DALTON COMMUNICATION

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

William Henderson,[a] Lip L. Koh,[b] John D. Ranford,*[b] Ward T. Robinson,[c] Jan O. Svensson,[c] Jagadese J. Vittal,[b] Yu M. Wang[b] and Yan Xu[d]

[a] *Department of Chemistry, University of Waikato, Private Bag 3105, Hamilton, New Zealand*
[b] *Department of Chemistry, National University of Singapore, Lower Kent Ridge Road, Singapore 119260. E-mail: chmjdr@nus.edu.sg*
[c] *University of Canterbury, Department of Chemistry, Private Bag 4800, Christchurch, New Zealand*
[d] *National Institute of Education, 469 Bukit Timah Road, Singapore 259756*

Received 14th June 1999, Accepted 4th August 1999

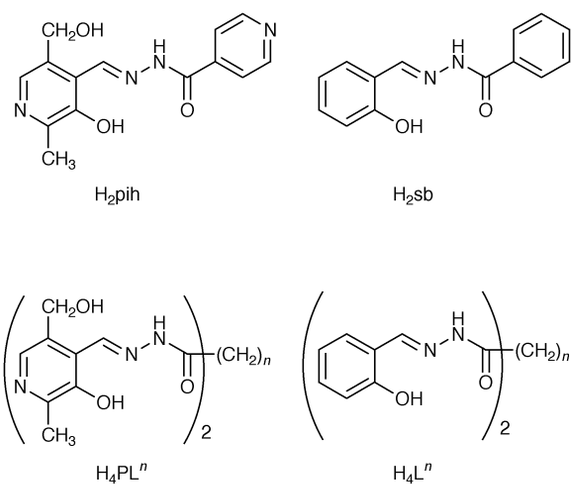
The tridentate metal binding domains of iron chelating agents have been tethered *via* a variable length methylene $(CH_2)_n$ linker (n = 1, 2, 4, 6, 8, 10, 14). The single-crystal X-ray structures of monomeric ($[Fe(HL^8)]\cdot 2.5H_2O$ $n = 8$; H_4L^8 is 1,10-decanedioyl di(salicylaldehyde hydrazone)) and dimeric ($[\{Fe(H_2PL^4)\}_2]Cl_2\cdot 2H_2O$ $n = 4$; H_4PL^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_2pih) looks promising.[6–8] Investigations into H_2pih are at a clinical stage and the *in vivo* iron chelating potential appears good.[9]

The simplest derivative of H_2pih, salicylaldehyde benzoylhydrazone (H_2sb), 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_2sa) has radioprotective[14] and cytotoxic properties.[15] Metal complexes of H_2pih, H_2sb and H_2sa 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_4PL^n [di(pyridoxal hydrazones): where n = 1, 2, 4, 8] and H_4L^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_4L^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 $[Fe(HL^8)]\cdot 2.5H_2O$ **1** and dimeric $[\{Fe(H_2PL^4)\}_2]Cl_2\cdot 2H_2O$ **2**, the first union between iron and this ligand type, and ESMS studies of cluster complexes.



The H_4L^n ligands were prepared as reported[18] with the pyridoxal analogues (H_4PL^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

†*Supplementary data available*: rotatable 3-D crystal structure diagram in CHIME format. See http://www.rsc.org/suppdata/dt/1999/3341/

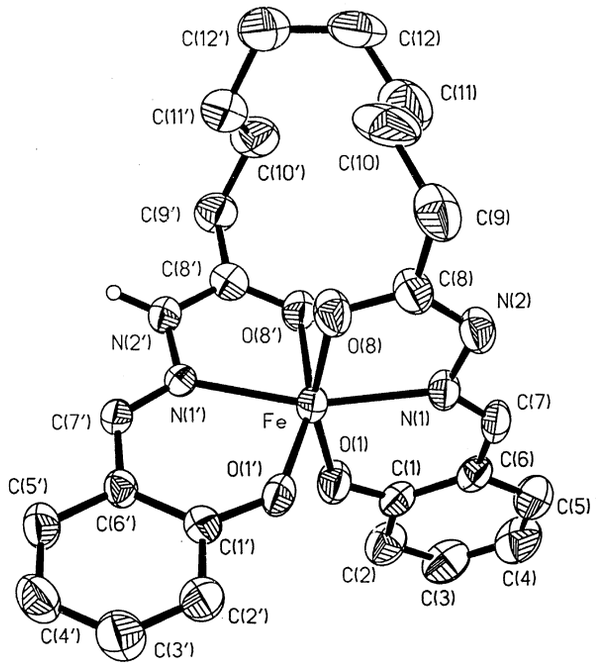


Fig. 1 Molecular structure of monomeric $[Fe(HL^8)]\cdot 2.5H_2O$ **1**. Lattice water molecules have been omitted for clarity. Selected bond distances (Å) and angles (°): Fe–O(1) 1.912(4), Fe–O(1′) 1.912(4), Fe–O(8) 2.026(4), Fe–O(8′) 2.085(4), Fe–N(1) 2.108(5), Fe–N(1′) 2.123(4); O(1)–Fe–O(8) 160.0(2), 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).

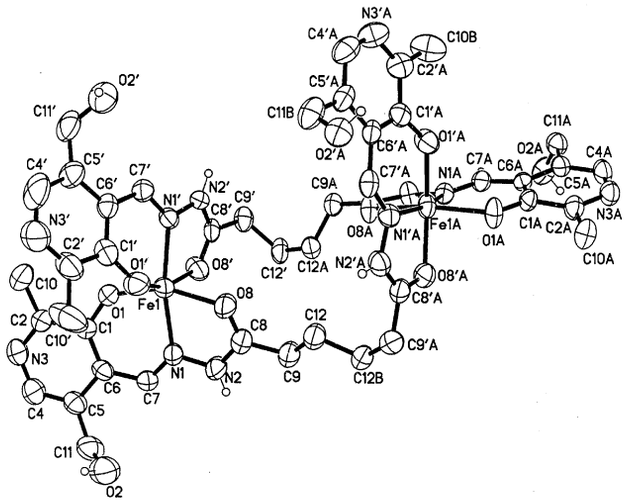


Fig. 2 Molecular structure of dimeric $[\{Fe(H_2PL^4)\}_2]Cl_2\cdot 2H_2O$ **2**. Lattice water molecules and chloride ions have been omitted for clarity. Selected bond distances (Å) and angles (°): Fe(1)–O(1) 1.927(5), Fe(1)–O(1′) 1.920(5), Fe(1A)–O(1A) 1.955(5), Fe(1A)–O(1′A) 1.937(5), Fe(1)–O(8) 2.079(5), 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).

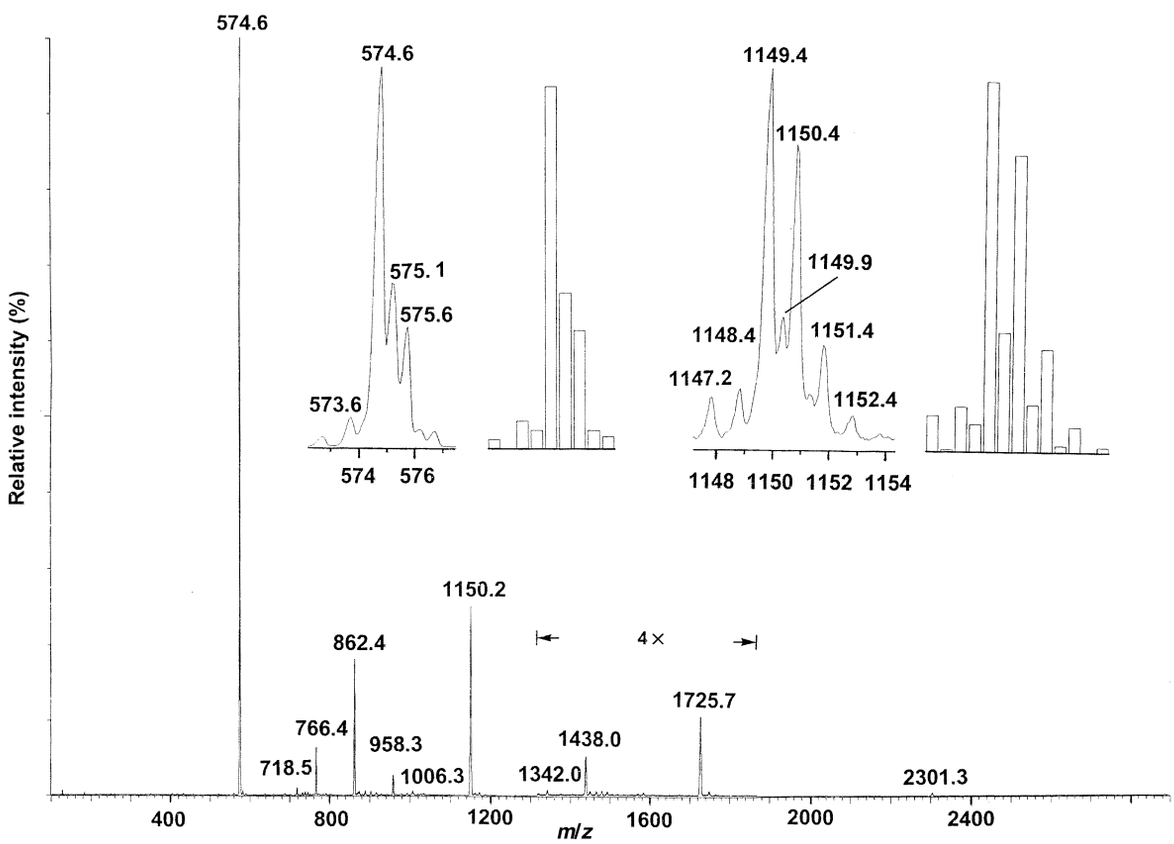


Fig. 3 ESMS spectrum of $[Fe(HL^{14})]\cdot 1.5H_2O$ showing peaks from monomer to heptamer with expansions and calculated patterns for monomer–dimer (3 : 4) and dimer–tetramer (7 : 3) peaks. Assignment of peaks (M = $[Fe(HL^{14})]$, 576 amu, 50 V): 575 $[M - H] + [M_2 - 2H]$ 3 : 4; 719 $[M_5 - 4H]$; 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.

This work was supported by a grant from the National University of Singapore (RP 970614).

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_4L^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*: $[Fe(HL^8)]\cdot 2.5H_2O$, $C_{24}H_{32}FeN_4O_{6.5}$, $M = 536.5$, 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_4PL^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*: $[\{Fe(H_2PL^4)\}_2]Cl_2\cdot 2H_2O$, $C_{44}H_{56}Cl_2Fe_2N_{12}O_{14}$, $M = 1159.6$, 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.

1 M. A. Engle, M. Erlandson and C. H. Smith, *Circulation*, 1964, **300**, 698.

2 P. J. Giardina, R. W. Grady, K. H. Ehlers, S. Burstein, J. H. Graziano, A. L. Marjenson and M. W. Hilgertner, *Ann. N. Y. Acad. Sci.*, 1990, **612**, 275.

3 *The Development of Iron Chelators for Clinical Use*, eds. R. J. Bergerson and G. M. Brittenham, CRC Press, London, 1994.

4 *Iron Transport and Storage*, eds. P. Ponka, H. M. Schulman and R. C. Woodworth, CRC Press, Boston, 1990.

5 *Progress in Iron Research: Advances in Experimental Medicine and Biology*, eds. C. Hershko, A. M. Konijn and P. Aisen, Plenum, New York, 1994, vol. 356.

6 A. Jacobs, *Brit. J. Haematol.*, 1979, **43**, 1; C. Hershko, S. Avramovici-Grisaru, G. Link, L. Gelfand and S. Sarel, *J. Lab. Clin. Med.*, 1981, **98**, 99.

7 P. Ponka, D. Richardson, E. Baker, H. M. Schulman and J. T. Edward, *Biochim. Biophys. Acta*, 1988, **967**, 122; P. Ponka, J. Borova, J. Neuwirt, O. Fuchs and E. Necas, *FEBS Lett.*, 1979, **97**, 317.

8 P. Ponka, R. W. Grady, A. Wilcznska and H. M. Schulman, *Biochim. Biophys. Acta*, 1984, **802**, 477.

9 G. M. Brittenham, V. R. Gordeuk, P. Ponka, P. Pootrakul, S. Fucharoen, P. Wasi and C. A. Finch, *Workshop on the Development of Oral Iron Chelating Agents*, Crete, 1987; G. M. Brittenham, *Semin. Hematol.*, 1990, **27**, 112.

10 P. Ponka, J. Borova, J. Neuwirt, O. Fuchs and E. Necas, *Biochim. Biophys. Acta*, 1979, **586**, 278.

11 D. K. Johnson, M. J. Pippard, T. B. Murphy and N. J. Rose, *J. Pharmacol. Exp. Ther.*, 1982, **221**, 339.

12 H. A. Offe, W. Siefken and G. Domagk, *Z. Naturforsch.*, *Teil B*, 1952, **7**, 446.

13 D. K. Johnson, T. B. Murphy, N. J. Rose, W. H. Goodwin and L. Pickart, *Inorg. Chim. Acta*, 1982, **67**, 159; L. Pickart, W. H. Goodwin, W. Burgua, T. B. Murphy and D. K. Johnson, *Biochem. Pharmacol.*, 1983, **32**, 3868; E. W. Ainscough, A. M. Brodie, W. A. Denny, G. J. Finlay, S. A. Gothe, J. D. Ranford and J. M. Waters, *J. Inorg. Biochem*., in the press.

14 O. V. Arapov, O. F. Alferva, E. I. Levocheskaya and I. Krasil'nikov, *Radiobiol.*, 1987, **27**, 843.

15 L. L. Koh, O. L. Kon, K. W. Loh, Y. C. Long, J. D. Ranford, A. L. C. Tan and Y. Y. Tjan, *J. Inorg. Biochem.*, 1998, **72**, 155.

16 T. B. Murphy, D. K. Johnson, N. J. Rose, A. Aruffo and V. Schomaker, *Inorg. Chim. Acta*, 1982, **66**, L67; T. B. Murphy, N. J. Rose, V. Schomaker and A. Aruffo, *Inorg. Chim. Acta*, 1985, **108**, 183; T. B. Murphy, D. K. Johnson, N. J. Rose, A. Aruffo and V. Schomaker, *Inorg. Chim. Acta*, 1982, **66**, L67; S. C. Chan, L. L. Koh, P.-H. Leung, J. D. Ranford and K. Y. Sim, *Inorg. Chim. Acta*, 1995, **236**, 101; E. W. Ainscough, A. M. Brodie, A. Dobbs, J. D. Ranford and J. M. Waters, *Inorg. Chim. Acta*, 1998, **267**, 27.

17 *Progress in Iron Research: Advances in Experimental Medicine and Biology*, eds. C. Hershko, A. M. Konijn and P. Aisen, Plenum, New York, 1994, vol. 356, p. 343.

18 J. D. Ranford, J. J. Vittal and Y. M. Wang, *Inorg. Chem.*, 1998, **37**, 1226.

19 B. B. Umarov, K. S. Khusenov, M. M. Ishankhodzhaeva, N. A. Parpiev and K. S. Gaibullaev, *Zh. Org. Khim.*, 1996, **32**, 93; R. C. Aggarawal and B. Singh, *Curr. Sci.*, 1978, **47**, 679; K. K. Narang and R. A. Lal, *Transition Met. Chem.*, 1977, **2**, 100; X.-M. Zhang and X.-Z. You, *Polyhedron*, 1996, **15**, 1793; L. Sacconi, *J. Chem. Soc.*, 1954, 1326; X. Wang, X. M. Zhang and H. X. Liu, *Polyhedron*, 1994, **13**, 2611; X. Wang, X. M. Zhang and H. X. Liu, *Acta Crystallogr.*, *Sect. C*, 1994, **50**, 1878; R. A. Lal, S. Das and R. K. Thapa, *Inorg. Chim. Acta*, 1987, **132**, 129; S. Das and R. A. Lal, *Indian J. Chem.*, *Sect. A*, 1988, **27**, 225; M. Husain, S. S. Bhattacharjee, K. B. Singh and R. A. Lal, *Polyhedron*, 1991, **10**, 779; K. K. Narang and R. A. Lal, *Curr. Sci.*, 1977, **46**, 401; R. A. Lal, K. N. Srivastava and S. Das, *Synth. React. Inorg. Met.-Org. Chem.*, 1988, **18**, 837.

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

Communication 9/04728J