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Synthesis, Physico-chemical and Iron(III)-Chelating Properties of Novel Hexadentate 3-Hydroxy-2(1*H*)pyridinone Ligands

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Abstract. Synthesis of hexadentate ligands *via* the *in situ* formation of 1-hydroxy benzotriazolyl active ester in the presence of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TBTU as a coupling agent is described. The pKa values and distribution coefficient values (1-octanol/water) of the ligands and the stability constants of their iron(III) complexes are reported.

INTRODUCTION

Hexadentate siderophore analogues can be constructed by derivatizing prototype bidentate hydroxypyridinones and attaching them to suitable molecular frameworks. Hexadentate ligands based on derivatizing the ring nitrogen of 3-hydroxy-[1,2,3] and 1-hydroxy-2(1*H*)pyridinones[4] have been reported with a view to using them as therapeutic chelating agents. Raymond and co-workers have developed an alternative strategy in order to synthesize HPO analogs with superior co-ordination geometries which lead to higher affinities for iron(III).[5,6] The method involves derivatization of the ring carbon of *N*-substituted bidentate hydroxypyridinone (HPO) with a carboxy group, ortho to the phenolic group, which is then attached to a suitable molecular framework using amide linkages.[5,6] Adopting yet another approach, Streater and co-workers have reported the preparation of tripodal hexadentate ligand *N*,*N*,*N*-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (**1**). This ligand possesses a relatively low log K_1 value for iron(III), namely 28.8, a value which is lower than the log β_3

Abbreviations. DCCI, dicyclohexylcarbodiimide; DFO, desferrioxamine; D, distribution coefficient; EDTA, ethylenediaminetetraacetic acid; HPO, hydroxypyridinone; MEM, methoxyethoxymethyl; MOPS, 3-(*N*-morpholino)propanesulfonic acid; TBDMS, tertiarybutyldimethylsilyl; TBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TRAM, 1,3,5-tris(aminomethyl)benzene; TREN, tris(2-aminoethyl)amine; TRMAM, 1,3,5-tris(methylaminomethyl)benzene.

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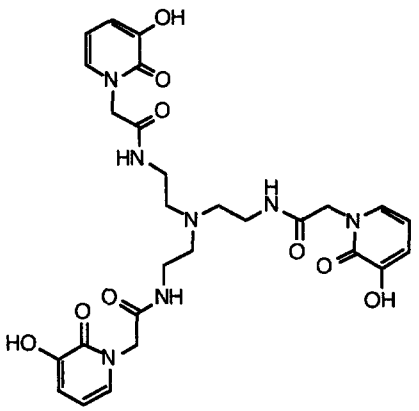

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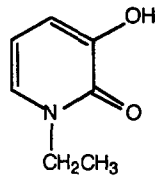

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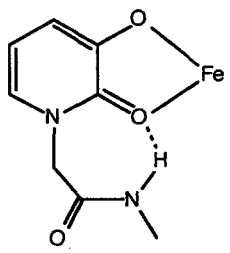
value for a corresponding bidentate analogue (**2**) 32.3.[7] The amide hydrogen atoms of the Fe(III) complex of **1** form intramolecular H-bonds between the amide and the three carbonyl oxygen atoms resulting in the formation of a seven membered ring (**3**).[8] Similar H-bonding has been observed in the Fe(III) complex of TRENCAM and enterobactin, in each case yielding a six membered ring (**4**).[9,10] In principle, the presence of such H-bonding is likely to weaken the interactions between iron(III) and chelating carbonyl oxygen atoms and therefore to possibly reduce the iron(III) binding affinity of the corresponding ligand. Such an effect offers a possible explanation for the log K_1 value of **1** for iron(III). Due to favourable entropic contributions resulting from the displacement of co-ordinated water molecules, an increase in the formation constants of up to 6 log units may be expected for a hexadentate ligand when compared to a similar bidentate unit[11] which would yield a value in the region of 36 to 38 for hexadentate pyridin-2-ones. There is therefore potential for increasing the affinity constants of hexadentate hydroxypyridin-2-ones for iron(III). If such H-bonding, as indicated in **3**, is avoided for instance by modifying the amide links, then a stronger interaction between iron(III) and chelating carbonyl oxygen atoms might be expected. Alternatively it is possible that unfavourable conformational changes occur in the hexadentate ligand upon formation of iron(III) complex. Significantly **1** lacks a preorganised conformation for metal binding. In order to establish which of the two possible explanations dominate, it was decided to synthesize two hexadentate ligands from 3-hydroxypyridin-2-ones using 1,3,5-tris(aminomethyl)benzene TRAM and 1,3.5-tris(methylaminomethyl)benzene TRMAM backbones, which have been employed in the construction of MECAM analogues.[12,13] Such a strategy provides analogous –NH and –NMe tripodal backbones for hexadentate ligands.



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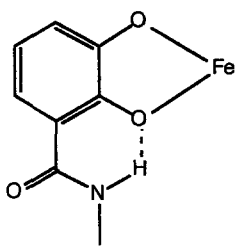

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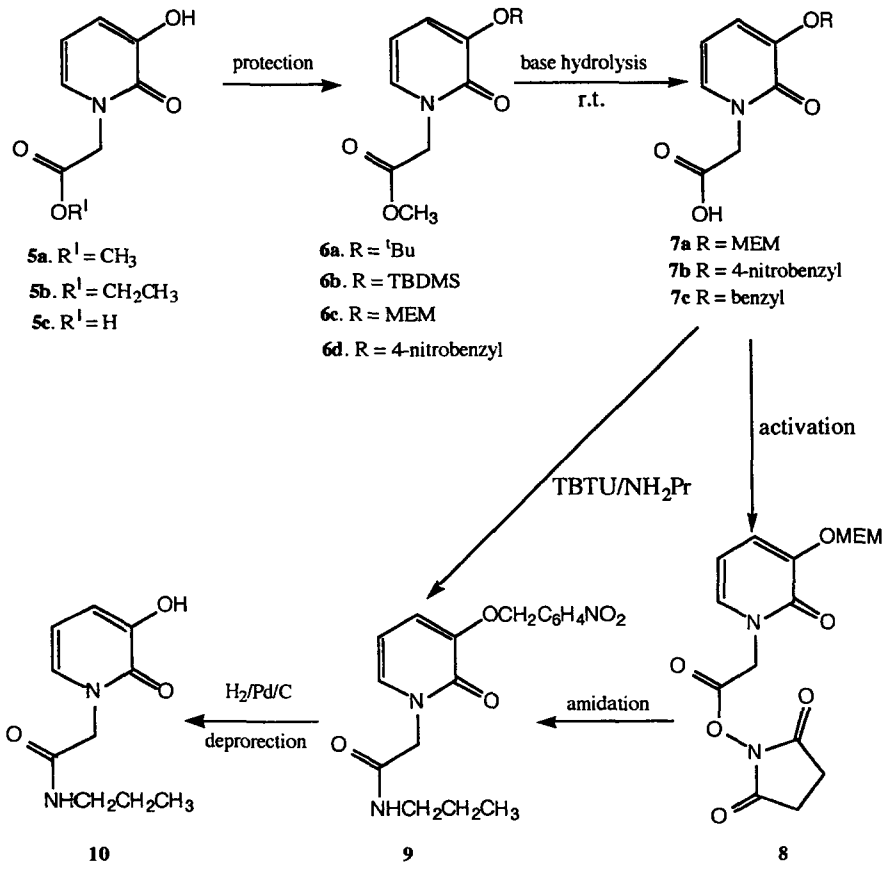
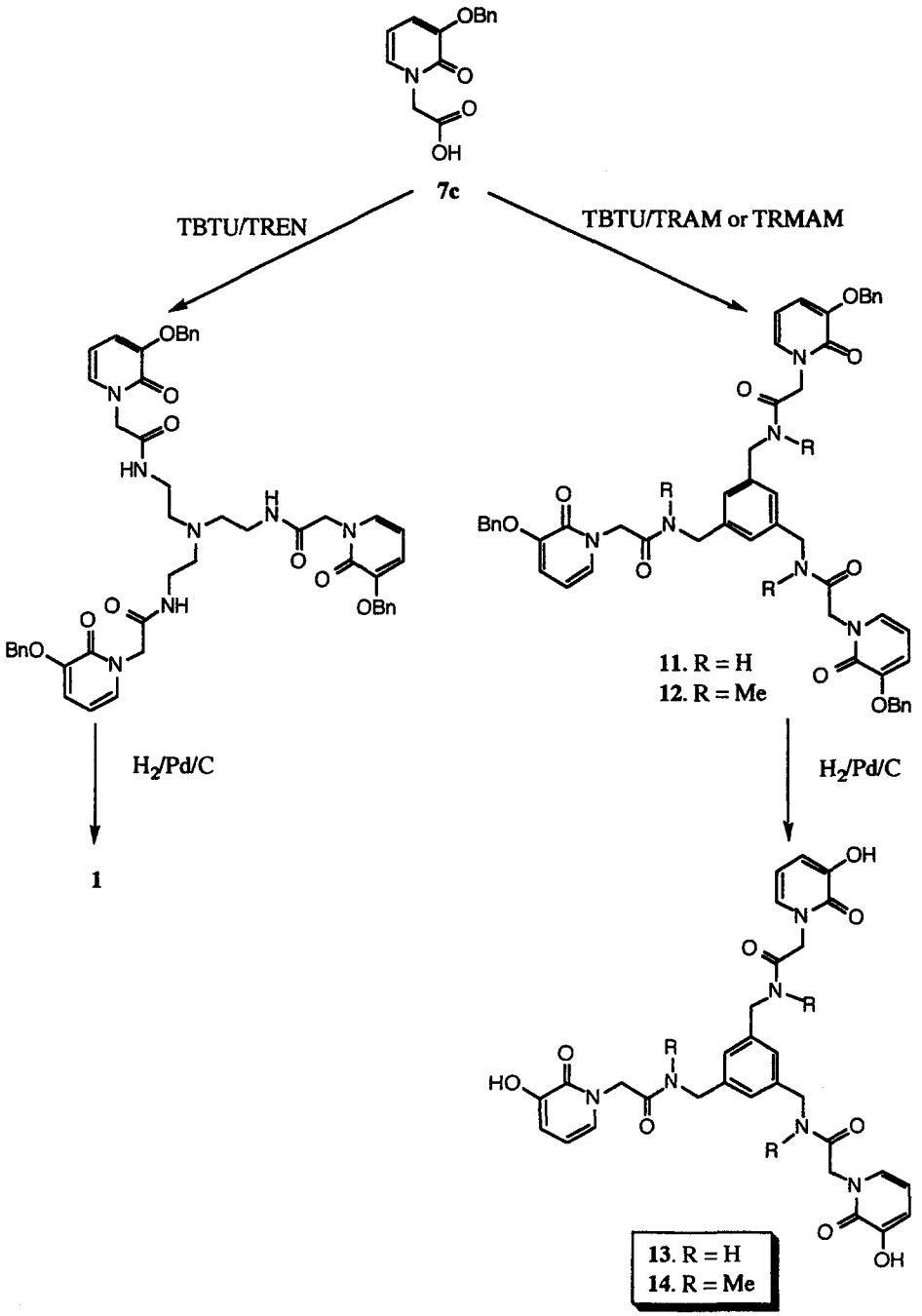
Synthesis of hexadentate 3-hydroxy-2(1*H*)pyridinone ligands

In order to optimise the yield of hexadentate ligands, different protecting groups were investigated. The behaviour of each protecting group was first examined by derivatizing a bidentate analogue **5** prior to the synthesis of hexadentate ligand in order to optimise the yield. The attempted protection of 3-hydroxyl function of the methyl ester **5a** by the ^{t}Bu group in the presence of t-butyltrichloroacetamidate in cyclohexane/dichloromethane and a catalytic amount of boron trifluoride etherate,[14] failed to give **6a** (Scheme 1).



Scheme 1

However the protection of 3-hydroxyl function of **5a** using the t-butyldimethylsilyl group with TBDMS chloride in the presence of imidazole as catalyst,[15] yielded the TBDMS- protected methyl ester **6b** as a viscous oil in a yield of 65%. However the controlled hydrolysis of **6b** was unsuccessful. Investigation for the protection of 3-hydroxyl function using the methoxyethoxymethoxy group, was attempted by reacting the methyl ester **5a** with MEM chloride in the presence of sodium hydride using dimethoxyethane as a solvent.[16] This afforded the MEM-protected ester **6c** as a viscous oil in a yield of 78%. Subsequent hydrolysis of **6c** in the presence of aqueous sodium hydroxide/methanol under conditions identical to those used for **6b** gave the MEM- protected carboxylic



Scheme 2. Synthesis of hexadentate ligands from 3-hydroxypyridin-2-ones.

acid **7a** as an oil in a yield of 93%. Activation of **7a** in the presence of DCCI and *N*-hydroxysuccinimide under the conditions as described by Streater and coworkers[7] yielded the succinimide ester **8** as a viscous oil in 22% yield. Subsequent reaction of **8** with amine did not produce a good yield of the corresponding amide. Investigation of the 4-nitrobenzyl group in the presence of 4-nitrobenzyl bromide in dimethylformamide and potassium carbonate at 50-60 °C (a modified method of Fukase *et al.*[17]), afforded the crystalline product of 4-nitrobenzyl protected methyl ester **6d** in a yield of 58%. However conversion of **7b** to the corresponding amide could not be achieved in good yield using NHSu. Coupling was also attempted *via* the *in situ* formation of 1-hydroxybenzotriazolyl activated ester by reacting **7b** with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TBTU[18] in the presence of *N*-methylmorpholine as a base and propylamine. This procedure yielded the 4-nitrobenzyl protected amide **9** in 92% yield. The 4-nitrobenzyl group was then removed by catalytic hydrogenation in the presence of Pd (5% w/w) to afford the deprotected propylamide **10**. It was planned therefore to use the 4-nitrobenzyl function as a protecting group during hexadentate ligand synthesis.

The synthesis of the hexadentate chelator **1** was investigated by reacting **7b** with TBTU, in the ratio of 3:1 with respect to the tetraamine, tris(2-aminoethyl)amine, TREN in dimethylformamide in the presence of *N*-methylmorpholine. After stirring the reaction mixture for 18 h, a precipitate was separated by filtration which was identified as the protected *N,N,N*-tris[2-(3-(4-nitrobenzyloxy)-2-oxo-1,2-dihydropyridin-1-yl)acetamido]-ethylamine. Unfortunately the crude product was difficult to crystallise and consequently the application of the 4-nitrobenzyl function offered no advantage over the substituted benzyl function previously reported by Streater and coworkers.[7] Thus the benzyl ether **7c** was finally adopted for the synthesis of the hexadentate ligands **13** and **14** (Scheme 2). 1,3,5-*N,N,N*-Tris[-*N*-((3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido)aminomethyl]-benzene **13** and 1,3,5-*N,N,N*-tris[*N*-methyl-*N*-((3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido)amino-methyl]benzene **14** were prepared using the tripodal amines 1,3,5-tris(aminomethyl)benzene, TRAM and 1,3,5-tris(methylaminomethyl)benzene, TRMAM respectively. These amines were prepared using the methodology described by Weitl and Raymond[12] and Pecoraro *et al.*[13] respectively.

RESULTS AND DISCUSSION

Ligand Properties. ***Ligand pKa Values.*** Owing to the limited aqueous solubility of hexadentate ligands ($<10^{-3}$ M at pH 7), the ligands **13** and **14** have been studied by spectrophotometric titration. For the ligand **13**, a 2.90×10^{-5} M solution in 0.1 M KCl was acidified by the addition of 0.2 M HCl and titrated with 300 μl of 0.2 M KOH. The resultant UV spectra (220-360 nm) over the pH range 4.38-10.98 are shown in Figure 1. The experimental spectrophotometric data (open circles) together with the best fit curve (λ_{max} 312 nm) over the pH range 5-11 is shown in Figure 2. The pKa value of the bidentate ligand **10** was also determined by spectrophotometric titration because this is a superior analogue of compounds 1 and **13** than the previously adopted compound **2**. The optimised pKa values obtained with the computer programme NONLIN15[19] are shown in Table 1 together with the corresponding data for the ligand **1**.[20] The pKa value for the bidentate ligand **10** corresponds to the dissociation of the hydroxyl proton. In contrast, three pKa values result from the three arms of the tripod in the hexadentate ligand series. Both the hexadentate ligands **13** and **14** are symmetrical and possess 3-point symmetry, their theoretical pKa values should therefore differ by log 3 (i.e. 0.4771). The

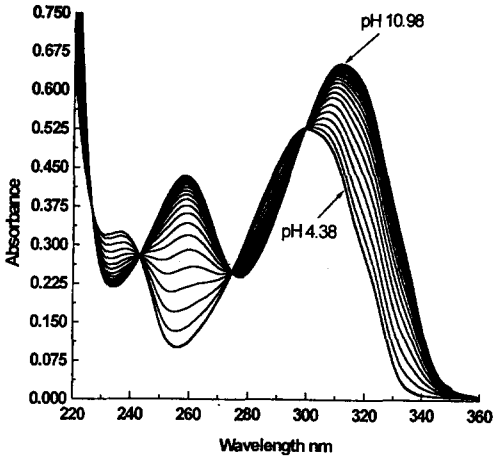


Figure 1. UV absorbance spectra (220 - 360 nm) of ligand **13** over the pH range 4.38 - 10.98.

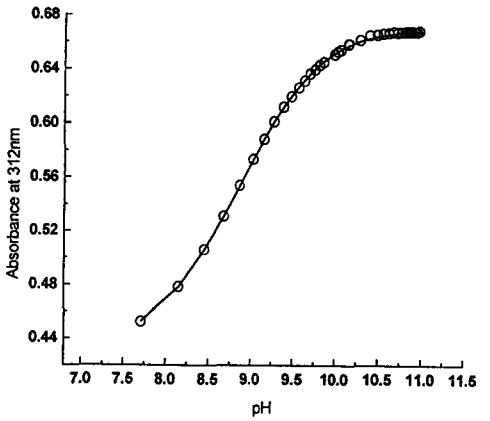


Figure 2. Spectrophotometric titration curve (λ_{max} = 312 nm) for ligand **13** over pH range 7.5 - 11.0.

observed spacing between pKa_1 and pKa_2, and between pKa_2 and pKa_3 are ~ 0.63 and ~ 0.60 for **13**. These values agree well with the observed spacing between the three identical ionisable groups of the hexadentate ligand **1** (0.55 and 0.56).[20] The larger differences between successive pKa values than that predicted by statistical

$$LH_3 \overset{pKa_1}{\rightleftharpoons} LH_2^- \overset{pKa_2}{\rightleftharpoons} LH^{-2} \overset{pKa_3}{\rightleftharpoons} L^{-3} \qquad \textbf{eq 1}$$

analysis is probably associated with the increased columbic interaction between these species (eq 1). The hexadentate ligand can be regarded as a trimer of a bidentate ligand and therefore it should possess an intrinsic site pKa similar to that of the bidentate ligand. The intrinsic site pKa of **13** (pKa_{int}) as calculated by equation 2,[21]

$$pKa_i = pKa_{int} + b(i - 2)\log 3 \qquad \textbf{eq 2}$$

is associated with the value 8.68 which is close to the pKa value of the corresponding bidentate ligand **10** namely 8.46 (Table 1). The ligand **14** gave analogous results. The spacing between the consecutive pKa values being 0.71 and 0.51. The intrinsic site pKa of **14** is 8.74 which again is comparable to bidentate analogue **10**.

The speciation plots from the derived protonation constants of the hexadentate ligands **13** and **14** (Figure 3) indicate that a significant fraction exists as the neutral species at pH 7.4 (86% for ligand **13** and 88% for ligand **14**). These values are comparable with the corresponding value for **1** which is 84%.

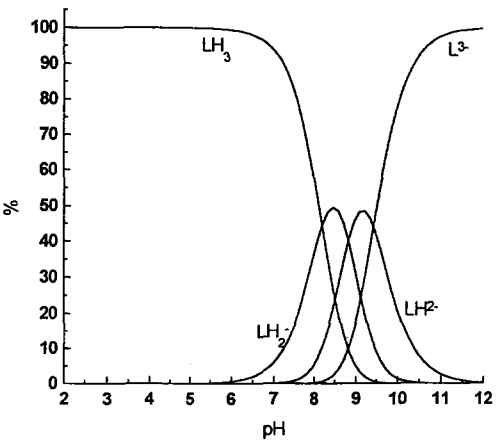


Figure 3. Speciation plot of ligand **13** over pH range 2 - 12.

The distribution coefficients for the ligands at pH 7.4 between 1-octanol/MOPS buffer were determined as 0.018±0.002, 0.030±0.002 for **13** and **14** respectively which compares well with the corresponding figure for **1**, namely 0.025. Thus the three ligands are relatively hydrophilic. This is in marked contrast to the analogous tris catecholato complexes, which possess very limited water solubilities.

Stability Constants of Iron(III) Complexes. The bidentate 3-hydroxy-2(1*H*)pyridinone ligands form a number of complexes with iron(III) so that in aqueous solution, they equilibrate to give mixtures in which the predominant species depend on the metal ion, ligand and hydrogen ion concentrations. The absolute (or cumulative) stability constant log β_3 for a bidentate ligand is obtained by summation of the logarithms of three stepwise equilibrium constants corresponding to the model shown in equation 3. This model has previously been

$$Fe^{3+} + L^- \rightleftharpoons FeL^{2+} \quad K_1$$
$$FeL^{2+} + L^- \rightleftharpoons FeL_2^+ \quad K_2$$
$$FeL_2^+ + L^- \rightleftharpoons FeL_3 \quad K_3 \qquad \textbf{eq 3}$$

Table 1. Distribution coefficients ($D_{7.4}$ values), pKa values, absolute stability constants (log β_3/K_1 values) and pM values for bidentate and hexadentate 3-hydroxy-2(1*H*)pyridinone ligands **1**, **2**, **10**, **13** and **14**.

ligand	$D_{7.4}$		pKas		log β_3/K_1	pM
	λ_{max}	free ligand	analytical wavelength λ_{max}/nm	spectrophotometric data		
2	–	1.57[7]	–	8.99±0.01[7]	32.3[7]	18.3[7]
10	285	0.59±0.001	310	8.46±0.002	29.10±0.003	17.0
1	285	0.025[7]	220	9.249±0.005, 8.686±0.005, 8.132±0.005, 5.993±0.005[20] Intrinsic site pKa 8.60[20]	28.8 [7]	25.8
13	285	0.018±0.002	312	9.43±0.008, 8.83±0.008, 8.20±0.008 Intrinsic site pKa 8.68	28.20±0.84	24.8
14	303	0.030±0.002	310	9.49±0.001, 8.98±0.001, 8.27±0.001 Intrinsic site pKa 8.74	28.70±0.64	25.1

The distribution coefficients were determined in octanol/MOPS buffer 0.01 M at pH 7.4 (n = 6), pKa values were determined spectrophotometrically for **10**, **13** and **14**; log β_3 value for **10** was determined by spectrophotometric titration, log K_1 values for **13** and **14** were determined spectrophotometrically by competition with EDTA and pM values were determined by calculating the equilibrium concentration of free hexaaquoiron(III) in a solution of pH 7.4 containing 10^{-6} M iron(III) and 10^{-5} M ligand.

shown to apply to the 3-hydroxy-2(1*H*)-pyridinones.[7] The β_3 value for the bidentate ligand **10** was obtained by spectrophotometric titration of a visible iron(III)-ligand using the automated system. This iron(III) complex was prepared in 10 : 1 molar ratio (ligand to Fe^{3+}) in acidic medium by adding 80 μl 30% HCl. The titrated data were obtained as an input to STABOPT program, a modified version of NONLIN15.[19] The determined log β_3 value was 29.1 (Table 1). This value is appreciably lower than that corresponding to **2** (Table 1), demonstrating the marked influence of the *N*-substituent on the electron density of ring and therefore the chelating ability of the 3 and 2 oxo functions. Clearly **10** is a superior bidentate analogue of the hexadentate pyridin-2-ones **1**, **13** and **14**. The hexadentate ligands only form one complex with iron(III) and the equilibrium constant corresponds to equation 4. Furthermore the extent of iron binding with the hexadentate ligand is such that the complexes are not

$$Fe^{3+} + L^{3-} \rightleftharpoons FeL \qquad K_1 \qquad \textbf{eq 4}$$

appreciably dissociated into free ligand and free metal above pH2.[7] Thus the conditional (proton dependent) stability constants of the hexadentate ligands **13** and **14** were determined spectrophotometrically by competition with EDTA at a given hydrogen ion concentration.[22] Solutions of the hexadentate ligand and $FeCl_3$ were mixed to give a concentration of ligand : Fe^{3+} in the ratio of 8 : 1 which was then titrated with EDTA. The optimised average log K_1 values of the hexadentate ligands **13** and **14** determined spectrophotometrically by competition with EDTA are presented in Table 1 together with the corresponding data for **1**.[7] The K_1 values for the ligands **1**, **13** and **14** are closely comparable, 28.8, 28.2 and 28.7 respectively.

The possibility of intramolecular H-bond formation between amide NH and the pyridinone C=O was eliminated in the ligand **14**, by replacing the amide proton by a *N*-methyl group. However no appreciable difference between the K_1 value of **13** and **14** was detected (Table 1). Thus intramolecular H-bonding is apparently not influential on the value of the iron(III) affinity constant. A similar observation was made with the ligands, MECAMS and Me_3MECAMS, the K_1 values being 41 and 40.6 respectively.[13,23] Thus the relatively low log K_1 value of **13** and **14** probably results from a lack of ligand predisposition in both cases.[7] Nevertheless, the pM values of the three hexadentate pyridinones **1**, **13** and **14** are 25.8, 24.8 and 25.1 respectively. These values are approximately 7 and 8 log units higher than those of the corresponding bidentate ligands **2** (pM value, 18.3) and **10** (pM value, 17.0) respectively and compare favourably with the corresponding value for DFO (26.6). Consequently these compounds warrant further investigation as iron chelating agents.

EXPERIMENTAL SECTION

General Procedure. Melting points are uncorrected. IR spectra are recorded on a Perkin Elmer 298. ^{1}H NMR spectra were recorded using a Perkin-Elmer R32 (90 MHz) or Bruker DRX (300 MHz) NMR Spectrometers. Mass spectra (EI) or positive ion fast atom bombardment (FAB) were recorded on a Jeol AX505W and a KRATOS MS890 MS. Elemental analysis were performed by Butterworth Laboratories Limited, Teddington, Middlesex or Micro analytical laboratories, Department of Chemistry. The University of Manchester, Manchester, M13 9PL.

1-[(Ethoxycarbonyl)methyl]-3-hydroxy-2(1H)pyridinone 5b. The compound **5b** was synthesised from 3-hydroxy-2(1*H*)-pyridinone by following the methodology as described by Streater *et al.*[7]

1-[(Methoxycarbonyl)methyl]-3-hydroxy-2(1H)pyridinone 5a. The compound **5b** (20 g, 0.1 mol) was mixed with dilute hydrochloric acid (pH 1) (200 ml) and heated under reflux for 12 h. The reaction mixture was filtered and then cooled at 0 °C for 2 h. The colourless needles formed were isolated by filtration, washed with acetone and dried to afford 1-carboxymethyl-3-hydroxy-2(1*H*)pyridinone **5c** (14.35 g, 84%); M.P. 220 °C, IR (nujol) 3220 (br, OH), 1700 (acid C=O), 1650 (pyridinone C=O), 1600 (ring C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz): δ 4.62 (2H, s, NCH_2), 6.07 (1H, t, J = 7.1, 6.9 Hz, 5–H), 6.75 (1H, dd, J = 7.1, 1.9 Hz, 4–H), 7.15 (1H, dd, J = 6.9, 1.9 Hz, 6–H), 7.0 (2H, br, 2OH). 1-Carboxymethyl-3-hydroxy-2(1*H*)pyridinone **5c** (10.2 g, 6 mmol) was mixed with distilled methanol (170 ml) saturated with hydrogen chloride gas. After refluxing the reaction mixture for 3 h, methanol was removed by rotary evaporation to give solid residue. Recrystallization from methanol yielded the compound **5a** as colourless plates (9.2 g, 83%); M.P. 160.5-161 °C; IR (nujol) 3200 (OH), 1740 (ester C=O), 1655 (pyridinone C=O), 1605 (ring C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz): δ 3.7 (3H, s, $COOCH_3$), 4.79 (2H, s, NCH_2), 6.15 (1H, t, J = 7.1, 6.9 Hz, 5–H), 6.77 (1H, dd, J = 7.1, 1.9 Hz, 4–H), 7.17 (1H, dd, J = 6.9, 1.9 Hz, 6–H), 9.11 (1H, s, OH). EIMS: m/z, 183 [$M^{+\cdot}$].

1-[(Methoxycarbonyl)methyl]-3-(4-nitrobenzyloxy)-2(1H)pyridinone 6d. To a suspension of methyl ester **5a** (5.5 g, 30 mmol) in dry dimethylformamide (100 ml) was added anhydrous potassium carbonate (4.1 g, 33 mmol) and 4-nitrobenzyl bromide (7.1 g, 33 mmol). The reaction mixture was then heated at 50-60 °C for 18 h. After removing dimethylformamide, the product was taken into dichloromethane (150 ml), washed with aqueous sodium bicarbonate (1% w/v, 3 x 100 ml), water (3 x 50 ml), dried over anhydrous sodium sulphate and filtered. Removal of the solvent under reduced pressure yielded yellow solid. Recrystallization from methanol afforded the compound **6d** as yellow crystals (5.5 g, 58%); M.P. 139-140 °C; IR (nujol) 1740 (ester C=O), 1660 (pyridinone C=O), 1600 (ring C=C), 1515 and 1345 (nitro N=O) cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz): δ 3.7 (3H, s, $COOCH_3$), 4.76 (2H, s, NCH_2), 5.22 (2H, s, $\mathbf{CH_2}C_6H_4NO_2$), 6.2 (1H, t, J = 7.1, 6.9 Hz, 5–H), 7.04 (1H, dd, J = 7.1, 1.9 Hz, 4–H), 7.34 (1H, dd, J = 6.9, 1.9 Hz, 6–H), 7.74 (2H, d, ArH, meta to NO_2), 8.26 (2H, d, ArH, ortho to NO_2); EIMS: m/z, 318 [$M^{+\cdot}$]; Anal. Calcd. for $C_{15}H_{14}N_2O_6$: C, 56.60; H, 4.43; N, 8.80 Found C, 56.51; H, 4.39; N, 8.73%.

1-[(Propylcarbamoyl)methyl]-3-hydroxy-2(1H)pyridinone 10. The 4-nitrobenzyloxy ester **6d** (2.1 g, 6.7 mmol) was mixed with methanol (50 ml) and aqueous sodium hydroxide (1 M. 50 ml) and was allowed to stir for 3 h at room temperature. Methanol was removed under reduced pressure, the aqueous solution was adjusted to pH 1 by the addition of concentrated hydrochloric acid. The solid precipitate formed was removed by filtration and recrystallised from ethanol to give pale yellow crystals of 1-carboxymethyl-3-(4-nitrobenzyloxy)-2(1*H*)pyridinone (7b) (1.8 g, 90%); M.P. 217-219 °C; IR (nujol) 1740 (acid C=O), 1655 (pyridinone C=O), 1585 (ring C=C), 1525 and 1340 (nitro N=O) cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz): δ 4.67 (2H, s, NCH_2), 5.22 (2H, s, $\mathbf{CH_2}C_6H_4NO_2$), 6.18 (1H, t, J = 7.1, 6.9 Hz, 5–H), 7.02 (1H, dd, J = 7.1, 1.9 Hz, 4–H), 7.24 (1H, dd, J = 6.9, 1.9 Hz, 6–H), 7.74 (2H, d, ArH, meta to NO_2), 8.26 (2H, d. ArH, ortho to NO_2); EIMS: m/z, 304 [$M^{+\cdot}$].

N-Methylmorpholine (0.8 g, 8 mmol) was added to a solution of 1-carboxymethyl-3-(4-nitrobenzyloxy)-2(1*H*)pyridinone 7b (1.2 g, 4 mmol) in dimethylformamide (50 ml) followed by the addition of 1-(1*H*-

benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 1.3 g, 4 mmol) under an atmosphere of nitrogen. After stirring the reaction mixture for 20 min at room temperature, propylamine (0.24 g, 4.2 mmol) was added dropwise and the mixture was stirred for 4 h. Dimethylformamide was removed under high vacuum and the product was taken into dichloromethane (50 ml). The organic fraction was washed with 5% aqueous sodium hydroxide (3 x 25 ml) and water (2 x 25 ml), dried over anhydrous sodium sulphate, filtered and concentrated to dryness by rotary evaporation to give solid residue. Recrystallization from absolute ethanol afforded **9** as colourless crystals (1.2 g, 92%); M.P. 192-193 °C; IR (nujol) 3270 (amide NH), 1650 (amide C=O), 1600 (ring C=C), 1520 and 1370 (nitro N=O) cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz): δ 0.85 (3H, t, $CH_2CH_2\mathbf{CH_3}$), 1.2-1.7 (2H, m, $CH_2\mathbf{CH_2}CH_3$), 3.05 (2H, q, $\mathbf{CH_2}CH_2CH_3$ coupled with NH), 4.56 (2H, s, NCH_2), 5.2 (2H, s, $\mathbf{CH_2}C_6H_4NO_2$), 6.12 (1H, t, J = 7.1, 6.9 Hz, 5–H), 6.95 (1H, dd, J = 7.1, 1.9, Hz, 4–H), 7.25 (1H, dd, J = 6.9, 1.9 Hz, 6–H), 7.74 (2H, d, ArH, meta to NO_2), 8.13 (1H, t, J = 5.4 Hz, CONH), 8.26 (2H, d, ArH, ortho to NO_2); Anal. Calcd: $C_{17}H_{19}N_3O_5$: C, 59.12; H, 5.55; N, 12.17 Found C, 59.29; H, 5.57; N, 12.01%.
The 4-nitrobenzyl protected amide **9** (1 g) in ethanol (50 ml) was hydrogenated over 5% Pd/C catalyst (100 mg) in the presence of catalytic amount of hydrochloric acid for 5 h. After filtration the solvent was removed by rotary evaporation to give oily product which was triturated with ethanol to give solid. Recrystallization from absolute ethanol afforded **10** as colourless plates (0.44 g, 75%), M.P. 207-208 °C [Lit,[7] 204-205 °C]; IR (nujol) 3280 (amide NH), 1650 (amide C=O), 1590 (ring C=C), cm^{-1}; ^1H NMR (CD_3OD, 90 MHz): δ 0.91 (3H, t, $CH_2CH_2\mathbf{CH_3}$), 1.3-1.75 (2H, m, $CH_2\mathbf{CH_2}CH_3$), 3.16 (2H, t, $\mathbf{CH_2}CH_2CH_3$), 4.63 (2H, s, NCH_2), 6.22 (1H, t, J = 7.1, 6.9 Hz, 5–H), 6.85 (1H, dd, J = 7.1, 1.9 Hz, 4–H), 7.05 (1H, dd, J = 6.9, 1.9 Hz, 6–H).

3-Benzyloxy-1-carboxymethyl-2(1H)-pyridinone ***7c***. The compound **7c** was prepared from **5b** as described by Streater *et al.*[7]

N,N,N-Tris[2-(3-hydroxy-2-oxo-1,2-dihydroxypyridin-1-yl)acetamido]-ethylamine ***1***. 3-Benzyloxy-1-carboxymethyl-2(1*H*)-pyridinone **7c** (3.9 g, 15 mmol) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 5.14 g, 16 mmol) were dissolved in dimethylformamide (100 ml) under nitrogen atmosphere and *N*-methylmorpholine (3 g, 30 mmol) was added. The corresponding active ester was immediately formed and the colour of the reaction mixture changed from colourless to light brown. After stirring for 20 min at room temperature, tris(2-aminoethyl)amine (TREN) (Fluka, 0.73 g, 5 mmol) was added. The mixture was then stirred at room temperature for 18 h. Dimethylformamide was removed under high vacuum and the product was taken into dichloromethane (100 ml), washed with 5% aqueous hydrochloric acid (2 x 50 ml), 5% aqueous sodium hydroxide (3 x 50 ml) and water (2 x 50 ml), dried over anhydrous sodium sulphate and filtered. The solvent was removed by rotary evaporation to give a colourless oil which solidified on standing at room temperature. Recrystallization from 95% ethanol yielded colourless crystals of *N,N,N*-tris[2-(3-benzyloxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (2.85 g, 66%); M.P. 163–164.5 °C; IR (KBr): 3311 (amide NH), 1652 (amide C=O), 1597 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz): δ 3.14 (6H, br, s, $CH_2\mathbf{CH_2}N$), 3.33 (6H, br, s, $\mathbf{CH_2}CH_2N$), 4.56 (6H, s, NCH_2CO), 4.99 (6H, s, CH_2Ph), 6.07 (3H, t, 5–H), 6.9 (3H, br. d, 4–H), 7.15 (3H, br, d, 6–H), 7.24–7.6 (15H, m, ArH), 8.04 (3H, br, t, NH); FABMS: m/z,

870 [M$^+$]; Anal Calcd. for $C_{48}H_{51}O_9N_7.1H_2O$: C, 64.93; H, 6.02; N, 11.04 Found C, 64.81; H, 6.06; N, 10.82%.

All the glassware used for hydrogenation reaction was washed with 2 M aqueous hydrochloric acid. *N,N,N*-Tris[2-(3-benzyloxy-2-oxo-1,2-dihydroxypyridin-1-yl)-acetamido]ethylamine (1.7 g, 2 mmol) was hydrogenated in ethanol (100 ml) and water (10 ml) in the presence of glacial acetic acid (2 ml) and 5% Pd on carbon catalyst (500 mg) for 18 h. The solution was filtered and evaporated to dryness. Recrystallization from absolute ethanol afforded the compound **1** as colourless powder (0.75 g, 65%); M.P. 181-183 °C [Lit, [7] 178–180 °C]; IR (KBr): 3268 (amide NH), 1654 (amide C=O), 1578 (C=C), 1549 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.31 (6H, br, s, CH_2N), 3.50 (6H, br, s, NHC**H**$_2$), 4.59 (6H, s, NCH_2CO), 6.09 (3H, t, J = 7.0, 6.9 Hz, 5–H), 6.73 (3H, d, 4–H), 7.09 (3H, d, 6–H), 8.54 (3H, br, s, NH), 9.07 (3H, br, s, OH); FABMS: m/z, 600 [M$^+$].

1,3,5-Triaminomethylbenzene trihydrochloride, TRAM **and** ***1,3,5-Tris[(N-methylamino)-methyl]benzene, TRMAM.*** The tripodal triamines TRAM and TRMAM were prepared as described by Weitl and Raymond[12] and Pecoraro *et al.*[13] respectively.

1,3,5-N,N,N-Tris[-N-((3-benzyloxy-2-oxo-1,2-dihydropyridin-1yl)acetamido)aminomethyl]-benzene 11. 3-Benzyloxy-1-carboxymethyl-2-(1*H*)-pyridinone **7c** (1.56 g, 6 mmol) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 1.93 g, 6 mmol) were dissolved in dimethylformamide (50 ml) and *N*-methylmorpholine (1.2 g, 12 mmol) was added under nitrogen, the resulting solution was stirred at room temperature for 30 min. 1,3,5-triaminomethylbenzene trihydrochloride, TRAM (0.55 g, 2 mmol) was added, followed by the dropwise addition of *N*-methylmorpholine (1.82 g, 18 mmol). After stirring at room temperature for 3 h, the reaction mixture was stirred at 60 °C for 20 h. Dimethylformamide was removed under high vacuum. The product was taken into chloroform (500 ml). The organic fraction was washed with 5% aqueous hydrochloric acid (2 x 100 ml), 5% aqueous sodium hydroxide (3 x 100 ml), water (100 ml), and brine (100 ml), dried over anhydrous sodium sulphate and filtered. The solvent was removed by rotary evaporation to give white solid. This solid was dissolved in a mixture of methanol and chloroform and finally crystallized by the addition of diethyl ether to afford **11** as white crystals (1.14 g, 64%); M.P. 235 °C; tlc (silica gel, MeOH : $CHCl_3$; 10 : 90; only one spot, Rf = 0.34); IR (KBr): 3267 (amide NH), 1654 (amide C=O), 1594 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.28 (6H, d, J = 5.7 Hz, $NHCH_2$), 4.62 (6H, s, NCH_2), 4.99 (6H, s, CH_2Ph), 6.08 (3H, t, J = 7.2, 6.9 Hz, 5–H), 6.89 (3H, d, 4–H), 7.08 (3H, s, ArH–central ring), 7.20 (3H, d, 6–H), 7.41–7.30 (15H, m, ArH), 8.65 (3H, t, J = 5.7, 5.4 Hz, NH); FABMS: m/z, 889 [M$^+$]; Anal. Calcd. for $C_{51}H_{48}N_6O_9$: C, 68.91; H, 5.44; N, 9.45 Found C, 69.00; H, 5.18; N, 9.56%.

1,3,5-N,N,N-Tris[N-methyl-N-((3-benzyloxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido)-aminomethyl]benzene 12. In an analogous procedure as in the preparation of **11**, use of 3-benzyloxy-1-carboxymethyl-2-(1*H*)-pyridinone **7c** (1.5 g, 6 mmol), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 1.93 g, 6 mmol) in dimethylformamide in the presence of *N*-methylmorpholine (2.2 g, 30 mmol) and 1,3,5-tris-[(*N*-methylamino)methyl]benzene trihydrochloride, TRMAM (0.63 g, 2 mmol) gave a viscous substance (1.9 g, 100%) on work up. The crude material was purified by column chromatography on

alumina (neutral aluminium oxide Fluka, eluent; EtOH : $CHCl_3$; 2 : 98; Rf = 0.37). Recrystallization from $CHCl_3/Et_2O$ afforded **12** as colourless power (1.23 g, 67%); M.P. 158–160 °C ; IR (KBr): 1656 (amide C=O), 1603 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.75–2.81 and 2.98–3.05 (9H, m, CH_3N), 4.49–4.66 (6H, unresolved m, $ArCH_2$), 4.85–4.97 (6H, m, NCH_2), 4.98 (6H, s, CH_2Ph), 6.02–6.09 (3H, m, 5–H), 6.87–7.19 (9H, complex m, ArH including central ring), 7.31–7.38 (15H, m, ArH); FABMS: m/z, 931 [M^+]; Anal. Calcd. for $C_{54}H_{54}N_6O_6.1H_2O$: C, 68.34; H, 5.94; N, 8.85 Found C, 68.59; H, 5.73; N, 8.73%.

1,3,5-N,N,N-Tris[-N-((3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido)aminomethyl]-benzene 13. All the glassware used for hydrogenation reaction was washed with 2 M aqueous hydrochloric acid, 1,3,5-*N*,*N*,*N*-tris[-*N*-((3-benzyloxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido)aminomethyl]benzene **11** (506 mg, 0.57 mmol) was dissolved in dimethylformamide (100 ml). The mixture was then hydrogenated in the presence of catalytic amount of 2 M hydrochloric acid and ~150 mg of 5% Pd/C catalyst for 18 h. The solution was filtered through a glass microfibre Whatman filter paper to remove the catalyst. Dimethylformamide was removed under high vacuum to give colourless solid. Recrystallization from methanol/water furnished the compound **13** as colourless crystals (234 mg, 67%); D.P. 175 °C; IR (KBr): 3278 (amide NH), 1655 (amide C=O), 1594 (C=C), 1554 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 4.28 (6H, d, J = 5.4 Hz, $CONHCH_2$), 4.64 (6H, s, NCH_2), 6.06 (3H, t, J = 6.9 Hz, 5–H), 6.69 (3H, dd, J = 7.2, 1.5 Hz, 4–H), 7.05–7.10 (6H, m, 6–H and ArH, central), 8.62 (3H, t, J = 5.7 Hz, CONH), 8.94 (3H, s, 3OH); Anal. Calcd. for $C_{30}H_{30}N_6O_9,0.75H_2O$: C, 57.00; H, 5.02; N, 13.29 Found C, 57.03; H, 5.21; N, 13.31%.

1,3,5-N,N,N-Tris[N-methyl-N-((3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido)-aminomethyl]benzene 14. Hydrogenation procedure was carried out as described in the preparation of **13** using 1,3,5-*N*,*N*,*N*-Tris[*N*-methyl-*N*-((3-benzyloxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido)aminomethyl]-benzene **12** (372 mg, 4 mmol) in dimethylformamide in the presence of Pd/C catalyst (~140 mg) under acidic condition which yielded a colourless solid on work up. Recrystallization from methanol/water furnished the compound **14** as colourless power in a yield of (161 mg, 60%); D.P. 160 °C; IR (KBr): 3278 (amide NH), 1655 (amide C=O), 1594 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.78– 2.80 and 3.02–3.09 (9H, m, CH_3N), 4.50–4.68 (6H, unresolved m, $ArCH_2$), 4.88–4.94 (6H, m, NCH_2), 6.04 (3H, t, J = 7.2, 6.9 Hz, 5–H), 6.68 (3H, d, 4–H), 6.98–7.11 (3H, m, Ar-central), 7.02 (3H, d, 6–H), 8.86 (3H, br, s, OH); FABMS: m/z, 661 [M^+]: Anal. Calcd. for $C_{33}H_{36}N_6O_9,3H_2O$: C, 55.46; H, 5.92; N, 11.72 Found C, 55.54; H, 5.72: N, 11.39%.

Automated computerised titration system for the determination of pKas and stability constants. The system utilises a combined automated spectrophotometric and potentiometric system controlled by an opus V286 computer. The programme was coded in Quic Basic ® v4.5.[19] This comprises a Corning Delta 255 pH meter, a Perkin-Elmer Lambda 5 UV/visible spectrophotometer and an autoburette interfaced to a PC computer. A blank titration of 0.1 M KCl 25 ml was carried out to determine the electrode zero using Gran's plot method.[24] A combined SIRIUS pH electrode, was used to calibrate the electrode zero. The solution (0.1 M KCl,

25 ml) contained in a jacketed titration cell, was acidified by 0.15 ml 0.2 M HCl. Titrations were carried out against 0.3 ml, 0.2 M KOH using 0.01 ml increments dispensed from a Metrohm 665 dosimat. Solutions were maintained at 25 ± 0.1 °C under an argon atmosphere. The above titration was repeated in the presence of ligand. The data obtained from titrations were analysed by the TITRFIT program, a modified version of NONLIN15.[19]

Competition studies with EDTA. The experimental conditions for the competition studies reported here ensured that the most associated species predominated. Absorbance at 525 nm was monitored for several hours until no further change was observed in order to establish that equilibrium was achieved. The value of Z was measured from equation 5, where A = absorbance of the competing system at equilibrium, A_{min} = absorbance of

$$Z = (A - A_{min}) / (A_{max} - A_{min}) \qquad \textbf{eq 5}$$

$$Fe + nLH \rightleftharpoons FeL^n + nH \qquad \textbf{eq 6}$$

FeEDTA in the absence of the sample ligand and A_{max} = absorbance of FeL_n in the absence of EDTA. The absolute stability constant of the equilibrium, (eq 6, charges omitted for clarity) is given by equation 7, where n is the number of ligands in the metal complex. E_T, L_T and M_T are total analytical concentrations of EDTA, ligand and

$$K_L = [Z / (1 - Z)][(E_T - (1 - Z)M_T) / (L_T - nZM_T)^n](\alpha_L{}^n / \alpha_E)K_E \qquad \textbf{eq 7}$$

$\alpha_L{}^n$ and α_E have the form $\alpha_L{}^n = 1 + \sum_{l=1}^{n} h^l / \prod_{j=1}^{l} Ka_j$ and $\alpha_E = 1 + \sum_{i=1}^{m} h^i / \prod_{j=1}^{i} Ka_j$, and h = hydrogen ion concentration, Ka_j are the three acid dissociation constants (n = 3) of pyridinone moieties or the four acid dissociation constants (m = 4) of EDTA.[26]

metal ion, respectively, and K_E is the absolute stability constant of EDTA complexed with iron(III). Experiments were performed at pH 7.5. Data were included in the final calculation when the Z value was between 0.2 and 0.8. The resulting spectrophotometric data were inserted into the COMPTI[25] a modified version of NONLIN15[19] to evaluate the affinity constants of the complex. The absolute stability constant log K_1 of the sample ligand was then calculated using the pKa values of the ligand and the literature value of the stability constant of EDTA with Fe(III) together with the pKa values of EDTA.[26]

Distribution coefficient of the free ligands were determined in 1-octanol/MOPS buffer pH 7.4 system using a filter probe device as described by Rai and coworkers.[27] The aqueous phase, 0.1M MOPS buffer pH 7.4 was saturated with the octanol phase before use.

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