A New Diazacyclooctane Dihydroxamate as a Siderophore Analogue — Synthesis and Equilibrium Studies of Iron(III) and Copper(II) Complexes

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A new siderophore analogue based on the mesocyclic diamine 1,5-diazacyclooctane (DACO) functionalized with two additional hydroxamate donor pendant groups, 1,5-diazacyclooctane-1,5-bis(N-methylacetohydroxamic acid) (DACODMAHA), has been synthesized from the corresponding diacetate derivative (DACODA). The complexation behaviour of this ligand towards Cu^{II} and Fe^{III} in aqueous solution has been studied by potentiometry, UV/Vis and EPR spectroscopy and magnetic moment measurements. The Cu^{II} com-

plexation involves the formation of a mononuclear species with a tetradentate mixed amine/hydroxamate coordination mode, whereas the Fe^{III} complexation involves a binuclear species with Fe_2L_3 stoichiometry and normal {O,O}-hydroxamate coordination mode. The redox potential of the iron complexes and the corresponding electron transfer mechanism are also evaluated.

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Introduction

Hydroxamic acid derivatives have been objects of interest for many years due to their natural occurrence and role as microbial iron-transporting compounds (siderophores).[1] Hydroxamate biomimetics have been developed with the aim of obtaining potential constituents of therapeutic agents, for use in chelating therapy (due to their ability to complex iron(III) and related metal ions)[2,3] or as metalloenzyme inhibitors (associated with their complexation with M^{II} transition metal ions).[4,5]

We have been involved in the development and study of a series of polyamine-polyhydroxamate acids as potential siderophore analogues. Their design has been based on macrocyclic or mesocyclic polyamine backbones with N-methylacetohydroxamic acids as pendant arms.[6–9] Some of those cyclic diamine-hydroxamate biomimetics — PIPDMAHA and DACHDMAHA (see Scheme 1) — proved to possess interesting properties as siderophore analogues of rhodotorulic acid, a naturally occurring dihydroxamate siderophore.[9] We therefore decided to extend this set of cyclic diamine-dihydroxamic acids by including a PIPDMAHA homologue with a larger backbone skeleton: 1,5-diazacyclooctane-1,5-bis(N-methylacetohydroxamic acid) (DACODMAHA, see Scheme 1). Its mesocyclic backbone, 1,5-diazacyclooctane (DACO), presents interesting

"boat/chair/crown" configurations and some of its derivatives, those bearing donor pendants such as acetate,[10] thiolate[11] and many other groups,[12–14] have attracted special attention in recent years.

Here we report the preparation of this new ligand (DACODMAHA) as well as the study of its acid—base and complexing properties towards iron(III) and copper(II), mostly by potentiometric and spectroscopic methods. In the absence of X-ray data, because of a lack of suitable crystals, molecular modelling calculations were performed to provide information about the probable topology of the binuclear iron(III)-DACODMAHA complex with 2:3 (metal: ligand) stoichiometry, which can be a mono- or a three-bridged dimer. Since the reduction of the Fe^{III} siderophore complexes has been proposed for the mechanism of microbial Fe release, the redox properties of the iron complexes were studied by voltammetric methods. This set of results is discussed and compared with previous reports on some of the corresponding synthetic or naturally occurring dihydroxamate siderophores.

Results and Discussion

Synthesis

1,5-diazacyclooctane-1,5-bis(N-methylacetohydroxamic acid) (DACODMAHA) was prepared by a two-step strategy, starting from the corresponding dicarboxylic acid derivative, 1,5-diazacyclooctane-N,N'-diacetic acid (DACODA), which was synthesized as described previously.[15] The carboxylic acid groups were condensed with O-benzyl-N-methylhydroxylamine, in the presence of 1-ethyl-3,3-di-

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PIPDMAHA

Rhodotorulic acid (RA)

DACODMAHA

DOCYDMAHA
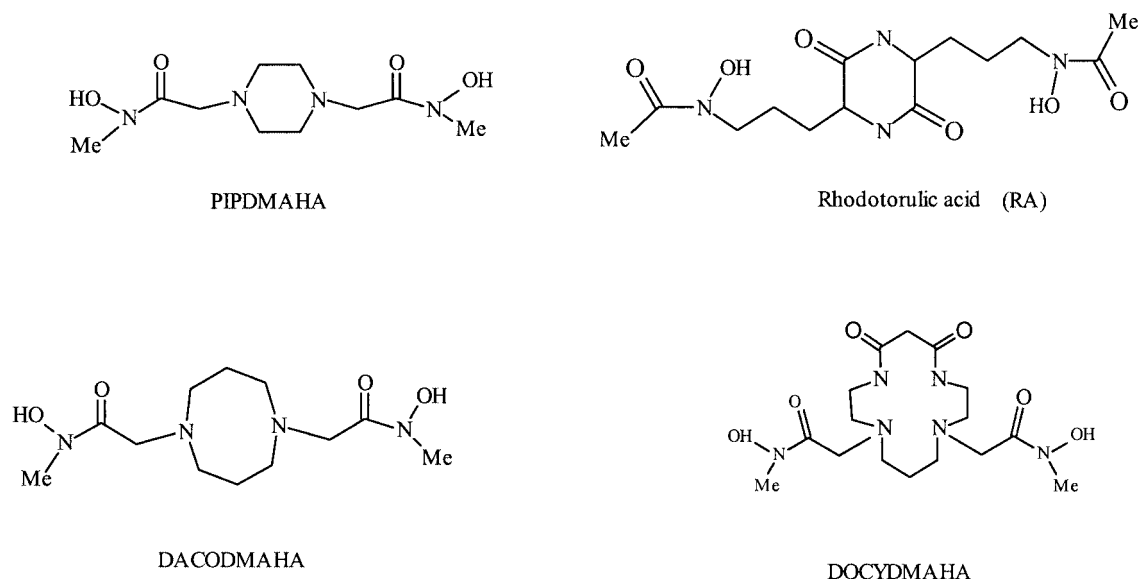
Scheme 1

methylaminopropyl-carbodiimide (EDC), at room temperature and pH 4.8. Next, standard catalytic hydrogenolysis ($P_{H_2} = 1$ atm) of this bis(O-benzylhydroxamate) derivative afforded the free dihydroxamate ligand (DACODMAHA). It is noteworthy that the synthetic scheme used for this ligand is quite different from that previously reported for the smaller size analogues (ex. PIPDMAHA),[6] which involved the coupling of the previously synthesized hydroxamate arms to the amine groups of the cyclic backbones. In the case of the current ligand, however, one of the amine groups in DACO is much more basic than the corresponding groups in the backbones of the previously reported ligands (see below), so the use of the same coupling reaction in the synthesis of DACODMAHA would require more drastic basic conditions, which would probably result in the subsequent hydrolytic scission of the hydroxamate peptidic bond.

Protonation Studies

The acid−base behaviour of this N,N'-bis(hydroxamate)-diazacyclooctane derivative (DACODMAHA) was studied by potentiometry. A representative pH titration curve for the ligand is presented in Figure 1. The fully protonated form of this ligand can release four protons, although only three protonation constants can be calculated from direct potentiometric data, since the first is too high to be accurately determined by this method (under our experimental conditions it is limited to the range 2−12).[16]

Thus, to achieve a full acid−base characterization of the ligand, a ^{1}H NMR titration was carried out (Figure 2). The indicated peak attribution was based on the corresponding integration areas and multiplicity. The ^{1}H NMR titrations curves corresponding to the three sets of methylenic protons show two well defined inflections at pD* ≈ 2 ($\Delta\delta_a = 0.47$ ppm, $\Delta\delta_c = 0.37$ ppm, $\Delta\delta_d = 0.40$ ppm) and pD* ≈ 13 ($\Delta\delta_a = 0.21$ ppm, $\Delta\delta_c = 0.38$ ppm, $\Delta\delta_d = 0.33$ ppm)
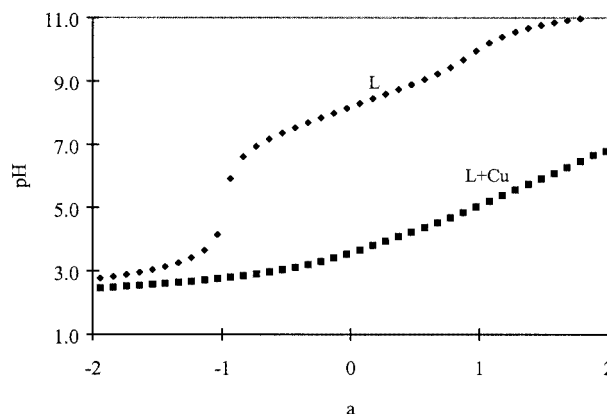

Figure 1. Potentiometric titration curves (experimental points) for the ligand H_2L (DACODMAHA) and for the copper(II)-H_2L system; $C_L = 2.4 \times 10^{-3}$ M, $C_L/C_{Cu} = 1:1$, $I = 0.1$ M (KNO$_3$); $T = 25.0$ °C

regions. A small inflection at pD* ≈ 8 ($\Delta\delta_a = 0.04$ ppm, $\Delta\delta_c = 0.05$ ppm, $\Delta\delta_d = 0.135$ ppm) region is due to the hydroxamate methyl protons, as would be expected. As in the cases studied previously,[17] attribution of the protonation processes (and stepwise protonation constants) to individual basic centres in the ligand is straightforward. The first and last constants (K_1 and K_4) are mainly associated with the protonation of the macrocyclic amines, while the remaining two (K_2 and K_3) are attributable to the protonation of the hydroxamate groups. Further analysis of the sequence of protonation of the four basic sites and calculation of the corresponding microconstants is beyond the scope of this work.

As mentioned above, only three protonation macroconstants (K_2, K_3, K_4) could be determined through fitting analysis of the potentiometric titration data (with the aid of the SUPERQUAD program[18]), while the first protonation constant (K_1) was obtained from fitting of the ^{1}H NMR
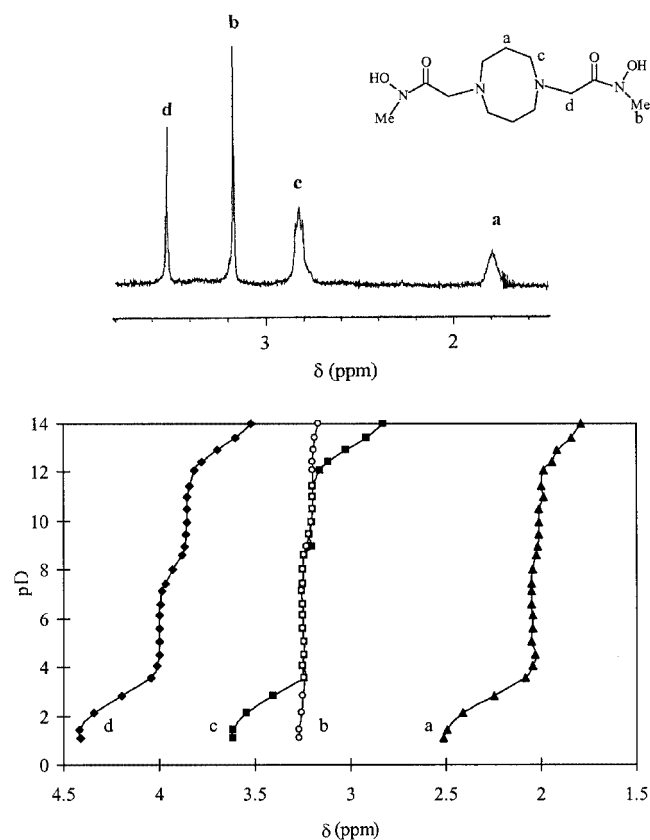
Figure 2. Proton NMR titration curves for DACODMAHA ($C_L = 2 \times 10^{-2}$ M); the pD* of the solution was adjusted with DCl and KOD

titration curves (Figure 2) with the aid of the PSEQUAD program.[16] The refined logarithmic stepwise protonation constants (log K) are listed in Table 1, together with the corresponding values for two structural analogues.

The first protonation constant of DACODMAHA (log $K_1 = 13.04$), attributable to the protonation of an amine group, is higher than the corresponding value for the cyclic backbone, DACO (log $K_1 = 10.74$).[19] This feature may be due to the formation of a very stable hydrogen bond be-

Table 1. Stepwise protonation constants of DACODMAHA, DACO and DACODA; global stability constants and spectroscopic data of the corresponding copper(II) complexes

Ligand	H+ log K_i	Cu2+ log $\beta_{Cu_pH_qL_r}$[a]	λ_{max}/nm (ε/M^{-1}cm^{-1})
DACODMAHA	13.0(3)	(1,2,1) = 29.7(1)	640 (112); pH = 2.99
	8.91(1)	(1,1,1) = 24.7(1)	595 (170); pH = 8.40
	7.45(2)		
	2.22(5)		
DACO	10.74[b]	(1,1,1) = 17.84[b]	637 (116)[c]
	7.25	(1,0,1) = 10.56	
DACODA	12.27[d]		
	4.70	(1,0,1) = 16.44[e]	621 (152)[c]
	1.84		

[a] The (*p,q,r*) symbolism means a species with stoichiometry (Cu$_p$H$_q$L$_r$) [b] From ref.[19] [c] From ref.[23] [d] From ref.[15] [e] From ref.[28]

tween the corresponding ammonium proton and the nearby nitrogen atom of the cyclic backbone, which should be stronger in DACODMAHA than in DACO. The corresponding dicarboxylic derivative, DACODA, showed similar behaviour (log $K_1 = 12.27$)[15] and it was proposed that the introduction of the acetate groups induces the "boat" as the major macrocyclic conformation, while DACO shows three conformations (boat, chair and crown). Furthermore, of the three conformations of the monoprotonated macrocycle, the "boat" presented the strongest $NH\cdots N$ hydrogen bond. Therefore, for similar reasons, the introduction of the hydroxamate arms should favour the "boat" conformation in DACODMAHA. On the other hand, there is an additional stabilization of the DACODMAHA monoprotonated species, due to the hydrogen bond between the ammonium proton and the deprotonated oxygen atom of the adjacent hydroxamate group, through a six-membered ring interaction. The quite low protonation constant of the second nitrogen atom of DACODMAHA (log $K_4 = 2.22$) supports the existence of such a strong hydrogen bond network, namely between the ammonium proton and the amine N atom ($>N^+$-$H\cdots N<$) as well as between the deprotonated amine nitrogen atom and the adjacent hydroxamic proton ($>N\cdots HO-$). The remaining protonation constants (log $K_2 = 8.91$, log $K_3 = 7.45$) agree with the expected values for hydroxamate groups (log $K = 8-10$),[20] although their values are slightly lowered by inductive/electrostatic effects of the α-amino/ammonium groups.

Copper(II) Complexation

The complexation behaviour of DACODMAHA toward copper(II) in aqueous solution was studied by potentiometry and UV/Vis spectrophotometry. A representative pH-metric titration curve of this binary system ($C_{Cu} = 2.4 \times 10^{-3}$ M, $C_L/C_{Cu} = 1:1$) is shown in Figure 1. Some precipitation was observed above pH 5. Analysis of the potentiometric titration curves shows that, in the presence of copper(II), the fully protonated form of the ligand is able to dissociate four protons. The global formation constants (Table 1) were calculated from fitting analysis of the titration curve by use of the SUPERQUAD computer program.[18] Below pH ≈ 5, the best model, included only two species, [CuH$_2$L]$^{2+}$ and [CuHL]$^+$; their overall stability constants (log β) are 29.7 and 24.7, respectively. For higher pH values, neutral species (ex. CuL) or mixed metal-ligand-hydroxo complexes are formed but they are insoluble under our experimental conditions. On the assumption that the last equivalent of base consumption corresponds mainly to the release of the last labile ligand proton, the drastic decrease in the corresponding pH (ca. 5−6) indicates that this proton is being pushed by the metal ion. A rough evaluation of the stability constant of the neutral species (log β$_{CuL}$ ≈ 19) indicates that it would be quite high and closer to the corresponding overall constants of the 1:2 complex (log β$_{CuL2}$) for α-aminohydroxamic acids (19.89 for glycine-hydroxamic acid)[21] than for simple hydroxamic acids (14.06 for acetohydroxamic acid).[22]
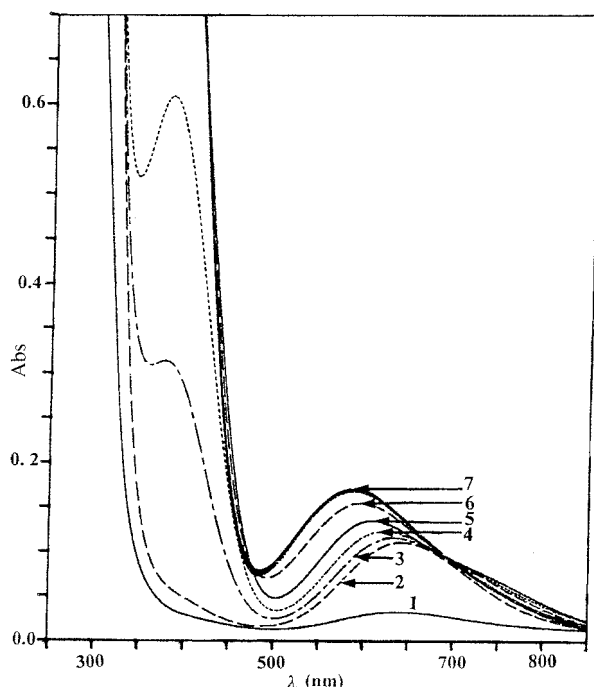
Figure 3. Absorbance spectra of Cu^{II}-DACODMAHA system as function of pH: 1.56 (1), 2.44 (2), 3.73 (3), 4.34 (4), 5.52 (5), 6.36 (6), 7.42−12.03 (7); $C_L = 3 \times 10^{-3}$ M, $C_L/C_M = 3:1$, $I = 0.1$ M (KNO_3), $T = 25.0\ °C$

In order to obtain further insight into the coordination modes involved in the Cu^{II}-DACODMAHA complexes, UV/Vis spectra were recorded for solutions with $C_L = 3 \times 10^{-3}$ M and 1:3 copper-to-ligand molar ratio at different pH values. We did not observe any precipitate in this study, probably due to the excess of ligand and/or to the higher dilution conditions. The Cu^{II}-DACODMAHA spectra (Figure 3) each exhibit a broad band in the visible region, attributable to the copper d-d transition energy (for pH = 1.6, $\lambda_{max} = 635$ nm, $\varepsilon_{max} = 48$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$; for pH = 7.4−12.0, $\lambda_{max} = 595$ nm, $\varepsilon_{max} = 170$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). At pH = 3.7, a charge transfer (c.t.) band started to appear at $\lambda_{max} = 400$ nm, its intensity increasing with the pH (for pH = 5.52, $\lambda_{max} = 400$ nm, $\varepsilon_{max} = 960$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). The isosbestic point at $\lambda = 680$ nm observed for $4 < pH < 7$ suggests the interconversion of two major chromophoric species in that pH range.

Conjugation of spectroscopic and potentiometric data was analysed with care, since different stoichiometries were used. Spectral analysis indicates that, in the highly acidic pH range (pH 1.6−2.4), there is only one major chromophoric species ($\lambda_{max} = 635-640$ nm), which should correspond to the CuH_2L species [or possibly $Cu(H_2L)_2$ for excess of ligand]. Spectra at pH 2.44 ($\lambda_{max} = 640$ nm and the absence of the c.t. band at 400 nm, but around 300 nm) suggest for that complex species a copper coordination sphere involving just one or two amine groups of the macrocycle and water molecules (see Scheme 2). In fact, the DACO-Cu^{II} complex, with Cu^{II} coordinated to the amine groups of the macrocycle, exhibits $\lambda_{max} = 637$ nm,[23] and

also some Cu^{II}-α-aminohydroxamic acid complexes with Cu^{II} coordinated to two nitrogen atoms and two water molecules, exhibit $\lambda_{max} = 647-650$ nm.[24] In the pH range around ca. 4−7 there is an interconversion of two species, which is accompanied by a probable change in the coordination mode. In fact the spectral changes that occurred along this pH range − the blue shift and the intensity increase in the d-d transition band, together with the appearance of the band at 400 nm − suggest an increase in the hydroxamate coordination with pH. For some aminohydroxamate ligands, UV bands identical to those seen in the oxygen-hydroxamate to Cu^{II} charge transfer were assigned.[8,25] On the other hand, visible bands with similar λ_{max} values were also found for Cu^{II} complexes with other cyclic polyaminopoly(N-methyl-hydroxamate) ligands, for which a mixed coordination of N-amine atoms of cyclic backbones and of O-hydroxamate atoms of the side arms was considered: $\lambda_{max} = 600$ nm for 1,4-diaazacycloheptane-N,N'-bis(N-methylacetohydroxamic acid);[26] $\lambda_{max} = 590$ nm for 4,8-(12,14-dioxo-1,4,8,11-tetraazacyclotetradecane)bis(N-methylacetohydroxamic acid) (DOCYDMAHA).[27] The UV/Vis spectroscopic data of copper(II) complexes with other DACO derivatives bearing different substituents also show absorption maxima around 594−637 nm, attributed to five-coordinate Cu^{II} complex, with distortion from square-pyramidal towards trigonal structures.[12] Furthermore, metal ion coordination through the amine nitrogen atoms and the acetate oxygen atoms has also been proposed for the DACODA-Cu^{II} complexes possessing acetate groups bonded to the macrocycle.[28] Thus, for the CuHL and CuL species, the coordination of Cu^{II} to the ligand should involve not only the mesocyclic amines but also one or two hydroxamate groups, respectively, and one water molecule that occupies the fifth coordination position. The absence of the sixth coordination position, as happens in other similar complexes with DACO derivatives, may also be assumed, consistently with the expected boat-chair conformation of the ligand. The central methylene C−H groups of the boat form in DACO shields the Cu^{II} centre, thus blocking the sixth coordination position.[13]

Electron Paramagnetic Resonance: To advance the study of the type of coordination involved in the DACODMAHA-Cu^{II} complexes further, EPR spectra were obtained over a wide pH range (2−10). No EPR-silent pH region was detected in that pH range, thus suggesting the absence of binuclear species under our experimental conditions. Two EPR spectra were selected (pH 2.99 and 8.40), because the major species under these pH conditions should be CuH_2L and CuL, respectively. All the recorded spectra (Figure 4) present the four lines due to the copper coupling.

The corresponding EPR parameters present $g_{//} > g_\perp$ ($g_{//} \cong 2.40$, $g_\perp \cong 2.08$, $A_{//} \cong 164 \times 10^{-4}$ cm^{-1}, $A_\perp \cong 35 \times 10^{-4}$ cm^{-1}, for pH = 2.99, and $g_{//} \cong 2.26$, $g_\perp \cong 2.07$, $A_{//} \cong 167 \times 10^{-4}$ cm^{-1}, $A_\perp \cong 30 \times 10^{-4}$ cm^{-1}, for pH = 8.40), indicating an axially elongated-tetragonal copper(II) environment, as is the case with most of the Cu^{II} complexes in solution. For pH = 2.99, the calculated spectral param-
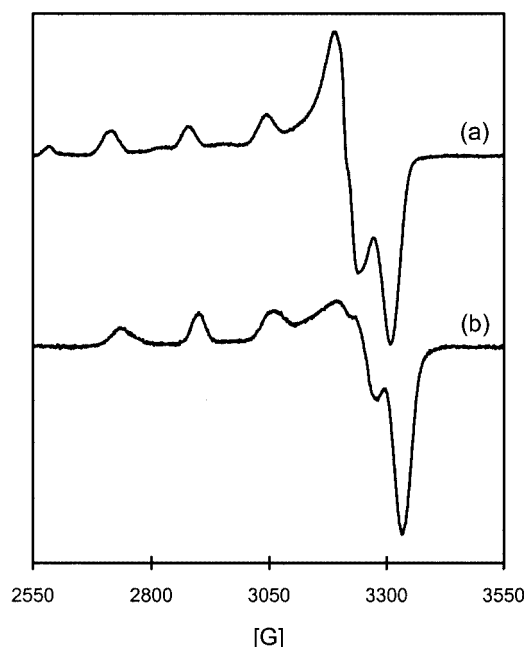
CuH₂L   CuHL   CuL
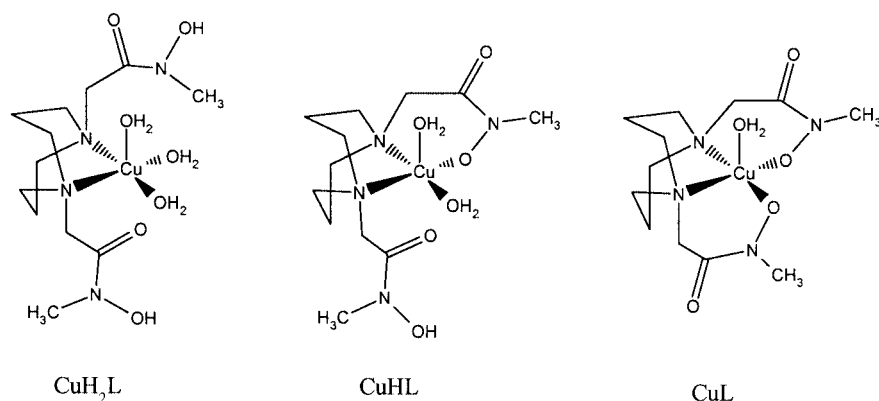
Scheme 2

Figure 4. EPR spectra of frozen D₂O solutions with 20% ethylene glycol containing the Cu^{II}-DACODMAHA complexes at: pD* = 2.99 (a), pD* = 8.40 (b) (C_{Cu} = 6.0 × 10⁻³ M, C_L/C_{Cu} = 1:1; T = 100 K; frequency 9.34 GHz and modulation frequency 100 kHz)

eters indicate complex formation. However, Cu^{II} still seems to be in equilibrium with water molecules ($g_{//}$ = 2.401 and $A_{//}$ = 146 × 10⁻⁴ cm⁻¹ for the Cu^{II} aquo complex)[29] and it is possible that the copper cation is coordinated by two ligand nitrogen atoms. At pD* = 8.40, there is one predominant species, for which the EPR parameters are close to those reported in the literature for some forms of Cu^{II}-α-aminohydroxamic acids complexes ($g_{//} \cong$ 2.33; $A_{//} \cong$ 167 × 10⁻⁴ cm⁻¹).[29] In this species Cu^{II} is coordinated through mixed (N,N) and (O,O) bonding modes. On the other hand, the parameters calculated for this major species, namely $A_{//}$, do not seem to give support to the hypothesis of Cu^{II} being coordinated by two hydroxamate groups ($g_{//} \cong$ 2.25−2.29; $A_{//} \cong$ 189−190 × 10⁻⁴ cm⁻¹).[30] Furthermore, the difference between $g_{//}$ and $g_⊥$ (Δg) is slightly higher at pH 2.99 (Δg = 0.23) than at pD* 8.40 (Δg =

0.21), thus suggesting that the presence of the hydroxamate groups in the copper coordination sphere induces less distortion from the square-planar geometry towards the tetragonal than its absence. In conclusion, the EPR spectra support the hypothesis suggested by the UV/Vis spectra, that DACODMAHA-Cu^{II} complexes have a five-coordinate structure with distorted square-pyramidal geometry. In the acid range, CuH₂L should have Cu^{II} coordinated only to the amine groups of the macrocycle and possibly three water molecules; at neutral pH, besides the coordination to the amine groups of the macrocycle, the metal centre should be also coordinated to one and two hydroxamate groups in CuHL and CuL, respectively, with one water molecule in the fifth apical position (see Scheme 2).

Iron Complexion

The interaction of DACODMAHA with iron(III) was studied by UV/Vis spectrophotometric titration (C_{Fe} = 1.80 × 10⁻⁴ M with tenfold ligand excess to avoid precipitation of hydroxo complexes). The set of visible spectra registered for the DACODMAHA-Fe^{III} system (Figure 5) shows that complex formation begins at a very low pH (pH < 2). There is a typical ferric-hydroxamate charge-transfer (c.t.) band, the intensity enhancement of which and its blue shift with increasing pH indicate greater coordination to the ferric ion (at pH 2.08, λ_{max} = 475 nm, ε = 1660 M⁻¹·cm⁻¹/Fe; at pH 8.5, $\lambda_{max.}$ = 420 nm, ε = 2666 M⁻¹·cm⁻¹/Fe). The isosbestic point at λ = 475 nm suggests the existence of only two major species in the pH range 3−5. For pH 5−9, $\lambda_{max.}$ and $\varepsilon_{max.}$ are practically pH-independent, indicating the existence of one predominant complex species. Its spectroscopic data is consistent with three hydroxamate moieties coordinated to Fe^{III} [31] (probably as a dimeric structure with Fe₂L₃ stoichiometry). At ca. pH 2.1, according to the spectroscopic data presented above, the major species should already have two hydroxamate moieties coordinated to each ferric centre (probably as a monomeric species). The isosbestic point should therefore be the result of an equilibrium between the monomeric and the dimeric species. Above
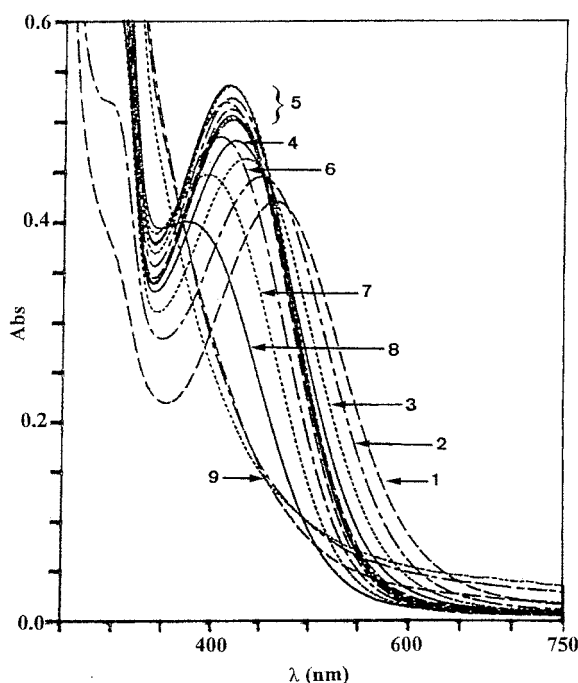
Figure 5. Absorbance spectra of Fe^{III}-DACODMAHA as a function of pH: 2.08 (1), 3.20 (2), 3.77 (3), 4.31(4), 4.91−8.97 (5), 9.38 (6), 9.92 (7), 10.48 (8), 10.92−11.83 (9). $C_{Fe} = 1.8 \times 10^{-4}$ M, $C_L/C_{Fe} = 10:1$; $I = 0.1$ (KNO$_3$); $T = 25.0$ °C
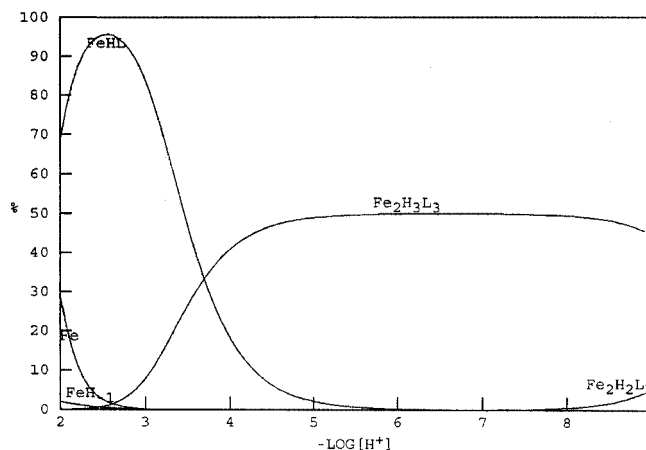

Figure 6. Distribution species diagram for the Fe^{III}-DACOD-MAHA system as a function of pH ($C_L/C_{Fe} = 10:1$; $C_{Fe} = 1.8 \times 10^{-4}$ M; $I = 0.1$ M (KNO$_3$); $T = 25$ °C)

pH = 9, the corresponding ferric complexes exhibit decreases in their $\varepsilon_{max.}$ and $\lambda_{max.}$ values (orange colour lightens to yellowish) with increasing pH, which is attributable to mixed hydroxo-hydroxamate complexes with only two hydroxamate moieties coordinated to each ferric centre. These results are similar to previously reported for rhodotorulic acid[32] and other synthetic dihydroxamic acids.[6,26,33]

The stability constants of the DACODMAHA-iron(III) complexes were determined by fitting analysis of the spectrophotometric titration data with the aid of the PSE-QUAD[16] program. In the pH range from 2−9, the best-fitting model includes one monomeric species [Fe(HL)]$^{2+}$ and two dimeric species ([Fe$_2$H$_3$L$_3$]$^{3+}$ and [Fe$_2$H$_2$L$_3$]$^{2+}$), the global stability constants of which are 28.91, 86.77 and 76.83, respectively. The corresponding species distribution diagram (Figure 6) is consistent with the spectral observations, namely the existence of the monomeric species (FeHL) in the acidic pH range and of the dimeric complex (Fe$_2$H$_3$L$_3$) in the neutral zone. The assigned Fe$_2$H$_2$L$_3$ species that started to form at ca pH 8 could also be attributable to a mixed Fe$_2$H$_3$L$_3$(OH) complex, or both may possibly occur, but these species are not distinguishable by the PSE-QUAD program. Although the first assumption seems consistent with the spectral analysis, above pH = 9, hydrolytic processes compete with the further deprotonation of the complexed ligand.

A brief comparative analysis of the iron affinity of DAC-ODMAHA and other related dihydroxamate ligands can be made from the set of results presented (Table 2), namely from the corresponding pFe values at pH = 7 (pFe = -log [Fe] for $C_L/C_{Fe} = 10$ and $C_L = 10^{-5}$ M), to take account of

the different proton concentration dependency of the different ligands. It is clearly shown that at neutral pH DACOD-MAHA has less affinity for Fe^{III} (pFe ≈ 17) than the related dihydroxamate derivatives (pFe ≈ 21 for PIPDMAHA and DOCYDMAHA).[9,33]

Since all these ligands form dimeric diiron complexes with the same stoichiometry and probably identical structures under these experimental conditions (the triply ligand-bridged coordination mode, see below), there are no relevant differences in the interaction between the two metal centres. Therefore, the ca 4 orders of magnitude difference in the pFe values should be attributable to overall charge differences due to pK_a differences. In fact, protonated DAC-ODMAHA is positively charged as a ligand, whereas the others are neutral Fe^{III} binding ligands. At neutral pH, the current complex (Fe$_2$H$_3$L$_3$) therefore has the ligand protonated with the concomitant Fe^{3+}-H^+ electrostatic repulsions and an overall decrease in the stability of the complex, while for the analogous ligands the corresponding complex species are deprotonated. Furthermore, comparison between the calculated pFe values associated with the ferric complexation with the current ligand and N-methyl-acetohydroxamic acid (pFe = 16)[34] indicates that the entropic factors associated with the polydenticity of the ligand in this study hardly overcome the destabilizing charge effects on the overall binding interaction. Furthermore, the weaker O-donor capacity of the hydroxamate groups is consistent with lower protonation constant found for one of the hydroxamate groups (log K_3 = 7.45), due to the electronic effect of the α-ammonium moiety.

Electron Paramagnetic Resonance: To obtain extra information on the dimeric complex of DACODMAHA with iron, the EPR spectrum of DACODMAHA-Fe^{III} system was registered at neutral pH (Figure 7), together with the corresponding spectra for DOCYDMAHA and PIPDMAHA iron complexes, for purposes of comparison.

Each of these spectra shows one single signal at $g = 4.3$, a characteristic value of high-spin octahedral ferric complexes, as previously found for the naturally occurring di-

Table 2. Global formation constants (log $\beta_{Fe_pH_qL_r}$), electronic spectroscopic data, pFe values, magnetic moments, and half-wave reduction potentials for the ferric complexes with DACODMAHA, DOCYDMAHA, PIPDMAHA and rhodotorulic acid

Ligand	log $\beta_{Fe_pH_qL_r}$[a]	λ_{max}/nm, (ε/$M^{-1}cm^{-1}$)	pFe[b]	μ_{Fe} (BM)	$E_{1/2}$ (mV)
DACODMAHA	(1,1,1) = 28.91(3)	420	16.6	5.8	−502
	(2,3,3) = 86.77(8)	(2666)			
	(2,2,3) = 76.83(8)				
DOCYDMAHA	(2,0,3) = 60.50[c]				
	(1,2,1) = 27.94				
	(1,1,1) = 24.50	425	21.1[c]	5.8[c]	−603[c]
	(1,0,1) = 21.33	(2995)[d]			
	(1,-2,1) = 5.07				
PIPDMAHA	(2,0,3) = 61.7[e]	425	21.5[c]	5.5[e]	−560[c]
		(2880)[e]			
Rhodotorulic acid[f]	(1,0,1) = 21.55	425	21.9	5.9	−601[c]
	(2,0,3) = 62.3	(2700)			

[a] The (p,q,r) symbology means a species with stoichiometry (Fe$_p$H$_q$L$_r$). [b] pFe = −log [Fe^{3+}] at pH = 7.4, C_{Fe}= 1 × 10^{-6} M, C_L = 1 × 10^{-5} M. [c] From ref.[9] [d] From ref.[33] [e] From ref.[6] [f] From ref.[35]

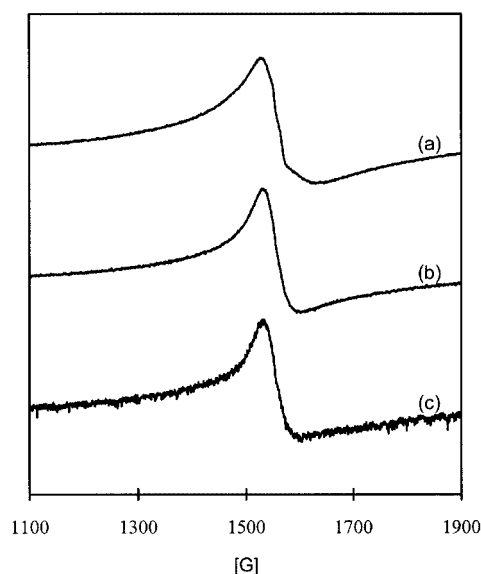Figure 7. EPR spectra of frozen D$_2$O solutions containing: (a) ferric-PIPDMAHA, (b) ferric-DOCYDMAHA and (c) ferric-DACODMAHA complexes (C_{Fe}/C_L = 2:3, pD* = 7; C_{Fe} = 1.00 × 10^{-3} M); T = 100 K; frequency 9.34 GHz and modulation frequency 100 kHz)

hydroxamate rhodotorulic acid.[35] The signal linewidth obtained for the dimeric ferric-DACODMAHA complex (72 G) is close to the value found for the DOCYDHAMA-Fe^{III} complex (70 G)[33] and for the ferric rhodotorulate in methanol (51 G), but much lower than the corresponding value for the PIPDMAHA-Fe complex (120 G). The ferric rhodotorulate complex has a distance of ca. 9−10 Å between the two ferric centres, which may be regarded as enough for the existence of significant metal interactions,[35] while the results obtained for the PIPDMAHA dimeric complex indicated dipole-dipole interactions between the metal centres.[6] This type of interaction has also been observed in ferric dimeric complexes of other dihydroxamate ligands, analogues of rhodotorulic acid.[31] These results therefore support the assumption that DACODMAHA forms di-

meric species with iron(III), although with weak magnetic interactions between the two metal centres.

Magnetic Properties: The molar magnetic susceptibility (χ_M) and magnetic moment (μ_{Fe}) of the DACODMAHA ferric complexes were measured in D$_2$O solution as a function of pH (pD*), by Evans' method.[36] In the pH 5−8 range (Fe$_2$H$_3$L$_3$ is the major species), the values found for the magnetic moments (μ_{Fe} = 5.83−5.74 BM) are consistent with the existence of a high-spin octahedral complex. They are just between the value for an octahedral unimolecular spin-free iron(III) complex (5.92 BM) and the literature value (5.7 BM) reported for some binuclear iron(III) complexes with very weak interactions between the ferric centres.[31] The ferric rhodotorulate and other dihydroxamate complexes have μ_{Fe} = 5.9 BM, while the ferric PIPDMAHA complex presented μ_{Fe} = 5.5 BM.[6] These results, together with the EPR spectra, suggest that, in the neutral pH range, the DACODMAHA diferric complex has the iron sites enough apart from one another and so only weak or negligible metal−metal interactions.

Molecular Simulation: In order to gain further insight into the Fe$_2$H$_3$L$_3$ complex structure, molecular mechanics modelling calculations were performed, through the use of the CERIUS-2 program[37] and also by a previously reported approach.[7] This study was aimed at determining the most probable configuration involved in the DACODMAHA-Fe^{III} dimeric complex, considering two different hypothesis (see Figure 8): (a) three-bridge conformation, similar to that found for the ferric-rhodotorulic acid complex,[38] in which the three ligand molecules each have a hydroxamate moiety coordinated to a different metal centre, and (b) one-bridge conformation, similar to that in the ferric-alcaligin complex,[39] in which only one ligand molecule is coordinated to two metal centres, while the remaining two ligand molecules have both the hydroxamate moieties with intramolecular coordination to the same metal ion.

These theoretical modelling studies showed that, for the dimeric ferric-DACODMAHA complex, conformation (a) is more stable than conformation (b), and that the distance
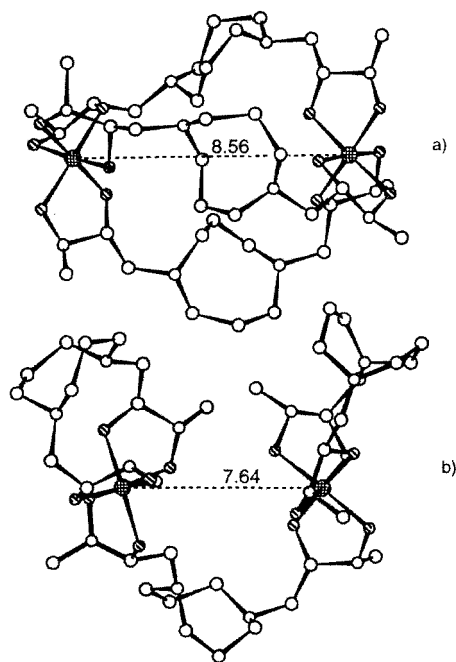
Figure 8. Low-energy conformations simulated for the dimeric complexes of the Fe^{III}/DACODMAHA system

between the ferric centres should be ca. 8.6 Å. This result supports the hypothesis that DACODMAHA forms a binuclear complex with weak interactions between the ferric centres. It is noteworthy that similar theoretical conformational preferences were found for DOCYDHAMA and PIPDHAMA ferric complexes, in which distances between the ferric centres were ca 8.5 Å and 7.7 Å, respectively. The lower stability associated with the structure with the mono-bridged coordination mode (b) should be mainly due to the existence of some steric strain involved in two ligand molecules, each one tetracoordinate to the same metal centre.

Electrochemistry of the Fe$_2$L$_3$ Complex: Cyclic voltammetry studies were carried out for the iron-DACODMAHA system in aqueous solution ($C_{Fe} = 2.5 \times 10^{-4}$ M) containing a 20-fold ligand excess, at pH = 7.0. A cathodic peak and the corresponding anodic counterpart were found. Analysis of the scan-rate effect on the voltammograms suggested a global mechanism involving an electrochemical reversible step (E) followed by an irreversible chemical reaction (C), perhaps a dissociation. In fact, the diagnostic criteria for (EC) global mechanisms are followed because: E_p^c shifts cathodically by around 30/n mV (16 mV) for a tenfold increase on v, for low scan rates; $i_p/v^{1/2}$ is virtually constant, in the absence of adsorption ($v < 5$ V/s) and the i_p^a/i_p^c ratio approximates to unity with increasing scan rate. The electrochemical reaction should involve a two-electron transfer because for $v = 20$ V/s, where the chemical reaction practically has no time to occur, $\Delta E_p = 30$ mV ($\Delta E_p = 60/n$ for a reversible reaction).[40,41]

This two-electron transfer agrees with Fe$_2$H$_3$L$_3$ being the major species at pH = 7 and the expected change of one electron per ferric centre. The fact that E_p^c presented a cathodic shift with increasing pH (pH = 5−8), as a linear

variation with a slope of ca 60 mV indicates that the global electrochemistry reaction involves the transfer of two electrons and two protons. On the other hand, the independence of E_p^a and E_p^c of the ligand concentration ($C_L/C_{Fe} = 10-30$) indicates that both the reduced and oxidized complexes have the same coordination number in relation to the ligand. Thus, the global electrochemical reaction can be represented by the equilibrium Equation (1). The overall stability constant for the reduced species was derived from Equation (2), by the same approach as reported previously.[6,42]

$$[Fe_2^{III}H_3L_3]^{3+} + 2H^+ + 2\overline{e} \rightleftharpoons [Fe_2^{II}H_5L_3]^{3+} \tag{1}$$
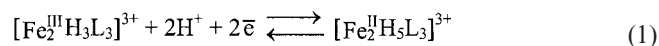
$$E_p^c - E_p^s = -\frac{59.15}{2} \times \log\frac{\beta^{III}_{Fe_2H_3L_3}}{\beta^{II}_{Fe_2H_5L_3}} - 59.15 \times pH \ (25\ ^\circ C) \tag{2}$$

The lower stability of the reduced complex (log $\beta^{II}_{Fe_2H_5L_3}$ = 67.01) relative to the oxidized one [log $\beta^{II}_{Fe_2H_5L_3}$ = 86.65] is consistent with the assumed easy dissociation of that species. The reduction potential obtained for the Fe^{III}-DACODMAHA system ($E_{1/2} = -502$ mV) is lower than the corresponding values for other dihydroxamate analogues and rhodotorulic acid[43] (see Table 2). This is not a surprising feature, however, in view of its lower affinity for the iron. Since this potential is closer to the range of physiological reducing agents such as NADH, the complex species is easily reduced and some differences in its possible biological activity are probable. Since many factors influence the biological activity of a siderophore, biological assays need to be done to evaluate the potential of this new siderophore model.

Conclusions

This study reports the preparation of a DACO derivative bearing two hydroxamate arms (DACODMAHA) and its complexation behaviour towards Cu^{II} and Fe^{III} in relation to that of previously reported analogues. With regard to its complexation with copper(II), this ligand can act as a tetradentate ligand, forming stable five-coordinate mononuclear complexes, with the involvement of amine/hydroxamate and H$_2$O coordination. In the case of iron(III), DACODMAHA-metal binding occurs exclusively through the hydroxamate groups, leaving the amine groups free for potential extra interactions. Although it can act as a tetradentate ligand towards Fe^{III}, forming a mononuclear species under acidic conditions, under conditions of ligand excess and around the physiological pH zone, the Fe^{III} coordination sphere can be completed by formation of a binuclear complex [Fe$_2$(HL)$_3$] with a triply ligand-bridged structure, in analogy with rhodotorulic acid. At neutral pH, that complex is a three-protonated species, with inherent charge effects, which should account for the lower stability of the DACODMAHA-Fe^{III} complexes, relative to other dihydro-

xamate siderophore analogues reported previously. This effect is further reflected in the redox potential, which becomes more positive and therefore closer to the range of the physiological reducing agents.

Experimental Section

Chemicals: Analytical grade reagents were used as supplied. The solvents were dried by standard methods.[44] 1,5-Diazacyclooctane-N,N'-diacetic acid was synthesized as described previously.[15]

Potentiometric Measurements: The pH potentiometric titrations were conducted at 25.0 ± 0.1 °C and at constant ionic strength (0.1 M, KNO_3) by use of a Crison Digital 517 instrument with an Ingold U1330 glass electrode and an Orion 90−00.11 Ag/AgCl reference electrode. The electrode calibration was carried out daily by titration of a strong acid (HNO_3, 0.1 M) with a strong base (KOH, 0.1 M) at the same ionic strength to ensure that we obtained adequate responses in the studied pH range and to control the exact concentration of the ligand (Gran's method).[45] The ligand was weighed directly into the potentiometric cell ($C_L = 2.4 \times 10^{-3}$ M) and the Cu^{II} solution was pipetted from a stock solution of 0.1 M $Cu(NO_3)_2$ in HNO_3 ($C_L/C_{Cu} = 1:1$). Calculations from potentiometric data were performed with the aid of the superquad[18] program.

Spectrophotometric Measurements: All spectra were measured with a Lambda 9 Perkin−Elmer spectrophotometer at 25 °C and at constant ionic strength ($I = 0.1$ M, KNO_3). Solutions of the metal complexes were generated in situ by addition, to an excess of the ligand, of a standard metal ion nitrate solution: 5×10^{-2} M $Cu(NO_3)_2$ in HNO_3 (1M) and 1000 ppm $Fe(NO_3)_3$ in HNO_3 (0.5 M). The pH measurements were carried out by use of a 420A Orion pH-meter, equipped with an Orion 91−03 glass calomel combination electrode. The stability constants for the iron(III) complexes were evaluated from the spectrophotometric titration data, by use of the PSEQUAD[16] computer program and speciation curves with the SPEA program.[46]

Electrochemical Measurements: Cyclic voltammograms were recorded with a three-electrode system with a hanging mercury drop working electrode, a platinum auxiliary electrode, and a saturated calomel reference electrode (SCE). All measurements were performed by use of an autolab ecochemie instrument coupled with a computer running the GPES-3 program. The complex in study was generated in situ, in a solution of iron(III) ($C_{Fe} = 2.5 \times 10^{-4}$ M) with a 20-fold excess of ligand, at pH 7.0, with constant ionic strength ($I = 1.0$ M, KNO_3), thermostatted at 25 °C and degassed with N_2. The pH measurements were performed with a 420A Orion pH meter equipped with an Orion 91−03 combined electrode.

Other Measurements: ^1H NMR spectra were recorded with a Varian Unity 300 spectrometer at 25 °C. Chemical shifts are reported in ppm (δ) from internal references {tetramethylsilane (TMS) in $CDCl_3$ solutions and sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionate (DSS) in D_2O solutions}. EPR studies were performed with a Bruker ESP ER 200D spectrometer (X-band) in frozen D_2O solutions with 20% ethylene glycol to ensure a good glass ($C_L/C_{Cu} = 1:1$, $C_{Cu} = 6.0 \times 10^{-3}$ M, $T = 100$ K, modulation frequency 100 kHz; $C_L/C_{Fe} = 3:2$, $C_{Fe} = 1.0 \times 10^{-3}$ M, pD* = 7.0, $T = 100$ K, modulation frequency 100 kHz). The spectra were simulated with the EPR program (version 1.0 by Frank Neese)[47] to calculate the g and A parameters. IR spectra were recorded with a Perkin−Elmer 683 spectrophotometer. Melting temperatures

were measured with a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were performed on a Fifons EA1108 CHNF/O instrument. Mass spectra were performed in a VG TRIO-2000 GC/MS instrument.

1,5-Diazacyclooctane-N,N'-bis(N-methyl-O-benzyl-acetohydroxamic Acid) (DACODMAHA-Bz): 1-Ethyl-3,3-(dimethylaminopropyl)-carbodiimide (3.60 g, 19 mmol) was added to a solution of 1,5-diazacyclooctane-N,N'-diacetic acid (1.10 g, 4.7 mmol) and O-benzyl-N-(methyl)hydroxylamine (2.40 g, 14 mmol) in THF/H_2O (2:1). The pH was adjusted to a constant 4.8 and the mixture was stirred at room temperature for 12 h. The end of the reaction was detected by TLC. The reaction mixture was dissolved in ethyl acetate, washed with saturated NaCl solution and dried with Na_2SO_4. Purification with a silica gel column yielded the pure final product (0.51 g, η = 25%) as a pale oil. IR (film): ṽ = 1675 cm^{-1} (C=O). ^1H NMR ($CDCl_3$/TMS): δ = 1.96 (t, 4 H, $NCH_2CH_2CH_2N$), 3.01 (br. t, 4 H, $NCH_2CH_2CH_2N$), 3.20 (s, 6 H, CH_3), 3.46 (br. t, 4 H, $NCH'_2CH_2CH'_2N$), 4.01 (s, 4 H, NCH_2CO), 4.95 (s, 4 H, CH_2Ph), 7.39−7.49 (m, 10 H, PhH) ppm. FAB-MS: m/z = 469 [M + 1].

1,5-Diazacyclooctane-N,N'-bis(N-methylacetohydroxamic Acid) (DACODMAHA): Pd/C (10%, 161 mg) was added to a solution of 1,5-diazacyclooctane-N,N'-bis(N-methyl-O-benzyl-acetohydroxamic acid) (0.48 g, 1.0 mmol) in dry methanol and the mixture was stirred under H_2 (1 atm) at room temperature for 4 h. After filtration of the solid residue, the solvent was evaporated under reduced pressure and the product was obtained as a white powder, which was then recrystallized from ethanol/acetonitrile (288 mg, η = 98%), m.p. 175 °C (dec.). IR (KBr): ṽ = 1640 cm^{-1} (C=O). ^1H NMR (D_2O/DSS): δ (ppm) 2.04 (m, 4 H, $NCH_2CH_2CH_2N$), 3.25 (br. s, 14 H, $NCH_2CH_2CH_2N$; CH_3), 4.00 (s, 4 H, NCH_2CO) ppm. (FAB-MS): m/z = 289 [M + 1]. $C_{12}H_{24}N_4O_4\cdot2H_2O$: calcd. C 44.43, H 8.70, N 17.27; found C 44.42, H 8.75, N 17.18.

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