Towards New Iron(III) Chelators: Synthesis and Complexing Ability of a Water-Soluble Tripodal Ligand Based on 2,2′-Dihydroxybiphenyl Subunits

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Received December 4, 1997

Keywords: Dihydroxybiphenyl / Iron chelation / Formation constants / Protonation constants

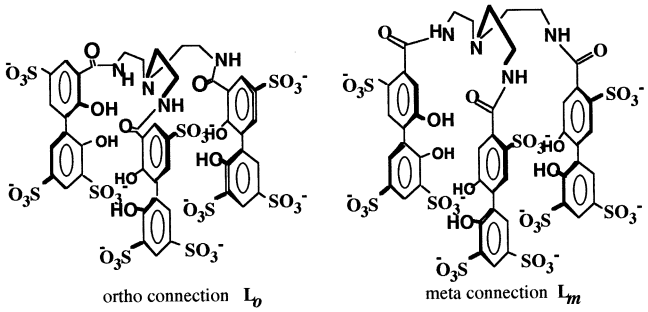
A new water-soluble iron(III) sequestering agent has been designed. The tris-bidentate tripodal ligand consists of three 2,2′-dihydroxybiphenyl subunits connected via amide linkages at their *meta* (4-) positions to a framework of the "tren" type. The key step of the synthesis involves the coupling of suitably substituted monophenyl moieties in order to obtain the biphenyl precursor. The deprotonation constants of the ligand, and the formation and deprotonation constants of the Fe^{III} complex have been determined from potentiometric and spectrophotometric measurements. The results are compared with those of a previously described homologous ligand in which the chelating subunits are attached to the tren framework via the *ortho* (3-) position of the biphenyl rather than the 4-position.

Introduction

Iron overloading is a significant health problem, for which iron chelation therapy is required[1]. A number of limitations to the use of desferrioxamine, the only approved drug for treatment of iron overload (parenteral administration, prohibitive price, short plasma half-life), underline the need for the development of a truly orally effective drug. The search for effective ferric ion chelating agents was originally centered on mimics of natural siderophores [tris(catecholate) or tris(hydroxamate) ligands][2]. Catechol-based ligands are the most powerful in the thermodynamic sense [enterobactin, a tris(catecholate) siderophore produced by *E. Coli* has been shown[3] to have a stability constant for Fe^{III} complexation of 10^{49}], but these ligands are extremely air-sensitive and they have been shown to promote bacterial infections. Hydroxamate-based ligands seem to suffer limitations inherent to all the hydroxamate chelating agents. Recently, potential drugs for iron chelation therapy incorporating "non-natural" chelating subunits have been studied, such as hydroxypyridinone[4], pyridoxal isonicotinoyl hydrazone[5], and 8-hydroxyquinoline[6].

In a previous publication[7], we described tripodal ligands based on three 2,2′-dihydroxybiphenyl subunits connected to a tris(2-aminoethyl)amine (tren) framework via the *ortho* (3-) positions of their phenolic groups (an example, L_o, is depicted in Figure 1). The results obtained with these water-soluble and air-stable ligands were promising[8]. Nevertheless, the complexing ability of these ligands was found to be lower than that of tris(catecholates) or tris(hydroxamates) on the basis of the respective pFe values. The interpretation of molecular modelling studies suggested that connection of the 2,2′-dihydroxybiphenyl chelating pendant arms to the tren framework via the 4-position, i.e. *meta* to the hydroxy group, rather than via the 3-position (*ortho* to the hydroxy group), should be more conducive to an octahedral arrangement of the ligation sites around the metal center. However, this new mode of connection renders impossible the so-called salicylate-like binding of the metal[9] (which generally occurs at low pH). On the other hand, the presence of an amide group at the position *ortho* to the hydroxy group has been shown by Shanzer et al.[10] to result in hydrogen bonding in the complex between the amide and the oxygen coordinated to Fe^{III}. These interactions suggest a preorganization of the ligand around the metal center that improves its binding properties. In order to examine the effect of the position of the amide linkage (*ortho* or *meta* to the phenolic group) on the complexing ability of the ligand, we report herein on the synthesis and iron(III) complexing properties of L_m, a tripodal ligand in which the biphenyl subunits are attached via their 4-positions (Figure 1).

Figure 1. Structural formulae of tris-2,2′-dihydroxybiphenyl ligands



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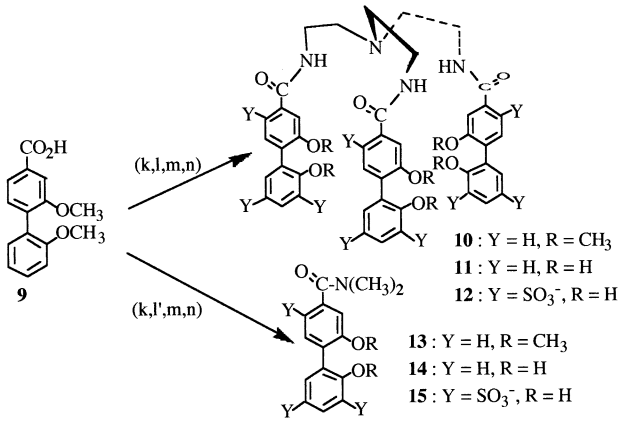
Results and Discussion

Synthesis of Ligands

The synthesis of tripodal ligands based on 2,2′-hydroxy-1,1′-biphenyl subunits, attached either via the 3-position[7a] or the 4-position (this work, Figure 2), is centered on the formation of amide links from carboxylate moieties, which are activated and then conjugated with the tetraamine, tris(2-aminoethyl)amine (tren). Coupling of the protected 2,2′-dimethoxy-1,1′-diphenyl-4-carboxylic acid **9** with tren was most conveniently accomplished using *N*,*N*′-carbonyldiimidazole (CDI).[18] For comparative complexation studies, simple bidentate ligands **14** and **15** were also prepared (Figure 2).

Figure 2. Synthesis of the ligands



(k) CDI, THF, nitrogen ; then (l) TREN ,(l') $HN(CH_3)_2$
(m) BBr_3, CH_2Cl_2, 0 °C ; (n) H_2SO_4, ,SO_3

Sulfonation, using H_2SO_4/oleum, of tripod **11** and of bidentate ligand **14** afforded the water-soluble products **12** and **15**, respectively.

In the previously studied[7a] series of ligands, in which the connection with the spacer tren was at the 3-position of the biphenyl group, the starting material for the synthesis was commercially available 2,2′-hydroxy-1,1′-biphenyl. Functionalization at the position *ortho* to the phenolic hydroxy group was readily and regiospecifically effected by various methods. The synthesis of 2,2′-dimethoxy-1,1′-diphenyl-4-carboxylic acid (**9**) involves heterocoupling[13][19][20] (Figure 3) of the iodo derivative **5** of methyl 2-methoxybenzoate (**4**) and 2-methoxyphenylboronic acid (**7**) (Figure 3B). The iodo derivative was obtained[12] from 5-methyl-2-nitroanisole (**1**) (Figure 3A).

Ligand Deprotonation Constants

The ligand L_m possesses six hydroxy groups and one tertiary amine function and is denoted LH_7^{8-} taking into account the negative charges of the nine sulfonate groups. The deprotonation constants were determined by potentiometric titration of the fully protonated ligand with NaOH in 0.1 M $NaClO_4$ at 25°C (Figure 4a). Analysis of the titration curve yielded only the following five pK_a values: 10.62 ± 0.07; 7.48 ± 0.11; 6.23 ± 0.12; 5.85 ± 0.13, and 4.88 ± 0.13.

Figure 3. Synthesis of 2,2′-dimethoxy-1,1′-diphenyl-4-carboxylic acid **9**

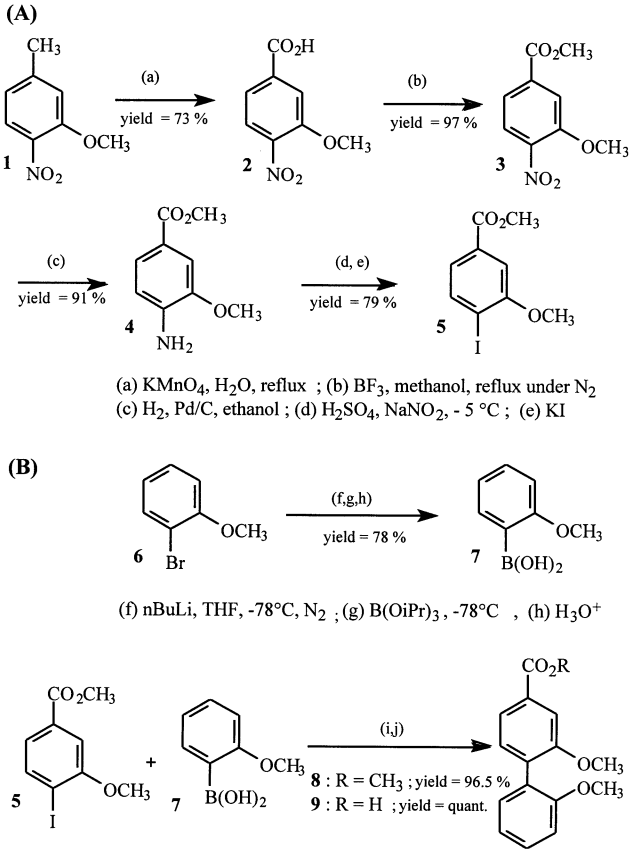
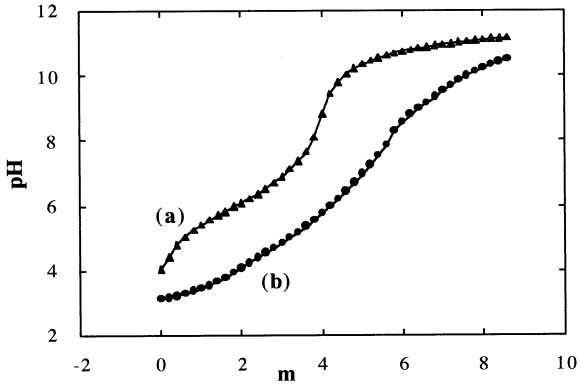


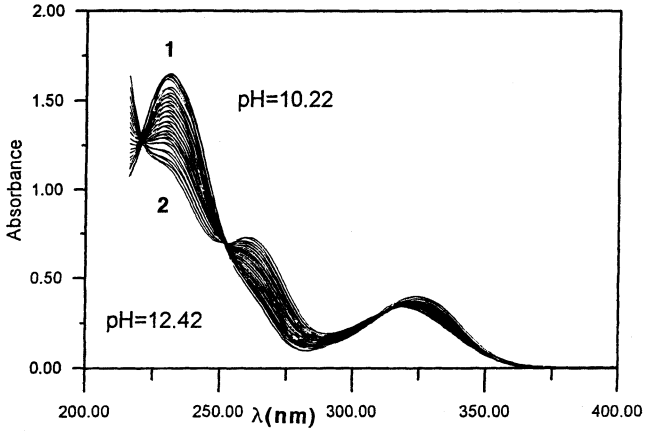
Figure 4. Potentiometric titration curves for (a) 0.5 mmol dm^{-3} ligand L_m; (b) ligand L_m + Fe^{3+} 1:1 0.5 mmol dm^{-3}; m = moles of base added per mol of ligand; all solutions were at 25°C and I = 0.1 mol dm^{-3} ($NaClO_4$); the deprotonation constants of the ligand and the complexation constants were calculated using the SUPERQUAD program[15]



The deprotonation constants of LH_2^{13-} and LH^{14-} species are too high to be accurately determined by direct potentiometry. Spectrophotometric titration was carried out at pH = 10.2−12.7 and revealed one buffer region. The corresponding UV spectra exhibit isosbestic behavior, indicating the presence of only two absorbing species (Figure 5). The data were analyzed using the LETAGROP-

SPEFO[16][17] program (absorbance values at 4 wavelengths). The model delineated involves a single-proton step for the deprotonation equilibrium, yielding a pK_a value of 11.70 ± 0.03. The pK_a values of the monomeric analogue **15** (5,3′,5′-trisulfonate derivative of compound **14**) were also determined: 5.50 ± 0.01 by potentiometry in the acidic range, and 11.68 ± 0.03 by UV/Vis spectrophotometry in the basic range. The value of 5.50 befits a hydroxy group *meta* to the electron-withdrawing amide group.

Figure 5. UV/Vis absorption spectra of the L_m ligand as a function of pH; 1: pH = 10.2; 2: pH = 12.4; $[L_m]$ = 0.05 mmol dm^{-3}; I = 0.1 mol dm^{-3} ($NaClO_4$)

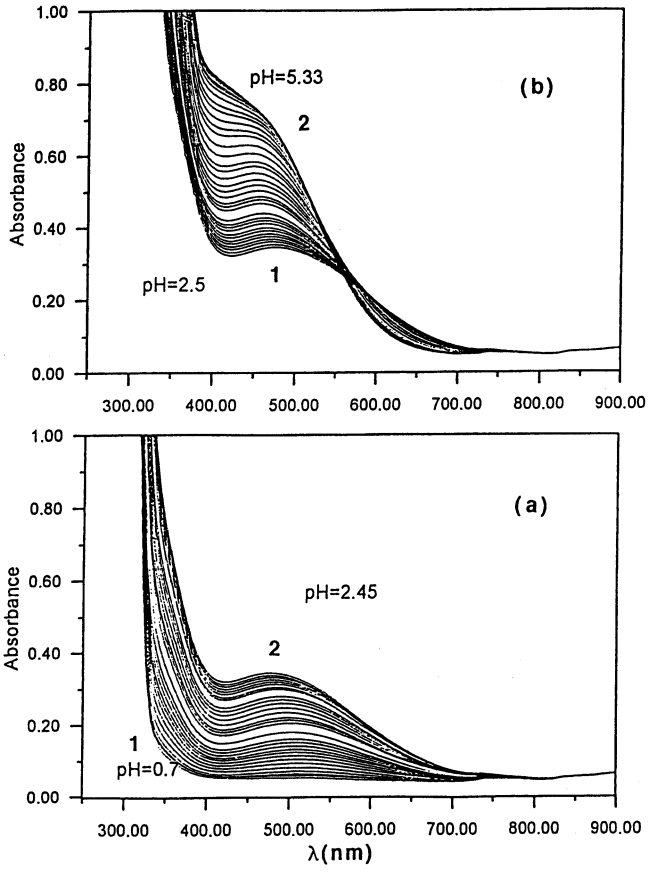


The three lower pK_a values (4.88, 5.85, and 6.23) and the two higher pK_a values (10.62 and 11.70) were assigned to hydroxy groups. The former reflect a roughly statistical separation (log 3 or 0.48), which is consistent with non-interacting arms if one allows for a difference up to one pK unit, as is encountered in several tripodal structures containing 2,2′-dihydroxybiphenyl[7b], catechol[21] or hydroxamate[22] binding units. It should be noted that the average value of these three pK_a′s, 5.65, is very close to the value of 5.50 determined for the monomeric analogue. A similar behavior is expected for the pK_a′s of the three remaining hydroxy groups, the value of 11.70 being very close to that of 11.68, measured for the monomeric analogue. Furthermore, it is assumed that the deprotonation of at least two OH groups, each borne by two different pendant arms of the tripod, induces a change in the UV spectrum. We can thus estimate the third pK_a in this range as having a value of 12.7 on the basis of a statistical separation (see above). The pK_a of 7.48 was attributed to the tertiary amine group by comparison with the value of 7.1 determined for tripodal ligands containing 2,2′-dihydroxybiphenyl subunits[7b].

Stability Constants of Ferric Complexes

Stability constants for the Fe^{III} complexes were determined by means of spectrophotometric and potentiometric titrations. The spectrophotometric behavior of the ferric complexes at a 1:1 metal-to-ligand molar ratio was investigated as a function of pH. The spectrum changed significantly over the pH range 0.7−5.3. The ligand reacts with Fe^{III} in acidic media, as indicated by the absorption spectra (Figure 6a). As the pH was increased from 1 to 2.5, the charge transfer band was found to shift from 520 nm ($\varepsilon \approx$ 600 mol^{-1} dm^3 cm^{-1}) to 470 nm ($\varepsilon \approx$ 1400 mol^{-1} dm^3 cm^{-1}). The absorbance data were processed with the LETAGROP-SPEFO program. The best fit was obtained by considering the formation of the $[FeLH_6]^{6-}$ and $[FeLH_5]^{7-}$ species, generating the values log β_{116}[23] = 61.9 ± 0.1 and log β_{115} = 60.0 ± 0.1, with ε_{max} = 580 mol^{-1} dm^3 cm^{-1} (λ = 520 nm) for $[FeLH_6]^{6-}$ and 1510 mol^{-1} dm^3 cm^{-1} (λ = 480 nm) for $[FeLH_5]^{7-}$. Increasing the pH to 5.3 led to an increase in the band intensity, which was accompanied by a shift to lower wavelength (Figure 6b). The best fitting model for the interpretation of spectrophotometric data was obtained by considering the equilibrium between $[FeLH_5]^{7-}$ and $[FeLH_3]^{9-}$. The calculated value of log β_{113} is 51.8 ± 0.1, with ε = 2600 mol^{-1} dm^3 cm^{-1} at 420 nm (shoulder). Only a marginal spectral change was observed over the pH range 5.3−7.5. At pH > 7.5, absorbances in the range 400−550 nm were found to decrease.

Figure 6. UV/Vis absorption spectra of Fe^{3+}−L_m ligand as a function of pH; (a) 1: pH = 0.7; 2: pH = 2.45; (b) 1: pH = 2.5; 2: pH = 5.3; $[Fe^{3+}]$ = $[L_m]$ = 0.25 mmol dm^{-3}; I = 0.1 mol dm^{-3} ($NaClO_4$)



Potentiometric titration on 1:1 solutions in ferric ion and ligand was also carried out over the pH range 3−10 (Figure 4b). Data were analyzed using the SUPERQUAD program by considering the $[FeLH_5]^{7-}$ species at the beginning of the titration and using the value of β_{115} calculated from the spectrophotometric titration. The model was best fitted (σ_{fit} = 2.9) with six Fe^{III} complex species $[FeLH_4]^{8-}$,

$[FeLH_3]^{9-}$, $[FeLH_2]^{10-}$, $[FeLH]^{11-}$, $[FeL]^{12-}$, and $[FeLOH]^{13-}$ giving the following values of log β_{11h}: log $\beta_{114} = 56.49 \pm 0.02$; log $\beta_{113} = 51.86 \pm 0.03$; log $\beta_{112} = 46.26 \pm 0.04$; log $\beta_{111} = 39.11 \pm 0.06$; log $\beta_{110} = 30.03 \pm 0.08$; log $\beta_{11-1} = 20.26 \pm 0.08$.

From these values, the following deprotonation constants can be deduced for the complexes: $pK_{FeLH5} = 3.51$; $pK_{FeLH4} = 4.63$; $pK_{FeLH3} = 5.60$; $pK_{FeLH2} = 7.15$; $pK_{FeLH} = 9.08$; $pK_{FeL} = 9.77$.

It should be noted that the value of log $\beta_{113} = 51.86$ is in good agreement with that of 51.8 determined from the spectrophotometric study, thus validating the proposed model.

Structural attributes of these complexes can be proposed on the basis of their spectral characteristics. For $[FeLH_5]^{7-}$, the band at 470 nm ($\varepsilon = 1400$ mol^{-1} dm^3 cm^{-1}) is indicative of a dihydroxylate mode of coordination involving one arm of the ligand, as is apparent from comparison with the system Fe^{III} sodium 2,2′-dihydroxybiphenyl-3,5,3′,5′-tetrasulfonate[9]. Over the pH range 2.5−5.3, the observed increase in absorbance and shift to shorter wavelength (shoulder at $\lambda = 430$ nm with $\varepsilon = 2600$ mol^{-1} dm^3 cm^{-1}) suggest a bis(dihydroxylate) coordination involving two arms of the ligand in the $[FeLH_3]^{9-}$ species. A region of constant specific absorbance at pH = 5.3−7.5 indicates no increased degree of coordination to the dihydroxylate groups. At pH > 7.5, a decrease in the absorption in the range 400−550 nm may correspond to the formation of hydroxo complexes. The successive deprotonation constants of the complex $[FeLH_3]^{9-}$ may thus be attributed to deprotonation of the tertiary amine group, of one hydroxy group of the uncoordinated arm, and of the two water molecules that complete the coordination sphere around Fe^{III}. The fact that only two arms of the ligand L_m are involved in coordination of Fe^{III} might be explained in terms of repulsive effects between the negative charges on the sulfonate groups in the 3′-positions. It is assumed that such interactions prevent the formation of a hexadentate coordination cavity involving all three arms.

The pFe ($-\log [Fe^{3+}]$) values have been calculated to provide a comparison of the relative ability of the ligands to complex Fe^{III} (under biologically reasonable conditions, i.e. pH = 7.4, $[L]_{tot} = 10^{-5}$ mol dm^{-3}, $[Fe]_{tot} = 10^{-6}$ mol dm^{-3}). The calculated value for ligand L_m is 19.7. This value shows that the ligand L_m is less effective than the analogous tripodal L_o (pFe = 22.8),[7b] in which the subunits are connected via their 3-positions, i.e. *ortho* to the hydroxy groups. This lower affinity of L_m for Fe^{III} can be partly related to the available coordination modes. The structure of the ligand L_o favours an initial coordination of the salicylate type (with oxygen atoms of the carbonyl and hydroxy groups). A preorganization of the ligand is thus expected for a complexation of the metal ion by the other arms. This cannot occur for L_m.

Conclusion

A multistep synthesis of the tris-bidentate tripodal ligand L_m has been described. This ligand incorporates three 2,2′-dihydroxybiphenyl subunits, which are attached to a central "tren" framework via amide linkages at their 4-positions, i.e. *meta* to the phenolic hydroxy groups. The aim of this work was to compare the complexing ability of L_m with that of L_o, a previously described iron chelator in which the chelating pendant arms are attached via their 3-positions, i.e. *ortho* to the phenolic groups.

The ligand deprotonation constants (six hydroxy groups and one tertiary amine function) and the stability constants β_{11h} of the ferric complexes (in a pH range of 1−10) have been determined. The pFe value of 19.7 calculated for L_m as compared to the value of 22.8 for L_o[7b] reveals an interesting structural effect. Since molecular models suggested that the *meta* connection (L_m) should be more conducive to the formation of an octahedral coordination cavity than the *ortho* connection (L_o), the observed result was unexpected. The structure of the ligand L_o favours an initial coordination of the salicylate type (with oxygen atoms of the carbonyl and hydroxy groups), which is assumed to "preorganize" the ligand for a complexation of the metal ion by the other arms. This is not possible for L_m, thus diminishing its complexing ability towards Fe^{III}. Furthermore, as the coordination changes from a salicylate to a dihydroxylate mode of bonding upon deprotonation of the complex, an amide conformational change is assumed to occur for the complexation of the metal center by L_o. Thus, hydrogen bonding between the amide group and the coordinated oxygen atom (six-membered ring) can occur, which is not possible in the complex of Fe^{III} with L_m. The importance of the preorganization of the ligand was emphasized by Shanzer et al.[10], and seems to play a major role in determining the complexing ability of a given ligand. Thus, for the design of novel ligands, geometry of the octahedral coordination sphere around the metal center is not the sole criterion for the prediction of complexation stability.

We are grateful to Dr. *Hakim Boukhalfa* (L.E.D.S.S., Grenoble) for performing the UV/Vis spectrophotometric measurements.

Experimental Section

Materials and Equipment: Solvents were purified by standard techniques; 5-methyl-2-nitroanisole and 2-bromoanisole are commercially available (Aldrich). The amine "tren" was distilled from sodium. All other compounds were of reagent grade and were used without further purification. Iron(III) stock solutions were prepared by dissolving appropriate amounts of ferric perchlorate hydrate (Aldrich) in standardized $HClO_4/NaClO_4$ solutions. The solutions were standardized for ferric ion spectrophotometrically by using a molar extinction coefficient of 4160 mol^{-1} dm^3 cm^{-1} at 240 nm[11]. − IR spectra were recorded with Perkin-Elmer 397 or Nicolet Impact 400 spectrometers. UV/Vis absorption spectra were recorded using 1.000-cm path length quartz cells with a Perkin-Elmer Lambda 2 spectrometer connected to a COMPAQ Deskpro 386s microcomputer. − Mass spectra were recorded with a NERMAG R 10 1 C mass spectrometer. − ^{1}H- and ^{13}C-NMR spectra were obtained using 5-mm tubes at 25°C with a Bruker AC 200 or a Bruker AM 400 spectrometer. − Microanalyses were performed by the Central Service of CNRS, Solaise (France). − Melting points were determined with a Büchi apparatus and are not corrected.

3-Methoxy-4-nitrobenzoic Acid (**2**): A mixture of 5-methyl-2-nitroanisole (**1**) (25.0 g, 0.15 mol), potassium permanganate (97.5 g, 0.62 mol), and sodium hydrogen carbonate (12.3 g, 0.12 mol) in 1.5 l of water was refluxed for 4 h. Thereafter, the MnO_2 formed was filtered off. The filtrate was cooled to 0°C and acidified with 20% sulfuric acid to pH = 1. The yellow solid thus produced was washed with water, dried, and purified by recrystallization from ethanol (white crystals, 21 g, 73%). – M.p. 226°C (ref.[12] 230–233°C). – ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 4.01 (s, 3 H, OCH_3), 7.71 (dd, J_1 = 8.3, J_2 = 1.4 Hz, 1 H), 7.79 (d, J = 1.4 Hz, 1 H), 7.83 (d, J = 8.3 Hz, 1 H). – ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 56.8 (OCH_3), 114.6 (CH), 121.3 (CH), 125.0 (CH), 135.8, 141.9, 151.5 (C–O), 165.8 (C=O). – IR (KBr): $\tilde{\nu}$ = 3250–2200 cm^{-1} [ν(OH)], 1680 [ν(C=O)].

Methyl 3-Methoxy-4-nitrobenzoate (**3**): 3-Methoxy-4-nitrobenzoic acid (**2**) (21.0 g, 0.11 mol) was dissolved in methanol (350 ml) under nitrogen. BF_3 (40 ml, 50% w/w in methanol) was added and the mixture was stirred under reflux for 12 h. The solvent was then removed, the residue was treated with water (300 ml), and the resulting suspension was filtered. The collected yellow solid was washed with water (500 ml) and then dried in vacuo (21.9 g, 0.10 mol, 91%). – M.p. 91°C (ref.[12] 90–91°C). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.97 (s, 3 H, CO_2CH_3), 4.02 (s, 3 H, OCH_3), 7.67 (dd, J_1 = 8.3, J_2 = 1.5 Hz, 1 H), 7.71 (d, J = 1.5 Hz, 1 H), 7.84 (d, J = 8.3 Hz, 1 H). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 52.8 (CO_2CH_3), 56.7 (OCH_3), 114.6 (CH), 121.4 (CH), 125.3 (CH), 134.8, 142.0, 152.4 (C–O), 165.2 (C=O). – IR (KBr): $\tilde{\nu}$ = 1740 cm^{-1} [ν(C=O)].

Methyl 4-Amino-3-methoxybenzoate (**4**): Compound **3** (10.0 g, 47.4 mmol) was dissolved in a mixture of dichloromethane (50 ml) and ethanol (300 ml). The resulting solution was stirred for 12 h under hydrogen in the presence of Pd/charcoal (10% Pd, 923 mg). The catalyst was subsequently removed by filtration and the solvent was evaporated. The yellow solid thus obtained was purified by recrystallization from ethanol (7.84 g, 43.3 mmol, 91%). – M.p. 128°C (ref.[12] 127–128°C). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.86 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 4.22 (m, 2 H, NH_2), 6.66 (d, J = 8.0 Hz, 1 H), 7.45 (d, J = 1.7 Hz, 1 H), 7.54 (dd, J_1 = 8.0, J_2 = 1.7 Hz, 1 H). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 51.6 (CO_2CH_3), 55.5 (OCH_3), 111.1 (CH), 113.1 (CH), 119.5, 124.0 (CH), 141.0, 146.1 (C–O), 167.3 (C=O). – IR (KBr): $\tilde{\nu}$ = 3450–3330 cm^{-1} [ν(NH_2)], 1670 [ν(C=O)].

Methyl 4-Iodo-3-methoxybenzoate (**5**): To a mixture of **4** (8.1 g, 44.7 mmol), water (110 ml) and 20% H_2SO_4 (24 ml), a solution of sodium nitrite (3.47 g, 49.6 mmol) in water (20 ml) was slowly added at 0°C. At the end of the reaction, the excess nitrous acid was destroyed by the addition of urea (800 mg). Then, 20 ml of aqueous KI (7.74 g, 46.6 mmol) was added dropwise and the mixture was stirred for 2 h at 50°C. To the resulting brown mixture was added an aqueous solution of $Na_2S_2O_5$. The product was isolated by extraction with diethyl ether, neutralization with 1 N NaOH (to pH = 5), washing with brine, and drying with Na_2SO_4. The solvent was evaporated and the iodo compound was purified by column chromatography (alumina, pentane containing 3% ethyl acetate), giving a white solid (10.4 g, 33.6 mmol, 72%). – M.p. 50–52°C (ref.[12] 52–53°C). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.92 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 7.36 (dd, J_1 = 8.1, J_2 = 1.8 Hz, 1 H), 7.44 (d, J = 1.8 Hz, 1 H), 7.84 (d, J = 8.1 Hz, 1 H). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 52.3 (CO_2CH_3), 56.5 (OCH_3), 92.6 (CI), 111.2 (CH), 123.3 (CH), 131.6, 139.5 (CH), 158.2 (C–O), 166.5 (C=O). – IR (KBr): $\tilde{\nu}$ = 1720 cm^{-1} [ν(C=O)].

2-Methoxyphenylboronic Acid (**7**): At –78°C, *n*BuLi (80 ml, 1.6 M in hexane, 0.128 mol) was added to a solution of 2-bromoanisole (**6**) (13.3 ml, 0.107 mol) in diethyl ether (100 ml) and the mixture was stirred at this temperature for 30 min. With a protective nitrogen atmosphere, the lithio derivative was then cannulated into a solution of triisopropyl borate (32 ml, 0.214 mol) in diethyl ether. After stirring at –78°C for 30 min, then at room temperature, the mixture was poured into aqueous HCl (10%, 300 ml). The product was extracted with diethyl ether, the combined extracts were washed with water (100 ml), and dried with Na_2SO_4. The oily, crude product was stored in a refrigerator for 24 h, giving white crystals. These somewhat unstable crystals were rapidly washed with cold pentane and then dried in vacuo (11 g, 0.072 mol, 68%). – M.p. 104–105°C (ref.[13] 105–106°C). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.85 (s, 3 H, OCH_3), 6.85 (d, J = 8.3 Hz, 1 H, ArH), 6.93–7.01 (m, 1 H, ArH), 7.34–7.43 (m, 1 H, ArH), 7.77 (dd, J_1 = 7.3, J_2 = 1.8 Hz, 1 H, ArH). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 55.3 (OCH_3), 109.9 (CH), 121.1 (CH), 132.7 (CH), 136.8 (CH), 164.4 (C–O).

Methyl 2,2′-Dimethoxy-1,1′-diphenyl-4-carboxylate (**8**): To a solution of **5** (1.0 g, 3.42 mmol) in freshly distilled DMF (15 ml) under nitrogen were added **7** (78 mg, 5.13 mmol), Et_3N (1.4 ml, 10.3 mmol), $Pd(OAc)_2$ (23 mg, 0.103 mmol), and P(*o*-Tol)$_3$ (65 mg, 0.212 mmol). The orange solution was heated at 100°C for 3 h, whereupon it became black. The solvent was evaporated and the residue was treated with dichloromethane and 10% ammonia. The organic phase was washed with water and then dried with Na_2SO_4. The orange oil was purified by column chromatography (silica gel; pentane/diethyl ether, 9:1). **8** was obtained as a white solid (0.9 g, 3.3 mmol, 97%). – M.p. 112°C. – R_f = 0.55 (pentane/diethyl ether, 2:1). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.33 (s, 3 H, CO_2CH_3), 3.39 (s, 3 H, OCH_3), 3.50 (s, 3 H, OCH_3), 6.53–6.62 (m, 2 H, ArH), 6.62–6.90 (m, 2 H, ArH), 6.87 (d, J = 7.7 Hz, 1 H), 7.20 (d, J = 1.4 Hz, 1 H), 7.25 (dd, J_1 = 7.7, J_2 = 1.4 Hz, 1 H). – ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 52.2 (CO_2CH_3), 55.4 and 55.6 (OCH_3), 111.3 (CH), 111.4 (CH), 120.2 (CH), 121.2 (CH), 126.3, 129.2 (CH), 130.7 (CH), 131.1, 131.4 (CH), 132.2, 156.6 (C–O), 156.7 (C–O), 167.2 (C=O). – IR (KBr): $\tilde{\nu}$ = 1710 cm^{-1} [ν(C=O)]. – MS (EI); *m/z*: 272 [M$^+$], 241, 225, 213, 198. – $C_{16}H_{16}O_4$: calcd. C 70.58, H 5.29; found C 70.40, H 5.47.

2,2′-Dimethoxy-1,1′-diphenyl-4-carboxylic Acid (**9**): Ester **8** (2.0 g, 7.34 mmol) was refluxed with ethanolic KOH (excess) for 3 h. The mixture was then concentrated and 10% HCl was added. The resulting precipitate was filtered off, washed with water, and dried in vacuo to give **9** as a white solid (1.9 g, quant.). – M.p. 170°C. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.78 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.98–7.07 (m, 2 H, ArH), 7.23–7.28 (m, 1 H, ArH), 7.33–7.41 (m, 1 H, ArH), 7.36 (d, J = 7.8 Hz, 1 H), 7.77 (d, J = 1.5 Hz, 1 H), 7.8 (dd, J = 7.8, 1.5 Hz, 1 H). – ^{13}C NMR (50 MHz, [D$_6$]DMSO): δ = 55.4 and 55.5 (OCH_3), 111.4 (CH), 111.5 (CH), 120.2 (CH), 121.4 (CH), 126.5, 129.1 (CH), 130.8 (CH), 131.1, 131.3 (CH), 132.2, 156.6 (C–O), 156.7 (C–O), 167.2 (C=O). – IR (KBr): $\tilde{\nu}$ = 3600–2300 cm^{-1} [ν(OH)], 1680 [ν(C=O)]. – MS (EI); *m/z*: 258 [M$^+$], 184.

N,N′,N″-(Nitrilotri-2,1-ethanediyl)tris(2,2′-dimethoxy-4-biphenylcarboxamide) (**10**): Under nitrogen, acid **9** (1.0 g, 3.87 mmol) was treated with CDI (690 mg, 4.26 mmol) in freshly distilled THF (50 ml) and the mixture was stirred for 12 h; tren (160 µl, 1.06 mmol) was added and the mixture was stirred overnight at room temperature. The solvent was then evaporated and the brown oil was purified by column chromatography (silica gel, dichloromethane containing 2% isopropylamine) giving **10** as a white solid (1.0 g, 3.50

mmol, 90%). − M.p. 116°C. − R_f = 0.6 (CH_2Cl_2/MeOH, 9:1). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.77 (br. s, 6 H, NCH_2), 3.61 (m, 24 H, NCH_2, OCH_3), 6.84−7.04 (m, 12 H, ArH), 7.24−7.44 (m, 9 H, ArH), 7.50 (br. s, 3 H, NH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 38.4 (CH_2), 54.7 (CH_2), 55.3 and 55.4 (OCH_3), 110.2 (CH), 110.9 (CH), 118.6 (CH), 120.1 (CH), 126.7, 128.7 (CH), 131.0 (CH), 131.2 (CH), 134.0, 156.6 (C−O), 156.9 (C−O), 167.8 (C=O). − IR (KBr): $\tilde{\nu}$ = 1635 cm^{-1} [ν(C=O)]. − MSM [FAB(+), NBA matrix]; *m/z*: 867 [M + H^+], 596, 284, 257, 241, 213. − $C_{51}H_{54}N_4O_9 \cdot 3H_2O$: calcd. C 66.64, H 6.36, N 6.10; found C 66.65, H 6.04, N 6.15.

N,N′,N′′-(Nitrilotri-2,1-ethanediyl)tris(2,2′-dihydroxy-4-biphenylcarboxamide) (**11**): A solution of BBr_3 in dichloromethane (1 M, 20 ml) was cooled to 0°C under nitrogen. Podand **10** (860 mg, 0.99 mmol) was added and the mixture was stirred for 12 h at room temperature, resulting in the deposition of a white precipitate. The mixture was treated with methanol and the solvents were evaporated. The residue was dissolved in 30 ml of 10% aq. NaOH. Subsequent addition of 10% HCl at 0°C gave **11** as a white precipitate, which was dried in vacuo (700 mg, 0.89 mmol, 90%). − M.p. 170°C. − ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 3.48 (br. s, 6 H, CH_2), 3.73 (br. s, 6 H, CH_2), 6.76−6.92 (m, 6 H, ArH), 7.10−7.21 (m, 9 H, ArH), 7.34−7.41 (m, 6 H, ArH), 8.81 (br. s, 3 H, NH), 9.37 (br. s, 3 H, OH), 9.50 (br. s, 3 H, OH). − ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 34.3 (CH_2), 52.1 (CH_2), 115.1, 115.8, 117.4, 118.8, 128.5, 129.3, 131.4, 133.8, 154.6 (C−O), 166.9 (C=O). − IR (KBr): $\tilde{\nu}$ = 3650−2300 cm^{-1} [ν(OH)], 1640 [ν(C=O)]. − MS [FAB(+), NBA matrix]; *m/z*: 783 [M + H^+], 613, 540, 528. − $C_{45}H_{42}N_4O_9 \cdot HBr \cdot H_2O$: calcd. C 61.35, H 5.15, N 6.36; found C 61.61, H 5.12, N 6.27.

N,N′,N″-(Nitrilotri-2,1-ethanediyl)tris(2,2′-dihydroxy-5,3′,5′-trisulfo-4-biphenylcarboxamide) (**12**): The hydroxylated tripod **11** (830 mg, 1.06 mmol) was dissolved in 60 ml of oleum (H_2SO_4 + 15% SO_3) at 0°C. The mixture was then stirred at 90°C for 3 h. After cooling to room temperature, the brown solution was cautiously poured onto crushed ice with stirring. With cooling, the solution was brought to pH = 4 by the slow addition of 10% aq. NaOH. Sodium sulfate was eliminated by repeated precipitations by adding methanol. − M.p. > 286°C. − ^{1}H NMR (200 MHz, D_2O): δ = 3.80 (m, 2 H, CH_2), 4.00 (m, 2 H, CH_2), 7.17 (s, 1 H), 7.91 (s, 1 H), 7.94 (d, *J* = 2.3 Hz, 1 H), 8.22 (d, *J* = 2.3 Hz, 1 H). − ^{13}C NMR (50 MHz, CD_3OD): δ = 37.4 (CH_2), 55.3 (CH_2), 118.6 (CH), 128.4 (CH), 129.4, 131.4, 134.1 (CH), 134.5, 134.9 (CH), 137.1, 138.0, 156.4 (C−O), 159.2 (C−O), 174.2 (C=O).

2,2′-Dimethoxy-4-(N,N-dimethylcarbamoyl)-1,1′-biphenyl (**13**): Under nitrogen at room temperature, acid **9** (0.9 g, 3.48 mmol) was treated with CDI (623 mg, 3.84 mmol) in THF (100 ml) for 12 h. The mixture was then cooled to 0°C, dimethylamine (5 ml) was added, and stirring was continued overnight at room temperature. The solvent was then evaporated and the residual brown oil was purified by column chromatography (silica gel, dichloromethane containing 2% isopropylamine) to afford **13** as a white solid (800 mg, 2.80 mmol, 80%). − M.p. 123°C. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.04 (s, 6 H, NCH_3), 3.69 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 6.89−6.98 (m, 4 H, ArH), 7.13−7.31 (m, 3 H, ArH). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 36.0 (NCH_3), 40.0 (NCH_3), 55.6 (OCH_3), 55.7 (OCH_3), 110.0 (CH), 111.1 (CH), 118.7 (CH), 120.3 (CH), 127.0, 128.8 (CH), 129.2, 131.1 (CH), 131.2 (CH), 136.3, 156.9 (C−OMe), 157.0 (C−OMe), 171.5 (C=O). − MS (EI): *m/z*: 285 [M], 257, 213.

2,2′-Dihydroxy-4-(N,N-dimethylcarbamoyl)-1,1′-biphenyl (**14**): To a solution of BBr_3 in dichloromethane (1 M, 20 ml) at 0°C under nitrogen, **13** (900 mg, 3.15 mmol) was added and the mixture was stirred for 12 h at room temperature. The mixture was then treated with methanol and the solvents were evaporated. The residue was redissolved in 20 ml of 10% aq. NaOH and subsequent addition of 10% HCl at 0°C gave **14** as a white precipitate, which was dried in vacuo (800 mg, 3.10 mmol, 98%). − M.p. 186°C. − ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 2.96 (s, 6 H, NCH_3), 6.77−6.90 (m, 4 H, ArH), 7.10−7.88 (m, 3 H, ArH), 9.23 (s, 1 H, OH), 9.43 (s, 1 H, OH). − ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 34.8 (NCH_3), 114.4 (CH), 115.7 (CH), 117.3 (CH), 118.8 (CH), 125.2, 127.0, 128.3 (CH), 131.3 (CH), 131.4 (CH), 136.2, 154.4 (C−OH), 154.6 (C−OH), 154.6 (C=O). − IR (KBr): $\tilde{\nu}$ = 3500−2300 cm^{-1} [ν(OH)], 1600 [ν(C=O)]. − MS (EI); *m/z*: 275 [M], 213.

2,2′-Dihydroxy-4-(N,N-dimethylcarbamoyl)-3,3′,5′-trisulfo-1,1′-biphenyl (**15**): Sulfonation of **14** was performed as for **12**. − M.p. > 286°C. − ^{1}H NMR (200 MHz, CD_3OD): δ = 2.87 (s, 3 H, CH_3), 3.03 (s, 3 H, CH_3), 6.64 (s, 1 H), 7.7 (d, *J* = 2.3 Hz, 1 H), 7.75 (s, 1 H), 8.16 (d, *J* = 2.3 Hz, 1 H). − ^{13}C NMR (50 MHz, CD_3OD): δ = 35.3 (CH_3), 39.8 (CH_3), 114.5 (CH), 126.3, 126.5 (CH), 127.2, 129.9, 132.6 (CH), 132.7 (CH), 133.5, 136.3, 137.0, 154.3 (C−N), 158.0 (C−N), 172.9 (C=O).

Potentiometric Experiments: All measurements were made at 25°C. Solutions were prepared with deionized, doubly distilled water. The ionic strength was fixed at 0.1 M with sodium perchlorate (PROLABO puriss). The pH measurements were performed using a TACUSSEL Ionoprocesseur-II millivoltmeter equipped with glass and calomel electrodes (TACUSSEL). The electrodes were calibrated to read p[H] according to the classical method[14]. Potentiometric titrations employed a TACUSSEL Electroburex burette and a pH meter (Ionoprocesseur-II) connected to a Hewlett Packard microcomputer. The titrations were automated using software developed in our laboratory. The ligands and their iron(III) complexes at concentrations of ca. 0.001 M were titrated with standardized 0.05 M sodium hydroxide. The titration data were refined by the nonlinear least-squares refinement program SUPERQUAD[15] in order to determine the equilibrium constants (protonation and complexation).

Spectrophotometric Experiments: The deprotonation constant of one hydroxy group was determined in the UV region (the ligand concentration in aqueous solution was ca. $5 \cdot 10^{-5}$ M). The ferric complexes were investigated by spectrophotometry: The UV/Vis spectrum of a solution containing equal amounts of ligand and Fe^{III} (10^{-4} M) was monitored as a function of pH over the range 1−10 (adjusted with $HClO_4$ or NaOH); an aliquot was taken from the solution after each adjustment of the pH (which was measured using a pH meter) and its spectrum was recorded. The ionic strength was fixed at 0.1 M with $NaClO_4$/$HClO_4$ for this second set of experiments. The spectrophotometric data were fitted with the LETAGROP-SPEFO program[16][17]. The program uses a nonlinear least-squares method and calculates the thermodynamic constants of the absorbing species and their corresponding electronic spectra. The calculations were performed on the basis of absorbance data at about 6−8 wavelengths (between 400 and 600 nm).

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[23] $\beta_{11n} = [FeLH_n]/[Fe^{3+}]\,[L]\,[H^+]^n$ is the equilibrium constant for $Fe^{3+} + L + nH^+ \rightleftharpoons FeLH_n$.

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