Clusters in bis-tridentate tethered domains of an iron chelating drug †

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The tridentate metal binding domains of iron chelating agents have been tethered *via* a variable length methylene $(CH_2)_n$ linker (n = 1, 2, 4, 6, 8, 10, 14). The single-crystal X-ray structures of monomeric ([Fe(HL^8)]·2.5H$_2$O n = 8; H$_4$L^8 is 1,10-decanedioyl di(salicylaldehyde hydrazone)) and dimeric ([{Fe(H$_2$PL^4)}$_2$]Cl$_2$·2H$_2$O n = 4; H$_4$PL^4 is 1,6-hexanedioyl di(pyridoxal hydrazone)) iron(III) complexes have been determined. The complexes exhibit oligomerism: with chain lengths of $n \geq 6$ monomers are prevalent whereas for shorter chains this is not possible and a series of clusters exist as shown by ESMS and molecular modelling.

Iron overload in humans due to genetic disorders such as β-thalassemia, or accidental ingestion, affects millions and may have life-threatening complications.[1] To date, the only clinically approved iron chelating drug is desferroximine B (DFO), a fungal extract which is expensive and needs to be administered by slow subcutaneous infusion.[2] There is an immediate need for an orally active iron chelating agent. To be clinically useful, there are a number of properties any iron chelating agent should have: iron specific; non-toxic; non-mutagenic/carcinogenic; high thermodynamic chelate stability; optimal solubility; cheap.[3,4] A number of potential agents are either being developed or in clinical trials, including pyridones, polycarboxylates and hydrazones.[3–5] Of the latter class, pyridoxal isonicotinoylhydrazone (H$_2$pih) looks promising.[6–8] Investigations into H$_2$pih are at a clinical stage and the *in vivo* iron chelating potential appears good.[9]

The simplest derivative of H$_2$pih, salicylaldehyde benzoylhydrazone (H$_2$sb), can mobilize iron[10] *in vitro* and *in vivo* in rats.[11] It is also bacteriostatic[12] and antitumour active,[13] with its Cu(II) complex being up to 100 times more potent than the metal free chelate. The Cu(II) complex of the closely related salicylaldehyde acetylhydrazone (H$_2$sa) has radioprotective[14] and cytotoxic properties.[15] Metal complexes of H$_2$pih, H$_2$sb and H$_2$sa have been studied extensively and this class of ligand act as planar, tridentate chelators.[15,16]

In order to minimize the possibility of production of reactive oxygen species, an iron chelating drug should preferably be hexadentate.[17] We have therefore linked two of the metal binding domains of this general ligand class to give the potentially hexadentate chelating agents H$_4$PL^n [di(pyridoxal hydrazones): where n = 1, 2, 4, 8] and H$_4$L^n [di(salicylaldehyde hydrazones): where n = 1, 2, 4, 6, 8, 10, 14 and is the length of the methylene linker between metal binding domains].[18]

With Cu(II), H$_4$L^n ligands bind one metal per tridentate domain.[18] However, for octahedral metals, there is the possibility of two domains binding to one metal: either from two different ligands[19] or, if the methylene chain is sufficiently long, from the two domains of one ligand. This opens up the possibility of cluster formation[20] with ring size controlled by both the nuclearity and n. The chelating properties, cluster formation and solubility of the ligands will be intimately coupled to the length of the linker. Here we report crystallographic studies of monomeric [Fe(HL^8)]·2.5H$_2$O 1 and dimeric [{Fe(H$_2$PL^4)}$_2$]-Cl$_2$·2H$_2$O 2, the first union between iron and this ligand type, and ESMS studies of cluster complexes.
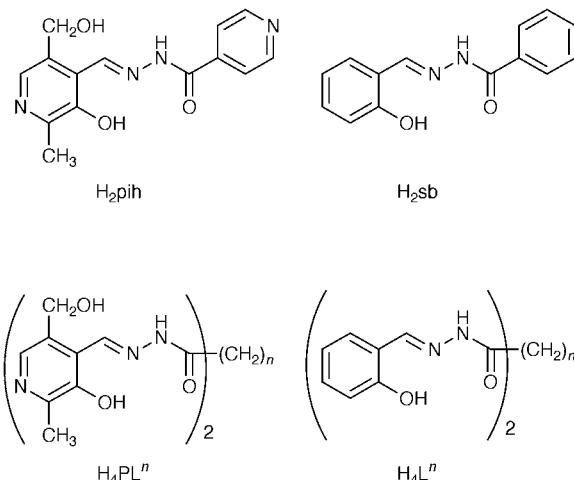
The H$_4$L^n ligands were prepared as reported[18] with the pyridoxal analogues (H$_4$PL^n) substituting pyridoxal for salicylaldehyde. One example of the general preparation for each class of complex will be given.‡

X-Ray analysis of 1§ shows the structure (Fig. 1) to be monomeric with the two inequivalent tridentate domains from the ligand coordinated to the Fe^{III} centre. The donors within a domain [O(1), N(1), O(8); O(1′), N(1′), O(8′)] are planar and perpendicular to each other (angle between domains 89.9°). As the complex is neutral, one domain is dianionic and the other monoanionic (dashed labels) with the extra proton on the amide N(2′). In spite of this, there are no significant differences in the bond lengths associated with the domains and the only noteworthy difference is for the angles N(1′)–N(2′)–C(8′) [116.2(5)°] and N(1)–N(2)–C(8) [110.2(5)°]. A 13-membered chelate ring has been formed incorporating the methylene chain. Other metric parameters may be considered normal.[15,16]

The X-ray structure of 2¶ (Fig. 2) is dimeric, with the non-symmetry related Fe^{III} centres coordinated by tridentate
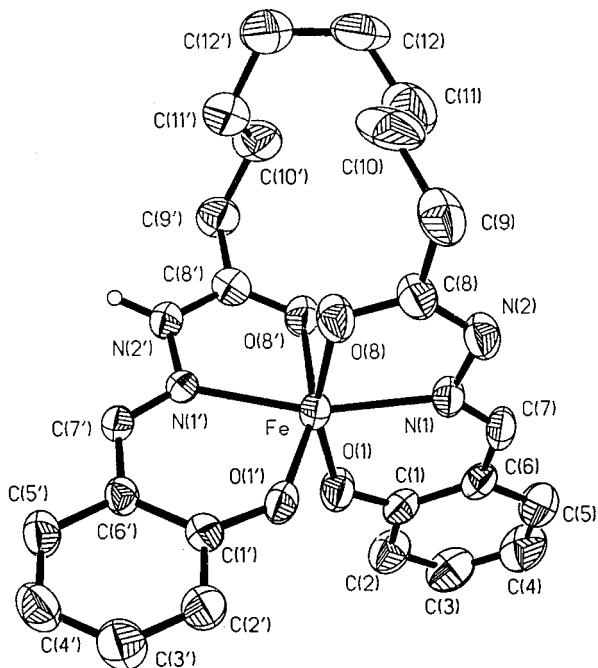
Fig. 1 Molecular structure of monomeric [Fe(HL^8)]·2.5H$_2$O **1**. Lattice water molecules have been omitted for clarity. Selected bond distances (Å) and angles (°): Fe–O(1) 1.912(4), Fe–O(1') 1.912(4), Fe–O(8) 2.026(4), Fe–O(8') 2.085(4), Fe–N(1) 2.108(5), Fe–N(1') 2.123(4); O(1)–Fe–O(8) 160.0(2), O(1)–Fe–O(1') 91.8(2), O(8)–Fe–O(1') 94.3(2), O(1)–Fe–O(8') 92.1(2), O(8)–Fe–O(8') 89.1(2), O(1')–Fe–O(8') 158.6(2), O(1)–Fe–N(1) 85.4(2), O(8)–Fe–N(1) 74.6(2), O(1')–Fe–N(1) 108.1(2), O(8')–Fe–N(1) 93.2(2), O(1)–Fe–N(1') 105.9(2), O(8)–Fe–N(1') 93.6(2), O(1')–Fe–N(1') 84.0(2), O(8')–Fe–N(1') 74.7(2), N(1)–Fe–N(1') 163.5(2).
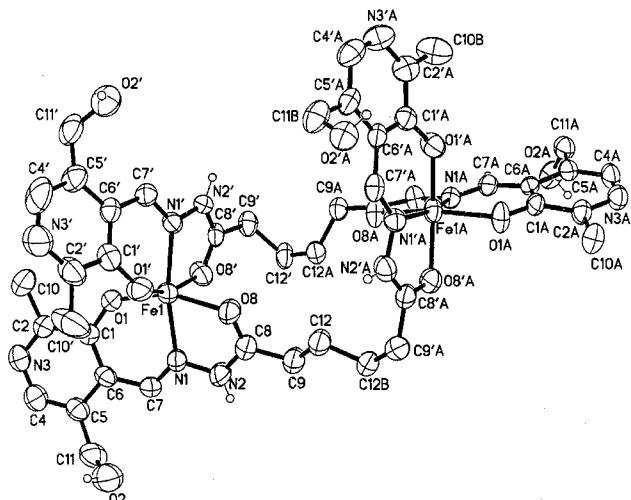

Fig. 2 Molecular structure of dimeric [{Fe(H$_2$PL^4)}$_2$]Cl$_2$·2H$_2$O **2**. Lattice water molecules and chloride ions have been omitted for clarity. Selected bond distances (Å) and angles (°): Fe(1)–O(1) 1.927(5), Fe(1)–O(1') 1.920(5), Fe(1A)–O(1A) 1.955(5), Fe(1A)–O(1'A) 1.937(5), Fe(1)–O(8) 2.079(5), Fe(1)–O(8') 1.989(5), Fe(1A)–O(8A) 1.987(4), Fe(1A)–O(8'A) 2.012(5), Fe(1)–N(1) 2.129(6), Fe(1)–N (1') 2.092(5), Fe(1A)–N(1A) 2.127(5), Fe(1A)–N (1'A) 2.129(5); O(1)–Fe(1)–O(8) 157.5(2), O(1')–Fe(1)–O(8') 157.3(2), O(8)–Fe(1)–O(1') 91.1(2), O(1)–Fe(1)–O(8') 97.1(2), O(1)–Fe(1)–O(1') 94.4(2), O(8)–Fe(1)–O(8') 85.7(2), O(8)–Fe(1)–N(1) 74.6(2), O(8')–Fe(1)–N(1') 74.3(2), O(1)–Fe(1)–N(1) 83.1(2), O(1')–Fe(1)–N(1') 84.1(2), O(1)–Fe(1)–N(1') 102.7(2), O(1')–Fe(1)–N(1) 110.8(2), O(8)–Fe(1)–N(1') 99.5(2), O(8')–Fe(1)– N(1') 90.0(2), N(1)–Fe(1)–N(1') 163.8(2), O(1A)–Fe(1A)–O(8A) 154.8(2), O(1'A)–Fe(1A)–O(8'A) 155.9(2), O(1A)–Fe(1A)–O(1'A) 93.8(2), O(8A)–Fe(1A)–O(8'A) 90.4(2), O(1A)–Fe(1A)–O(8'A) 85.7(2), O(1'A)–Fe(1A)–O(8A) 99.7(2), O(1A)–Fe(1A)–N(1A) 82.8(2), O(1'A)–Fe(1A)–N(1'A) 83.3(2), O(1A)–Fe(1A)–N(1'A) 112.9(19), O(1'A)–Fe(1A)–N(1A) 94.9(2), O(8A)–Fe(1A)–N(1A) 75.0(2), O(8'A)–Fe(1A)–N(1'A) 74.8(2), O(8A)–Fe(1A)–N(1'A) 99.5(2), O(8'A)–Fe(1A)–N(1A) 109.0(2), N(1A)–Fe(1A)–N(1'A) 164.2(2).
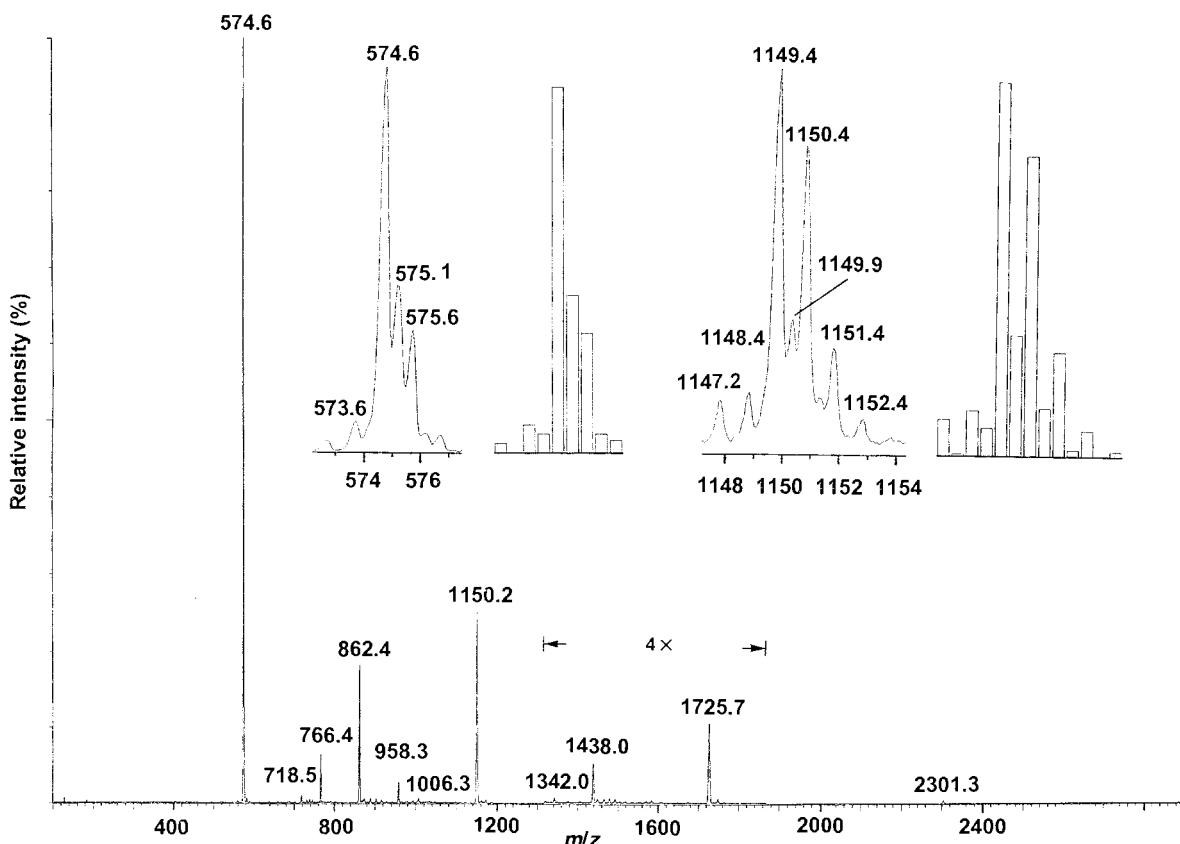

Fig. 3 ESMS spectrum of [Fe(HL^{14})]·1.5H$_2$O showing peaks from monomer to heptamer with expansions and calculated patterns for monomer–dimer (3:4) and dimer–tetramer (7:3) peaks. Assignment of peaks (M = [Fe(HL^{14})], 576 amu, 50 V): 575 [M − H] + [M$_2$ − 2H] 3:4; 719 [M$_5$ − 4H]; 766 [M$_4$ − H]; 862 [M$_3$ − 2H]; 958 [M$_5$ − 3H]; 1006 [M$_7$ − 4H]; 1150 [M$_2$ − H] + [M$_4$ − 2H] 7:3; 1342 [M$_7$ − 3H]; 1438 [M$_5$ − 2H]; 1726 [M$_3$ − H]; 2301 [M$_4$ − H].

domains from the different ligands. Both pairs of tridentate ONO metal binding domains are perpendicular (88.0°). The exact protonation state of domains cannot be determined, however the angle N(1)–N(2)–C(8) of 115.0(6)° is consistent with the amide being protonated whereas the other three equivalent angles are not (av. 109.5°).∥ As the ligands bridge the Fe^{III} centres an 18-membered chelate ring incorporating both methylene chains and metals now exists. There is an extensive H-bonding network involving the pyridine, and amide nitrogens and pyridoxal methanol, phenolato and solvent oxygen centres.

ESMS reveals that all complexes exist in solution as a distribution of clusters. When n is 1, 2, 4 and 6 dimers dominate the spectra with oligomers up to pentamers identifiable, and no monomer present. For $n = 8$, 10 and 14 the predominant species is the dimer with major amounts of monomer and lesser quantities of higher nuclearity species (Fig. 3). Changing the cone voltage had little effect on spectra showing that aggregation was minimal. In general, monomers will form chelate rings, incorporating the methylene chain, of size ($n + 5$). Molecular mechanics calculations of total energy and modelling for differing values of n suggest that this will be the case for $n \geq 6$. For $n < 6$, oligomeric cluster complexes must exist.

Notes and references

‡ Satisfactory elemental analyses were obtained.
§ Preparation of complex **1**. Addition of FeCl$_3$ (81 mg, 0.50 mmol) in ethanol (7 ml) to a solution of H$_4$L^8 (219 mg, 0.50 mmol) and LiOH (48 mg, 2.00 mmol) in DMF (10 ml) gave a black solution which was stirred for 2 h. The product was filtered off and washed with ethanol then diethyl ether prior to drying *in vacuo*. For **1**, crystals suitable for X-ray analysis were collected from the filtrate. Yields 65–78%. C,H,N: calc.: C 53.7, H 6.0, N 10.5; found: C 53.7, H 6.0, N 10.6%. *Crystal data*: [Fe(HL^8)]·2.5H$_2$O, C$_{24}$H$_{32}$FeN$_4$O$_{6.5}$, $M = 536.5$, black polyhedra, monoclinic, space group $P2_1/c$, $a = 11.135(2)$, $b = 19.350(4)$, $c = 12.405(2)$ Å, $\beta = 98.48(3)°$, $U = 2643.6(8)$ Å^3, $D_c = 1.355$ g cm^{-3}, $Z = 4$, $F(000) = 1124$, μ(Mo-Kα) = 0.619 mm^{-1}; $R = 0.065$, $R_w = 0.064$ using 2639 unique reflections with $I > 4\sigma(I)$. CCDC reference number 186/1608. See http://www.rsc.org/suppdata/dt/1999/3341/ for crystallographic files in .cif format. *Negative-ion ESMS data (mass, %)*: (50 V; pyridine : methanol) M − H(490.1, 100); M$_2$ − H(981.2, 8); M$_3$ − H(1472.3, 1); M$_4$ − H(1963.4, 0.2) where M$_2$ is dimer, M$_3$ is trimer *etc*.
¶ Preparation of complex **2**. FeCl$_3$ (81 mg, 0.50 mmol) was added to a suspension of H$_4$PL^4 (236 mg, 0.50 mmol) and LiOH (48 mg, 2.00 mmol) in ethanol (20 ml). The deep brown solution was stirred with warming for 0.5 h and left to evaporate slowly (4–5 weeks). The complex was filtered and given a cursory wash with ethanol then diethyl ether prior to drying *in vacuo*. Crystals of **2** suitable for X-ray analysis were collected from the reaction mixture. Yields 37–50%. C,H,N: calc.: C 45.5, H 4.9, N 14.5, Cl 6.1; found: C 45.7, H 4.8, N 14.4, Cl 5.9%. *Crystal data*: [{Fe(H$_2$PL^4)}$_2$]Cl$_2$·2H$_2$O, C$_{44}$H$_{56}$Cl$_2$Fe$_2$N$_{12}$O$_{14}$, $M = 1159.6$, black polyhedra, triclinic, space group $P\bar{1}$, $a = 11.9265(3)$, $b = 13.6996(3)$, $c = 18.6798(2)$ Å, $\alpha = 72.145(1)$, $\beta = 85.219(1)$, $\gamma = 87.090(1)°$, $U = 2893.9(1)$ Å^3, $D_c = 1.331$ g cm^{-3}, $Z = 2$, $F(000) = 1204$, μ(Mo-Kα) = 0.661 mm^{-1}; $R = 0.089$, $R_w = 0.231$ using 9630 unique reflections with $I > 2\sigma(I)$. *Negative-ion ESMS data (mass, %)*: (50 V; pyridine:methanol) M$_2$ − 2H(524.1, 100); M$_3$ − 2H(786.7, 1); M$_2$ − H(1049.2, 19); M$_2$ − 2H + Li(1055.2, 15).
∥ The waters and chloride are disordered and the exact location of protons on pyridine rings and/or water is not certain.

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20 See *e.g.* D. L. Caulder and K. N. Raymond, *J. Chem. Soc., Dalton Trans.*, 1999, 1185 and refs. therein.

Communication 9/04728J