

Iron(III) Chelation: Tuning of the pH Dependence by Mixed Ligands

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The iron(III) chelating properties of two heteropodands with 8-hydroxyquinoline and catechol binding groups were examined and compared to those of the corresponding homopodal analogues, O-TRENSEX and TRENCAMS. Like the parent homopodands, the two heteropodands are based on the TREN scaffold and the chelating units are connected by amide groups, TRENSEX2CAMS having two 8-hydroxyquinoline and one catechol arms and TRENSEXCAMS2 one 8-hydroxyquinoline and two catechol moieties. The aqueous coordination chemistry of these ligands was examined by po-

tentiometric and spectrophotometric methods in combination with ¹H NMR spectroscopy. The respective pFe^{III} values showed a cooperative effect of the mixed chelating units. Moreover, the pFe^{III} dependence on pH showed that the mixed ligands exhibit a higher complexing ability than the parent ligands over the pH range 5–9 which is of biological relevance. This result could be of great interest for medical applications.

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Introduction

Siderophores are iron chelating agents that are excreted by microorganisms to render iron soluble in the environment and favour the uptake of this metal. Iron chelation by some natural or synthetic chelators can be applied to human diseases characterised by iron overload. Although the coordination chemistry of a great number of water-soluble synthetic iron chelators has been described,^[1] the pH dependence of the coordination abilities has not been thoroughly investigated. Most of the comparative data in the literature are allowed by the use of the pFe^{III} values (calculated for [Fe^{III}]_{tot} = 1 μM, [L]_{tot} = 10 μM at pH = 7.4) and, of course, pFe^{III} reflects only the complexing ability of a given ligand at physiological pH. Owing to the fact that pH can vary in a relatively large range between the different biological compartments, an understanding of the pH dependence of the pFe^{III} value may be a decisive criterion for the understanding of the in vivo behaviour of a given iron chelator. On the other hand, the design and the synthesis of iron chelators with high pFe^{III} values in a large range of

pH, constitutes an interesting challenge for chemists. We previously reported the dependence of pFe^{III} on pH for two homopodands based on catechol and on 8-hydroxyquinoline bidentate units.^[2,3] We showed that the tris(catechol) ligand TRENCAMS is a stronger ferric chelator than the tris(hydroxyquinoline) analogue O-TRENSEX at pH values greater than 7 and that the opposite sequence is observed at pH values lower than 7. In order to obtain an efficient chelator of iron(III) over a large range of pH, we have synthesised mixed tripodal siderophores with both catechol and 8-hydroxyquinoline moieties. Recently, the synthesis of mixed tripodal ligands based on (i) two hydroxypyridinone and one catechol moieties, and (ii) two hydroxypyridinone and one 2-hydroxyisophthalamide groups has been published.^[4] We report in this paper the synthesis, acid-base properties, and ferric complexation of two monotopic tripodal mixed ligands TRENSEX2CAMS (**L**¹) and TRENSEXCAMS2 (**L**²; Figure 1). These molecules belong to a family of tripods with a tris(2-aminoethyl)amine group anchoring arms that bear bidentate coordination subunits (namely either a 5-sulfo-8-hydroxyquinoline-7-carbamoyl group or 5-sulfo-2,3-dihydroxybenzoyl unit). The ligands differ by the number of catecholyamide groups, none for ligand **L**⁰ (O-TRENSEX), one for ligand **L**¹, two for ligand **L**² and three for ligand **L**³ (TRENCAMS; Figure 1). Combining potentiometric and absorption spectrophotometric titrations with ¹H NMR spectroscopy we were able to characterise in water the protonated and ferric species formed with ligands **L**¹ and **L**² over a large pH range.

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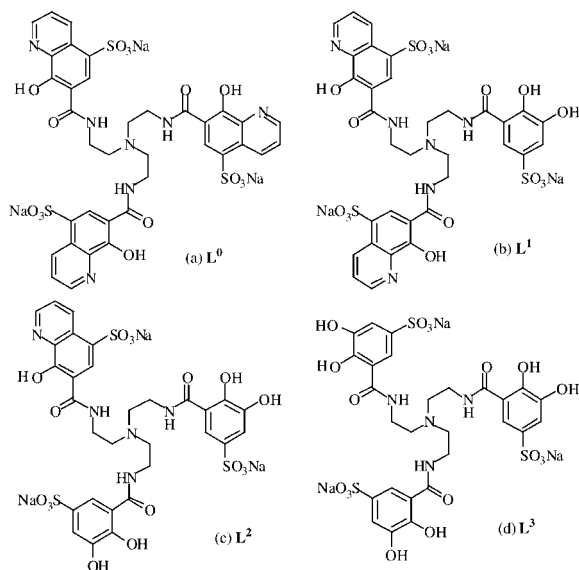


Figure 1. Chemical formulae of the tripodal ligands: (a) **L**⁰ (O-TRENSEX), (b) **L**¹ (TRENSEX2CAMS), (c) **L**² (TRENSEX-CAMS2), (d) **L**³ (TRENEXAMS)

Results and Discussion

Synthesis

The synthesis of the heterotripodal hexadentate ligands **L**¹ and **L**² is presented in Figure 2. The two catecholate subunits of ligand **L**² were connected to the tripodal scaffold by direct condensation^[5] of two equivalents of 2,3-dimethoxybenzoic chloride with one equivalent of tris(2-aminoethyl)amine (TREN) [path (a')], then the oxinate subunit was grafted onto the free primary amine group by coupling with activated (CDI) 7-carboxy-8-hydroxyquinoline. The synthesis of **L**¹ required protection of one arm of TREN with a trityl group [paths (a, b, c)]. Phenol groups were protected with benzyl groups (oxine) and methyl groups (catechol), respectively; deprotections were performed with BBr₃. Finally, regiospecific sulfonation in position 5 of both oxinate and catecholate subunits afforded the hydrosoluble ligands **L**¹ and **L**².

Acid-Base Properties of Ligands

The protonation constants of **L**¹ and **L**² were determined by combination of potentiometric and spectrophotometric

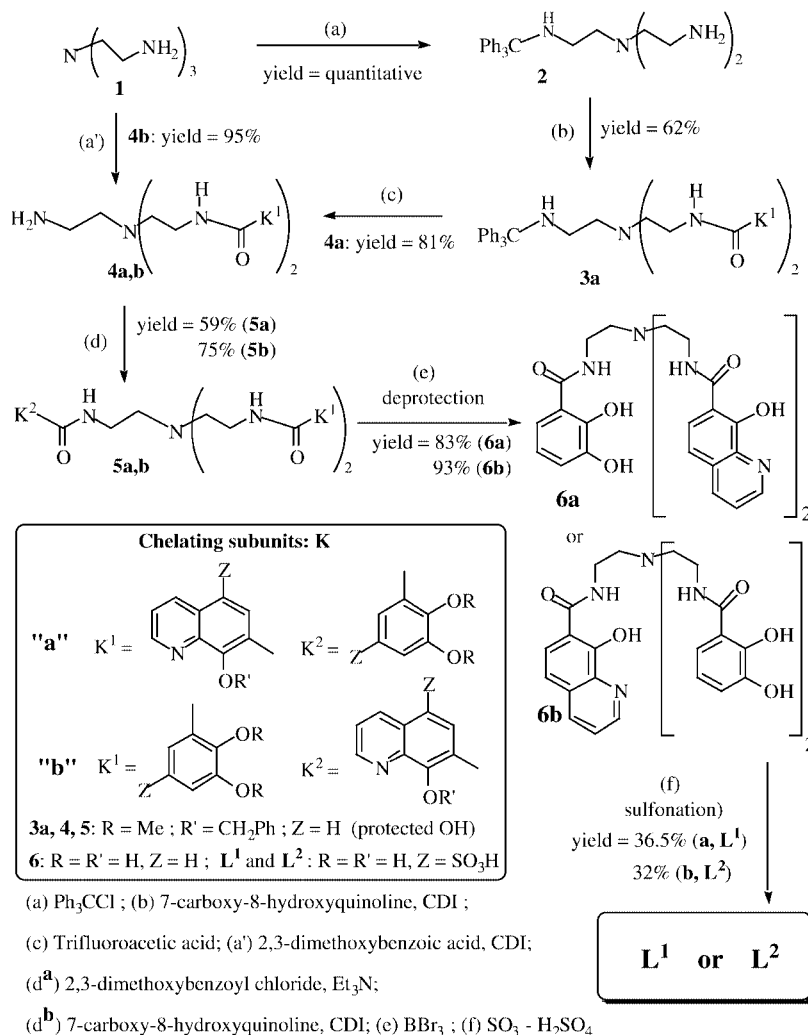


Figure 2. Synthesis of the heterotripodal ligands **L**¹ and **L**²

titrations (see details in Exp. Sect.). Both ligands possess a total of ten protonable functions. However, protonation of the three sulfo groups that are moderately strong acids ($\log K^H < 2$)^[6] was not considered likely under our conditions. Seven stepwise protonation constants were determined for each ligand. The corresponding values and statistical errors are given in Table 1. Speciation diagrams are presented in Figures S1 and S2 of the supporting information.

Table 1. Assignment of the successive protonation constants of various heterotripodal ligands with 8-hydroxyquinoline and/or catechol subunits; solvent: water, $I = 0.10$ M (NaClO_4), $T = (25.0 \pm 0.2)^\circ\text{C}$; numbers in parentheses correspond to statistical errors (3σ) on the last digit

Function	L^0 [a]	L^1	L^2	L^3 [b]
NH pyridinium	1.8(1) 2.55(8) 3.01(4)	1.8(2) 2.89(15)	2.34(6)	
OH quinoline	7.44(4) 8.18(5) 8.62(4)	7.85(9) 8.78(3)	7.93(2)	
OH catechol (<i>ortho</i>)		5.62(6)	5.55(4) 7.35(2)	5.57(5) 6.32(4) 7.05(4)
OH catechol (<i>meta</i>)		11.5(2)	11.41(8) ^[c] 12.5(3) ^[c]	12.05(10) ^[d] 8.01(2)
NH ammonium	6.36(3)	6.96(6)	6.56(3)	

[a] Ref.^[2] [b] Ref.^[3] [c] Spectrophotometric determinations under basic conditions. [d] Average value for the three *meta* catechol OH groups.

The protonation constants of L^1 were determined by potentiometry except for $\log K_1^H = 11.5$ and $\log K_7^H = 1.8$, which were determined spectrophotometrically. As for L^2 , the values in Table 1 represent averages of values determined by both methods, except for $\log K_5^H = 6.56$ (determined by potentiometry) and $\log K_1^H = 12.5$ (determined by spectrophotometry).

The protonation constants were attributed to L^1 and L^2 by comparison with the $\log K^H$ values of the ligand L^0 , bearing three hydroxyquinoline groups,^[2] and with those of the tris(catecholate) ligand L^3 ^[3] (Table 1). The highest protonation constants (11.5 for L^1 ; 12.5 and 11.41 for L^2) can be assigned to the *meta* hydroxyl groups of the sulfocatecholyl moieties. These values are in agreement with those reported under similar conditions for some sulfocatechol derivatives such as 1,2-dihydroxybenzene-4-sulfonic acid ($\log K^H = 12.16$)^[7], 1,2-dihydroxybenzene-3,5-disulfonic acid (Tiron; $\log K^H = 12.5$)^[8] or *N,N*-dimethyl-2,3-dihydroxy-5-sulfonatobenzamide (DMBS; $\log K^H = 11.5$)^[9]. The values of the lowest protonation constants (1.8 and 2.89 for L^1 ; 2.34 for L^2) correspond to the pyridine nitrogen of the sulfhydroxyquinoline moieties. Compared to 8-hydroxyquinoline-5-sulfonic acid ($\log K^H = 3.93$)^[10] the protonation constants determined for L^1 and L^2 are two orders of magnitude lower. This could be explained by the electron-withdrawing effect of the carbonyl linker in position 7, which is similar to that of the nitro group in 7-nitro-8-

hydroxyquinoline-5-sulfonic acid ($\log K^H = 2.01$)^[11]. The hydroxyl protonation constant of 8-hydroxyquinoline-5-sulfonic acid^[10] is equal to 8.42 and those of L^0 vary between 7.44 and 8.62.^[2] We attribute the values 8.78 and 7.85 for L^1 and 7.93 for L^2 to the protonation of the 8-hydroxyquinoline OH groups.

Based on the fact that the species L^2H_3 and L^2H_4 have the same absorption spectra, suggesting that protonation does not occur on a chromophore, the protonation constant $\log K_5^H = 6.56$ was attributed to the tertiary amine nitrogen of L^2 anchor. This was confirmed by the ^1H NMR titration of L^2 which showed an inflection point at $\text{pD} \approx 6$ and a significant upfield shift of the methylene protons, characteristic of the protonation of its closest acid-base site, i.e. the tertiary amine (Figure 3). Similarly for L^1 , the protonation constant $\log K_4^H = 6.96$ was attributed to the tertiary amine.

The values of $\log K^H$ of the tertiary amine anchor determined for L^1 and L^2 are close to that of L^0 ($\log K^H = 6.36$)^[2]. They are, however, much lower than that of the tris(catechol) ligand L^3 ($\log K^H = 8.01$)^[3]. It has already been reported^[12] that the protonation of this site is sensitive to the ability of the tertiary amine to form hydrogen bonds with substituents in the β -position of the TREN scaffold. Formation of a hydrogen bond between the lone electron pair of the tertiary amine nitrogen and the amide hydrogen

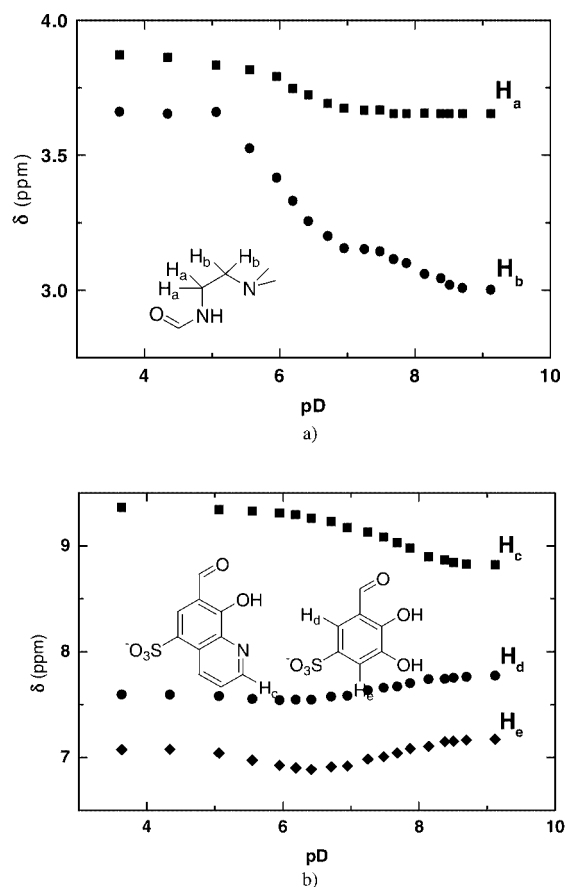


Figure 3. ^1H NMR chemical shift variation as a function of pD for the methylene (a) and aromatic (b) protons of L^2 ; solvent: D_2O , $[L^2] = 5$ mM

decreases strongly its basicity, providing a sufficient stabilisation of the deprotonated form of the amine, as observed with the tripodal ligands bearing 8-hydroxyquinoline groups.

The interactions of the amide linkers with the tertiary amine nitrogen can be further affected by the protonation state of the *ortho*-hydroxyl groups either of the 8-hydroxyquinoline or the catechol subunits. Indeed, the protonation of these sites could induce a different geometry around the TREN anchor, as suggested by the shoulder at pD = 7–8.5 in the curve H_b during the ¹H NMR titration of ligand L² (Figure 3). The isomer shift of the H_c as well as H_d and H_e (Figure 3) observed for L² increases from pD = 6.5 to 9 with an inflection point at pD = 7.5–8 in relation to the protonation of hydroxyl groups (log K^H = 7.35 and 7.93). This indicates that such a protonation is responsible for a conformational change around the amide-catechol axis, which induces a shift of the proton resonance.

The remaining protonation constants (7.35 and 5.55 for L² and 5.62 for L¹) are attributed to the catechol hydroxyl groups *ortho* with respect to the amide linker. These values are in agreement with those of other tripodal catecholyamide-type ligands.^[13]

The calculated electronic spectra of protonated species of L¹ and L² are given in Figure 4. The spectral properties of L¹ and L² are qualitatively in agreement with those of other 8-hydroxyquinoline and/or catechol based ligands. Catechol derivatives show a $\pi \rightarrow \pi^*$ transitions in the UV region (300–400 nm) with the maximum of the absorption band shifting to longer wavelengths and the molar absorption coefficient increasing as the hydroxyl groups are deprotonated.^[14] Similarly, 8-hydroxyquinoline-5-sulfonic acid displays spectral variations in this region upon protonation.^[15] It should be noted that molar absorption coefficients of the heterotripodal ligands ($\approx 2 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at around 340 nm and $\approx 5\text{--}6 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at around 270 nm for totally deprotonated L¹ and L²) are higher than the value obtained by addition of molar absorption coefficients of simple 8-hydroxyquinolinolate or catecholate ligands.^[15,16]

Characterisation of the Ferric Complexes

Both ligands are hexadentate and form only 1:1 ferric complexes. The potentiometric titration curves recorded for a 1:1 metal-to-ligand ratio (Figure 5) show a large pH jump at $a = 7$, indicating that all the ligand protons are released when the ferric ion is bound. Since both L¹ and L² form ferric complexes totally at pH values less than 3 the global stability constant log β_{FeL} could not be determined from the potentiometric data.

The spectra obtained during the spectrophotometric titrations of ferric complexes of L¹ and L² are shown in Figures S3 and S4. The absorbance data were refined with the Letagrop-Spefo^[17,18] and Specfit^[19–22] programs and provided values of global stability constants of ferric complexes of both L¹ and L². The spectrophotometric data obtained from the competition for ferric ion between L² and EDTA were treated with the Specfit program allowing the determi-

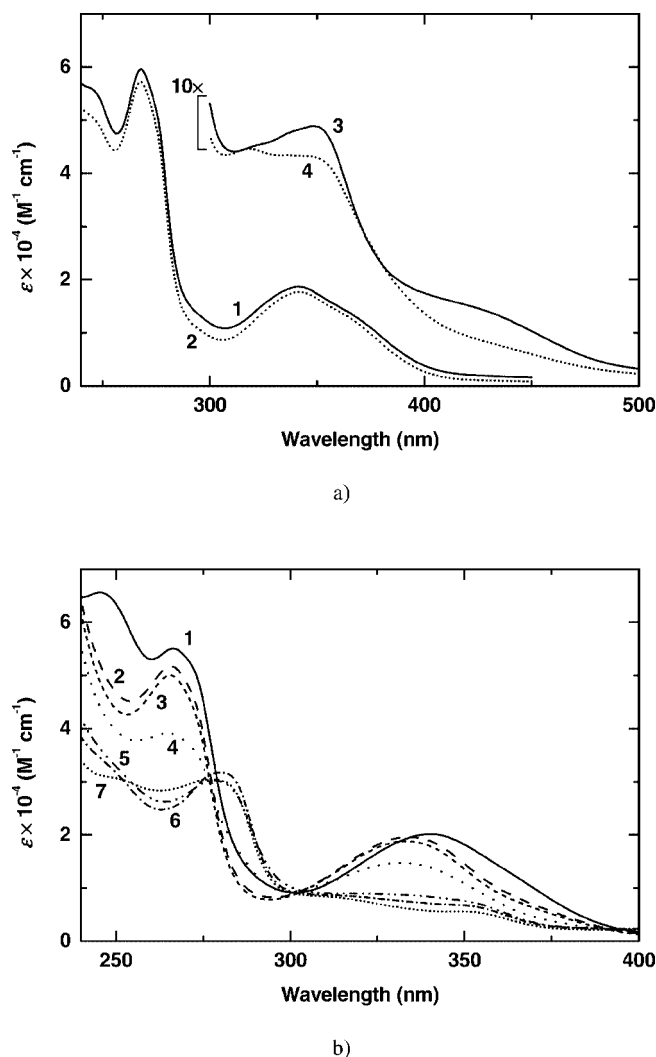


Figure 4. Calculated electronic spectra of the protonated species of heterotripodal ligands; solvent: water, $I = 0.10 \text{ M}$ (NaClO_4), $T = (25.0 \pm 0.2)^\circ\text{C}$: (a) ligand L¹: (1) L¹H₇, (2) L¹H₆, (3) L¹H, (4) L¹; (b) ligand L²: (1) L², (2) L²H, (3) L²H₂, (4) L²H₃ = L²H₄, (5) L²H₅, (6) L²H₆, (7) L²H₇; charges are omitted for the sake of clarity

nation of the stability constant log β_{FeL} . All the results are summarised in Table 2.

The electronic spectra of de-, mono- and polyprotonated ferric complexes of L¹ and L² calculated from the refinement of absorbance data are shown in Figure 6; speciation diagrams are presented in Figure 7.

The ferric complexes of catechol derivatives generally show a strong ligand-to-metal charge transfer (LMCT) band with a maximum at about 700 nm for mono(catecholate) species, at about 560 nm for bis(catecholate) species and finally at about 480 nm for tris(catecholate) complexes. The charge-transfer bands observed are due to electronic transitions from the highest valence orbitals of the ligand to the 3d orbitals of iron(III).^[23] As the maximum moves to shorter wavelengths with increasing number of coordinating ligands the molar absorption coefficient increases. Electron-withdrawing substituents on the aromatic rings of the catechol moieties increase the molar absorption coefficient but have little effect on the wavelength of the maximum. Ferric

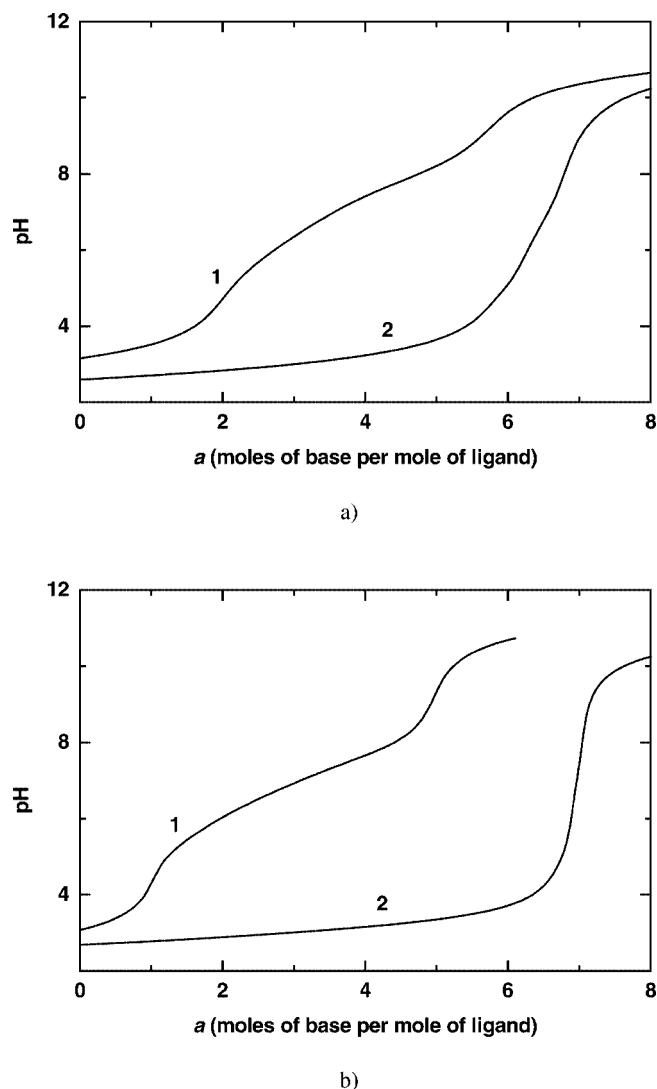


Figure 5. Potentiometric titration curves for the heterotripodal ligands and their ferric complexes; solvent: water, $I = 0.10$ M (NaClO_4), $T = (25.0 \pm 0.2)^\circ\text{C}$: (a) titration of L^1 (1 mM): (1) free ligand, (2) 1:1 ferric complex; (b) titration of L^2 (1 mM): (1) free ligand, (2) 1:1 ferric complex

complexes of 8-hydroxyquinoline-5-sulfonic acid are characterised by two absorption maxima in the visible region. The maximum at 443 nm is assigned to an LMCT band belonging to the phenolic oxygen, while the band at 595 nm is due to charge transfer from the pyridine nitrogen to the metal cation.^[2] It should be noted that in common with L^0 and L^3 , both L^1 and L^2 form protonated ferric complexes, indicating that the coordination sites of the complexes can be protonated without losing the ferric ion. This behaviour can be attributed to a change of catecholate or oxinate bonding mode to the salicylate mode (coordination with carbonyl and *o*-hydroxyl oxygens).

It is of interest to consider the different multiprotonated ferric complexes of L^1 and L^2 by examining their electronic spectra (Table 2) as this can provide information on the coordination modes and their successive deprotonation constants $\text{p}K_{\text{FeLH}_n}$ (defined in Table 3). These values are calculated from the difference between the values of $\log \beta_{\text{FeLH}_n}$

Table 2. Cumulative stability constants β_{FeLH_n} , pFe^{III} values and UV/Vis spectral characteristics of ferric complexes formed with the tripodal ligands; solvent: water, $I = 0.10$ M (NaClO_4), $T = (25.0 \pm 0.2)^\circ\text{C}$; numbers in parentheses correspond to statistical errors (3σ) on the last digit; an incertitude of 5% is estimated for the molar absorption coefficients

Ligand	Species	$\log \beta_{\text{FeLH}_n}^{[a]}$	$\text{pFe}^{\text{III}[b]}$	λ_{max} (nm) ^[c]	ε_{max} ($\text{M}^{-1}\cdot\text{cm}^{-1}$)
L^0 [2]	FeL^0H_5	42.2(1)	29.5	435	8200
				525*	3500
	FeL^0H	36.5(2)		443	5200
				595	5200
L^1	FeL^1	30.9(1)		443	5400
				595	5400
	FeL^1H_5	48.6(2)	31.6	440*	6900
				540*	3700
	FeL^1H_3	46.2(4)		560	4000
	FeL^1H_2	44.3(3)		580	3900
L^2	FeL^2	41.6(2)		560	4500
				550	4500
	FeL^2H_5	55.91(3)	32.3	409	4700
				520*	3300
	FeL^2H_4	53.7(1)		573	3500
	FeL^2H_2	47.7(3)		542	4500
L^3 [3]	FeL^3	44.7(6)		525	4800
				525	4800
	FeL^3H_5	64.05(7)	29.6	495	4300
				520	4800
	FeL^3H_3	57.4(1)		500	5000
	FeL^3H	49.1(1)		488	5300

[a] $\beta_{\text{FeLH}_n} = [\text{FeLH}_n]/([\text{Fe}^{\text{III}}] \times [\text{L}] \times [\text{H}^+]^n)$. [b] Calculated for $[\text{Fe}^{\text{III}}]_{\text{tot}} = 10^{-6}$ M, $[\text{L}]_{\text{tot}} = 10^{-5}$ M, $\text{pH} = 7.4$. [c] * = shoulder. [d] Species have identical electronic spectra.

and are reported in Table 3 together with the values from tris(catecholate) and tris(8-hydroxyquinolate) complexes for comparison. The deprotonation constants of ferric L^1 and L^2 complexes have been attributed by considering that the deprotonation of an 8-hydroxyquinoline group involved in the ferric coordination occurs at a pH much lower than that of the deprotonation of the catechol group. This is supported by the $\text{p}K_{\text{FeLH}_n}$ values recently determined for tripodal ferric complexes containing a C-pivot scaffold with either an 8-hydroxyquinoline (ligand COX)^[24] or a catechol subunit (ligand CacCAM).^[25] In particular, the values of $\text{p}K_{\text{FeLH}}$ (2.12 for Fe-COX and 6.59 for Fe-CacCAM) have been unambiguously attributed to the coordination of the third chelating arm.

The electronic spectrum of FeL^1H_5 , which exhibits two shoulders at 440 nm ($\varepsilon = 6940 \text{ M}^{-1}\cdot\text{cm}^{-1}$) and 540 nm ($\varepsilon = 3680 \text{ M}^{-1}\cdot\text{cm}^{-1}$), is similar to that of the bis(salicylate) complex of L^0 [2] involving a coordination with two 8-hydroxyquinoline arms of the ligand through the oxygen atoms of the carbonyl and hydroxyl groups. The spectrum of the FeL^1H_3 complex exhibits an absorption band at $\lambda_{\text{max}} = 560$ nm ($\varepsilon = 3970 \text{ M}^{-1}\cdot\text{cm}^{-1}$), in agreement with the bis(oxinate) coordination. The oxygen and pyridine nitrogen atoms of two 8-hydroxyquinoline arms are involved in the coordination. The value $\text{p}K_{\text{FeL}^1\text{H}_5} = 2.4$ ($\text{FeL}^1\text{H}_5/\text{FeL}^1\text{H}_3$ equilibrium) corresponds to the deprotonation of both 8-hydroxyquinoline nitrogens in a two-proton step. The small

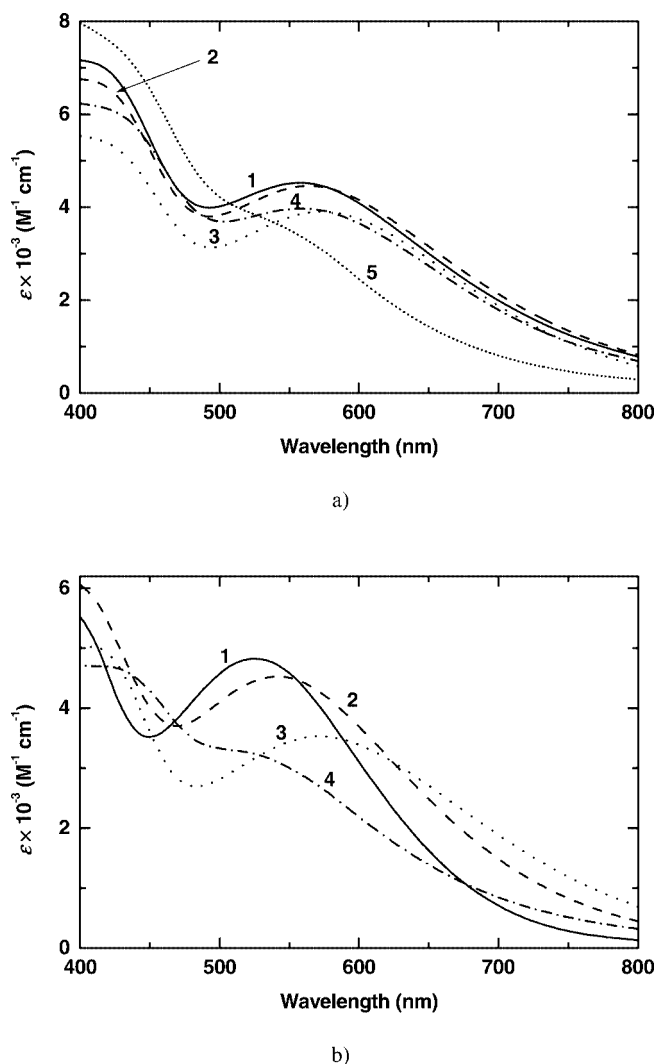


Figure 6. Calculated electronic spectra of the ferric complexes formed with the heterotripodal ligands; solvent: water, $I = 0.10$ M (NaClO_4), $T = (25.0 \pm 0.2)^\circ\text{C}$: (a) ferric L^1 species: (1) FeL^1 , (2) FeL^1H , (3) FeL^1H_2 , (4) FeL^1H_3 , (5) FeL^1H_5 ; (b) ferric L^2 species: (1) $\text{FeL}^2 \equiv \text{FeL}^2\text{H}$, (2) FeL^2H_2 , (3) FeL^2H_4 , (4) FeL^2H_5 ; charges are omitted for the sake of clarity

red-shift of λ_{max} to 580 nm for the formation of FeL^1H_2 without significant change of ϵ ($3930 \text{ M}^{-1}\cdot\text{cm}^{-1}$) might be attributed to the deprotonation of the ammonium nitrogen. The value $\text{p}K_{\text{FeL}^1\text{H}_3} = 1.9$ shows a strong stabilisation of the deprotonated tertiary amine nitrogen by intramolecular hydrogen bonds. The increase of ϵ to $4490 \text{ M}^{-1}\cdot\text{cm}^{-1}$ with the concomitant blue shift of λ_{max} to 560 nm for the formation of FeL^1H is in agreement with the coordination of the catechol arm in a salicylate mode. The last deprotonation equilibrium is accompanied with a spectral change ($\lambda_{\text{max}} = 550 \text{ nm}$, $\epsilon = 4560 \text{ M}^{-1}\cdot\text{cm}^{-1}$) that is indicative of a shift to a catecholate coordination and formation of the FeL^1 species. It should be pointed out that the values of $\text{p}K_{\text{FeL}^1\text{H}_2}$ and $\text{p}K_{\text{FeL}^1\text{H}}$ (2.7 and 4.8) are significantly lower than those observed for tris(catecholate) complexes: 8.34 (two-proton step) and 5.5 for L^3 , and 4.1 and 5.74 for MECAMS^[9] (Table 3), while the $\text{p}K_{\text{a}}$'s of the free ligand are of the same order of magnitude.

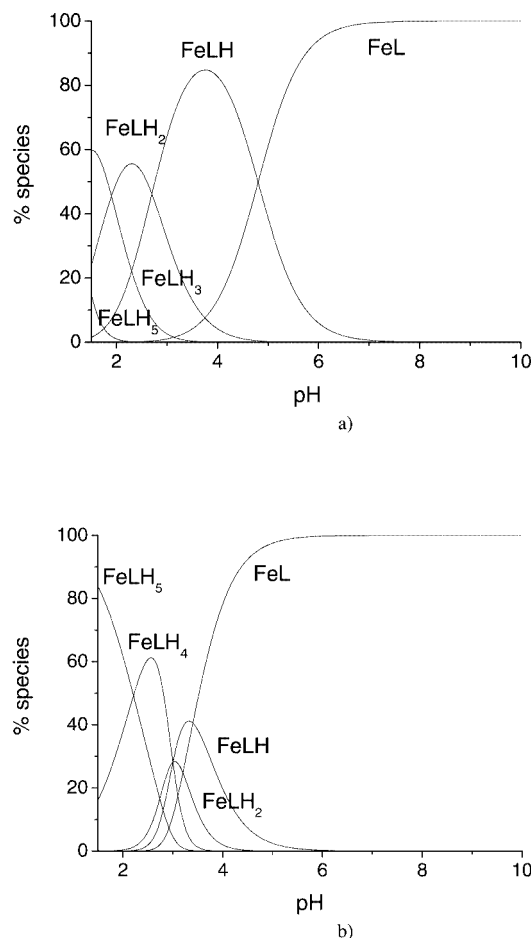


Figure 7. Speciation diagrams for (a) ferric- L^1 complexes, (b) ferric- L^2 complexes

Table 3. Successive deprotonation constants of ferric complexes;^[a] solvent: water, $I = 0.10$ M (NaClO_4), $T = (25.0 \pm 0.2)^\circ\text{C}$

Ligand ^{[a][b]}	$\text{p}K_{\text{FeLH}_5}$	$\text{p}K_{\text{FeLH}_4}$	$\text{p}K_{\text{FeLH}_3}$	$\text{p}K_{\text{FeLH}_2}$	$\text{p}K_{\text{FeLH}}$
L^0 [2]	5.7 ^[c]				5.6
L^1	2.4 ^[d]		1.9	2.7	4.8
L^2	2.2	6.0 ^[d]		3.0	3.4
L^3 [3]	3.1	3.5	8.34 ^[d]		5.5
Enterobactin ^[26]			2.5	3.52	4.95
MECAMS ^[9]			3.46	4.10	5.74
COX ^[24]		4.8 ^[e]			2.12
CacCAM ^[25]				5.15	6.59

^[a] $K_{\text{FeLH}_n} = ([\text{FeLH}_{n-1}] \times [\text{H}])/[\text{FeLH}_n]$. ^[b] MECAMS: 1,3,5-tris{[(2,3-dihydroxy-5-sulfobenzoyl)amino]methyl}benzene; COX: 1,1,1-tris[3-(8-hydroxyquinoline-7-carboxamido)propyl]-(polyethylene glycol_{<43>}methyl ether); CacCAM: 2,2,2-tris[3-(2,3-dihydroxybenzamido)propyl]acetic acid. ^[c] $K_{\text{FeLH}_n} = ([\text{FeLH}] \times [\text{H}]^4)/[\text{FeLH}_5]$. ^[d] $K_{\text{FeLH}_n} = ([\text{FeLH}_{n-2}] \times [\text{H}]^2)/[\text{FeLH}_n]$. ^[e] $K_{\text{FeLH}_n} = ([\text{FeLH}] \times [\text{H}]^3)/[\text{FeLH}_4]$.

The spectral properties of the FeL^2H_5 species ($\epsilon = 4700 \text{ M}^{-1}\cdot\text{cm}^{-1}$ at $\lambda_{\text{max}} = 409 \text{ nm}$ and a shoulder at 520 nm with $\epsilon = 3260 \text{ M}^{-1}\cdot\text{cm}^{-1}$) are intermediate between the bis(salicylate) coordination of ferric L^3 (two catechols)^[3] and ferric L^0 (two 8-hydroxyquinolines).^[2] This confirms that L^2 forms a bis(salicylate) complex with one catechol and one

8-hydroxyquinoline coordinated to the ferric ion. For the formation of FeL^2H_4 the λ_{max} is red-shifted to 573 nm, consistent with the change of coordination from a salicylate to an oxinate mode for the 8-hydroxyquinoline arm. There is only a slight change in ϵ ($3530 \text{ M}^{-1}\cdot\text{cm}^{-1}$), indicating that iron is still coordinated by two arms. The value $\text{p}K_{\text{FeL}^2\text{H}_5} = 2.2$ of the $\text{FeL}^2\text{H}_5/\text{FeL}^2\text{H}_4$ equilibrium corresponds well to the pyridinium nitrogen deprotonation. The formation of the FeL^2H_2 and FeL^2H species is accompanied by spectral shifts of λ_{max} from 573 nm to 542 nm and 525 nm, respectively, and an increase of ϵ to $4530 \text{ M}^{-1}\cdot\text{cm}^{-1}$ and $4820 \text{ M}^{-1}\cdot\text{cm}^{-1}$ is observed. The equilibrium between FeL^2H_4 and FeL^2H_2 ($\text{p}K_{\text{FeL}^2\text{H}_4} = 6.0$) is attributed to the coordination of the third arm (catechol) in a salicylate mode and the change of coordination from salicylate to catecholate for the coordinated catechol arm. The equilibrium between FeL^2H_2 and FeL^2H corresponds to the shift of bonding mode from salicylate to catecholate of the third catechol arm ($\text{p}K_{\text{FeL}^2\text{H}_2} = 3.0$). As for FeL^1 complexes, the deprotonation of the catechol groups upon complexation occurs at lower pH than for tris(catechol) ligands cited above. The last equilibrium leading to FeL^2 species does not induce any spectral changes. It is likely that the deprotonation of the tertiary amine nitrogen is involved. The value of $\text{p}K_{\text{FeL}^2\text{H}} = 3.4$ is higher than the corresponding constant for ferric L^1 complex ($\text{p}K_{\text{FeL}^1\text{H}_3} = 1.9$).

pFe^{III} Values

Since the ligands are weak acids, proton competition occurs depending on their protonation constants and the pH. The pFe^{III} value ($-\log [\text{Fe}^{\text{III}}]$) is thus a better measure of the relative efficiency of the ligands under given conditions of pH, $[\text{Fe}^{\text{III}}]_{\text{tot}}$ and $[\text{L}]_{\text{tot}}$. The pFe^{III} values have been calculated for pH values over the range 2–10 and are reported as the plot of pFe^{III} vs. pH presented in Figure 8, together with the plots calculated for L^0 and L^3 .

The important result, as seen from Figure 8, is that the mixed ligands L^1 and L^2 are stronger iron chelating agents than L^0 and L^3 over the pH range 5–9. This shows that

mixed ligands lead to enhanced affinity for the ferric ion at pH above 5 (L^1) or 6 (L^2) relative to ligands with three identical arms. This is related to the different basicities of the binding subunits. Catechol is more basic than 8-hydroxyquinoline, which leads to a stronger competition of protons with ferric ions for catechol ligands at low pH. Hence, 8-hydroxyquinoline is a better chelating agent at low pH, while catechol is a stronger chelator at high pH as observed for L^0 [tris(8-hydroxyquinoline) ligand] and L^3 [tris(catechol) ligand]. Our results with L^1 (one catechol group) and L^2 (two catechol groups) show that a greater $\text{p}K_{\text{a}}$ difference between the proton dissociation of the catechol in the free ligand and that of the same group in the complex is observed in comparison to other tripodal ligands containing only catechol groups (see Table 3). This suggests that the initial coordination of the 8-hydroxyquinoline arm with Fe^{III} favours proton loss of the free catechol and thus increases the affinity of this group for Fe^{III} . This can explain that the highest values for pFe^{III} are obtained with L^1 and L^2 over the pH range 5–9, which is biologically relevant, as shown by the plot in Figure 8. We have also reported in Table 2 the pFe^{III} values calculated under standard conditions (pH = 7.4, $[\text{L}]_{\text{tot}} = 10^{-5} \text{ M}$, $[\text{Fe}^{\text{III}}]_{\text{tot}} = 10^{-6} \text{ M}$) for L^1 (31.6) and for L^2 (32.3). These values are higher than those determined for L^0 (29.5) and for L^3 (29.6) and among the highest values determined for an iron sequestering agent.

Conclusion

Iron(III) complexation by two heterotripodal ligands L^1 and L^2 has been characterised. The results are compared to the parent homotripodal ligands allowing the study of the variation of the ligand properties from tris(8-hydroxyquinoline) to tris(catechol) ligand. The thermodynamic and spectroscopic study reveals that in the acidic medium the complex formation starts with two arms coordinating the ferric ion in the salicylate coordination mode. Both 8-hydroxyquinoline subunits are involved in the case of L^1 , and one 8-hydroxyquinoline and one catecholate subunit in the case of L^2 . This indicates that 8-hydroxyquinoline is a better ligand than catechol at low pH values. Despite the fact that catechol oxygens are better donors than the oxygen and nitrogen atoms of 8-hydroxyquinoline, the competition between H^+ and the metal cation “balances” the complexation efficiency. The striking feature of the mixed ligands is their higher complexing ability than that of the parent ligands over a range of biologically relevant pH values, and especially at neutral pH. This can be explained by the finely tuned complexation power of 8-hydroxyquinoline and catechol groups. It should be noted that the complexation efficiency of the tris(8-hydroxyquinoline) ligand L^0 is less sensitive to pH than the tris(catechol) ligand L^3 over the pH range 2–10. The results described in this work illustrate the great interest of the mixed ligands approach, which, via a synergistic action, leads to a more efficient iron complexing agent than the homopodate ligands.

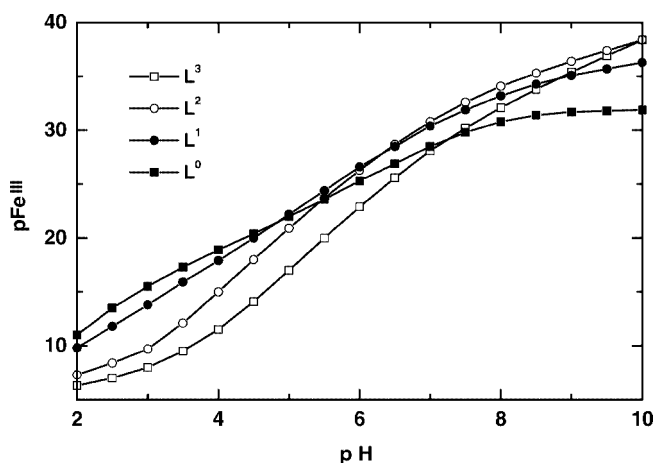


Figure 8. Plot of pFe versus pH for the tripodal ligands; calculated for $[\text{L}]_{\text{tot}} = 10^{-5} \text{ M}$ and $[\text{Fe}^{\text{III}}]_{\text{tot}} = 10^{-6} \text{ M}$

Experimental Section

Materials and Equipment: Solvents were purified by standard techniques. The amine TREN was distilled from over CaH_2 . All other compounds were of reagent grade and were used without further purification. Mass spectra were obtained on NERMAG R 10 10C or Thermo Finnigan Polaris Q or Bruker Esquire-LC 1.6n (electrospray) mass spectrometers. ^1H and ^{13}C NMR spectra were recorded in 5 mm tubes at 25 °C with Bruker AC 200 or WM 250 or Avance 300 spectrometers (δ ppm, TMS reference). Microanalyses were performed by the Central Service of CNRS, Solaise (France). Melting points were determined with a Büchi apparatus and are not corrected.

***N*¹,*N*¹-bis(2-aminoethyl)-*N*²-(trityl)-1,2-ethanediamine (2):** Under argon at room temperature, triphenylchloromethane (2.65 g, 9.5 mmol) in dry CH_2Cl_2 (150 mL) was added dropwise to a solution of tris(2-aminoethyl)amine (1; 5.56 g, 38 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred overnight and then successively washed with 10% NaOH (ca. 100 mL) and brine. The organic phase was dried over sodium sulfate and concentrated under vacuum to give **2** as a yellowish solid (3.7 g, yield: 9.5 mmol, 100%). M.p. 63–65 °C. ^1H NMR (200 MHz, CDCl_3): δ = 7.5 (m, 6 H, ArH), 7.3–7.1 (m, 9 H, ArH), 2.9 (br. s, 5 H, NH), 2.7 (t, $^3J_{\text{H,H}}$ = 6 Hz, 4 H, CH_2), 2.6 (t, $^3J_{\text{H,H}}$ = 6 Hz, 2 H, CH_2), 2.4 (t, $^3J_{\text{H,H}}$ = 6 Hz, 4 H, CH_2), 2.2 (t, $^3J_{\text{H,H}}$ = 6 Hz, 2 H, CH_2) ppm. ^{13}C NMR (250 MHz, CDCl_3): δ = 146.1 (Cq), 128.5 (CH), 127.7 (CH), 126.1 (CH), 70.6 (Cq), 57.4 (CH_2), 55.0 (CH_2), 40.8 (CH_2), 39.9 (CH_2) ppm. MS (DCI, NH_3 /isobutane): m/z = 389 [$\text{M} + \text{H}$]⁺, 243 [$\text{C}_{19}\text{H}_{15}$]⁺.

Compound 3a: Under a stream of argon in order to remove CO_2 as and when it is produced, a solution of 8-benzyloxy-7-carboxyquinoline (3.65 g, 13 mmol) in dry THF (150 mL) was treated with a solution of carbonyldiimidazole (CDI; 2.2 g, 13.6 mmol) in THF (40 mL), under reflux for 2 h. Tripod **2** (2.535 g, 6.5 mmol) in THF (50 mL) was then added dropwise and the mixture was stirred overnight under reflux. The solvent was evaporated off and CH_2Cl_2 was added to the residue. The solution was washed successively with saturated NH_4Cl and brine and then dried. Concentration afforded a dark oil which was chromatographed (silica, cyclohexane/ CH_2Cl_2 gradient, then CH_2Cl_2 /methanol 1 → 2%) to give **3a** as a yellow foam (3.7 g, 4 mmol, 62%). ^1H NMR (250 MHz, CDCl_3): δ = 8.95 (dd, $^3J_{\text{H,H}}$ = 1.6, 4.0 Hz, 2 H, ArH), 8.14 (m, 4 H, ArH), 8.07 (br. t, $^3J_{\text{H,H}}$ = 4.8 Hz, 2 H, NH amide), 7.6–7.0 (m, 29 H, ArH), 5.5 (s, 4 H, CH_2 benzyl), 3.2 (dt, $^3J_{\text{H,H}}$ = 6.3, 4.8 Hz, 4 H, CH_2), 2.47 (t, $^3J_{\text{H,H}}$ = 5.5 Hz, 2 H, CH_2), 2.21 (t, $^3J_{\text{H,H}}$ = 6.3, 4.8 Hz, 4 H, CH_2), 2.13 (t, $^3J_{\text{H,H}}$ = 5.3 Hz, 2 H, CH_2), 2.0 (br. s, 1 H, NH) ppm. ^{13}C NMR (250 MHz, CDCl_3): δ = 165.1 (CO), 153.8 (Cq), 149.5 (CH), 146.1 (Cq), 142.6 (Cq), 136.7 (Cq), 136.0 (CH), 131.3 (Cq), 128.8–128.5 (unresolved CH's), 127.6 (unresolved CH's), 126.0 (CH), 125.3 (Cq), 123.1 (CH), 122.3 (CH), 78.3 (CH_2), 70.7 (Cq), 53.9 (CH_2), 52.5 (CH_2), 40.5 (CH_2) ppm. MS (DCI, NH_3 /isobutane): m/z = 911 [MH]⁺, 243 [$\text{C}_{19}\text{H}_{15}$]⁺, 91 [C_7H_7]⁺. $\text{C}_{59}\text{H}_{54}\text{N}_6\text{O}_4$ (911.1): calcd. C 77.78, H 5.97, N 9.22; found C 77.40, H 5.92, N 9.31.

Compound 4a: Under argon at room temperature, a solution of **3a** (3.61 g, 4 mmol) in CH_2Cl_2 (100 mL) was stirred with $\text{CF}_3\text{CO}_2\text{H}$ (3.7 g, 32 mmol) for 12 h. The mixture was treated with 10% NaOH, then washed with brine, dried and concentrated. Column chromatography (silica, CH_2Cl_2 then CH_2Cl_2 /methanol 5%/isopropylamine 1%) afforded **4a** as a beige foam (2.15 g, 81%). ^1H NMR (300 MHz, CDCl_3 , 22 °C, TMS): δ = 8.95 (dd, $^3J_{\text{H,H}}$ = 1.7, 4.1 Hz, 2 H, Hquin), 8.22 (br. t, $^3J_{\text{H,H}}$ = 5.6 Hz, 2 H, NH amide), 8.11

(dd, $^3J_{\text{AB}}(\text{H-H})$ = 1.7, 4.1 Hz, 2 H, Hquin), 7.97 (d, $^3J_{\text{H,H}}$ = 8.6 Hz, 2 H, Hquin), 7.5–7.3 (m, 14 H, ArH and Hquin), 5.5 (s, 4 H, CH_2 benzyl), 3.25 (dt, $^3J_{\text{H,H}}$ = 5.6, 6.4 Hz, 4 H, CH_2), 3.2 (br. t, 2 H, NH_2), 2.59 (m, 2 H, CH_2), 2.50 (m, 2 H, CH_2), 2.36 (t, $^3J(\text{H-H})$ = 6.4 Hz, 4 H, CH_2) ppm. ^{13}C NMR (300 MHz, CDCl_3): δ = 165.5 (CO), 153.7 (Cq), 149.6 (CH), 142.4 (Cq), 136.7 (Cq), 136.1 (CH), 131.3 (Cq), 128.9 (CH), 128.8 (CH), 128.7 (CH), 127.3 (CH), 125.3 (Cq), 123.2 (CH), 122.3 (CH), 78.5 (CH_2), 55.1 (CH_2), 53.5 (CH_2), 38.9 (CH_2), 37.9 (CH_2) ppm. MS (DCI, NH_3 /isobutane): m/z = 669 [$\text{M} + \text{H}$]⁺.

Compound 5a: 2,3-Dimethoxybenzoic acid (0.62 g, 3.4 mmol) was dissolved in SOCl_2 (15 mL) and the mixture was stirred overnight under argon. The solution was then evaporated to dryness to give 2,3-dimethoxybenzoic chloride (0.685 g). Compound **4a** (2.27 g, 3.4 mmol), triethylamine (0.35 g, 3.5 mmol) and dry CH_2Cl_2 (150 mL) were added and the mixture was stirred at room temperature under argon. The mixture was treated successively with 25% NaOH and brine and then dried and the solvent was evaporated off. Column chromatography (silica, CH_2Cl_2 then CH_2Cl_2 /methanol 1 → 5%) gave **5a** as a white foam (1.665 g, 2 mmol, yield = 59%). ^1H NMR (250 MHz, CDCl_3): δ = 8.94 (dd, $^3J_{\text{H,H}}$ = 1.6, 4.0 Hz, 2 H, Hquin), 8.2–8.1 (m, 7 H, Hquin + NH amide), 7.6–7.3 (m, 15 H, ArH), 7.01 (t, $^3J_{\text{H,H}}$ = 7.9 Hz, 1 H, H catech.), 6.89 (dd, $^3J_{\text{H,H}}$ = 1.6, 6.4 Hz, 1 H, H catech.), 5.55 (s, 4 H, CH_2 benzyl), 3.80 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.41–3.25 (m, 6 H, CH_2), 2.6 (m, 2 H, CH_2), 2.4 (m, 4 H, CH_2) ppm. ^{13}C NMR (250 MHz, CDCl_3): δ = 165.2 (CO), 165.0 (CO), 153.8 (Cq), 152.3 (Cq), 149.5 (CH), 147.4 (Cq), 142.4 (Cq), 136.7 (Cq), 135.9 (CH), 131.2 (Cq), 128.6 (unresolved CH's), 127.4 (CH), 126.8 (Cq), 125.0 (Cq), 123.9 (CH), 123.0 (CH), 122.4 (CH), 122.2 (CH), 114.9 (CH), 78.3 (CH_2), 61.1 (CH_3), 55.7 (CH_3), 52.7 (CH_2), 52.4 (CH_2), 37.5 (CH_2), 37.2 (CH_2) ppm. MS (FAB, NBA matrix): m/z = 833 [$\text{M} + \text{H}$]⁺, 541, 305 [$\text{C}_{19}\text{H}_{17}\text{N}_2\text{O}_2$]⁺. $\text{C}_{49}\text{H}_{48}\text{N}_6\text{O}_7$ (832.9): calcd. C 70.66, H 5.81, N 10.09; found C 70.21, H 5.83, N 9.96.

Compound 6a: Under argon at 0 °C, BBr_3 (5.3 g, 21 mmol) in CH_2Cl_2 (30 mL) was added dropwise to a solution of **5a** (1.52 g, 1.8 mmol) in CH_2Cl_2 (250 mL). After stirring overnight at room temperature, the mixture was cooled to 0 °C and treated with methanol (50 mL). After 4 h the solution was concentrated and then repeatedly evaporated with methanol to remove the borate. The residue was treated with 4 N NaOH and extracted with CH_2Cl_2 to remove the impurities. The aqueous solution was acidified with 4 N HCl to give a beige precipitate which was washed with water and then dried under vacuum to give pure **6a** (0.937 g, 1.5 mmol, 83%). ^1H NMR (300 MHz, DMSO): δ = 9.0–8.7 (m, 5 H, NH + ArH), 8.3–8.1 (m, 2 H, ArH), 8.0–7.8 (m, 2 H, ArH), 7.6–7.5 (m, 2 H, ArH), 7.3–7.1 (m, 4 H, ArH), 6.9–6.8 (m, 1 H, ArH), 3.5–3.3 (m, 6 H, CH_2), 2.8–2.7 (m, 6 H, CH_2) ppm. ^{13}C NMR (250 MHz, CDCl_3): δ = 169.6 (CO), 167.7 (CO), 156.7 (Cq), 151.2 (Cq), 149.4 (Cq), 148.7 (CH), 146.2 (Cq), 139.1 (Cq), 135.9 (CH), 130.4 (Cq), 125.2 (CH), 118.5 (CH), 117.7 (CH), 117.6 (CH), 116.6 (CH), 112.8 (Cq), 52.9 (CH_2), 52.8 (CH_2), 37.3 (CH_2), 37.2 (CH_2) ppm. MS (DCI, NH_3 /isobutane): m/z = 625 [$\text{M} + \text{H}$]⁺.

Compound 1^l: In portions, **6a** (0.7 g, 1.1 mmol) was added to oleum ($\text{SO}_3/\text{H}_2\text{SO}_4$; 15 mL) while stirring vigorously. After stirring overnight at room temperature, the mixture was carefully poured onto ice to give a beige precipitate. Filtration and washing with cold water gave the product, which was recrystallised from a minimum amount of water. The pure product (acidic form) was thoroughly dried under vacuum at 30 °C. A yellow powder was obtained (0.347 g, 0.4 mmol, 36.5%). ^1H NMR (300 MHz, D_2O /NaOD): δ = 8.65 (dd, $^3J_{\text{H,H}}$ = 1.6, 8.6 Hz, 2 H, Hquin), 8.56 (dd,

$^3J_{\text{H,H}} = 1.5, 4.1 \text{ Hz}$, 2 H, Hquin), 8.40 (s, 2 H, Hquin), 7.63 (d, $^3J_{\text{H,H}} = 2.5 \text{ Hz}$, 1 H, Hcatech.), 7.47 (dd, $^3J_{\text{H,H}} = 4.1, 8.6 \text{ Hz}$, 2 H, Hquin), 7.03 (d, $^3J_{\text{H,H}} = 2.6 \text{ Hz}$, 1 H, Hcatech.), 3.54 (m, 6 H, CH_2), 2.85 (m, 6 H, CH_2) ppm. ^{13}C NMR (300 MHz, D_2O -NaOD): $\delta = 171.3$ (Cq), 170.7 (Cq), 170.2 (Cq), 161.3 (Cq), 148.9 (Cq), 147.4 (CH), 145.1 (Cq), 134.6 (CH), 129.1 (CH), 127.8 (Cq), 125.9 (Cq), 123.9 (CH), 119.6 (Cq), 119.0 (CH), 116.1 (Cq), 111.4 (Cq), 111.0 (CH), 53.2 (CH_2), 36.9 (CH_2) ppm. MS (Electrospray, methanol/water, +ve mode): $m/z = 865$ [$\text{M} + \text{H}$] $^+$. $\text{C}_{33}\text{H}_{32}\text{N}_6\text{O}_{16}\text{S}_3 \cdot 3.5\text{H}_2\text{O}$: calcd. C 42.72, H 4.24, N 9.06, S 10.37; found C 42.51, H 3.85, N 8.49, S 9.62.

Compound 4b: Under argon, a mixture of 2,3-dimethoxybenzoic acid (5.28 g, 29 mmol) and CDI (5.16 g, 31.9 mmol) in dry CH_2Cl_2 (350 mL) was refluxed for 1.5 h. A solution of TREN (2.11 g, 14.5 mmol) in CH_2Cl_2 (40 mL) was added dropwise and reflux was maintained overnight. The solution was filtered and washed with 4 N NaOH then brine. After drying over anhydrous Na_2SO_4 , the solvent was eliminated to afford an oil. Chromatography (silica, CH_2Cl_2 /methanol 1 $\rightarrow$ 8% and $i\text{PrNH}_2$ 1%) gave **4b** as a pale yellow oil (6.53 g, 13.7 mmol, 95%) pure enough for the following steps. ^1H NMR (200 MHz, CDCl_3): $\delta = 8.22$ (br. t, $^3J_{\text{H,H}} = 5 \text{ Hz}$, 2 H, NH), 7.61 (dd, $^3J_{\text{H,H}} = 1.6, 8 \text{ Hz}$, 2 H, ArH), 7.11 (t, $^3J_{\text{H,H}} = 8 \text{ Hz}$, 2 H, ArH), 7.01 (dd, $^3J_{\text{H,H}} = 1.6, 8 \text{ Hz}$, 4 H, ArH), 3.88 (s, 6 H, OCH_3), 3.87 (s, 6 H, OCH_3), 3.60 (dt, $^3J_{\text{H,H}} = 5.8, 6.2 \text{ Hz}$, 4 H, CH_2), 2.75 (m, 8 H, CH_2), 2.64 (m, 2 H, CH_2), 2.2 (br. s, 2 H, NH_2) ppm. ^{13}C NMR (200 MHz, CDCl_3): $\delta = 165.3$ (CO), 152.3 (Cq), 147.2 (CH), 126.7 (Cq), 124.1 (CH), 122.3 (CH), 115.1 (CH), 61.1 (OCH_3), 57.0 (CH_2), 55.87 (OCH_3), 53.5 (CH_2), 39.7 (CH_2), 37.6 (CH_2) ppm. MS (DCI, NH_3 /isobutane): $m/z = 475$ [$\text{M} + \text{H}$], 432, 280, 208, 182.

Compound 5b: Under argon a solution of 7-carboxy-8-hydroxyquinoline (2.30 g, 12 mmol) and CDI (2.27 g, 14 mmol) in dry THF (200 mL) was refluxed for 3 h. Compound **4b** (6 g, 12 mmol) in THF (80 mL) was added dropwise and the reflux was maintained during 12 h. The THF was evaporated off and the residue was dissolved CH_2Cl_2 and treated with NH_4Cl and brine. Drying and evaporation of the solvent afforded an orange foam which was chromatographed (silica, $i\text{PrNH}_2$ 1%, CH_2Cl_2 , MeOH 1 $\rightarrow$ 5%) to give **4b** (5.8 g, 9 mmol, 75%) pure enough for the following step. ^1H NMR (20 MHz, CDCl_3): $\delta = 8.82$ (dd, $^3J_{\text{H,H}} = 1.6, 4.2 \text{ Hz}$, 1 H, Hquin), 8.19 (br. t, 3 H, NH), 8.11–8.02 (m, 1 H, Hquin), 7.56–7.44 (m, 3 H, ArH + Hquin), 7.27–6.86 (m, 6 H, ArH + Hquin), 3.83 (s, 6 H, OCH_3), 3.76 (s, 6 H, OCH_3), 3.65–3.56 (m, 6 H, CH_2), 2.94–2.82 (m, 6 H, CH_2) ppm. ^{13}C NMR (250 MHz, CDCl_3): $\delta = 167.2$ (CO), 165.5 (CO), 154.4 (Cq), 152.2 (Cq), 148.1 (CH), 147.1 (Cq), 138.9 (Cq), 135.6 (Cq), 130.1 (Cq), 126.7 (Cq), 126.1 (CH), 124.0 (CH), 122.8 (CH), 122.2 (CH), 116.7 (CH), 114.9 (CH), 112.8 (Cq), 61.1 (COCH_3), 55.7 (COCH_3), 53.7 (COH_2), 53.2 (CH_2), 37.8 (CH_2), 37.7 (CH_2) ppm.

Compound 6b: Under argon at 0 °C, BBr_3 (9.04 g, 36 mmol) in CH_2Cl_2 (30 mL) was added dropwise to a solution of **5b** (1.94 g, 3 mmol) in CH_2Cl_2 (300 mL). After stirring overnight at room temperature, the mixture was treated with methanol (50 mL) at 0 °C. The solution was concentrated and then repeatedly evaporated with methanol. The residue was treated with 4 N NaOH and extracted with CH_2Cl_2 . The aqueous solution was then acidified to pH 7.6 with 4 N HCl to give a beige precipitate which was washed with water and dried under vacuum to give **6b** pure enough for the following step (1.65 g, 2.8 mmol, 93%). ^1H NMR (200 MHz, DMSO): $\delta = 8.9$ (m, 1 H, Hquin), 8.7 (m, 3 H, NH), 8.32 (d, $^3J_{\text{H,H}} = 8.2 \text{ Hz}$, 1 H, Hquin), 7.94 (d, $^3J_{\text{H,H}} = 8.0 \text{ Hz}$, 1 H, Hquin), 7–6.3 (dd, $^3J_{\text{H,H}} = 4.80, 4 \text{ Hz}$, 1 H, Hquin), 7.37 (d, $^3J_{\text{H,H}} = 28.2 \text{ Hz}$, 1

H, Hquin.), 7.20 (d, $^3J_{\text{H,H}} = 7.4 \text{ Hz}$, 2 H, Hcat.), 6.84 (d, $^3J_{\text{H,H}} = 7.4 \text{ Hz}$, 2 H, Hcat.), 6.54 (t, $^3J_{\text{H,H}} = 7.4 \text{ Hz}$, 2 H, Hcat.), 3.42 (m, 6 H, CH_2), 2.77 (m, 6 H, CH_2) ppm. ^{13}C NMR (250 MHz, DMSO): $\delta = 169.7$ (CO), 167.9 (CO), 156.4 (Cq), 149.4 (Cq), 149.0 (CH), 146.1 (Cq), 139.0 (Cq), 136.1 (CH), 130.5 (Cq), 125.3 (CH), 123.4 (CH), 118.8 (CH), 117.9 (CH), 117.3 (CH), 116.8 (CH), 115.1 (Cq), 112.7 (Cq), 52.6 (CH_2), 36.8 (CH_2) ppm. MS (DCI, NH_3 /isobutane): $m/z = 590$ [$\text{M} + \text{H}$].

Compound L²: In portions, **6b** (1.49 g, 2.5 mmol) was added to oleum ($\text{SO}_3/\text{H}_2\text{SO}_4$) (20 mL) while stirring vigorously. After stirring at room temperature for 12 h, the mixture was carefully poured onto ice to give a brown precipitate. Filtration and washing with cold water afforded the product, which was recrystallised from a minimum amount of water. The pure product (acidic form) was dried under vacuum at 30 °C. A yellowish powder was obtained (0.672 g, 0.8 mmol, 32%). ^1H NMR (250 MHz, D_2O /NaOD): $\delta = 8.48$ (m, 1 H, Hquin), 8.48 (m, 1 H, Hquin), 8.23 (s, 1 H, Hquin), 7.30 (m, 1 H, Hquin), 7.14 (m, 2 H, Hcatech), 6.49 (m, 2 H, Hcatech), 3.30 (m, 6 H, CH_2), 2.62 (m, 6 H, CH_2) ppm. ^{13}C NMR (250 MHz, D_2O -NaOD): $\delta = 174.3$ (Cq), 174.0 (Cq), 173.1 (Cq), 170.2 (Cq), 163.2 (Cq), 150.1 (CH), 147.8 (Cq), 137.0 (CH), 131.6 (CH), 130.3 (Cq), 128.6 (CH), 126.5 (Cq), 122.1 (Cq), 117.6 (Cq), 115.5 (CH), 115.4 (CH), 113.8 (Cq), 55.9 (CH_2), 39.3 (CH_2), 39.2 (CH_2) ppm. MS (Electrospray, methanol/water, +ve mode): $m/z = 830$ [$\text{M} + \text{H}$] $^+$, 750 [$\text{M} - \text{SO}_3$]. $\text{C}_{30}\text{H}_{31}\text{N}_5\text{O}_{17}\text{S}_3 \cdot 5\text{H}_2\text{O}$: calcd. C 39.17, H 4.49; N 7.61; S 10.46; found C 39.29, H 4.59, N 7.64, S 9.65.

Potentiometric and Spectrophotometric Measurements: The solutions were prepared with boiled deionised water, de-oxygenated and flushed continuously with argon (purified by a Sigma Oxiclear cartridge) in order to exclude CO_2 and O_2 . The ionic strength was maintained at 0.1 M with sodium perchlorate (Prolabo, Puriss or Merck, p.a.) and all measurements were carried out at (25.0 ± 0.2) °C. Perchloric acid (70%, Fluka; approx. 0.1 M) and sodium hydroxide (Normex, Carlo Erba; approx. 0.1 M) concentrations were determined respectively by titration of sodium tetraborate solution (puriss. p. a., ACS, Fluka, approx. 0.1 M) in the presence of methyl red as indicator (Pointet Girard, France) and potassium hydrogenphthalate (puriss. p. a., Fluka, approx. 0.1 M), with phenolphthalein as indicator (Prolabo, France). The ligands **L¹** and **L²** were dissolved in aqueous 0.1 M NaClO_4 medium and their concentrations were calculated by weight. Stock iron(III) solutions [$\text{Fe}(\text{ClO}_4)_3 \cdot 9\text{H}_2\text{O}$, pract., Fluka or Aldrich; approx. 0.01 M], prepared under acidic conditions (0.1 M HClO_4), were back titrated with $\text{Th}(\text{NO}_3)_4 \cdot 5\text{H}_2\text{O}$ (Merck, approx. 0.1 M) in the presence of excess EDTA (Titrisol, Merck, approx. 0.1 M) and xylenol orange (Merck) as end-point indicator.^[27] The concentration was also controlled spectrophotometrically by using the molar extinction coefficient^[28] of $4160 \text{ M}^{-1}\text{cm}^{-1}$ at 240 nm in 0.1 M HClO_4 . The acid in excess was evaluated by a potentiometric titration with standardised 0.1 M NaOH solution using quantitative formation of a complex with maltol (99%, Aldrich).^[29] **L¹** and **L²** ferric complexes were prepared by mixing adequate volumes of stock ferric perchlorate and stock ligand solutions. The free hydrogen concentrations were measured with a glass-Ag/AgCl combined electrode (Metrohm or Tacussel High Alkalinity, filled with 0.1 M NaCl and saturated with AgCl). The electrode was calibrated in order to read the pH according to the classical method (neutralisation of 0.1 M HClO_4 by 0.1 M NaOH).^[30]

The potentiometric titrations were performed into a jacketed cell, thermostatted by the water flow of a Haake thermostat, using either a digital millivoltmeter (Tacussel Isis 20,000) with a piston-

fitted microburette (Gilmont) or an automatic titrator system (Metrohm, DMS Titrino 716) connected to an IBM Aptiva microcomputer. The titrations of the ligands ($[L] \approx 10^{-3}$ M, $2.5 < \text{pH} < 10.5$ for L^1 and $3.9 < \text{pH} < 9.8$ for L^2) and of their iron(III) complexes ($[\text{Fe}^{\text{III}}]_{\text{tot}} = [L]_{\text{tot}} \approx 10^{-3}$ M, $2.2 < \text{pH} < 11.0$ for L^1 and $2.3 < \text{pH} < 8.9$ for L^2) were carried out by addition of known volumes of standardised sodium hydroxide. The data obtained during the potentiometric titrations were fitted with the Superquad^[31] or Hyperquad^[32] software packages.

Spectrophotometric measurements were carried out on double-beam UV/Vis spectrophotometers, either Uvikon 941 (Kontron) or Perkin–Elmer (Lambda 2), equipped with a thermostating cell holder (Perkin–Elmer PTP-1) and connected to an IBM PC 340 microcomputer for data acquisition (UV Winlab software, Perkin–Elmer). Absorption spectra of the free ligands ($[L]_{\text{tot}} \approx 10^{-4}$ M, $240 \text{ nm} < \lambda < 400 \text{ nm}$, $2.5 < \text{pH} < 10.5$ for L^1 and $2.6 < \text{pH} < 12.5$ for L^2) and of their iron(III) species ($[L]_{\text{tot}} = [\text{Fe}^{\text{III}}]_{\text{tot}} \approx 10^{-4}$ M, $400 \text{ nm} < \lambda < 800 \text{ nm}$, for L^1 $2.5 < \text{pH} < 10.5$ and $1.0 < \text{pH} < 9.0$ for L^2) were measured at different pH values using quartz spectrophotometric cells of 0.2 or 1 cm optical path length (Hellma) against 0.1 M aqueous NaClO_4 solution as a reference. Absorption spectra recorded as a function of pH for ferric complexes of L^1 and L^2 are given in the supporting information (Figures S3 and S4).

A batch competition titration between the ferric L^2 complex and EDTA (99.5%, A.C.S. reagent, Aldrich) was carried out under conditions of $[L^2]_{\text{tot}} = 5.4 \times 10^{-5}$ M, $[\text{Fe}^{\text{III}}]_{\text{tot}} = 4.5 \times 10^{-5}$ M, $[\text{EDTA}]_{\text{tot}} = 9.21 \times 10^{-4}$ M and the pH values adjusted with perchloric acid over the range 3.9 to 5.5 (in steps of 0.1 pH unit). The solution was equilibrated for two days and then the absorption spectra were recorded (400–800 nm).

The spectrophotometric data using absorbance values from 20 wavelengths (between 400 and 800 nm) were processed with both the Specfit^[19–22] and Letagrop-Spefo^[17,18] programs in order to calculate the thermodynamic constants of the absorbing species and their corresponding electronic spectra. The range of values for the residual-squares sum $[\Sigma(A_{\text{exp}} - A_{\text{calcd}})^2]$ of the fits was over the range 10^{-2} to 10^{-3} . The calculated electronic spectra are presented in Figure 6.

NMR Titration: The proton NMR titration was carried out for L^2 over the pD range 3.5–9.2 in order to attribute protonation constants to different protonable sites of the ligand. The ligand was dissolved in D_2O . The pD was adjusted with DCl or NaOD solutions. pH measurements were performed with a Tacussel PHN 850 apparatus equipped with a microelectrode Radiometer XC61. pD values were calculated according to $\text{pD} = \text{pH}_{\text{meas}} + 0.4$.^[33]

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