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Synthesis and iron chelating properties of hydroxypyridinone and hydroxypyranone hexadentate ligands

Tao Zhou,*[a] Xiao-Le Kong[b] and Robert C Hider *[b]

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Chelation therapy has become an important therapeutic approach for some diseases. In attempt to identify clinically useful chelators, four hexadentate ligands were synthesized by conjugating the corresponding bidentate ligands (3-hydroxypyridin-4-one (3,4-HOPO), 3-hydroxypyridin-2-one (3,2-HOPO), 1-hydroxypyridin-2-one (1,2-HOPO), and 3-hydroxypyran-4-one) each with a free amino group to a tripodal acid. Their pK_a values and affinities for iron(III) were investigated. The pFe^{3+} values of the hexadentate pyridinones **1** (3,4-HOPO), **3** (3,2-HOPO) and **4** (1,2-HOPO), and the pyranone **2** was found to follow the sequence **1** > **4** ≫ **3** > **2**, which is different to the pFe^{3+} value sequence of the corresponding bidentate forms (3,4-HOPO ≫ 3,2-HOPO > 1,2-HOPO > 3-hydroxypyranone). Hexadentate 3,4-HOPOs and 1,2-HOPOs have the greatest potential as iron scavenging agents.

Introduction

Metal ions play important roles in living processes, and thus chelation therapy has become an important therapeutic approach for some diseases.[1] One example is the therapeutic application of iron chelators for the treatment of iron overload disorders associated with β-thalassaemia and sickle cell anaemia. Thus deferioxamine B (DFO),[2] deferiprone[3,4] and deferasirox[5,6] are each widely used for this purpose. Iron chelators also have potential for the treatment of microbial infections[7–10] and neurodegenerative disorders,[11] such as Parkinson's disease,[12] Alzheimer's disease[13] and Friedreich's Ataxia.[14] A wide range of bidentate chelators, such as 3-hydroxypyridin-4-one (3,4-HOPO), 1-hydroxypyridin-2-one (1,2-HOPO), 3-hydroxypyridin-2-one (3,2-HOPO), 3-hydroxypyranone, catechol and hydroxamate (Fig. 1) possess relatively high affinities for iron(III).[15] Hydroxypyridinones (HOPOs) combine the characteristics of both hydroxamate and catechol groups, forming 5-membered chelate rings in which the metal is coordinated by two vicinal oxygen atoms.[16] Among the three classes of HOPOs, 3,4-HOPO possesses the highest affinity for iron(III) ($\log K_1$ = 14.2, $\log \beta_3$ = 37.2), 3,2-HOPO the next ($\log K_1$ = 11.7, $\log \beta_3$ = 32), and 1,2-HOPO the lowest ($\log K_1$ = 10.3, $\log \beta_3$ = 27).[16] 3-Hydroxypyranones, for instance maltol, possess a relatively low $\log \beta_3$ value for iron(III), typically 28. However, the metal affinity of their hexadentate analogues has not been reported. In order to facilitate a direct comparison of iron(III) affinity constants, hexadentate ligands of these four bidentate ligands were synthesized by conjugation onto the same tripodal scaffold (Fig. 2).

Experimental section

General

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer with TMS as an internal standard. Electrospray ionization (ESI) mass spectra were obtained by infusing samples into an LCQ Deca XP ion trap instrument. High resolution mass spectra (HRMS) were determined on a QTOFMicro (Waters, USA) by direct infusing samples into the

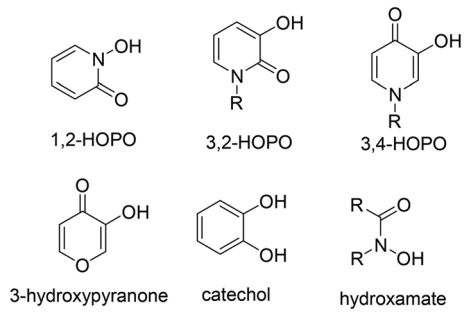


Fig. 1 Bidentate metal ligands.

[a]School of Food Science and Biotechnology, Zhejiang Gongshang University, 18 Xuezheng Street, Xiasha, Hangzhou, Zhejiang 310018, P. R. China. E-mail: taozhou@zjgsu.edu.cn
[b]Institute of Pharmaceutical Sciences, King's College London, 150 Stamford Street, London, SE1 9NH, UK. E-mail: robert.hider@kcl.ac.uk

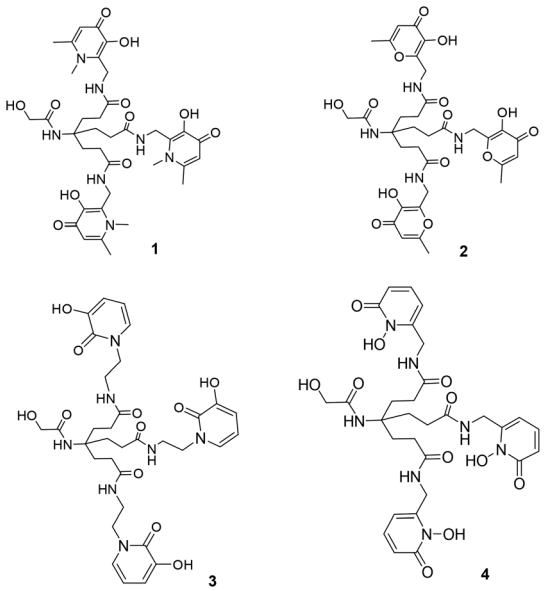


Fig. 2 Structures of hexadentate ligands (**1**–**4**).

ESI source. Di-*tert*butyl 4-amino-4-[2-(*tert*-butoxycarbonyl)ethyl]-heptanedioate (**5**) was prepared as reported.[17] 2-(Aminomethyl)-3-(benzyloxy)-1,6-dimethylpyridin-4(1*H*)-one (**8**) and 3-(benzyloxy)-2-(hydroxymethyl)-6-methyl-4*H*-pyran-4-one (**10**) were prepared according to previously published work.[18] All other chemicals were purchased from Aldrich or Merck Chemical Company and used without any further purification.

Synthetic procedures

Di-*tert*-butyl-4-(2-(benzyloxy)acetamido)-4-(3-*tert*-butoxy-3-oxopropyl)heptanedioate (6). To a mixture of amine **5** (14.94 g, 36 mmol), triethylamine (3.636 g, 36 mmol) and anhydrous dichloromethane (150 mL) cooled in an ice-bath was added dropwise 2-(benzyloxy)acetyl chloride (6.092 g, 33 mmol). The mixture was stirred at room temperature for 6 h. Then the reactant was washed with cold 10%HCl, brine, 10% $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by chromatography using EtOAc/hexane (1 : 4 to 1 : 2) to provide product **6** (16.7 g, 89.8%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 27H, CH_3), 1.98 (m, 6H, CH_2), 2.18 (m, 6H, CH_2), 3.87 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 6.32 (s, 1H, NH), 7.32–7.38 (m, 5H, Ph). ESI-MS: *m*/*z* 564 ($[M + H]^+$).

4-(2-(Benzyloxy)acetamido)-4-(2-carboxyethyl)heptanedioic acid (7). A solution of **6** (15.6 g) in 96% formic acid (50 mL) was stirred at room temperature overnight. After removal of the solvent, product **7** was obtained as a white solid in quantitative yield. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.86 (m, 6H, CH_2), 2.13 (m, 6H, CH_2), 3.85 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 7.01 (s, 1H, NH), 7.27–7.38 (m, 5H, Ph), 12.12 (br, 3H, COOH). ESI-MS: *m*/*z* 396 ($[M + H]^+$), 418 ($[M + Na]^+$).

***N*^{1},*N*^{7}-Bis((3-(benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-(3-((3-(benzyloxy)-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methylamino)-3-oxopropyl)-4-(2-(benzyloxy)acetamido)heptanediamide (9).** A mixture of **7** (1.58 g, 4 mmol), amine **8** (3.715 g, 14.4 mmol), HOBt (1.946 g, 14.4 mmol), DCC (2.966 g, 14.4 mmol) and DMF (25 ml) was stirred at room temperature for 2 days. After removal of the solvent, the residue was purified by chromatography using $CHCl_3$/MeOH (8 : 1 to 4 : 1) to provide product **9** (3.8 g, 85% yield) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.83 (m, 6H, CH_2), 2.05 (m, 6H, CH_2), 2.24 (s, 9H, CH_3), 3.38 (s, 9H, CH_3), 3.81 (s, 2H, CH_2), 4.33 (d, *J* = 4.8 Hz, 6H, CH_2), 4.49 (s, 2H, CH_2), 5.06 (s, 6H, CH_2), 6.18 (s, 3H, C5–H in pyridinone), 6.98 (s, 1H, NH), 7.23–7.43 (m, 20H, Ph), 8.08 (t, *J* = 4.8 Hz, 3H, NH). ESI-MS: *m*/*z* 1116.5 ($[M + H]^+$), 558.8 ($[M + 2H]^{2+}$).

***N*^{1},*N*^{7}-Bis((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl)-4-(3-((3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methylamino)-3-oxopropyl)-4-(2-hydroxyacetamido)heptanediamide trihydrochloride (1).** A mixture of **9** (1.85 g), 5% Pd/C (0.6 g), 1.25 M HCl methanolic solution (3 ml) and methanol (50 ml) was shaken under an atmosphere of hydrogen (30 psi) at room temperature for 2 days. After filtration, the solvent was removed under reduced pressure, the residue was redissolved in methanol (5 ml), acetone was added to form a white precipitate, and left at 4 °C overnight. The white product **1** was isolated as a trihydrochloric acid salt (1.32 g, 92% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.85 (m, 6H, CH_2), 2.10 (m, 6H, CH_2), 2.57 (s, 9H, CH_3), 3.69 (s, 2H, CH_2), 3.88 (s, 9H, CH_3), 4.56 (d, *J* = 5.1 Hz, 6H, CH_2), 7.26 (s, 3H, C5–H in pyridinone), 7.34 (s, 1H, NH), 8.90 (t, *J* = 5.1 Hz, 3H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 20.96 (*C*H_3), 29.59 (*C*H_2), 30.50 *C*H_2), 31.06 (*C*H_3), 35.09 *C*H_2), 57.08 (*C*), 61.81 *C*H_2), 113.08 (*C*–5H in pyridinone), 140.23(*C*-2 in pyridinone), 143.06 (*C*-3 in pyridinone), 148.93 (*C*-6 in pyridinone), 159.95 (*C*-4 in pyridinone), 171.64 (*C*O), 173.63 (*C*O). MS (ESI): *m*/*z* 756.3 ($[M + H]^+$), 378.7 ($[M + 2H]^{2+}$); HRMS: calcd for $C_{36}H_{50}N_7O_{11}$ 756.3568 ($[M + H]^+$), 378.6824 ($[M + 2H]^{2+}$); found 756.3560, 378.6814, respectively.

***N*^{1},*N*^{7}-Bis((3-(benzyloxy)-6-methyl-4-oxo-4*H*-pyran-2-yl)methyl)-4-(3-((3-(benzyloxy)-6-methyl-4-oxo-4*H*-pyran-2-yl)methylamino)-3-oxopropyl)-4-(2-(benzyloxy)acetamido)heptanediamide (12).** To a mixture of triacid **7** (1.58 g, 4 mmol), amine **11** (3.528 g, 14.4 mmol) and DMF (25 ml) cooled to 20 °C was added DIPEA (3.722 g, 28.8 mmol). Then HCTU (5.957 g, 14.4 mmol) was added to this stirred solution in three portions. The stirring was continued overnight. The reactant was concentrated and the residue was purified by chromatography using EtOAc/MeOH (9 : 1 to 4 : 1) to provide product **12** (4.15 g, 96.3% yield) as a pale yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96 (m, 6H, CH_2), 2.03 (m, 6H, CH_2), 2.19 (s, 9H, CH_3), 3.86 (s, 2H, CH_2), 4.12 (d, *J* = 5.9 Hz, 6H, CH_2), 4.56 (s, 2H, CH_2), 5.16 (s, 6H, CH_2), 5.71 (t, *J* = 5.9 Hz, 3H, NH), 6.15 (s, 3H, C5–H in pyridinone), 6.57 (s, 1H, NH), 7.30–7.38 (m, 20H, Ph). ESI-MS: *m*/*z* 1077.4 ($[M + H]^+$), 1099.3 ($[M + Na]^+$).

***N*^{1},*N*^{7}-Bis((3-hydroxy-6-methyl-4-oxo-4*H*-pyran-2-yl)methyl)-4-(3-((3-hydroxy-6-methyl-4-oxo-4*H*-pyran-2-yl)methylamino)-3-oxopropyl)-4-(2-hydroxyacetamido)heptanediamide (2).** To a solution of **12** (1.56 g) in anhydrous DCM (20 ml) cooled with ice-bath under nitrogen was added dropwise boron trichloride

(1 M in DCM, 17.4 ml). The mixture was stirred at room temperature for 2 days. The reaction was quenched with methanol. After removal of the solvent, the residue was redissolved in methanol (5 ml). Acetone was added to form a precipitate which was left in a fridge overnight. The product **2** was obtained as a pale yellow solid (0.97 g, 93% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.73 (m, 6H, CH_2), 1.95 (m, 6H, CH_2), 2.09 (s, 9H, CH_3), 3.60 (s, 2H, CH_2), 4.09 (d, J = 5.4 Hz, 6H, CH_2), 6.07 (s, 3H, C5–H in pyranone), 6.68 (s, 1H, NH), 8.20 (t, J = 5.4 Hz, 3H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 19.58 (CH_3), 29.76 (CH_2), 30.71 CH_2), 35.78 CH_2), 57.08 (C), 61.79 CH_2), 111.70 (C–5H in pyranone), 141.99 (C-2 in pyranone), 147.40 (C-3 in pyranone), 164.88 (C-6 in pyridinone), 171.53 (CO), 172.81 (C-4 in pyridinone), 173.93 (CO). ESI-MS: m/z 717.2 ($[M + H]^+$), 739.2 ($[M + Na]^+$). HRMS: calcd for $C_{33}H_{41}N_4O_{14}$ 717.2619 ($[M + H]^+$), found 717.2616; calcd for $C_{33}H_{40}N_4O_{14}Na$ 739.2439 ($[M + H]^+$), found 739.2434.

***tert*-Butyl 2-(3-methoxy-2-oxopyridin-1(2*H*)-yl)ethylcarbamate (14).** To a solution of **13** (4.5 g, 36 mmol) in dry DMF (40 mL) was added sodium hydride (1.728 g, 60% in mineral oil, 43.2 mmol). The mixture was stirred at room temperature for 30 min, then heated to 70 °C. A solution of *tert*-butyl 2-bromoethylcarbamate (9.274 g, 41.4 mmol) in 30 ml DMF was added dropwise. The stirring was continued at 70 °C overnight. After removal of the solvent, the residue was purified by chromatography using EtOAc/MeOH (9.5 : 0.5) to provide product **14** (7.1 g, 73.6% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9H, CH_3), 3.47 (q, J = 5.9 Hz, 2H, CH_2), 3.82 (s, 3H, CH_3), 4.13 (t, J = 5.9 Hz, 2H, CH_2), 5.10 (br, 1H, NH), 6.12 (t, J = 7.1 Hz, 1H, C5–H in pyridinone), 6.63 (dd, J = 7.4 Hz, 1.6 Hz, 1H, C4–H in pyridinone), 6.89 (d, J = 6.7 Hz, 1H, C6–H in pyridinone). ESI-MS: m/z 269 ($[M + H]^+$).

2-(3-Methoxy-2-oxopyridin-1(2*H*)-yl)ethanaminium 2,2,2-trifluoroacetate (15). To a solution of **14** (3.5 g) in dichloromethane (30 mL) cooled with ice-bath was added trifluoroacetic acid (20 mL). Then the mixture was stirred at room temperature for 3 h. After trifluoroacetic acid was completely removed *in vacuo*, compound **15** was obtained as a white solid in quantitative yield. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.14 (t, J = 5.6 Hz, 2H, CH_2), 3.71 (s, 3H, CH_3), 4.14 (t, J = 6.2 Hz, 2H, CH_2), 6.21 (t, J = 7.2 Hz, 1H, C5–H in pyridinone), 6.83 (dd, J = 7.5 Hz, 1.5 Hz, 1H, C4–H in pyridinone), 7.21 (dd, J = 7.5 Hz, 1.6 Hz, 1H, C6–H in pyridinone), 8.03 (br, 3H, NH_3^+). ESI-MS: m/z 169 ($[M + H]^+$).

4-(2-(Benzyloxy)acetamido)-*N*^{1},*N*^{7}-bis(2-(3-methoxy-2-oxopyridin-1(2*H*)-yl)ethyl)-4-(3-(2-(3-methoxy-2-oxopyridin-1(2*H*)-yl)ethylamino)-3-oxopropyl)heptanediamide (16). To a mixture of **7** (0.553 g, 1.4 mmol), **15** (1.42 g, 5.04 mmol) and DMF (15 ml) was added DIPEA (1.95 g, 15.12 mmol). Then HCTU (2.085 g, 5.04 mmol) was added to the above stirred solution. The stirring was continued overnight. The reactant was concentrated and the residue was purified by chromatography using DCM/MeOH (9 : 1 to 3 : 1) to provide product **16** (0.93 g, 78.5% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.79 (m, 6H, CH_2), 1.96 (m, 6H, CH_2), 3.31 (q, J = 5.8 Hz, 6H, CH_2), 3.67 (s, 9H, CH_3), 3.84 (s, 2H, CH_2), 3.92 (t, J = 6.0 Hz, 6H, CH_2), 4.53 (s, 2H, CH_2), 6.10 (t, J = 7.1 Hz, 3H, C5–H in pyridinone), 6.76 (dd, J = 7.5 Hz, 1.5 Hz, 3H, C4–H in pyridinone), 6.95 (s, 1H, NH), 7.07 (dd, J = 6.9 Hz, 1.6 Hz, 3H, C6–H in pyridinone), 7.29–7.38 (m, 5H, Ph), 7.98 (t, J = 5.7 Hz, 3H, NH). ESI-MS: m/z 846.3 ($[M + H]^+$), 868.5 ($[M + Na]^+$).

N^1,N^7-Bis(2-(3-hydroxy-2-oxopyridin-1(2*H*)-yl)ethyl)-4-(3-(2-(3-hydroxy-2-oxopyridin-1(2*H*)-yl)ethylamino)-3-oxopropyl)-4-(2-hydroxyacetamido)heptanediamide (3). To a solution of **16** (0.78 g) in anhydrous DCM (20 ml) cooled with ice-bath under nitrogen was added dropwise boron trichloride (1 M in DCM, 12 ml). The mixture was stirred at room temperature for 6 days. The reaction was quenched with methanol. After removal of the solvent, the residue was redissolved in methanol (5 ml), acetone was added to form the precipitate, left in fridge overnight. The product **3** was obtained as a pale brown solid (0.61 g, 80.3% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.78 (m, 6H, CH_2), 1.97 (m, 6H, CH_2), 3.34 (q, J = 5.8 Hz, 6H, CH_2), 3.73 (s, 2H, CH_2), 3.96 (t, J = 5.9 Hz, 6H, CH_2), 4.53 (s, 2H, CH_2), 6.08 (t, J = 7.0 Hz, 3H, C5–H in pyridinone), 6.70 (dd, J = 7.3 Hz, 1.7 Hz, 3H, C4–H in pyridinone), 6.77 (s, 1H, NH), 7.01 (dd, J = 6.8 Hz, 1.7 Hz, 3H, C6–H in pyridinone), 8.08 (t, J = 5.6 Hz, 3H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 29.54 (CH_2), 30.32 (CH_2), 37.40 (CH_2), 48.43 (CH_2), 56.61 (C), 61.41 (CH_2), 105.19 (C–5H in pyridinone), 114.94 (C–4H in pyridinone), 128.65 (C–6H in pyridinone), 146.59 (C-3 in pyridinone), 157.71 (C-2 in pyridinone), 171.05 (CO), 172.41 (CO). ESI-MS: m/z 714.3 ($[M + H]^+$), 736.5 ($[M + Na]^+$). HRMS: calcd for $C_{33}H_{44}N_7O_{11}$ 714.3099 ($[M + H]^+$), found 714.3091; calcd for $C_{33}H_{43}N_7O_{11}Na$ 736.2918 ($[M + Na]^+$), found 736.2907.

1-Hydroxy-6-oxo-1,6-dihydropyridine-2-carboxylic acid (18). To a solution of **17** (7.3 g, 52.5 mmol) in trifluoroacetic acid (45 mL) and acetic acid (30 mL) was added dropwise CH_3CO_3H (22 ml, 36–40% in acetic acid). The mixture was stirred at room temperature for 1 h, and then stirred at 80 °C overnight. After cooled in fridge, filtered, washed with cold methanol, crude product **18** was obtained as pale yellow solid (6.35 g, 78%). The crude product was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.65 (dd, J = 7.0 Hz, 1.7 Hz, 1H, C3–H in pyridinone), 6.72 (dd, J = 9.0 Hz, 1.7 Hz, 1H, C5–H in pyridinone), 7.45 (dd, J = 9.0 Hz, 7.0 Hz, 1H, C4–H in pyridinone). ESI-MS: m/z 156.07 ($[M + H]^+$).

1-(Benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxylic acid (19). A mixture of **18** (6.3 g, 40.6 mmol), K_2CO_3 (11.21 g, 81.29 mmol), benzyl chloride (6.17 g, 48.72 mmol) and methanol (100 ml) was refluxed overnight. After filtration, the filtrate was concentrated to dryness. The residue was redissolved in 20 ml water, acidified with 6N HCl to pH 2, filtered, washed with cold water to provide product **19** as a off-white solid (8.45 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.29 (s, 2H, CH_2), 6.56 (dd, J = 6.8 Hz, 1.7 Hz, 1H, C3–H in pyridinone), 6.74 (dd, J = 9.3 Hz, 1.7 Hz, 1H, C5–H in pyridinone), 7.41–7.52 (m, 6H, Ph and C4–H in pyridinone).

Methyl 1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxylate (20). To 100 ml of methanol at −5 °C was added 10 ml of

thionyl chloride at such a rate that the temperature did not reach 10 °C. The clear, colorless solution was cooled to −5 °C. **19** (8.21 g) was added, the mixture was gradually warmed to room temperature, and stirred for 3 days. After removal of the solvent, the crude product **20** was obtained. ESI-MS: *m*/*z* 260 ($[M + H]^+$), 282 ($[M + Na]^+$).

1-(Benzyloxy)-6-(hydroxymethyl)pyridin-2(1*H*)-one (21). A mixture of crude **20** (obtained from last step), $NaBH_4$ (7.49 g) in THF (150 ml) was stirred at 70 °C for 2 h. Methanol (15 mL) was added. After cooled to room temperature, the reaction was quenched with saturated NH_4Cl (100 ml). The stirring was continued for 1.5 h. The aqueous layer was extracted with DCM (2 × 150 ml). The combined organic layers were dried over Na_2SO_4. After removal of the solvent, the residue was purified by chromatography using DCM/MeOH (9.5 : 0.5) to provide product **21** (3.8 g, 50% yield overall two steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.98 (br, 1H, OH), 4.48 (d, J = 3.6 Hz, 2H, CH_2), 5.30 (s, 2H, CH_2), 6.21 (m, 1H, C3–H in pyridinone), 6.60 (dd, J = 9.2 Hz, 1.7 Hz, 1H, C5–H in pyridinone), 7.29 (m, 1H, C4–H in pyridinone), 7.38 (m, 3H, Ph), 7.45 (m, 2H, Ph). ESI-MS: *m*/*z* 232.1 ($[M + H]^+$), 254.1 ($[M + Na]^+$).

2-((1-(Benzyloxy)-6-oxo-1,6-dihydropyridin-2-yl)methyl)isoindoline-1,3-dione (22a). To a mixture of **21** (3.7 g, 16 mmol), triphenylphosphine (5.04 g, 19.2 mmol), phthalimide (2.83 g, 19.2 mmol) and THF (60 mL) cooled with ice-bath was added dropwise DIPAD (4.13 g, 19.2 mmol) at a period of 30 min. The temperature was slowly raised to room temperature, and stirring was continued overnight. After filtration, the product was washed with a small amount of THF, **22a** (5.04 g, 87.5% yield) was obtained as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.67 (s, 2H, CH_2), 5.44 (s, 2H, CH_2), 5.83 (m, 1H, C3–H in pyridinone), 6.63 (dd, J = 9.2 Hz, 1.6 Hz, 1H, C5–H in pyridinone), 7.19 (dd, J = 9.2 Hz, 6.9 Hz, 1H, C4–H in pyridinone), 7.43 (m, 3H, Ph), 7.56 (m, 2H, Ph), 7.76 (m, 2H, Ph), 7.88 (m, 2H, Ph). ESI-MS: *m*/*z* 361.1 ($[M + H]^+$), 383.3 ($[M + Na]^+$).

6-(Aminomethyl)-1-(benzyloxy)pyridin-2(1*H*)-one (22). To a solution of **22a** (4.96 g, 13.78 mmol) in ethanol (50 ml) was added hydrazine (0.843 g in 10 ml H_2O, 16.54 mmol). After being refluxed for 3 h, the reaction mixture was chilled to 0 °C, adjusted to pH 1 with concentrated hydrochloric acid, filtered, and washed with a plenty of water. The filtrate was adjusted pH to 12 with 10 N NaOH, and extracted with DCM (3 × 150 ml). The combined organic layers were dried over Na_2SO_4. After removal of the solvent, the residue was purified by chromatography using DCM/MeOH (9 : 1) to provide product **22** (3.03 g, 95.6% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.67 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 6.07 (m, 1H, C3–H in pyridinone), 6.61 (dd, J = 9.2 Hz, 1.7 Hz, 1H, C5–H in pyridinone), 7.28 (m, 1H, C4–H in pyridinone), 7.39 (m, 3H, Ph), 7.47 (m, 2H, Ph).

***N*^{1},*N*^{7}-Bis((1-(benzyloxy)-6-oxo-1,6-dihydropyridin-2-yl)methyl)-4-(3-((1-(benzyloxy)-6-oxo-1,6-dihydropyridin-2-yl)methylamino)-3-oxopropyl)-4-(2-(benzyloxy)acetamido)heptanediamide (23).** A mixture of **7** (1.185 g, 3 mmol), **22** (2.484 g, 10.8 mmol), HOBt (1.458 g, 10.8 mmol) and DMF (20 ml) was stirred at room temperature for 20 min, and then EDC (2.070 g, 10.8 mmol) was added. Stirring was continued for 2 days. After removal of the solvent, the residue was purified by chromatography using DCM/MeOH (14 : 1) to provide product **23** (2.55 g, 82.3% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.90 (m, 6H, CH_2), 2.08 (m, 6H, CH_2), 3.78 (s, 2H, CH_2), 4.16 (d, J = 6.0 Hz, 6H, CH_2), 4.50 (s, 2H, CH_2), 5.24 (s, 6H, CH_2), 5.96 (dd, J = 6.9 Hz, 1.4 Hz, 3H, C5–H in pyridinone), 6.53 (dd, J = 9.2 Hz, 1.6 Hz, 3H, C3–H in pyridinone), 6.57 (s, 1H, NH), 6.89 (t, J = 6.0 Hz, 3H, NH), 7.16 (dd, J = 9.2 Hz, 6.9 Hz, 3H, C4–H in pyridinone), 7.27–7.44 (m, 20H, Ph). MS (ESI): *m*/*z* 1032 ($[M + H]^+$).

***N*^{1},*N*^{7}-Bis((1-hydroxy-6-oxo-1,6-dihydropyridin-2-yl)methyl)-4-(3-((1-hydroxy-6-oxo-1,6-dihydropyridin-2-yl)methylamino)-3-oxopropyl)-4-(2-hydroxyacetamido)heptanediamide (4).** To a solution of **23** (1.2 g) in anhydrous DCM (20 ml) cooled with ice-bath under nitrogen was added dropwise borontrichloride (1 M in DCM, 14 ml). The mixture was stirred at room temperature for 2 days. The reaction was quenched with methanol. After removal of the solvent, the residue was redissolved in methanol (5 ml), acetone was added to form a precipitate which was allowed to stand at 4 °C overnight. The product **4** was obtained as a white solid (0.75 g, 96% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.93 (m, 6H, CH_2), 2.20 (m, 6H, CH_2), 3.77 (s, 2H, CH_2), 4.29 (d, J = 5.8 Hz, 6H, CH_2), 6.06 (dd, J = 7.0 Hz, 1.0 Hz, 3H, C5–H in pyridinone), 6.42 (dd, J = 9.0 Hz, 1.3 Hz, 3H, C3–H in pyridinone), 6.90 (s, 1H, NH), 7.33 (dd, J = 9.0 Hz, 7.1 Hz, 3H, C4–H in pyridinone), 8.51 (t, J = 6.0 Hz, 3H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 29.46 (*C*H_2), 30.27 (*C*H_2), 37.38 (*C*H_2), 55.79 (*C*), 61.46 (*C*H_2), 102.04 (*C*–5H in pyridinone), 115.80 (*C*–3H in pyridinone), 137.18 (*C*–4H in pyridinone), 145.61 (*C*-6 in pyridinone), 158.02 (*C*-2 in pyridinone), 171.20 (*C*O), 172.59 (*C*O). MS (ESI): *m*/*z* 672.2 ($[M + H]^+$), 694.3 ($[M + Na]^+$). HRMS: calcd for $C_{30}H_{38}N_7O_{11}$ 672.2629 ($[M + H]^+$), found 672.2630; calcd for $C_{30}H_{37}N_7O_{11}Na$ 694.2449 ($[M + Na]^+$), found 694.2446.

Physicochemical characterisation of chelators 1–4

p*K*$_a$ determination. The pK_a values of chelators **1–4** were determined by spectrophotometric titration.[19–21] The automatic titration system used in this study comprised an autoburette (Metrohm Dosimat 765 liter ml syringe) and Mettler Toledo MP230 pH meter with Metrohm pH electrode (6.0133.100) and a reference electrode (6.0733.100). 0.1 M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic jacketed titration vessel at 25 °C ± 0.1 °C by using a Techne TE-8J temperature controller. The solution under investigation was stirred vigorously during the experiment. A Gilson Mini-plus#3 pump with speed capability (20 ml min^{-1}) was used to circulate the test solution through a Hellem quartz flow cuvette. For the stability constant determinations, a 50 mm path length cuvette was used, and for pK_a determinations, a cuvette path length of 10 mm was used. The flow cuvette was mounted on an HP 8453 UV-visible spectrophotometer. All instruments were interfaced to a computer and con-

trolled by a Visual Basic program. Automatic titration and spectral scans adopted the following strategy: the pH of a solution was increased by 0.1 pH unit by the addition of KOH from the autoburette; when pH readings varied by <0.001 pH unit over a 3 s period, an incubation period was activated. For pK_a determinations, a period of 1 min was adopted; for stability constant determinations, a period of 5 min was adopted. At the end of the equilibrium period, the spectrum of the solution was then recorded. The cycle was repeated automatically until the defined end point pH value was achieved. All the titration data were analyzed with the pHab program.[22] The species plot was calculated with the HYSS program.[23] Analytical grade reagent materials were used in the preparation of all solutions.

Iron(III) affinity determination. Iron(III) affinities of these four hexadentate ligands were determined by competition with EDTA. Solutions of Iron(III), hexadentate ligand (**1–4**), and EDTA were added to KCl solution (0.1 M) and alkalimetrically titrated. The pH observation was taken after standing for periods up to 4 h to achieve equilibrium. The data were analyzed using pHab software to obtain the iron(III) affinity constants. The pFe^{3+} value was calculated by HYSS program[23] based on the iron(III) affinity constants determined and pK_a values of the hexadentate ligands. The conditions for the calculation were pH = 7.4, $[Fe^{3+}]_{Total} = 10^{-6}$ M, $[Ligand]_{Total} = 10^{-5}$ M.

Results and discussion

Chemistry

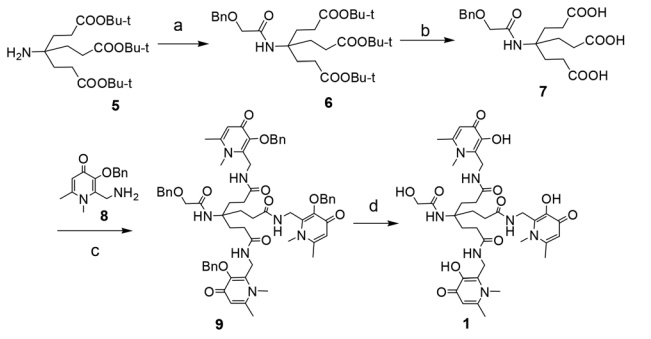
Hexadentate ligands can be constructed by connecting three bidentate ligands onto a suitable tripodal backbone. However, in order for the ligand to adopt the correct geometry for metal binding, the backbone should be linked to the ring at the *ortho* position relative to one of the coordinating oxygen anions.[24,25] This design has been adopted in the present work.

Synthesis of hexadentate 3-hydroxypyridin-4-one (1). The synthetic route for hexadentate 3,4-HOPO (**1**) is presented in Scheme 1. Acylation of amine **5**[17] with 2-(benzyloxy)acetyl chloride provided compound **6** in good yield. Treatment of **6** with formic acid yielded triacid **7**, which was used in the next step reaction without purification. Protected bidentate 3,4-HOPO containing a free amino group (**8**),[18] was then coupled to triacid **7** in the presence of *N*-hydroxybenzotriazole (HOBt) and *N*,*N*′-dicyclohexylcarbodiimide (DCC), generating the protected hexadentate 3,4-HOPO (**9**) in 85% yield. Deprotection of **9** was achieved by hydrogenation in the presence of Pd/C, providing the trichloride of hexadentate 3,4-HOPO (**1**) as a white solid in excellent yield.

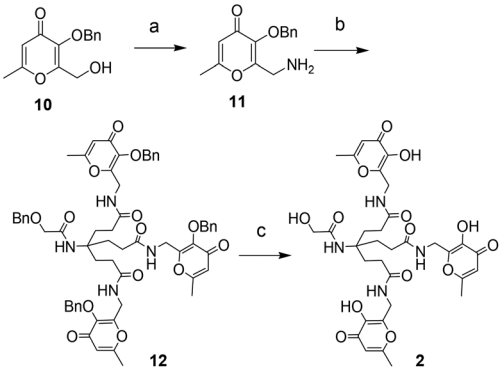
Synthesis of hexadentate 3-hydroxypyran-4-one (2). The protected 3-hydroxypyran-4-one (**11**) was prepared from compound **10**[17] by converting the hydroxyl group to an amino group using the Mitsunobu reaction in the presence of triphenylphosphine (Ph_3P), phthalimide, and diisopropyl azodicarboxylate (DIPAD), followed by hydrazination (Scheme 2). Conjugation of amine **11** to the tripodal acid **7** was achieved using DCC/HOBt as coupling agent, providing the protected hexadentate pyranone **12**, which was subjected to the treatment with boron trichloride, yielding the hexadentate 3-hydroxypyran-4-one (**2**) in excellent yield.

Synthesis of hexadentate 3-hydroxypyran-2-one (3). The synthetic route for hexadentate 3,2-HOPO (**3**) is shown in Scheme 3. Reaction of 3-methoxypyridin-2(1*H*)-one (**13**) with *tert*-butyl 2-bromoethylcarbamate using sodium hydride as a base produced compound **14** in 73.6% yield. Treatment of 14 with trifluoroacetic acid (TFA) gave the TFA salt of amine (**15**), which was coupled to triacid **7** in the presence of *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HCTU) to form the protected hexadentate 3-hydroxypyran-2-one (**16**) in 78.5% yield. Treatment of **16** with boron trichloride generated hexadentate 3-hydroxypyran-2-one (**3**) in 80.3% yield.

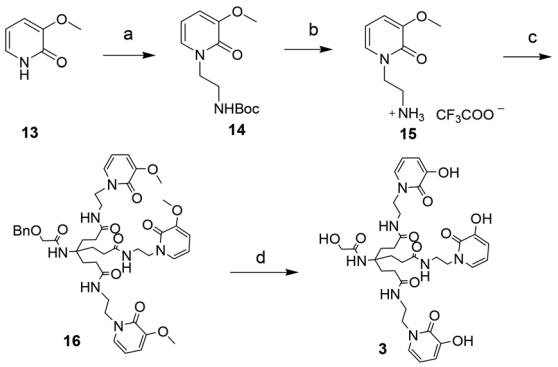
Synthesis of hexadentate 1-hydroxypyran-2-one (4). The synthetic route for hexadentate 1,2-HOPO (**4**) is shown in Scheme 4. Oxidation of 6-hydroxypyridine-2-carboxylic acid (**17**) with peracetic acid produced compound **18** in 78% yield using trifluoroacetic acid as a solvent. This yield is similar to



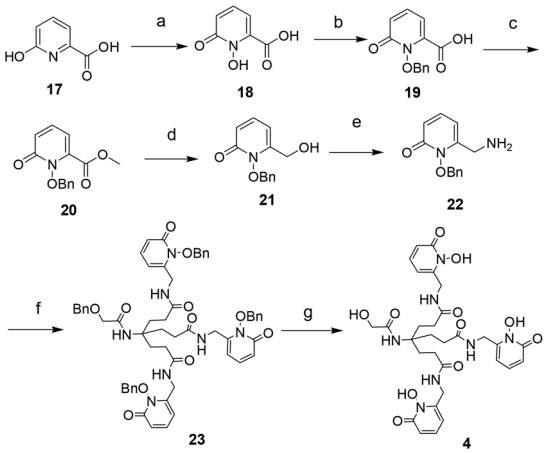
Scheme 1 Reagents and conditions: (a) $BnOCH_2COCl$, Et_3N, 0 °C then rt; (b) HCOOH, rt, overnight; (c) HOBt, DCC, DMF, rt; (d) H_2, Pd/C, MeOH, HCl, rt.



Scheme 2 Reagents and conditions: (a) (i) $(Ph_3)P$, phthalimide, DIPAD, 0 °C to rt; (ii) NH_2NH_2, reflux; (b) **7**, HCTU, DIPEA, DMF, rt; (c) BCl_3, DCM, 0 °C to rt.



Scheme 3 Reagents and conditions: (a) $BocNHCH_2CH_2Br$, NaH, DMF, rt then 70 °C; (b) TFA, rt; (c) **7**, HCTU, DIPEA, DMF, rt; (d) BCl_3, DCM, 0 °C to rt.



Scheme 4 Reagents and conditions: (a) CH_3CO_3H, rt; (b) BnCl, MeOH, reflux; (c) $SOCl_2$, MeOH; (d) $NaBH_4$, THF, MeOH, 70 °C; (e) (i) $(Ph_3)P$, phthalimide, DIPAD, 0 °C to rt; (ii) NH_2NH_2, reflux; (f) **7**, HOBt, EDC, DMF, rt; (g) BCl_3, DCM, 0 °C to rt.

that reported by Workman *et al*. using acetic acid as a solvent.[26] Benzylation of **18** gave compound **19**, which on esterification in the presence of thionyl chloride in methanol produced **20**. Reduction of ester bond in **20** was achieved by the treatment with sodium borohydride in tetrafuran (THF), followed by the addition of methanol,[26] yielding compound **21** in a reasonable yield. In the absence of methanol, ester reduction does not occur. Amine **22** was prepared from **21** using the Mitsunobu reaction in the presence of Ph_3P, phthalimide and DIPAD, followed by hydrazination. Conjugation of **22** with triacid **7** in the presence of EDC and HOBt provided protected hexadentate 1-hydroxypyran-2-one (**23**), which was treated with boron trichloride, thereby generating the hexadentate 1-hydroxypyran-2-one (**4**). In the present work, the strategy for the preparation of the hexadentate 1,2-HOPO by using a methylene linker between the 1.2-HOPO coordinating group and the molecular scaffold was similar to that reported by Workman *et al*.[27]

Physico-chemical characterization

Determination of pK_a values. The pK_a values of the hexadentate chelators **1–4** were determined by spectrophotometric titration with an automated titration system.[19–21] All the titration data were analyzed using the pHab software.[22] The UV spectra of **1–4** were pH dependent. As an example, the UV spectra of **1** is presented in Fig. 3, which was recorded between 250 and 350 nm over the pH range 1.64–11.2 for the free ligand. The speciation spectra demonstrate a clear shift in λ_{max} from 280 to 310 nm, which reflects the pH dependence of the ligand ionization equilibrium.

The pK_a values of **1** obtained from nonlinear least-squares regression analysis are 2.44, 3.09, 3.73, 8.90, 9.47 and 10.01. Of the six pK_a values, the lower three values correspond to the 4-oxo functions and the higher three correspond to the 3-hydroxyl functions. There are three pK_a values for each of chelators **2**, **3** and **4** in the pH range 2-10 (Table 1). These values are typical for each individual pyridinone class. It should be noted that the pK_a values of **4** are lower than those of the other three compounds and as a result at pH 7.0 would be largely unprotonated. This provides an indication of less competition with protons for iron complexation.

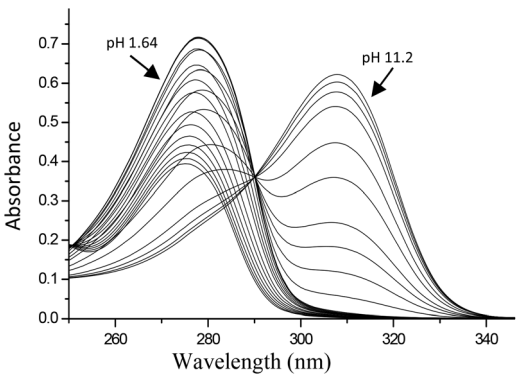


Fig. 3 pH dependence of UV spectra of **1**. [**1**] = 17.83 μM, pH was changed from 1.64 to 11.2 by the addition of KOH.

Table 1 pK_a values of hexadentate ligands **1–4** and their affinity constants for Fe(III) and Cu(II)

Ligands	pK_a	Log K (Fe(III))	pFe^{3+}
1	10.01 ± 0.006, 9.47 ± 0.008 8.90 ± 0.005, 3.73 ± 0.006 3.09 ± 0.004, 2.44 ± 0.003	32.8 ± 0.04	27.6
2	8.97 ± 0.005, 8.35 ± 0.007 7.70 ± 0.008	25.6 ± 0.02	23.5
3	9.83 ± 0.006, 8.96 ± 0.009 8.10 ± 0.005	27.7 ± 0.02	23.9
4	6.51 ± 0.007, 5.68 ± 0.002 4.87 ± 0.004	25.9 ± 0.01	26.8

pFe^{3+} is under the conditions: $[Fe^{3+}]_{Total}$ = 1 μM, $[ligand]_{Total}$ = 10 μM, pH = 7.4.

Iron(III) complexation. All the four compounds form stable coloured complexes with iron(III). Chelators **1** and **2** form red-yellow iron complexes, whereas chelators **3** and **4** form a purple colour and a faint yellow colour iron complex at pH 7.0, respectively. The iron complexes of **3** and **2** are more soluble than the other two, which precipitate at neutral pH values at concentrations above 0.1 mM. The stability constants were determined for the four compounds by competition with EDTA. The results are presented in Fig. 4. The rate of exchange of iron(III) between hexadentate ligands was found to be very slow for **2** and **3** (a 3 day period was adopted to guarantee the solution reached equilibrium), but relatively fast for chelators **1** and **4** (a period of 1 hour was used to achieve equilibrium). Because there is quite a wide range in affinities for iron(III) amongst the four compounds, slightly different conditions were adopted for each of the compounds in order to favour partitioning of iron(III) to EDTA. For chelators **2** and **3**, a relatively low level of EDTA was used (2 mM) at pH 7.24. The log K values of iron(III) complexes of **2** and **3** were determined to be 25.6 and 27.7, respectively (Table 1). For chelators **1** and **4**, a higher EDTA concentration was employed (98.6 mM) and the competition was performed at a more acidic pH value (6.4). Both changes favour EDTA in the competition for iron(III). The log K values of iron(III) complexes of **1** and **4**, being 32.8 and 25.9, respectively (Table 1). Thus the affinities for iron(III) cover the range 32.8 to 25.6. These differences become apparent in the pFe^{3+}-pH plots under the conditions of 1 μM iron(III) and 10 μM ligand (Fig. 5).

The sequence of pFe^{3+}(7.4) values of chelators **1–4** under the conditions of 1 μM iron(III) and 10 μM ligand are 27.6, 23.5, 23.9, and 26.8, respectively. Chelator **1**, the 3,4-HOPO, binds iron(III) with the highest affinity. Chelator **4**, the 1,2-HOPO, is the next highest, despite possessing a much lower log $K(Fe^{III})$ value. The relatively high pFe^{3+} value results from the low pK_a values of the free ligand. The iron(III) affinity of compound **1** is higher than that of desferrioxamine (pFe^{3+} = 26.6), a hexadentate siderophore containing three hydroxamate moieties, and the iron(III) affinity of **4** is similar to that of desferrioxamine, but **2** and **3** are lower than desferrioxamine. In comparison to enterobactin, a tricatecholate hexadentate ligand, with the extremely high pFe^{3+} value of 35.5, hexadentate ligands **1–4** have appreciably lower pFe^{3+} values. However, the effectiveness of enterobactin to scavenge iron at pH 7.0 is limited by its weak acidity and the required loss of six protons on binding iron(III).[28]

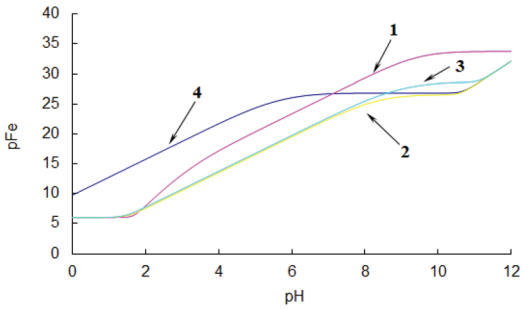


Fig. 5 pFe-pH plots for the condition 1 μM iron(III) and 10 μM ligand.

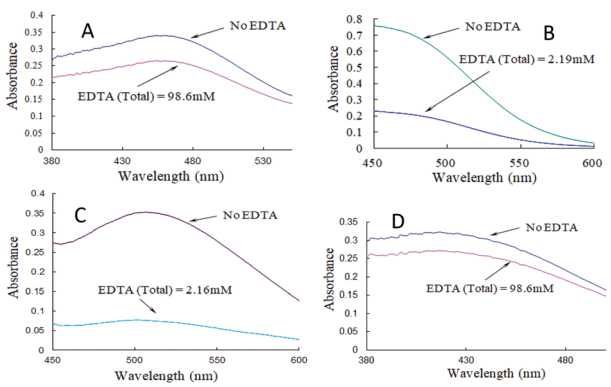


Fig. 4 Iron(III) binding competition between hexadentate chelators **1–4** and EDTA, and iron(III) binding in the absence of EDTA. (A) Chelator **1**, $[L]_{Total}$ = 67 μM; $[Fe]_{Total}$ = 53.4 μM; pH 6.431. (B) Chelator **2**, $[L]_{Total}$ = 815 μM; $[Fe]_{Total}$ = 741 μM; pH 7.24. (C) Chelator **3**, $[L]_{Total}$ = 1.02 mM; $[Fe]_{Total}$ = 0.73 mM; pH 7.24. (D) Chelator **4**, $[L]_{Total}$ = 65.9 μM; $[Fe]_{Total}$ = 53.4 μM; pH 6.43.

Conclusions

With regards to the iron(III) affinities of the hexadentate ligands **1**, **2** **3** and **4**, the pFe^{3+} values follow the sequence **1** > **4** ≫ **3** > **2**, which is different to the pFe^{3+} value sequence of the corresponding bidentate molecules, namely 3,4-HOPO ≫ 3,2-HOPO > 1,2-HOPO > 3-hydroxypyranone.[15] The major difference in behaviour of the hexadentate ligands when compared to their respective bidentate analogues was found with **4**. Thus whereas there are 4 orders of magnitude difference in the pFe^{3+} values of deferiprone and 1,3-dihydroxypyridin-2-one, the pFe^{3+} values of **1** and **4** are almost identical (Table 1). Clearly there is a large advantage in forming hexadentate 1,3-dihydroxypyridin-2-ones judging from the differences in the pFe^{3+} values of the corresponding bidentate and hexadentate analogues (1,2-HOPO and **4**) and this probably accounts for the large number of such oligodentate 1,2-HOPO ligands that have been investigated.[16,29]

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 T. Zhou, Y. M. Ma, X. L. Kong and R. C. Hider, *Dalton Trans.*, 2012, **41**, 6371–6389.

2 C. Hershko, A. M. Konijn and G. Link, *Br. J. Haematol.*, 1998, **101**, 399–406.
3 R. C. Hider and A. V. Hoffbrand, *N. Engl. J. Med.*, 2018, **379**, 2140–2150.
4 J. A. B. Balfour and R. H. Foster, *Drugs*, 1999, **58**, 553–578.
5 H. Nick, P. Acklin, R. Lattmann, P. Buehlmayer, S. Hauffe, J. Schupp and D. Alberti, *Curr. Med. Chem.*, 2003, **10**, 1065–1076.
6 M. D. Cappellini, *Best Pract. Res., Clin. Haematol.*, 2005, **18**, 289–298.
7 D. H. Qiu, Z. L. Huang, T. Zhou, C. Shen and R. C. Hider, *FEMS Microbiol. Lett.*, 2011, **314**, 107–111.
8 Y. J. Zhou, M. X. Zhang, R. C. Hider and T. Zhou, *FEMS Microbiol. Lett.*, 2014, **355**, 124–130.
9 Y. J. Zhou, M. S. Liu, Al R. Osamah, X. L. Kong, S. Alsam, S. Battah, Y. Y. Xie, R. C. Hider and T. Zhou, *Eur. J. Med. Chem.*, 2015, **94**, 8–21.
10 Y. Y. Xie, M. S. Liu, P. P. Hu, X. L. Kong, D. H. Qiu, J. L. Xu, R. C. Hider and T. Zhou, *Med. Chem. Res.*, 2013, **22**, 2351–2359.
11 R. C. Hider, S. Roy, Y. M. Ma, X. L. Kong and J. Preston, *Metallomics*, 2011, **3**, 239–249.
12 A. Martin-Bastida, R. J. Ward, R. Newbould, P. Piccini, D. Sharp, C. Kabba, M. C. Patel, M. Spino, J. Connelly, F. Tricta, R. R. Crichton and D. T. Dexter, *Sci. Rep.*, 2017, **7**, 1398, DOI: 10.1038/s41598-017-01402-2.
13 M. A. Santos, K. Chand and S. Chaves, *Coord. Chem. Rev.*, 2016, **327**, 287–303.
14 N. Boddaert, K. H. Le Quan Sang, A. Rötig, A. Leroy- Willig, S. Gallet, F. Brunelle, D. Sidi, J. C. Thalabard, C. Thalabard and Z. I. Cabantchik, *Blood*, 2007, **110**, 401–408.
15 T. Zhou, G. Winkelmann, Z. Y. Dai and R. C. Hider, *J. Pharm. Pharmacol.*, 2011, **63**, 893–903.
16 A. Cilibrizzi, V. Abbate, Y. L. Chen, Y. M. Ma, T. Zhou and R. C. Hider, *Chem. Rev.*, 2018, **118**, 7657–7701.
17 G. R. Newkome, R. K. Behera, C. N. Moorefield and G. R. Baker, *J. Org. Chem.*, 1991, **56**, 7162–7167.
18 Z. D. Liu, R. Kayyali, R. C. Hider, J. B. Porter and A. E. Theobald, *J. Med. Chem.*, 2002, **45**, 631–639.
19 Z. D. Liu, H. H. Khodr, D. Y. Liu, S. L. Lu and R. C. Hider, *J. Med. Chem.*, 1999, **42**, 4814–4823.
20 P. S. Dobbin, R. C. Hider, A. D. Hall, P. D. Taylor, P. Sarpong, J. B. Porter, G. Xiao and D. van der Helm, *J. Med. Chem.*, 1993, **36**, 2448–2458.
21 B. L. Rai, L. S. Dekhordi, H. H. Khodr, Y. Jin, Z. D. Liu and R. C. Hider, *J. Med. Chem.*, 1998, **41**, 3347–3359.
22 P. Gans, A. Sabatini and A. Vacca, *Ann. Chim.*, 1999, **89**, 45–49.
23 L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca, *Coord. Chem. Rev.*, 1999, **184**, 311–318.
24 B. O'Sullivan, J. Xu and K. N. Raymond, in *Iron Chelators, New Development Strategies*, ed. D. G. Badman, R.J. Bergeron, G. M. Brittenham, The Saratoga Group, Florida, 2000, pp. 177–208.
25 T. Zhou, R. C. Hider and X. Kong, *Chem. Commun.*, 2015, **51**, 5614–5617.
26 D. G. Workman, A. Tsatsanis, F. Lewis, J. P. Boyle, M. Mousadoust, N. T. Hettiarachchi, M. Hunter, C. S. Peers, D. Tetard and J. A. Duce, *Metallomics*, 2015, **7**, 867–876.
27 D. G. Workman, M. Hunter, L. G. Dover and D. Tétard, *J. Inorg. Biochem.*, 2016, **160**, 49–58.
28 K. Abu-Dari and K. N. Raymond, *Inorg. Chem.*, 1991, **30**, 519–524.
29 P. W. Durbin, B. Kullgren, J. Xu and K. N. Raymond, *Int. J. Radiat. Biol.*, 2000, **76**, 199–214.

