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

Anne-Marie Albrecht-Gary,*[a] Sylvie Blanc,[a] Frederic Biaso,[b] Fabrice Thomas,[b] Paul Baret,[b] Gisele Gellon,[b] Jean-Louis Pierre,[b] and Guy Serratrice*[b]

Keywords: Tripodal ligands / Iron / Thermodynamics / UV/Vis spectroscopy / Siderophores

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-TRENSOX and TRENCAMS. Like the parent homopodands, the two heteropodands are based on the TREN scaffold and the chelating units are connected by amide groups, TRENSOX2CAMS having two 8-hydroxyquinoline and one catechol arms and TRENSOXCAMS2 one 8-hydroxyquinoline and two catechol moieties. The aqueous coordination chemistry of these ligands was examined by potentiometric and spectrophotometric methods in combination with ^{1}H 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.

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(hydroxyquinolinate) analogue O-TRENSOX 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 hydroxypiridinone and one catechol moieties, and (ii) two hydroxypiridinone and one 2-hydroxyisophtalamide groups has been published.[4] We report in this paper the synthesis, acid-base properties, and ferric complexation of two monotopic tripodal mixed ligands TRENSOX2CAMS (**L^1**) and TRENSOXCAMS2 (**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 catecholylamide groups, none for ligand **L^0** (O-TRENSOX), 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 ^{1}H 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.

[a] Laboratoire de Physico-Chimie Bioinorganique, UMR CNRS 7509, European School of Chemistry, 25 rue Becquerel, 67200 Strasbourg, France,
Fax: (internat.) +33-(0)3/90 24 26 39
E-mail: amalbre@chimie.u-strasbg.fr
[b] Laboratoire de Chimie Biomimétique, LEDSS, UMR CNRS 5616, Université Joseph Fourier,
BP 53, 38041 Grenoble Cedex 9, France
Fax: (internat.) +33-(0)4/76 51 48 36
E-mail: guy.serratrice@ujf-grenoble.fr
Supporting information for this article is available on the WWW under http://www.eurjic.org or from the author.

  DOI: 10.1002/ejic.200200643

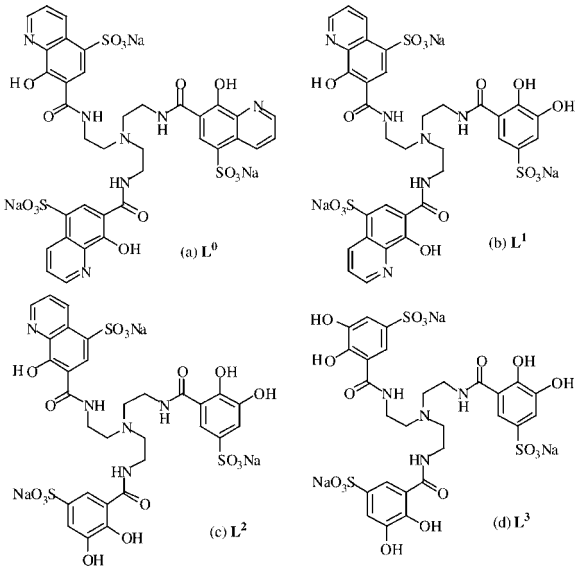


Figure 1. Chemical formulae of the tripodal ligands: (a) **L⁰** (O-TRENSOX), (b) **L¹** (TRENSOX2CAMS), (c) **L²** (TRENSOX-CAMS2), (d) **L³** (TRENCAMS)

Results and Discussion

Synthesis

The synthesis of the heterotripodal hexadentate ligands **L¹** and **L²** is presented in Figure 2. The two catecholate subunits of ligand **L²** 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 **L¹** 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 BBr_3. Finally, regiospecific sulfonation in position 5 of both oxinate and catecholate subunits afforded the hydrosoluble ligands **L¹** and **L²**.

Acid-Base Properties of Ligands

The protonation constants of **L¹** and **L²** were determined by combination of potentiometric and spectrophotometric

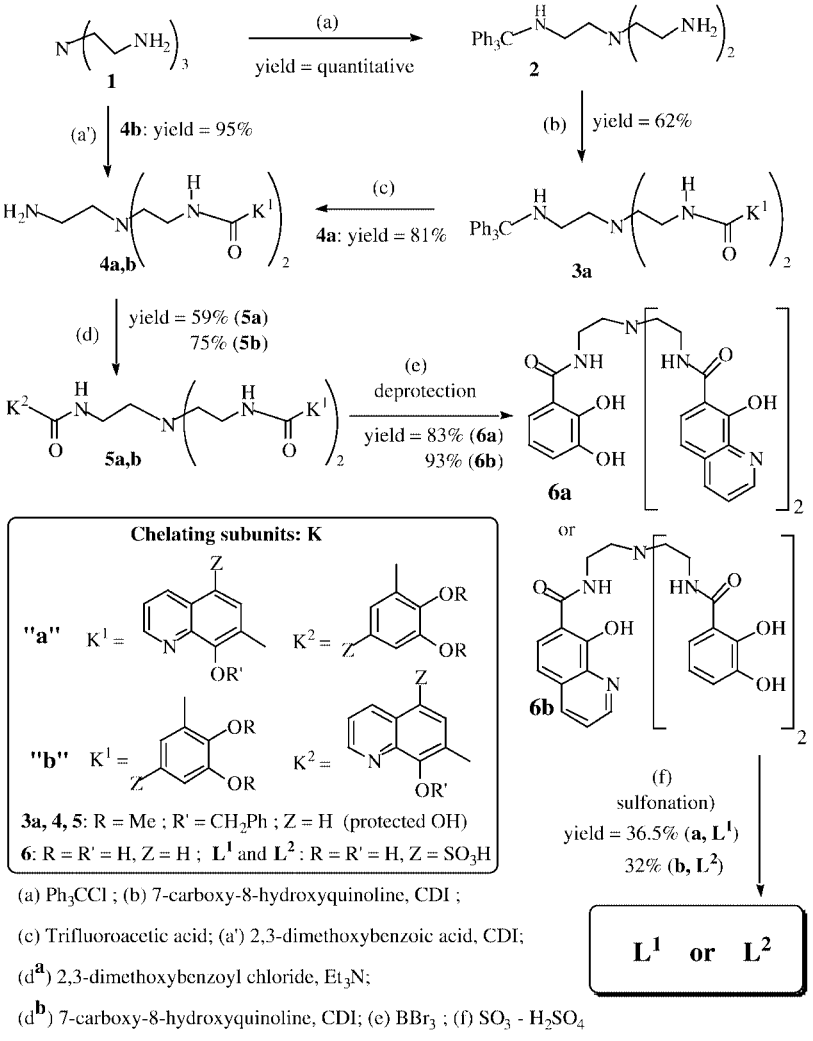


Figure 2. Synthesis of the heterotripodal ligands **L¹** and **L²**

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 ($NaClO_4$), $T = (25.0 \pm 0.2)$ °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 (*ortho*)		5.62(6)	5.55(4) 7.35(2)	5.57(5) 6.32(4) 7.05(4)
OH catechol (*meta*)		11.5(2)	11.41(8)[c] 12.5(3)[c]	12.05(10)[d]
NH ammonium	6.36(3)	6.96(6)	6.56(3)	8.01(2)

[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 $\mathbf{L^1}$ 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 $\mathbf{L^2}$, 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 $\mathbf{L^1}$ and $\mathbf{L^2}$ by comparison with the log K^H values of the ligand $\mathbf{L^0}$, bearing three hydroxyquinoline groups,[2] and with those of the tris(catecholate) ligand $\mathbf{L^3}$ [3] (Table 1). The highest protonation constants (11.5 for $\mathbf{L^1}$; 12.5 and 11.41 for $\mathbf{L^2}$) 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 $\mathbf{L^1}$; 2.34 for $\mathbf{L^2}$) correspond to the pyridine nitrogen of the sulfohydroxyquinoline moieties. Compared to 8-hydroxyquinoline-5-sulfonic acid (log $K^H = 3.93$),[10] the protonation constants determined for $\mathbf{L^1}$ and $\mathbf{L^2}$ 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 $\mathbf{L^0}$ vary between 7.44 and 8.62.[2] We attribute the values 8.78 and 7.85 for $\mathbf{L^1}$ and 7.93 for $\mathbf{L^2}$ to the protonation of the 8-hydroxyquinoline OH groups.

Based on the fact that the species $\mathbf{L^2}H_3$ and $\mathbf{L^2}H_4$ 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 $\mathbf{L^2}$ anchor. This was confirmed by the ^{1}H NMR titration of $\mathbf{L^2}$ which showed an inflection point at pD ≈ 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 $\mathbf{L^1}$, 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 $\mathbf{L^1}$ and $\mathbf{L^2}$ are close to that of $\mathbf{L^0}$ (log $K^H = 6.36$).[2] They are, however, much lower than that of the tris(catechol) ligand $\mathbf{L^3}$ (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

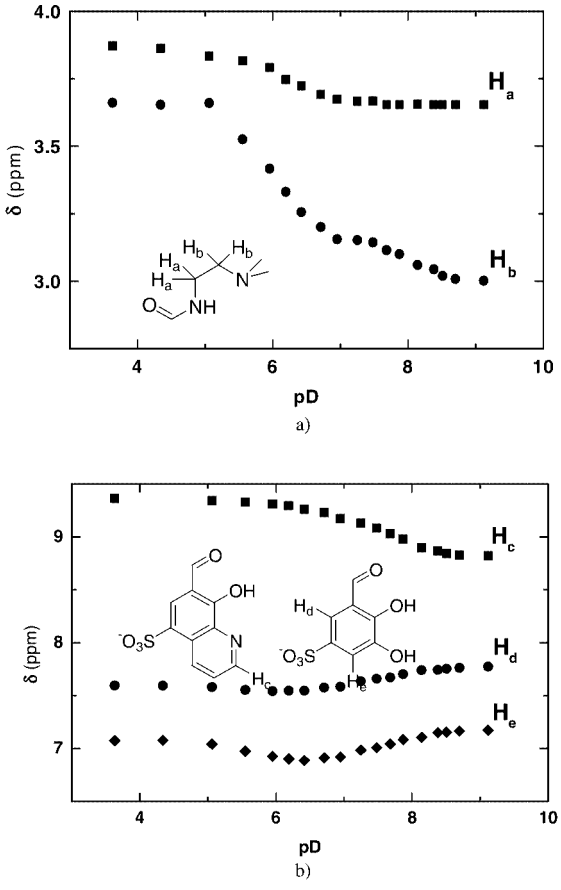


Figure 3. ^{1}H NMR chemical shift variation as a function of pD for the methylene (a) and aromatic (b) protons of $\mathbf{L^2}$; solvent: D_2O, $[\mathbf{L^2}] = 5$ mM

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 ^{1}H 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 catecholylamide-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 (≈2 × 10^4 M^{-1}·cm^{-1} at around 340 nm and ≈5−6 × 10^4 M^{-1}·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-hydroxyquinolinate or catecholate ligands.[15,16]

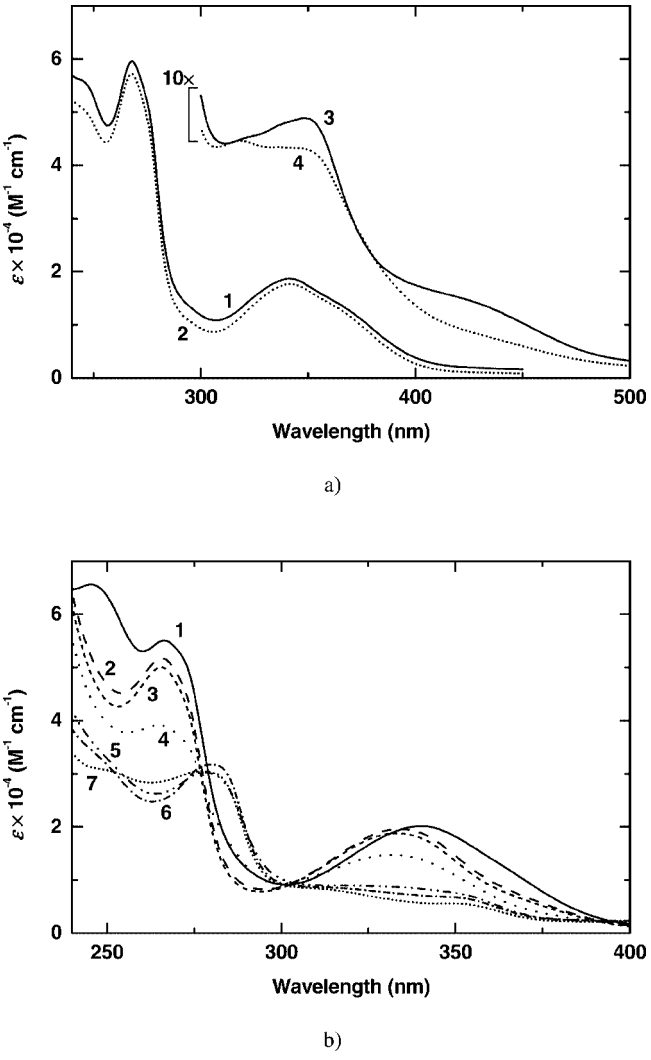


Figure 4. Calculated electronic spectra of the protonated species of heterotripodal ligands; solvent: water, I = 0.10 M ($NaClO_4$), T = (25.0 ± 0.2) °C: (a) ligand **L¹**: (1) $\mathbf{L^1H_7}$, (2) $\mathbf{L^1H_6}$, (3) $\mathbf{L^1H}$, (4) **L¹**; (b) ligand **L²**: (1) **L²**, (2) $\mathbf{L^2}$ H, (3) $\mathbf{L^2H_2}$, (4) $\mathbf{L^2H_3} \equiv \mathbf{L^2H_4}$, (5) $\mathbf{L^2H_5}$, (6) $\mathbf{L^2H_6}$, (7) $\mathbf{L^2H_7}$; charges are omitted for the sake of clarity

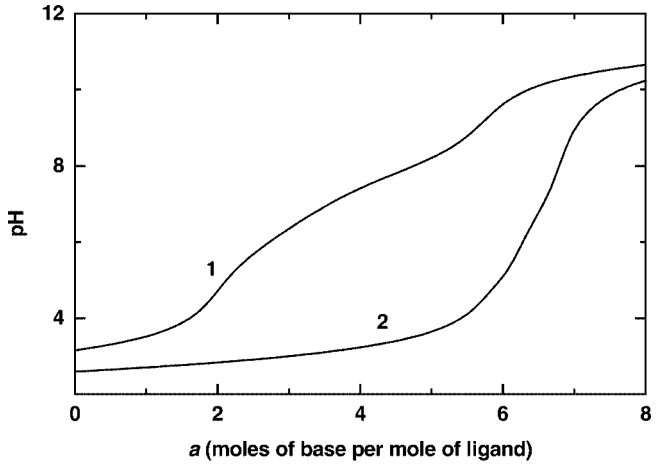
Characterisation of the Ferric Complexes

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.

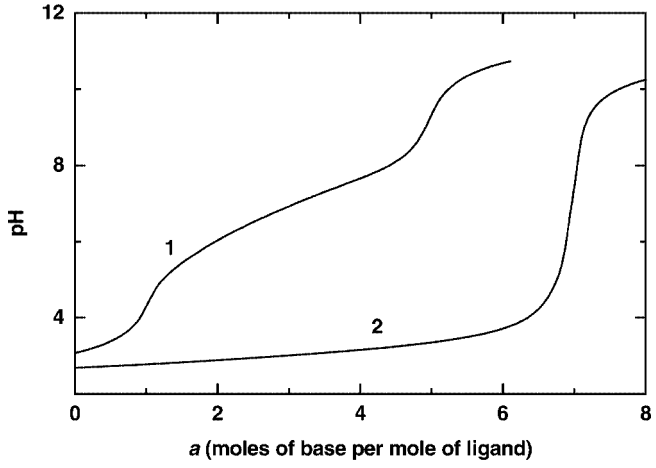
The spectra obtained during the spectrophotometric titrations of ferric complexes of **L¹** and **L²** are shown in Figures S3 and S4. The absorbance data were refined with the Letagrop-Spefo[17,18] and Specfit[19−22] programs and provided values of global stability constants of ferric complexes of both **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 determination 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



a)



b)

Figure 5. Potentiometric titration curves for the heterotripodal ligands and their ferric complexes; solvent: water, I = 0.10 M ($NaClO_4$), T = (25.0 ± 0.2) °C: (a) titration of $\mathbf{L^1}$ (1 mM): (1) free ligand, (2) 1:1 ferric complex; (b) titration of $\mathbf{L^2}$ (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 $\mathbf{L^0}$ and $\mathbf{L^3}$, both $\mathbf{L^1}$ and $\mathbf{L^2}$ 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 $\mathbf{L^1}$ and $\mathbf{L^2}$ by examining their electronic spectra (Table 2) as this can provide information on the coordination modes and their successive deprotonation constants pK_{FeLH_n} (defined in Table 3). These values are calculated from the difference between the values of log β_{FeLH_n}

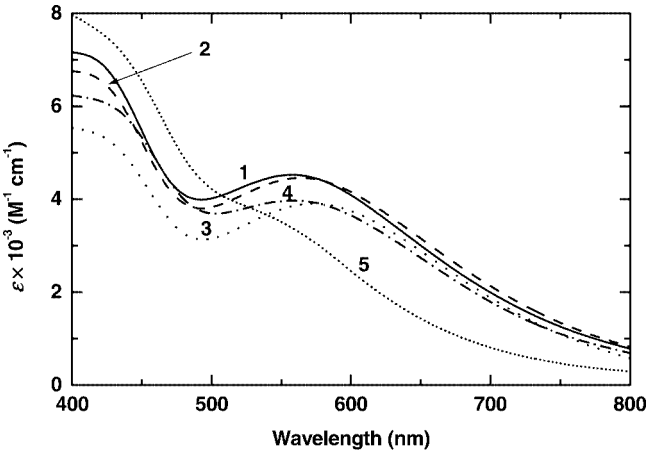
Table 2. Cumulative stability constants β_{FeLHn}, pFe^{III} values and UV/Vis spectral characteristics of ferric complexes formed with the tripodal ligands; solvent: water, I = 0.10 M ($NaClO_4$), T = (25.0 ± 0.2) °C; numbers in parentheses correspond to statistical errors (3σ) on the last digit; an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

Ligand	Species	log β_{FeLH_n}[a]	pFe^{III}[b]	λ_{max} (nm)[c]	ε_{max} ($M^{-1}\cdot cm^{-1}$)
$\mathbf{L^0}$ [2]	$Fe\mathbf{L^0}H_5$	42.2(1)	29.5	435	8200
				525*	3500
	$Fe\mathbf{L^0}H$	36.5(2)		443	5200
				595	5200
	$Fe\mathbf{L^0}$	30.9(1)		443	5400
				595	5400
$\mathbf{L^1}$	$Fe\mathbf{L^1}H_5$	48.6(2)	31.6	440*	6900
				540*	3700
	$Fe\mathbf{L^1}H_3$	46.2(4)		560	4000
	$Fe\mathbf{L^1}H_2$	44.3(3)		580	3900
	$Fe\mathbf{L^1}H$	41.6(2)		560	4500
	$Fe\mathbf{L^1}$	36.8(4)		550	4500
$\mathbf{L^2}$	$Fe\mathbf{L^2}H_5$	55.91(3)	32.3	409	4700
				520*	3300
	$Fe\mathbf{L^2}H_4$	53.7(1)		573	3500
	$Fe\mathbf{L^2}H_2$	47.7(3)		542	4500
	$Fe\mathbf{L^2}H$[d]	44.7(6)		525	4800
	$Fe\mathbf{L^2}$ [d]	41.3(6)		525	4800
$\mathbf{L^3}$ [3]	$Fe\mathbf{L^3}H_5$	64.05(7)	29.6	495	4300
	$Fe\mathbf{L^3}H_3$	57.4(1)		520	4800
	$Fe\mathbf{L^3}H$	49.1(1)		500	5000
	$Fe\mathbf{L^3}$	43.6(1)		488	5300

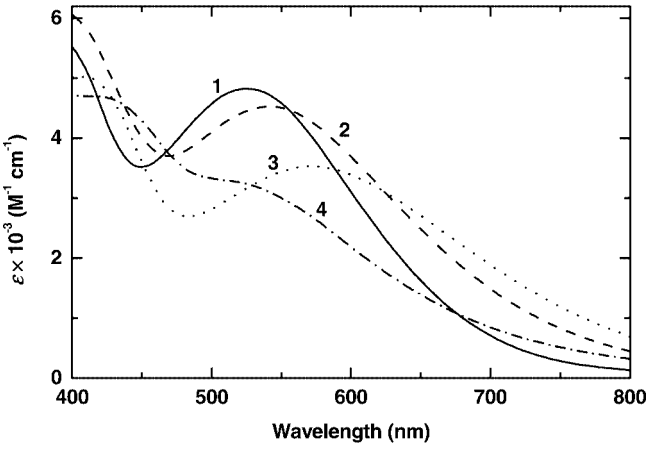
[a] $\beta_{FeLHn} = [FeLH_n]/([Fe^{III}] \times [L] \times [H^+]n)$. [b] Calculated for $[Fe^{III}]_{tot} = 10^{-6}$ M, $[L]_{tot} = 10^{-5}$ M, pH = 7.4. [c] * = shoulder. [d] Species have identical electronic spectra.

and are reported in Table 3 together with the values from tris(catecholate) and tris(8-hydroxyquinolinate) complexes for comparison. The deprotonation constants of ferric $\mathbf{L^1}$ and $\mathbf{L^2}$ 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 pK_{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 pK_{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 $Fe\mathbf{L^1}H_5$, which exhibits two shoulders at 440 nm (ε = 6940 $M^{-1}\cdot cm^{-1}$) and 540 nm (ε = 3680 $M^{-1}\cdot cm^{-1}$), is similar to that of the bis(salicylate) complex of $\mathbf{L^0}$ [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 $Fe\mathbf{L^1}H_3$ complex exhibits an absorption band at λ_{max} = 560 nm (ε = 3970 $M^{-1}\cdot cm^{-1}$), 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 $pK_{FeL^1H_5}$ = 2.4 ($Fe\mathbf{L^1}H_5/Fe\mathbf{L^1}H_3$ equilibrium) corresponds to the deprotonation of both 8-hydroxyquinoline nitrogens in a two-proton step. The small



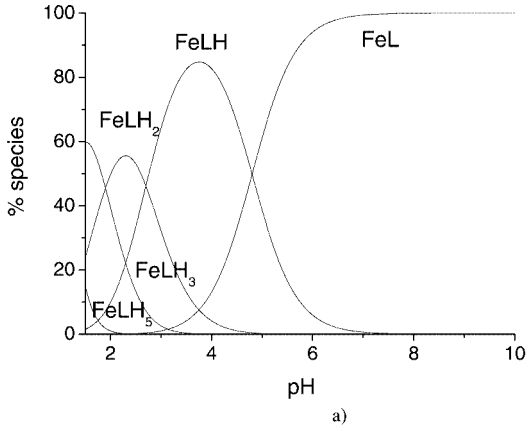
a)



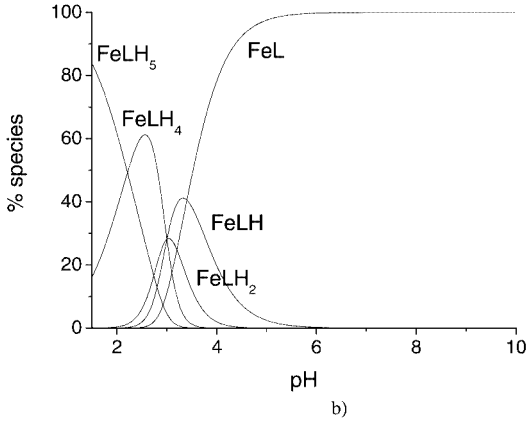
b)

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 ± 0.2) °C: (a) ferric $\mathbf{L^1}$ species: (1) Fe$\mathbf{L^1}$, (2) Fe$\mathbf{L^1}$H, (3) Fe$\mathbf{L^1}H_2$, (4) Fe$\mathbf{L^1}H_3$, (5) Fe$\mathbf{L^1}H_5$; (b) ferric $\mathbf{L^2}$ species: (1) Fe$\mathbf{L^2}$ ≡ Fe$\mathbf{L^2}$ H, (2) Fe$\mathbf{L^2}H_2$, (3) Fe$\mathbf{L^2}H_4$, (4) Fe$\mathbf{L^2}H_5$; charges are omitted for the sake of clarity

red-shift of λ_{max} to 580 nm for the formation of Fe$\mathbf{L^1}H_2$ without significant change of ε (3930 $M^{-1}\cdot cm^{-1}$) might be attributed to the deprotonation of the ammonium nitrogen. The value $pK_{FeL^1H_3}$ = 1.9 shows a strong stabilisation of the deprotonated tertiary amine nitrogen by intramolecular hydrogen bonds. The increase of ε to 4490 $M^{-1}\cdot cm^{-1}$ with the concomitant blue shift of λ_{max} to 560 nm for the formation of Fe$\mathbf{L^1}$H is in agreement with the coordination of the catechol arm in a salicylate mode. The last deprotonation equilibrium is accompanied with a spectral change (λ_{max} = 550 nm, ε = 4560 $M^{-1}\cdot cm^{-1}$) that is indicative of a shift to a catecholate coordination and formation of the Fe$\mathbf{L^1}$ species. It should be pointed out that the values of pK_{FeLH_2} and pK_{FeLH} (2.7 and 4.8) are significantly lower than those observed for tris(catecholate) complexes: 8.34 (two-proton step) and 5.5 for $\mathbf{L^3}$, and 4.1 and 5.74 for MECAMS[9] (Table 3), while the pK_a's of the free ligand are of the same order of magnitude.



a)



b)

Figure 7. Speciation diagrams for (a) ferric-$\mathbf{L^1}$ complexes, (b) ferric-$\mathbf{L^2}$ complexes

Table 3. Successive deprotonation constants of ferric complexes;[a] solvent: water, I = 0.10 M ($NaClO_4$), T = (25.0 ± 0.2) °C

Ligand[a][b]	pK_{FeLH_5}	pK_{FeLH_4}	pK_{FeLH_3}	pK_{FeLH_2}	pK_{FeLH}
$\mathbf{L^0}$ [2]	5.7[c]				5.6
$\mathbf{L^1}$	2.4[d]		1.9	2.7	4.8
$\mathbf{L^2}$	2.2	6.0[d]		3.0	3.4
$\mathbf{L^3}$ [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_{FeLHn} = ([$FeLH_{n-1}$] × [H])/[$FeLH_n$]. [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_{FeLHn} = ([FeLH] × $[H]^4$)/[$FeLH_5$]. [d] K_{FeLHn} = ([$FeLH_{n-2}$] × $[H]^2$)/[$FeLH_n$]. [e] K_{FeLHn} = ([FeLH] × $[H]^3$)/[$FeLH_4$].

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

8-hydroxyquinoline coordinated to the ferric ion. For the formation of Fe$\mathbf{L^2}H_4$ 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 $M^{-1}\cdot cm^{-1}$), indicating that iron is still coordinated by two arms. The value $pK_{FeL^2H_5}$ = 2.2 of the Fe$\mathbf{L^2}H_5$/ Fe$\mathbf{L^2}H_4$ equilibrium corresponds well to the pyridinium nitrogen deprotonation. The formation of the Fe$\mathbf{L^2}H_2$ and Fe$\mathbf{L^2}H$ 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 $M^{-1}\cdot cm^{-1}$ and 4820 $M^{-1}\cdot cm^{-1}$ is observed. The equilibrium between Fe$\mathbf{L^2}H_4$ and Fe$\mathbf{L^2}H_2$ ($pK_{FeL^2H_4}$ = 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 Fe$\mathbf{L^2}H_2$ and Fe$\mathbf{L^2}H$ corresponds to the shift of bonding mode from salicylate to catecholate of the third catechol arm ($pK_{FeL^2H_2}$ = 3.0). As for Fe$\mathbf{L^1}$ 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 Fe$\mathbf{L^2}$ species does not induce any spectral changes. It is likely that the deprotonation of the tertiary amine nitrogen is involved. The value of pK_{FeL^2H} = 3.4 is higher than the corresponding constant for ferric $\mathbf{L^1}$ complex ($pK_{FeL^1H_3}$ = 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 [Fe^{III}]) is thus a better measure of the relative efficiency of the ligands under given conditions of pH, $[Fe^{III}]_{tot}$ and $[L]_{tot}$. 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 $\mathbf{L^0}$ and $\mathbf{L^3}$.

The important result, as seen from Figure 8, is that the mixed ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ are stronger iron chelating agents than $\mathbf{L^0}$ and $\mathbf{L^3}$ over the pH range 5−9. This shows that mixed ligands lead to enhanced affinity for the ferric ion at pH above 5 ($\mathbf{L^1}$) or 6 ($\mathbf{L^2}$) 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 $\mathbf{L^0}$ [tris(8-hydroxyquinoline) ligand] and $\mathbf{L^3}$ [tris-(catechol) ligand]. Our results with $\mathbf{L^1}$ (one catechol group) and $\mathbf{L^2}$ (two catechol groups) show that a greater pK_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 $\mathbf{L^1}$ and $\mathbf{L^2}$ 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, $[L]_{tot} = 10^{-5}$ M, $[Fe^{III}]_{tot} = 10^{-6}$ M) for $\mathbf{L^1}$ (31.6) and for $\mathbf{L^2}$ (32.3). These values are higher than those determined for $\mathbf{L^0}$ (29.5) and for $\mathbf{L^3}$ (29.6) and among the highest values determined for an iron sequestering agent.

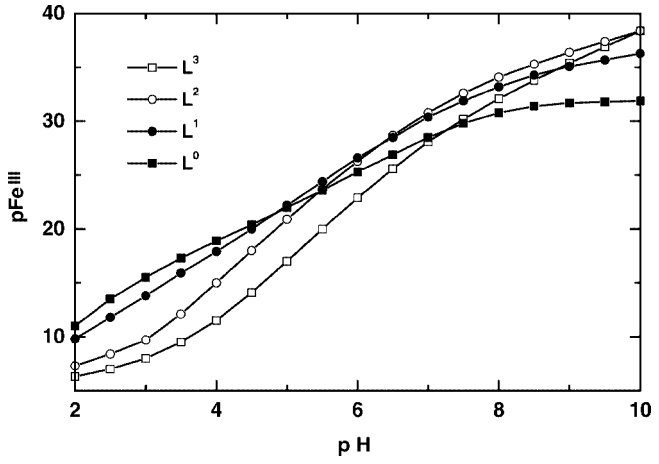


Figure 8. Plot of pFe versus pH for the tripodal ligands; calculated for $[L]_{tot} = 10^{-5}$ M and $[Fe^{III}]_{tot} = 10^{-6}$ M

Conclusion

Iron(III) complexation by two heterotripodal ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ 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 $\mathbf{L^1}$, and one 8-hydroxyquinoline and one catecholate subunit in the case of $\mathbf{L^2}$. 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 $\mathbf{L^0}$ is less sensitive to pH than the tris(catechol) ligand $\mathbf{L^3}$ 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.

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, $^3J_{H,H}$ = 6 Hz, 4 H, CH_2), 2.6 (t, $^3J_{H,H}$ = 6 Hz, 2 H, CH_2), 2.4 (t, $^3J_{H,H}$ = 6 Hz, 4 H, CH_2), 2.2 (t, $^3J_{H,H}$ = 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 $[M + H]^+$, 243 $[C_{19}H_{15}]^+$.

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, $^3J_{H,H}$ = 1.6, 4.0 Hz, 2 H, ArH), 8.14 (m, 4 H, ArH), 8.07 (br. t, $^3J_{H,H}$ = 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, $^3J_{H,H}$ = 6.3, 4.8 Hz, 4 H, CH_2), 2.47 (t, $^3J_{H,H}$ = 5.5 Hz, 2 H, CH_2), 2.21 (t, $^3J_{H,H}$ = 6.3, 4.8 Hz, 4 H, CH_2), 2.13 (t, $^3J_{H,H}$ = 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 $[C_{19}H_{15}]^+$; 91 $[C_7H_7]^+$. $C_{59}H_{54}N_6O_4$ (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 CF_3CO_2H (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, $^3J_{H,H}$ = 1.7, 4.1 Hz, 2 H, Hquin), 8.22 (br. t, $^3J_{H,H}$ = 5.6 Hz, 2 H, NH amide), 8.11 (dd, $^3J_{AB}$(H-H) = 1.7, 4.1 Hz, 2 H, Hquin), 7.97 (d, $^3J_{H,H}$ = 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, $^3J_{H,H}$ = 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, ^{3}(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 $[M + H]^+$.

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, CD Cl_3): δ = 8.94 (dd, $^3J_{H,H}$ = 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, $^3J_{H,H}$ = 7.9 Hz, 1 H, H catech.), 6.89 (dd, $^3J_{H,H}$ = 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 $[M + H]^+$, 541, 305 $[C_{19}H_{17}N_2O_2]^+$. $C_{49}H_{48}N_6O_7$ (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 $[M + H]^+$.

Compound L¹: In portions, **6a** (0.7 g, 1.1 mmol) was added to oleum (SO_3/H_2SO_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, $^3J_{H,H}$ = 1.6, 8.6 Hz, 2 H, Hquin), 8.56 (dd,

$^3J_{H,H}$ = 1.5, 4.1 Hz, 2 H, Hquin), 8.40 (s, 2 H, Hquin), 7.63 (d, $^3J_{H,H}$ = 2.5 Hz, 1 H, Hcatech.), 7.47 (dd, $^3J_{H,H}$ = 4.1, 8.6 Hz, 2 H, Hquin), 7.03 (d, $^3J_{H,H}$ = 2.6 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): δ = 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 $[M + H]^+$. $C_{33}H_{32}N_6O_{16}S_3 \cdot 3.5H_2O$: 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 → 8% and $iPrNH_2$ 1%) gave **4b** as a pale yellow oil (6.53 g, 13.7 mmol, 95%) pure enough for the following steps. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.22 (br. t, $^3J_{H,H}$ = 5 Hz, 2 H, NH), 7.61 (dd, $^3J_{H,H}$ = 1.6, 8 Hz, 2 H, ArH), 7.11 (t, $^3J_{H,H}$ = 8 Hz, 2 H, ArH), 7.01 (dd, $^3J_{H,H}$ = 1.6, 8 Hz, 4 H, ArH), 3.88 (s, 6 H, OCH_3), 3.87 (s, 6 H, OCH_3), 3.60 (dt, $^3J_{H,H}$ = 5.8, 6.2 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, $CDCl_3$): δ = 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 [M + 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, $iPrNH_2$ 1%, CH_2Cl_2, MeOH 1 → 5%) to give **4b** (5.8 g, 9 mmol, 75%) pure enough for the following step. ^{1}H NMR (20 MHz, CD Cl_3): δ = 8.82 (dd, $^3J_{H,H}$ = 1.6, 4.2 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, 0 CH_3), 3.76 (s, 6 H, 0 CH_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, $CDCl_3$): δ = 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%). ^{1}H NMR (200 MHz, DMSO): δ = 8.9 (m, 1 H, Hquin), 8.7 (m, 3 H, NH), 8.32 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, Hquin), 7.94 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, Hquin), 7−6.3 (dd, $^3J_{H,H}$ = 4.80, 4 Hz, 1 H, Hquin), 7.37 (d, $^3J_{H,H}$ = 28.2 Hz, 1 H, Hquin.), 7.20 (d, $^3J_{H,H}$ = 7.4 Hz, 2 H, Hcat.), 6.84 (d, $^3J_{H,H}$ = 7.4 Hz, 2 H, Hcat.), 6.54 (t, $^3J_{H,H}$ = 7.4 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): δ = 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 [M + H].

Compound L^2: In portions, **6b** (1.49 g, 2.5 mmol) was added to oleum (SO_3/H_2SO_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%). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 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): δ = 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 $[M + H]^+$, 750 $[M - SO_3]$. $C_{30}H_{31}N_5O_{17}S_3$ $5H_2O$: 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 (purris. p. a., ACS, Fluka, approx. 0.1 M) in the presence of methyl red as indicator (Pointet Girard, France) and potassium hydrogenphthalate (purris. p. a., Fluka, approx. 0.1 M), with phenolphthalein as indicator (Prolabo, France). The ligands L^1 and L^2 were dissolved in aqueous 0.1 M $NaClO_4$ medium and their concentrations were calculated by weight. Stock iron(III) solutions [$Fe(ClO_4)_3 \cdot 9H_2O$, pract., Fluka or Aldrich; approx. 0.01 M], prepared under acidic conditions (0.1 M $HClO_4$), were back titrated with $Th(NO_3)_4 \cdot 5H_2O$ (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 $M^{-1} \cdot cm^{-1}$ 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] L^1 and L^2 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 ($[\mathbf{L}] \approx 10^{-3}$ M, $2.5 < pH < 10.5$ for $\mathbf{L^1}$ and $3.9 < pH < 9.8$ for $\mathbf{L^2}$) and of their iron(III) complexes ($[Fe^{III}]_{tot} = [\mathbf{L}]_{tot} \approx 10^{-3}$ M, $2.2 < pH < 11.0$ for $\mathbf{L^1}$ and $2.3 < pH < 8.9$ for $\mathbf{L^2}$) 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 thermostatting 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 ($[\mathbf{L}]_{tot} \approx 10^{-4}$ M, 240 nm $< \lambda <$ 400 nm, $2.5 < pH < 10.5$ for $\mathbf{L^1}$ and $2.6 < pH < 12.5$ for $\mathbf{L^2}$) and of their iron(III) species ($[\mathbf{L}]_{tot} = [Fe^{III}]_{tot} \approx 10^{-4}$ M, 400 nm $< \lambda <$ 800 nm, for $\mathbf{L^1}$ $2.5 < pH < 10.5$ and $1.0 < pH < 9.0$ for $\mathbf{L^2}$) 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 $\mathbf{L^1}$ and $\mathbf{L^2}$ are given in the supporting information (Figures S3 and S4).

A batch competition titration between the ferric $\mathbf{L^2}$ complex and EDTA (99.5%, A.C.S. reagent, Aldrich) was carried out under conditions of $[\mathbf{L^2}]_{tot} = 5.4 \times 10^{-5}$ M, $[Fe^{III}]_{tot} = 4.5 \times 10^{-5}$ M, $[EDTA]_{tot} = 9.21 \times 10^{-4}$ 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 $[\Sigma(A_{exp} - A_{calcd.})^2]$ 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 $\mathbf{L^2}$ 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 $pD = pH_{meas} + 0.4$.[33]

Acknowledgments

This work has been supported by the Centre National de la Recherche Scientifique (UMR, 7509 and 5616, PCV program), the Faculty of Chemistry, University Louis Pasteur, Strasbourg (France) and the University Joseph Fourier, Grenoble (France). The authors thank Professor C. Béguin (LEDSS) for his interest in these studies.

[1] A. M. Albrecht-Gary, A. L. Crumbliss, *Iron Transport and Storage in Microorganisms, Plants and Animals*, in Metal Ions in Biological Systems (Eds.: A. Sigel and H. Sigel), M. Dekker **1998**; vol. 35, pp. 239–327.
[2] G. Serratrice, H. Boukhalfa, C. Béguin, P. Baret, C. Caris, J. L. Pierre, *Inorg. Chem.* **1997**, *36*, 3898–3910.
[3] F. Thomas, C. Béguin, J. L. Pierre, G. Serratrice, *Inorg. Chim. Acta* **1999**, *291*, 148–157.
[4] S. M. Cohen, B. O'Sullivan, K. N. Raymond, *Inorg. Chem.* **2000**, *39*, 4339–4346.
[5] S. M. Cohen, K. N. Raymond, *Inorg. Chem.* **2000**, *39*, 3624–3631.
[6] L. E. Maley, D. P. Mellor, *Austr. J. Sci. Res.* **1949**, 579–594.
[7] Y. Murakami, K. Nakamura, M. Tokunaga, *Bull. Chem. Soc. Jpn.* **1963**, *36*, 669–675.
[8] D. H. Zhu, M. J. Kappel, K. N. Raymond, *Inorg. Chim. Acta* **1988**, *147*, 115–121.
[9] W. R. Harris, K. N. Raymond, F. L. Weitl, *J. Am. Chem. Soc.* **1981**, *103*, 2667–2675.
[10] P. Letkeman, A. E. Martell, R. J. Motekaitis, *J. Coord. Chem.* **1980**, *10*, 47–52.
[11] S. A. Abbasi, *Pol. J. Chem.* **1983**, *57*, 355–361.
[12] S. M. Cohen, M. Meyer, K. N. Raymond, *J. Am. Chem. Soc.* **1998**, *120*, 6277–6286.
[13] S. J. Rodgers, L. Chi-Woo, Y. N. Chiu, K. N. Raymond, *Inorg. Chem.* **1987**, *26*, 1622–1625.
[14] T. M. Garrett, P. W. Miller, K. N. Raymond, *Inorg. Chem.* **1989**, *28*, 128–133.
[15] R. Näsänen, A. Ekman, *Acta Chem. Scand.* **1952**, *6*, 1384–1390.
[16] V. L. Pecoraro, F. L. Weitl, K. N. Raymond, *J. Am. Chem. Soc.* **1981**, *103*, 5133–5140.
[17] L. G. Sillen, *Acta Chem. Scand.* **1964**, *18*, 1085–1098.
[18] L. G. Sillen, B. Warnqvist, *Ark. Kemi.* **1968**, *31*, 377–390.
[19] H. Gampp, M. Maeder, C. J. Meyer, A. D. Zuberbühler, *Talanta* **1985**, *32*, 95–101.
[20] F. J. C. Rossoti, H. S. Rossoti, R. J. Whewell, *J. Inorg. Nucl. Chem.* **1971**, *33*, 2051–2065.
[21] H. Gampp, M. Maeder, C. J. Meyer, A. D. Zuberbühler, *Talanta* **1985**, *32*, 257–264.
[22] H. Gampp, M. Maeder, C. J. Meyer, A. D. Zuberbühler, *Talanta* **1986**, *33*, 943–951.
[23] T. B. Karpishin, M. S. Gebhard, E. I. Solomon, K. N. Raymond, *J. Am. Chem. Soc.* **1991**, *113*, 2977–2984.
[24] D. Imbert, P. Baret, D. Gaude, I. Gautier-Luneau, G. Gellon, F. Thomas, G. Serratrice, J. L. Pierre, *Chem. Eur. J.* **2002**, *8*, 1091–1100.
[25] D. Imbert, F. Thomas, P. Baret, G. Serratrice, D. Gaude, J. L. Pierre, J. P. Laulhère, *New J. Chem.* **2000**, *24*, 281–288.
[26] L. D. Loomis, K. N. Raymond, *Inorg. Chem.* **1991**, *30*, 906–911.
[27] *Méthodes d'analyses complexométriques avec les Titriplex*, Merck, Darmstadt, 1990.
[28] R. Bastian, R. Weberling, F. Palilla, *Anal. Chem.* **1956**, *28*, 459–462.
[29] C. Gerard, R. P. Hugel, *J. Chem. Research (M)* **1980**, 3927–3957.
[30] A. E. Martell, R. J. Motekaitis, in *Determination and Use of Stability Constants*, VCH Weinheim, 1988, Chapter 1, pp. 7–19.
[31] P. Gans, A. Sabatini, A. Vacca, *J. Chem. Soc., Dalton Trans.* **1985**, 1195–1200.
[32] P. Gans, A. Sabatini, A. Vacca, *Talanta* **1996**, *43*, 1739–1753.
[33] P. K. Glasoe, A. Long, *J. Phys. Chem.* **1960**, *64*, 188–190.

Received November 25, 2002

