1.7 (4 H, m), 2.31 (2 H, t, *J* 6.8), 2.60 (2 H, t, *J* 5.9), 3.37 (2 H, q, *J* 5.9), 3.64 (3 H, s), 3.68 (2 H, t, *J* 6.8), 4.78 (2 H, s), 5.2 (1 H, br s) and 7.3 (5 H, s).

Ethyl 6-[N-benzyloxy-3-(t-butyloxycarbonylamino)propanamido]hexanoate **3d** was obtained as an *oil* in 30% yield (Found: C, 63.1; H, 8.35; N, 6.3. $C_{23}H_{36}N_2O_6$ requires C, 63.3; H, 8.3; N, 6.4%); ν_{max}(neat)/cm^{-1} 3360, 1725, 1700, 1655, 750 and 700; δ($CDCl_3$) 1.24 (3 H, t, *J* 6.85), 1.43 (9 H, s), 1.2–1.4 (2 H, m), 1.5–1.75 (4 H, m), 2.28 (2 H, t, *J* 6.6), 2.61 (2 H, t, *J* 6.8), 3.38 (2 H, q, *J* 6.6), 3.63 (2 H, t, *J* 6.8), 4.12 (2 H, q, *J* 6.85), 4.79 (2 H, s), 5.23 (1 H, br s) and 7.38 (5 H, s).

During these *N*-alkylation reactions, *O*-alkylation products **4b–d** were separated by column chromatography.

Ethyl 4-[*N*-benzyloxy-3-(t-butoxycarbonylamino)propanimidoyloxy]butanate **4b** was obtained as an oil in 20% yield; ν_{max}(neat)/cm^{-1} 3350, 1730, 1710, 750 and 700; δ($CDCl_3$) 1.24 (3 H, t, *J* 6.9), 1.43 (9 H, s), 1.97 (2 H, quint, *J* 6.8), 2.35 (2 H, t, *J* 5.9), 2.42 (2 H, t, *J* 6.8), 3.31 (2 H, q, *J* 5.9), 4.12 (2 H, q, *J* 6.9), 4.14 (2 H, t, *J* 6.8), 4.95 (2 H, s), 4.98 (1 H, br s) and 7.2–7.4 (5 H, m).

Methyl 5-[*N*-benzyloxy-3-(t-butyoxycarbonylamino)propanimidoyloxy]pentanoate **4c** was obtained as an oil in 18% yield; ν_{max}(neat)/cm^{-1} 3320, 1720, 1675, 735 and 700; δ($CDCl_3$) (a mixture of *E* and *Z*) 1.43 (9 H, s), 1.6–1.75 (4 H, m), 2.2–2.7 (3.1 H, m), 2.59 (0.9 H, t, *J* 6.6), 3.2–3.4 (2 H, m), 3.67 (3 H, s), 3.96 (0.9 H, t, *J* 6.1), 4.10 (1.1 H, t, *J* 6.1), 4.85 (1 H, br s), 4.92 (0.9 H, s), 4.95 (1.1 H, s) and 7.25–7.4 (5 H, m).

Ethyl 6-[*N*-benzyloxy-3-(t-butoxycarbonylamino)propanimidoyl]hexanoate **4d** was obtained as an oil in 16% yield; ν_{max}(neat)/cm^{-1} 3370, 1735, 1710, 750 and 700; δ($CDCl_3$) (a mixture of *E* and *Z*) 1.26 (3 H, t, *J* 6.9), 1.42 (9 H, s), 1.3–1.5 (2 H, m), 1.55–1.8 (4 H, m), 2.25–2.3 (3.7 H, m), 2.52 (0.3 H, t, *J* 6.6), 3.25–3.45 (2 H, m), 4.0–4.2 (2 H, m), 4.12 (2 H, q, *J* 6.9), 4.97 (2 H, s), 5.06 (1 H, br s) and 7.25–7.4 (5 H, m).

General Procedure for Compounds **7a–d**: *a Typical Example, Tris-(2-{3-[N-benzyloxy-3-(t-butoxycarbonylamino)propanamido]-propanamido}ethyl)amine* **7a**.—Methyl ester **3a** (1.2 g, 3.15 mmol) was treated with 1 mol dm^{-3} NaOH (4 cm^3, 4 mmol) in MeOH (15 cm^3) at room temperature for 1.5 h to give the carboxylic acid **5a** (1.08 g, 93%). Compound **5a** (0.95 g, 2.6 mmol) was allowed to react with HONSu (0.6 g, 5.2 mmol) in the presence of EDC·HCl (1 g, 5.2 mmol) at −10 °C, and the mixture was stirred overnight at room temperature to afford HONSu ester **6a** (1.2 g, 100%), which was used without purification.

A mixture of compound **6a** (1.2 g, 2.6 mmol) and tris-(2-aminoethyl)amine (0.12 g, 0.8 mmol) in DMF (30 cm^3) was stirred for 40 h at 38 °C. DMF was removed under reduced pressure, and the residual oil was dissolved in AcOEt (200 cm^3). The organic phase was washed with water (80 cm^3 × 2), dried (Na_2SO_4), and then evaporated. The residue was purified by gel chromatography to afford the product **7a** as an amorphous solid (0.69 g, 73%); ν_{max}(KBr)/cm^{-1} 3320, 1705, 1695, 1650, 750 and 700; δ($CDCl_3$) 1.42 (27 H, s), 2.3–2.5 (12 H, m), 2.56 (6 H, t, *J* 6.2), 3.15 (6 H, q, *J* 6.2), 3.33 (6 H, q, *J* 5.1), 3.94 (6 H, t, *J* 7.4), 4.77 (6 H, s), 5.32 (3 H, br s), 7.01 (3 H, br s) and 7.37 (15 H, s).

Tris-(2-{4-[N-benzyloxy-3-(t-butoxycarbonylamino)propanamido]butanamido}ethyl)amine **7b**. Similar coupling of compound **6b** (obtained from **3b** in 90% yield) gave the product **7b** as an amorphous solid in 71% yield; ν_{max}(KBr)/cm^{-1} 3275, 1710, 1695, 1655, 745 and 695; δ($CDCl_3$) 1.41 (27 H, s), 1.94 (6 H, quint, *J* 6.3), 2.20 (6 H, t, *J* 5.6), 2.5–2.7 (12 H, m), 3.2–3.35 (12 H, m), 3.6–3.8 (6 H, m), 4.80 (6 H, m), 5.36 (3 H, br s), 7.07 (3 H, br s) and 7.37 (15 H, m).

Tris-(2-{5-[N-benzyloxy-3-(t-butoxycarbonylamino)propanamido]pentanamido}ethyl)amine **7c**. Similarly compound **6c** (obtained from **3c** in 98% yield) gave the product **7c** as an oil in 74% yield; ν_{max}(KBr)/cm^{-1} 3300, 1710, 1695, 1650, 750 and 700; δ($CDCl_3$) 1.42 (27 H, s), 1.5–1.7 (12 H, m), 2.21 (6 H, t, *J* 5.4), 2.51 (6 H, t, *J* 5.4), 2.60 (6 H, t, *J* 5.1), 3.22 (6 H, q, *J* 5.1), 3.36 (6 H, q, *J* 5.4), 3.55–3.7 (6 H, m), 4.78 (6 H, s), 5.22 (3 H, br s), 6.78 (3 H, br s) and 7.37 (15 H, m).

Tris-(2-{6-[N-benzyloxy-3-(t-butoxycarbonylamino]propanamido]hexanamido}ethyl)amine **7d**. Compound **6d** (obtained from **3d** in 89% yield) gave the product **7d** as an oil in 66% yield; ν_{max}(neat)/cm^{-1} 3315, 1700, 1670, 1650, 750 and 700; δ($CDCl_3$) 1.25–1.5 (6 H, m), 1.42 (27 H, s), 1.55–1.85 (12 H, m), 2.17 (6 H, t, *J* 7.1), 2.54 (6 H, 5, *J* 5.4), 2.59 (6 H, t, *J* 5.8), 3.24 (6 H, q, *J* 5.4), 3.35 (6 H, q, *J* 5.8), 3.62 (6 H, t, *J* 6.4), 4.78 (6 H, s), 5.21 (3 H, br s), 6.62 (3 H, br s) and 7.37 (15 H, s).

General Procedure for Compounds **8a–d**: *a Typical Example, Tris-(2-{3-[3-(t-butoxycarbonylamino)-N-hydroxypropanamido]propanamido}ethyl)amine* **8a**.—A mixture containing compound **7a** (0.65 g, 0.55 mmol) and 10% Pd–C (80 mg) in

MeOH (25 cm^3) was subjected to hydrogenation with H_2 at room temperature for 10 h. After filtration of the catalyst, the solvent was evaporated to give the *product* **8a** as an amorphous solid, which was purified by gel chromatography (0.43 g, 85%) (Found: C, 48.6; H, 7.65; N, 14.6. $C_{39}H_{72}N_{10}O_{15}\cdot 2H_2O$ requires C, 48.95; H, 8.0; N, 14.65%); ν_{max}(neat)/cm^{-1} 3300, 1690 and 1645; δ([$^2H_6$]DMSO at 40 °C) 1.38 (27 H, s, CMe_3), 2.38 (6 H, t, *J* 7.1, HNCOCH_2), 2.4–2.55 [12 H, m, NCH_2 and (HO)NCOCH_2], 3.12 (6 H, t, *J* 6.8, CH_2NHCO$_2$), 3.15 (6 H, t, *J* 6.4, CH_2NHCO), 3.69 [6 H, t, *J* 7.1, CH_2(HO)NCO], 6.59 (3 H, br s, HNCO$_2$), 7.80 (3 H, br s, HNCO) and 9.64 (3 H, br s, HO).

Tris (2-{4-[3-(*t-butoxycarbonylamino*)-N-*hydroxypropanamido*]*butanamido*}*ethyl*)*amine* **8b** was similarly obtained in 94% yield (Found: C, 51.4; H, 8.15; N, 14.1. $C_{42}H_{78}N_{10}O_{15}\cdot H_2O$ requires C, 51.4; H, 8.2; N, 14.3%); ν_{max}(KBr)/cm^{-1} 3280, 1690 and 1635; δ([$^2H_6$]DMSO at 50 °C) 1.37 (27 H, s, CMe_3), 1.75 (6 H, quint, *J* 6.7, $CH_2CH_2CH_2$), 2.09 (6 H, t, *J* 7.3, HNCOCH_2), 2.4–2.6 [12 H, m, NCH_2 and (HO)NCOCH_2], 3.0–3.2 (12 H, m, CH_2NHCO and CH_2NHCO$_2$), 3.48 [6 H, t, *J* 6.8, CH_2(HO)NCO], 6.55 (3 H, br s, HNCO$_2$), 7.65 (3 H, br s, HNCO) and 9.65 (3 H, br s, HO).

Tris-(2-{5-[3-(*t-butoxycarbonylamino*)-N-*hydroxybutanamido*]*pentanamido*}*ethyl*)*amine* **8c** was similarly obtained in 77% yield (Found: C, 52.9; H, 8.4; N, 13.6. $C_{45}H_{84}N_{10}O_{15}\cdot H_2O$ requires C, 52.8; H, 8.45; N, 13.7%); ν_{max}(KBr)/cm^{-1} 3315, 1690 and 1635; δ([$^2H_6$]DMSO at 50 °C) 1.37 (27 H, s, CMe_3), 1.4–1.6 (12 H, m, $CH_2CH_2CH_2CH_2$), 2.09 (6 H, t, *J* 6.2, HNCOCH_2), 2.4–2.6 (12 H, m, NCH_2 and CH_2NHCO), 3.0–3.2 (12 H, m, CH_2NHCO and CH_2NHCO$_2$), 3.47 [6 H, t, *J* 5.9, CH_2(HO)NCO], 6.5 (3 H, br s, HNCO$_2$), 7.6 (3 H, br s, HNCO) and 9.52 (3 H, br s, HO).

Tris-(2-{6-[3-(*t-butoxycarbonylamino*)-1-*hydroxypropanamido*]*hexanamido*}*ethyl*)*amine* **8d** was similarly obtained in 68% yield (Found: C, 53.0; H, 8.5; N, 13.25. $C_{48}H_{90}N_{10}O_{15}\cdot 2H_2O$ requires C, 53.2; H, 8.75; N, 12.95%); ν_{max}(KBr)/cm^{-1} 3330, 1690 and 1640; δ([$^2H_6$]DMSO at 40 °C) 1.15–1.35 (6 H, m, $[CH_2]_2CH_2[CH_2]_2$), 1.38 (27 H, s, CMe_3), 1.45–1.6 (12 H, m, $CH_2CH_2CH_2CH_2CH_2$), 2.07 (6 H, *t*, *J* 7.5, NHCOCH_2) 2.4–2.6 [12 H, m, NCH_2 and (HO)NCOCH_2], 3.11 (6 H, t, *J* 6.8, CH_2NHCO$_2$), 3.14 (6 H, t, *J* 6.6, CH_2NHCO), 3.46 [6 H, t, *J* 7.3, CH_2(HO)NCO], 6.57 (3 H, br s, HNCO$_2$), 7.61 (3 H, br s, HNCO) and 9.53 (3 H, br s, HO).

Spectral Determination of Iron(III) *Complexes.*—The measurement was carried out by the method described previously.[1]

Iron(III)-*exchange Reactions with EDTA.*—These were carried out by the procedure reported.[1]

Biological Assay.—The biological activity of **8a–d**–iron(III) complexes was evaluated by the standard paper-disc method[2c] by using *Aureobacterium flavescens*. On nutrient agar (ATCC Medium 424) (0.7% agar) containing the strain (~10^6 cells cm^{-3}) laid over nutrient agar plates (1.5% agar) were placed filter paper discs (6 mm diam.). Each disc was impregnated with aliquots (15 mm^3) of 23 or 230 μmol dm^{-3} complex solutions in water. The diameter of halo of growth was measured after incubation at 25 °C for 4 days.

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