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Methyl-Hydroxypyridinecarboxylic Acids as Possible Bidentate Chelating Agents for Aluminium(III): Synthesis and Metal–Ligand Solution Chemistry

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In view of a possible application to aluminium chelation therapy, 1-methyl-3-hydroxy-4-pyridinecarboxylic acid (1M3H4P) and 1-methyl-4-hydroxy-3-pyridinecarboxylic acid (1M4H3P) were synthesized, and their chemical interactions with aluminium(III) were investigated in aqueous 0.6 m (Na)Cl at 25 °C by means of potentiometric titrations. The qualitative and quantitative results obtained were confirmed by UV spectrophotometry and ^{1}H NMR spectroscopy. For both ligands, the species AlL^{2+}, AlL_2^+, AlL_3, and AlL_2H_{-1} were identified, with $\log\beta_{1,1,0} = 7.66$, $\log\beta_{1,2,0} = 14.27$, $\log\beta_{1,3,0} = 19.099$, and $\log\beta_{1,2,-1} = 7.00$ for 1M3H4P, $\log\beta_{1,1,0} = 7.21$, $\log\beta_{1,2,0} = 13.41$, $\log\beta_{1,3,0} = 18.15$, and $\log\beta_{1,2,-1} = 6.4$ for 1M4H3P. The chelation strength of 1M3H4P and 1M4H3P is lower than that of other available aluminium(III) chelators, e.g. deferiprone (pAl difference at physiological pH: ca. 3 log units). The octanol/aqueous partitioning values of 1M3H4P and 1M4H3P were 0.0054 and 0.0015, respectively, showing high hydrophilicity. The efficiencies of the ligands to chelate aluminium(III) were evaluated at physiological pH and ion strength "in vitro". 1M3H4P and 1M4H3P were more effective than their non-methylated analogs 3H4P and 4H3P.

Introduction

The therapy for metal-overload pathologies usually involves the administration of suitable chelating agents to selectively remove the metal from the body. Regarding aluminium(III) and iron(III), there is a need for new, safe, inexpensive, efficient, and orally effective chelators,[1–3] because the existing ones, i.e. desferrioxamine and deferiprone, have a number of drawbacks, including oral inefficacy and the high cost for the former,[4–6] and several toxic side effects and controversial efficiency for the latter.[5–8]

In a previous work[9] we proposed hydroxypyridinecarboxylic acids (HPs) as new possible chelating agents for aluminium(III) in view of several promising properties such as their lack of toxicity (references in ref.[9]), their low molecular weight (which is required for oral activity), and their very low affinity towards essential metal ions like Zn^{2+}.[10] Our results showed that the unsubstituted derivatives, i.e. 3H4P and 4H3P, cannot be regarded as possible alternatives to desferrioxamine and deferiprone, because they do not adequately complex aluminium(III) at physiological pH. As a strategy to improve the Al^{III} complexation ability, without affecting the positive properties, alkylation of the pyridinic ring was proposed, because the electron-donor properties of the alkyl substituents can be expected to increase the basicity of chelating oxygen atoms and therefore to increase the affinity towards hard metal ions, such as aluminium(III) and iron(III).

In this work, the *N*-methyl derivatives of 3H4P and 4H3P, i.e. 1-methyl-3-hydroxy-4-pyridinecarboxylic acid (1M3H4P) and 1-methyl-4-hydroxy-3-pyridinecarboxylic acid (1M4H3P) (Figure 1), have been synthesized, and the formation of complexes between the ligands and aluminium(III) studied in aqueous solutions by means of potentiometric titrations. The speciation data obtained has been confirmed by UV/Vis and ^{1}H NMR measurements. The partitioning of the ligands in an octanol/aqueous system was determined with UV/Vis spectroscopy. The efficiency of the ligands to chelate aluminium(III) has been determined at physiological conditions (pH and ion strength) in vitro.

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Results and Discussion

Synthesis of the Ligands

To synthesize the new ligands, initially 3H4P and 4H3P (which are not commercially available) were prepared as de-

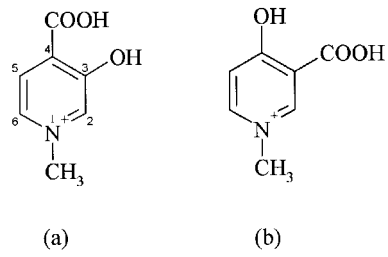

Figure 1. (**a**) 1-Methyl-3-hydroxy-4-pyridinecarboxylic acid (1M3H4P), (**b**) 1-methyl-4-hydroxy-3-pyridinecarboxylic acid (1M4H3P), both shown as the H_2L^+ forms.

scribed.[9] Next, the hydroxyacids were *N*-methylated by use of a conventional method (CH_3I in dimethylformamide).[11]

Solution Equilibria

Acidity Constants of the Ligands

The potentiometric titration of the ligands alone allowed the computation of the pK_a value for the HL form (for simplicity, charges will be generally omitted from the formulae, except in the Tables), corresponding to the deprotonation of the OH group (Table 1). Phenolic oxygen atoms in unsubstituted HPs, e.g. in 3H4P and 4H3P, generally have pK_a values around 10–11:[9] the drastic (10^4–10^5-fold) increase of the acidity of this group in 1M3H4P and 1M4H3P can be explained by the strong inductive effect exerted by the positively charged nitrogen atom, and by the fact that the molecule is uncharged at the pH where OH deprotonates. In comparison, for 3H4P and 4H3P the nitrogen is neutral and the molecule is charged –1. For 1M4H3P the acidity is even higher because the positive charge of the nitrogen may exert also a resonance effect. To the best of our knowledge, a similar effect in a phenolic oxygen atom was observed only in a naphthyridine compound by Agafonova et al.:[12] for 8-hydroxy-1,6-naphthyridine the pK_a was 8.33, whereas for 8-hydroxy-6-methyl-1,6-naphthyridinium it was 4.34.

Table 1. Acidic properties of 1M3H4P and 1M4H3P.[a]

Species	**1M3H4P**		**1M4H3P**		Technique used to obtain the data
	pK_a	n	pK_a	n	
H_2L^+	0.28 ± 0.05	4	0.12 ± 0.02	2	UV
HL	6.6326 ± 0.0008	12	5.9578 ± 0.0006	8	potentiometry

[a] For potentiometric data, n is the number of experiments from which the data were obtained. Values are shown as the mean and standard deviation of the mean. For UV data, n is the number of wavelengths from which the data were obtained; a weighted mean and the corresponding weighted standard deviation were calculated from the data.

Accurate potentiometric measurements at pH values lower than ca. 1.5–2 cannot be executed, because in these conditions the pH modification is negligible because of the acid–base equilibria. Therefore, the more acidic pH region has been explored with UV measurements, which allowed the determination of the pK_a value for the H_2L form, corresponding to the deprotonation of the COOH group (Table 1). Its high acidity is in agreement with values previously observed for other HPs.[9]

Metal–Ligand Complexes – Potentiometric Results

For both ligands, the potentiometric titrations of solutions containing the metal and the ligand allowed the identification of the complexes AlL, AlL_2, AlL_3, and AlL_2H_{-1} (where HL represents either 1M3H4P or 1M4H3P in the neutral form). The values of their stability constants are reported in Table 2. The distribution diagrams for the two systems, as computed for a solution containing 10^{-3} m Al^{III} and $4\cdot10^{-3}$ m ligand, are reported in Figure 2.

Table 2. Stability constants for Al–L complexes, at 25 °C in aqueous (Na)Cl 0.6 m (reactions: $m\,Al^{3+} + l\,L^- + h\,H^+ \rightleftharpoons Al_mL_lH_h^{3m-l+h}$; the p$K_a$ value refers to the equilibrium $AlL_2^+ \rightleftharpoons AlL_2H_{-1} + H^+$).

Species	**1M3H4P**			**1M4H3P**		
	log β	pK_a	n	log β	pK_a	n
AlL^{2+}	7.66 ± 0.03		12	7.21 ± 0.06		12
AlL_2^+	14.27 ± 0.04	7.27	12	13.41 ± 0.03	7.0	12
AlL_2H_{-1}	7.00 ± 0.07		8	6.4 ± 0.1		8
AlL_3	19.099 ± 0.008		8	18.15 ± 0.03		8

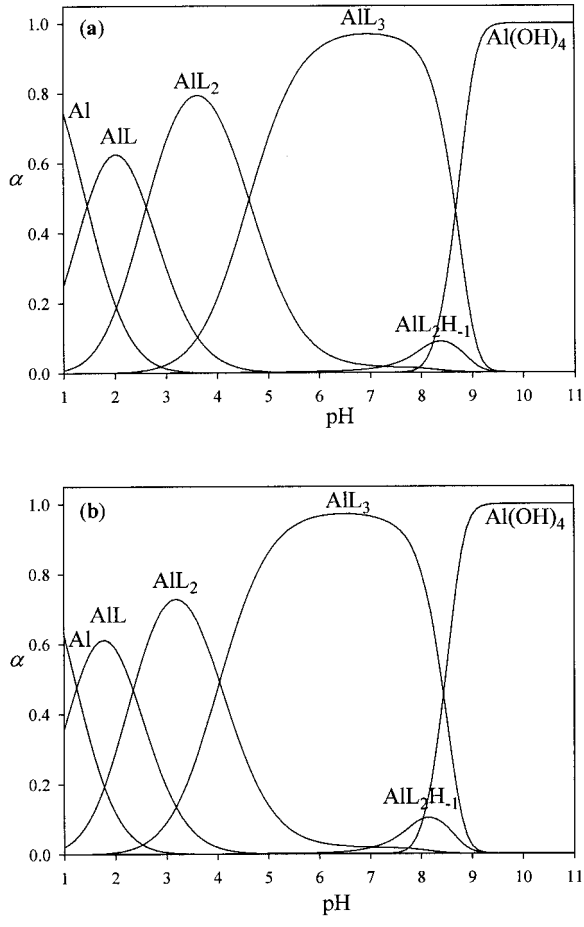

Figure 2. Distribution diagrams of the aluminium complexes in the presence of 1M3H4P (**a**) and 1M4H3P (**b**) in aqueous (Na)Cl 0.6 m, T = 25 °C; $[Al]_0 = 10^{-3}$ m, $[ligand]_0 = 4\cdot10^{-3}$ m.

The free metal ion predominates only at very acidic pH values, i.e. below 1.5, wheras upon increasing the pH the

sequential formation of AlL, AlL_2 and AlL_3 is observed. A water molecule coordinated to the metal center in AlL_2 may deprotonate to form AlL_2H_{-1}. At basic pH values, all the complexes are destabilized owing to the formation of $Al(OH)_4$. In the investigated concentration range, the precipitation of aluminium hydroxide was not observed in either system in the presence of a threefold or higher excess ligand concentration, because of the high affinity of the ligands towards the metal ion.

The acidity of $AlL_2(H_2O)_2$ (pK_a values around 7 for both ligands, see Table 2) is much lower than that of $Al(H_2O)_6$ ($pK_a = 5.52$[13]). This accounts for the negligible concentration of AlL_2H_{-1} at acidic and neutral pH values. The lower acidity is due in part to statistical reasons [a smaller number of deprotonating water molecules in $AlL_2(H_2O)_2$ than in $Al(H_2O)_6$] and in part to a relatively high electronic density around the chelating oxygen atoms of the ligand, higher than that on the oxygen of water,[14] which destabilizes the deprotonation of the coordinated water molecules in $AlL_2(H_2O)_2$.

Metal–Ligand Complexes – UV Results

Selected UV spectra for the Al/1M3H4P and the Al/1M4H3P solutions at acidic-to-neutral pH values are reported in Figure 3. All spectra show the typical absorption of substituted pyridinic rings. The increase of pH causes a bathochromic shift of the main peak and a general increase of the absorption coefficients. These effects are in agreement with the gradual formation of less positively charged, thus electron-richer, species (AlL^{2+}, AlL_2^{+}, AlL_3) at higher pH values.

In the elaboration of the UV data for both systems the formation constant of AlL was kept constant at the value determined from potentiometric titrations, as this species is already formed at the most acidic pH values investigated. The values for the stability constant of AlL_2 could be optimised: $\log \beta_{1,2,0} = 14.2 \pm 0.1$ for Al/1M3H4P, $\log \beta_{1,2,0} = 13.2 \pm 0.1$ for Al/1M4H3P. The $\log \beta$ values of AlL_2 compare reasonably well with the corresponding potentiometric ones ($\log \beta_{1,2,0} = 14.27 \pm 0.04$ and 13.41 ± 0.03, respectively). This agreement suggests the absence of systematic errors in the results.

Metal–Ligand Complexes – ^{1}H NMR Results

In the ^{1}H NMR spectra of 1M3H4P in D_2O, the singlet of the CH_3 protons is at $\delta = 4.37$, and the signals due to the protons H(2), H(5), and H(6), are observed at $\delta = 8.52$ (s), $\delta = 8.28$ (d), and $\delta = 8.20$ (d), respectively. The signals do not show appreciable pD-dependent shift, at least in the investigated pD range (2–7). For 1M4H3P at acidic pD values (pD < 4), the singlet of the CH_3 protons appears at $\delta = 4.16$, and H(2), H(5), and H(6) are observed respectively at $\delta = 8.89$ (s), $\delta = 7.20$ (d), and $\delta = 8.31$ (d). At pD = 7.4, all signals appear at higher fields ($\delta = 3.94$, $\delta = 8.20$, $\delta = 6.70$, and $\delta = 7.89$, respectively), because of the deprotonation of the phenolic oxygen.

In the presence of aluminium(III), the peaks of the free ligands are observed only in acidic solutions, and new groups of peaks due to the complexes appear at all investigated pD values.

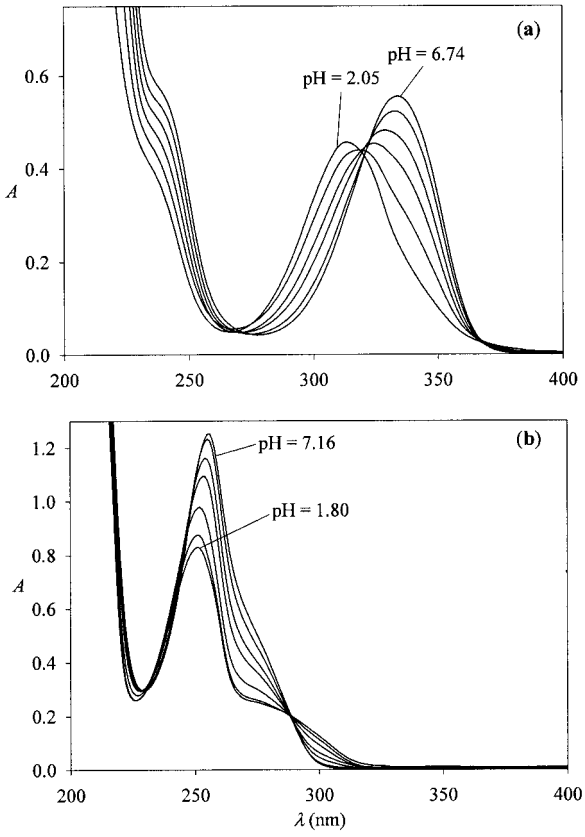

Figure 3. (a) UV spectra of Al/1M3H4P, (b) Al/1M4H3P at various pH values.

For Al/1M3H4P (Figure 4), at pD = 1.9 two additional peaks appear at $\delta = 4.30$ and $\delta = 4.28$, which represent the CH_3 protons of two complexes, i.e. AlL and AlL_2 according to the potentiometric data. The expected quantitative distribution suggests the attribution of the most intense signal ($\delta = 4.28$) to AlL_2. In the aromatic region AlL and AlL_2 give rise to two additional peaks, one more intense than the other, at $\delta = 8.05$ and $\delta = 8.00$, respectively, the other peaks of the complexes overlapping those of the free ligand. Upon increasing the pD to 4.0, the complexes give rise to four CH_3 signals at $\delta = 4.30$ (very low intensity, AlL), $\delta = 4.28$ (AlL_2), $\delta = 4.26$ and $\delta = 4.24$ (AlL_3, as discussed below). The peaks in the aromatic region show a complicated pattern in which only some peaks of the free ligand ($\delta = 8.52$ and 8.28 ppm) can be easily assigned. At pD = 6.5, even though only the species AlL_3 exists in solution in significant amount, four CH_3 signals are observed between 4.19 and 4.22 ppm, together with a fifth less intense peak at $\delta = 4.23$ ppm. This small signal is probably produced by AlL_2 and AlL_2H_{-1}, which at this pD value are about twenty times less concentrated than AlL_3. The other four signals can be explained considering that the ligand is not symmetrical and may chelate the metal, forming AlL_3, in two different spatial configurations (Figure 5). If the three ligands of AlL_3 chelate the metal ion in the same configurations, a

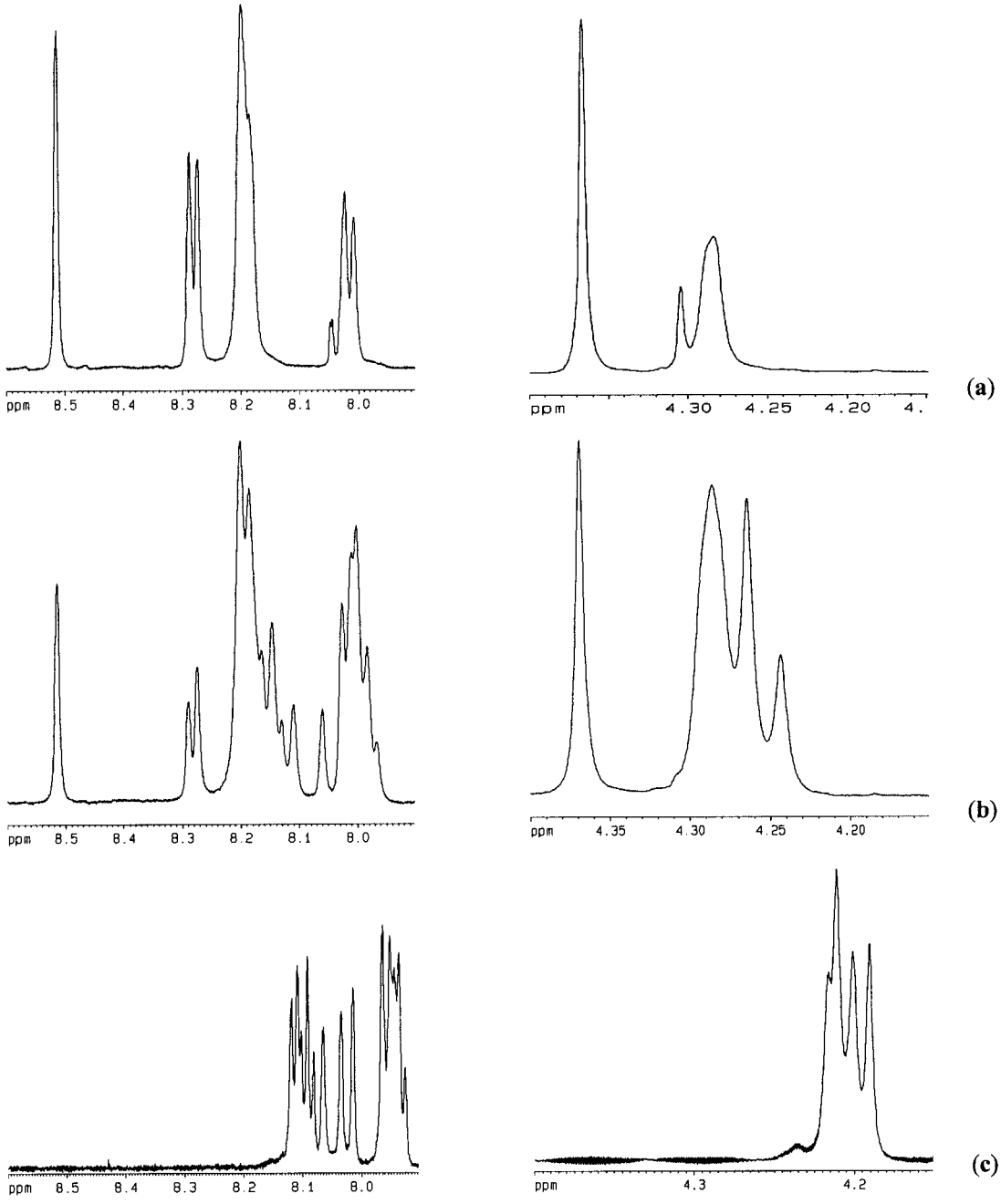

Figure 4. ^1H NMR spectra of D_2O solutions containing Al^{III} ($6.5 \cdot 10^{-3}$ m) and 1M3H4P ($1.9 \cdot 10^{-2}$ m), pD = 1.9 (a), 4.0 (b), 6.5 (c). For the spectrum at pD = 6.5, v_0 = 600 MHz. The sensitivity of spectra in the aromatic zone was enhanced by a factor 4.

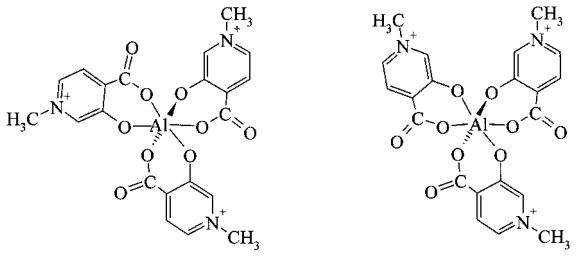

Figure 5. The two spatial diastereoisomers of AlL_3 (L = 1M3H4P).

symmetrical diastereoisomer (AlL_{3-sym}) forms, and the three CH_3 groups have the same NMR response. If one ligand chelates differently from the other two, an unsymmetrical diastereoisomer ($AlL_{3\text{-}unsym}$) forms, and the three CH_3 groups give rise to different signals in the NMR spectrum. In the aromatic region four signals are expected as well for each proton. The quite broad peaks at δ = 8.07, δ = 8.03, and δ = 8.01 do not show COSY couplings with other signals, and between them no reasonable coupling constant exists. Thus, they can be attributed to the H(2) protons of the two AlL_3 species. The fourth H(2) signal is

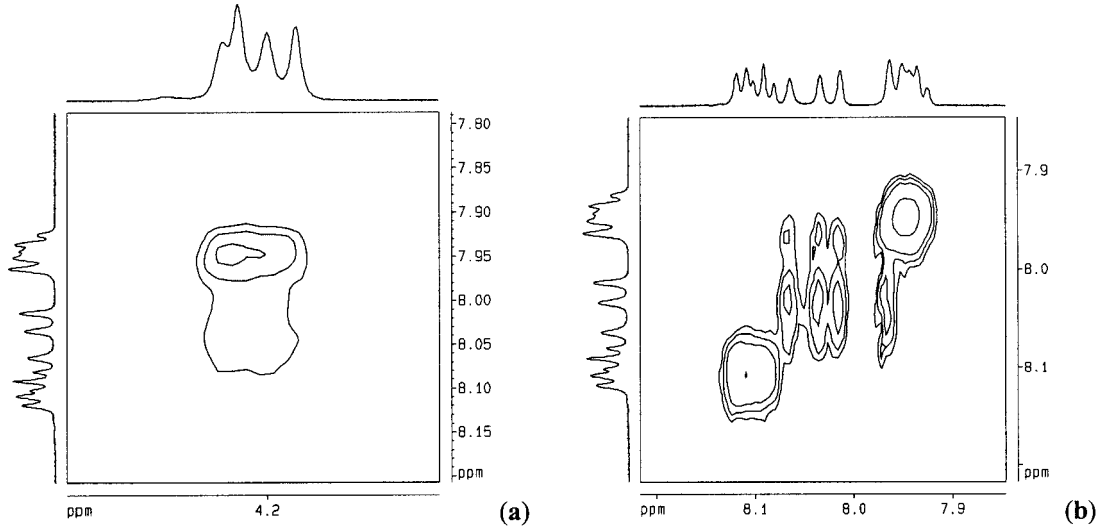

Figure 6. ^1H –^1H phase sensitive NOESY spectra of a D_2O solution containing Al^{III} $6.5\cdot10^{-3}$ m and 1M3H4P $1.9\cdot10^{-2}$ m, pD = 6.46 (ν_o, 600 MHz). (a) NOESY correlations between the aromatic (F1) and CH_3 (F2) protons. (b) *In phase* (exchange) correlations between the aromatic protons.

probably the singlet which appears at δ = 7.96 ppm. The four signals of H(5) and H(6) (centered at δ ca. 8.1 and δ ca. 7.95, respectively, COSY data) cannot be distinguished from each other due to strong overlap. The NOESY interactions between CH_3 and H(6) are significantly more intense than those between CH_3 and H(2) (Figure 6, a). This is in agreement with a distortion of the aromatic ring in the complex, which brings the CH_3 protons closer to H(6) than to H(2). In addition, NOESY spectra show strong correlation peaks between the aromatic protons which are in phase with the diagonal (Figure 6, b): this finding is in agreement with relatively fast chemical exchanges of the various ligand molecules in AlL_3.[15–17]

For Al/1M4H3P (Figure 7), at pD = 1.1 an additional CH_3 peak, and two H(5) and H(6) multiplets, are visible at higher fields. The pattern is in agreement with the formation of only one complex, which according to the potentiometric data is AlL [its H(2) signal overlaps with the corresponding signal of the free ligand at δ = 8.89 ppm]. In addition to the peaks of AlL and of the free ligand, at pD = 2.8 new broad signals of CH_3, H(2), H(5), and H(6) appear (e.g. at δ = 7.05 ppm) due to the formation of AlL_2. The broadness of these signals cannot be due to fast ligand exchanges, because the peaks of the free ligand are narrow. It may be explained as chemical-shift dispersion by the formation of four possible spatial isomers of AlL_2. At pD = 7.4, where only the species AlL_3 exists in solution in a significant amount, a complicated pattern is observed. The signal centered at δ = 6.95 ppm shows clearly four doublets having identical coupling constants (^3J = 7.25 Hz), and as for Al/1M3H4P this pattern can be explained by assuming the formation of $AlL_{3\text{-sym}}$ and $AlL_{3\text{-unsym}}$. For this system it can be seen that the four doublets are equally intense, indicating that $[AlL_{3\text{-unsym}}] = 3\cdot[AlL_{3\text{-sym}}]$, as statistically expected. The independence of the relative intensities of these four doublets from pD was confirmed by another spectrum obtained at pD = 6.4 (not shown). The broad band at δ = 7.05 ppm, as well as the peak at 8.9 ppm, are produced by the minor species AlL_2 and AlL_2H_{-1} (these two peaks do not couple with the peaks of AlL_3 centered at δ = 8.85, 8.20 and 6.95 ppm, as demonstrated by a COSY spectrum). The signals of the complexes, differently from those of the free ligand, do not show an appreciable pD-dependent shift, because they do not undergo any deprotonation (except AlL_2 to a small extent) in the entire pD range.

Generally, the use of D_2O instead of H_2O does not modify the qualitative results, i.e. the species existing in H_2O should be present in D_2O as well. However, heavy water introduces isotopic effects, which may modify the quantitative distribution of the species, especially of those bearing acidic protons.[18,19] In order to obtain NMR quantitative data directly comparable with the potentiometric results, the system Al/1M3H4P was investigated in H_2O. The spectra are identical to the corresponding ones in D_2O (except that all peaks are broader). The integration values of the signals of the free ligand and of the complexes give the relative amount of free and complexed ligand. The results obtained are reported in Table 3, together with the corresponding values calculated from the stability constants reported in Table 1 and Table 2. The agreement between the two sets of data is very good at all pH values, suggesting the absence of systematic errors in the results.

Metal–Ligand Complexes – Comparison with Previous Results

As previously mentioned, the methylation of the pyridinic ring is expected to increase the affinity of the ligand towards aluminium(III). To verify this, pAl plots[20] vs. pH for 1M3H4P, 1M4H3P, and their corresponding unmethylated compounds were computed (Figure 8): higher pAl values indicate stronger metal–ligand complexes. The plot for

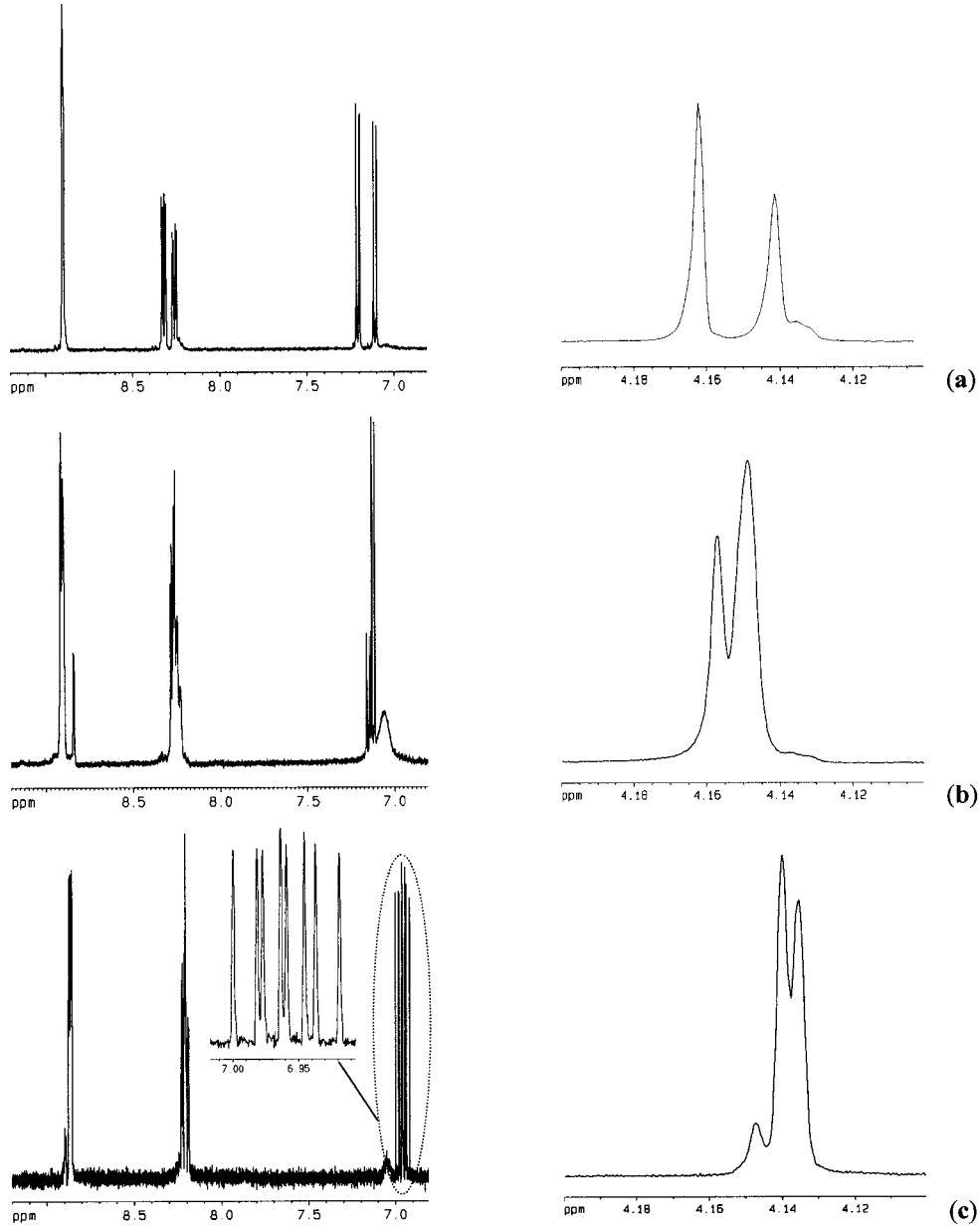

Figure 7. ^1H NMR spectra of D_2O solutions containing Al^{III} ($6.5 \cdot 10^{-3}$ m) and 1M4H3P ($1.1 \cdot 10^{-2}$ m), pD = 1.1 (a), 2.8 (b). Spectrum of Al^{III} ($6.5 \cdot 10^{-3}$ m) + 1M3H4P ($2.0 \cdot 10^{-2}$ m), pD = 7.4 (c). The sensitivity of spectra in the aromatic zone was enhanced by a factor 4.

Table 3. Percentages of free and complexed ligand (Al/1M3H4P spectra in H_2O).

pH	From NMR data		From potentiometric results	
2.08	free ligand	54.9	$H_2L^+ + HL$	54.1
	compl. ligand	45.1	$AlL^{2+} + 2\ AlL_2^+$	45.9
3.05	free ligand	40.1	HL	40.0
	compl. ligand	59.9	$AlL^{2+} + 2\ AlL_2^+ + 3\ AlL_3$	60.0
4.04	free ligand	25.0	HL	26.2
	compl. ligand	75.0	$AlL^{2+} + 2\ AlL_2^+ + 3\ AlL_3$	73.8
5.04	free ligand	12.0	$HL + L^-$	14.7
	compl. ligand	88.0	$2\ AlL_2^+ + 3\ AlL_3$	85.3
6.12	free ligand	8.6	$HL + L^-$	8.5
	compl. ligand	91.4	$2\ AlL_2^+ + 2\ AlL_2H_{-1} + 3\ AlL_3$	91.5

deferiprone (speciation data from ref.[21]) is reported for comparison. The HPs 3H4P, 4H3P, 1M3H4P, and 1M4H3P, are weaker aluminium(III) chelators than deferiprone at all pH values. However, it is important to note that 1M3H4P forms significantly more stable complexes with aluminium(III) than 3H4P does, especially at slightly acidic and neutral pH values: the pAl difference is up to two log units. On the other hand, the same methyl substitution in 4H3P did not produce any increase in chelation strength, as pAl values of 1M4H3P are almost identical to those of 4H3P. We do not yet have an explanation for the different effect of the *N*-methyl substitution in the two isomers. The

study of other alkylated HPs, now in progress, should better clarify this point.

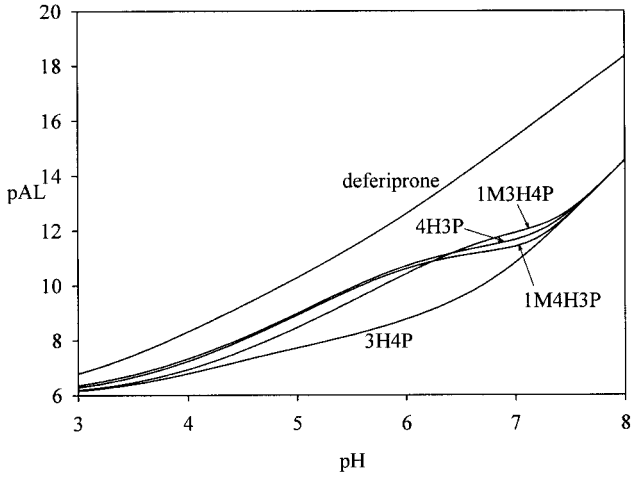

Figure 8. pAl for several ligands (see text); $[Al]_0 = 10^{-6}$ m, $[ligand]_0 = 5 \cdot 10^{-5}$ m.

n-Octanol/Water Distribution and Chelation Efficacy

The octanol/water distribution coefficients of 1M3H4P, 1M4H3P and deferiprone were 0.0054 ± 0.0042, 0.0015 ± 0.0013, and 0.031 ± 0.019, respectively (mean ± standard deviation of 3 experiments, each conducted with duplicate observations), which shows the high hydrophilicity of these new ligands. In the presence of 1M3H4P and 1M4H3P, the concentration of Al in the octanol phase was very much lower than in the aqueous phase, which indicates very high hydrophilicity of the metal–ligand complexes. The results with deferiprone are reasonably similar to those previously published.[22]

The chelation-efficacy results obtained for deferiprone, 1M3H4P and 1M4H3P are summarized in Table 4. The efficiency of Al chelation of deferiprone (74%) is similar to that previously reported (65%).[22] As for 1M3H4P and 1M4H3P the results of the potentiometric studies show a 1:3 Al–ligand complex at pH 7.4 (Figure 2), the efficiency was calculated to show the percentage of ligand associated with aluminium in a 1:3 Al–ligand complex. Values for 3H4P and 4H3P are reported as well for comparison. An appreciable increase in efficiency can be observed for the 1-methyl derivatives. Statistical comparison of the efficiency of 3H4P, 4H3P, 1M3H4P and 1M4H3P by 1-way ANOVA followed by a Neuman–Keuls multiple comparison test shows a significant difference between 3H4P and 1M3H4P. Efficacy results are in agreement with thermodynamic data predictions at physiological pH, as the values at pH = 7.4 found in this study are in the same order as the pAl values are (Figure 8), i.e. 1M3H4P > 4H3P > 1M4H3P > 3H4P.

Conclusions

Both 1M3H4P and 1M4H3P form strong complexes in solution with aluminium(III), so that in the investigated concentration range ($3 \cdot 10^{-4}$ m < $[Al]_0$ < 10^{-3} m, 10^{-3} m < $[L]_0$ < $4 \cdot 10^{-3}$ m) no metal hydroxide precipitates at neutral pH values in the presence of a threefold or higher ligand excess over aluminium(III). For both ligands the species AlL, AlL_2, AlL_3 and the minor species AlL_2H_{-1} are formed in solution. UV spectra studies confirmed the stability constants for AlL_2 that were obtained in the potentiometric studies. ^1H NMR spectra in D_2O at acidic pD values confirmed that several complexes coexist in solution. At neutral pD values the spectra allowed the detection of two different diastereoisomers of AlL_3, which could be quantified for 1M4H3P. For 1M3H4P the spectra indicated that in AlL_3 the aromatic ring of the ligand is distorted and that metal–ligand exchanges occur. For Al/1M3H4P, NMR spectra in H_2O confirmed the quantitative ratio between free and complexed ligand at several pH values. The chelation efficiencies are quite high, and they are in agreement with thermodynamic data at physiological pH.

Table 4. Al^{III} chelation efficiencies of some hydroxypyridinecarboxylic acids. The values for DFO and for deferiprone are reported for comparison. Values reported are mean ± standard deviation of three experiments with DFO, deferiprone, 3H4P and 4H3P, and six experiments with 1M3H4P and 1M4H3P, each conducted in duplicate.

Ligand	Chelation efficiency (%)	Reference
DFO	112 ± 29	this work
deferiprone	74 ± 6	this work
1M3H4P	50 ± 16	this work
1M4H3P	37 ± 15	this work
3H4P	20 ± 2	[9]
4H3P	43 ± 6	[9]

The metal–ligand complexes are very hydrophilic at physiological pH. This should be regarded as a positive property: although chelating agents having $D_{o/w}$ values around 0.2 were hypothesized to be best suitable,[23] in our evaluation of a series of 3,4-hydroxypyridinones we found that some very hydrophilic compounds were among the most orally bioavailable and effective chelators of Al.[24–26]

On the other hand, 1M3H4P and 1M4H3P form weaker complexes than deferiprone does at all pH values. Therefore, these derivatives are expected to not be as effective in vivo. The synthesis of HPs with stronger affinity towards aluminium(III) is advisable.

1M3H4P forms significantly stronger complexes with aluminium(III) than does 3H4P. This suggests that further alkylation of the pyridinic ring can be a good strategy towards the synthesis of stronger aluminium chelators. For 1M4H3P no increase of stability was observed with respect to 4H3P. It is therefore not clear whether the alkylation of the pyridinic ring can be effective for all HPs. Other derivatives should be investigated in order to clarify the effect of the alkyl substitution. In order not to affect significantly the high hydrophilicity of the complexes, the simple methyl derivatives seem to be more promising. Towards this goal, the syntheses of 2-methyl and 1,2-dimethyl derivatives of 3H4P and 4H3P, and the thermodynamic and in vitro studies of the complexation with aluminium(III), are now in progress.

Experimental Section

Synthesis of the Ligands

All chemicals and solvents were obtained from Aldrich Chimica, Lancaster or Acros. The identity and purity of the intermediates and of the final compounds were checked by melting point (m.p.) measurements (Gallenkamp MFB 595010 M/B capillary m.p. apparatus), ^{1}H NMR spectra (Bruker 300 MHz), TLC (Merck silica gel 60F-254 glass plates), mass spectrometry (Mat, 112 Varian Mat Bremen mass spectrometer) and elemental analysis (Perkin–Elmer Elemental Analyzer Model 240B).

1M3H4P: 3H4P (0.5 g, 3.62 mmol) was dissolved in 30 mL DMF by adding NaOH 10% dropwise up to pH = 10, then CH_3I (1.12 mL, d = 2.280 g·cm^{-3}, 17.99 mmol) was added. The mixture was heated at 100 °C for 2 h and after cooling it was acidified with concd. HCl and the solvents evaporated to dryness. The residue was re-crystallized twice from ethanol and glacial CH_3COOH giving 0.84 g (83% yield) of 1M3H4P·HI (*N*-methyl-3-hydroxy-4-carboxypyridinium iodide); m.p. 258 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 4.24 (s, 3 H, CH_3), 7.97 [d, $^3J_{5,6}$ = 5.92 Hz, 1 H, H(5)], 8.05 [dd, $^3J_{5,6}$ = 5.92 and $^4J_{6,2}$ = 0.95 Hz, 1 H, H(6)], 8.42 [d, $^4J_{2,6}$ = 0.95 Hz, 1 H, H(2)] ppm.

The iodide salt was transformed into the 3-hydroxy-*N*-methylpyridinium-4-carboxylate zwitterion (betaine). To this aim, the iodide salt (0.984 g) was dissolved in water (10 mL), acidified at pH = 2 with concd. HCl, and added to IRA-400-Cl amberlite resin (2.21 g, previously conditioned with HCl 1 M). The mixture was stirred for 2 h and then the resin was filtered off, the filtrate evaporated and the residue re-crystallized from glacial CH_3COOH. The collected precipitate was washed with acetone and dried (0.44 g, yield 83%). M.p. 252 °C (dec.). IR (KBr): $\tilde{\nu}$ = 3387, 2857, 2510, 1722 cm^{-1}. ^{1}H NMR ([D$_6$]DMSO): δ = 4.19 (s, 3 H, CH_3), 8.02 [dd, $^3J_{5,6}$ = 5.8 Hz, 2 H, H(5) and H(6)], 8.44 [s, 1 H, H(2)] ppm. HR MS: m/z = [MH]$^+$ 154.11. $C_7H_7NO_3$ (153.15): calcd. C 54.90, H 4.61, N 9.15; found C 54.85, H 4.62, N 9.10.

Finally, the betaine product was transformed into the corresponding chloride salt (1M3H4P·HCl), by dissolution in ethyl acetate and addition of concd. HCl. Then the resulting solution was evaporated to dryness (0.54 g, yield 100%). M.p. 220 °C. ^{1}H NMR (CD_3OD): δ = 4.21 (s, 3 H, CH_3), 8.30 [d, $^3J_{5,6}$ = 6.3 Hz, 1 H, H(5)], 8.42 [d, $^3J_{5,6}$ = 6.4 Hz, 1 H, H(6)], 8.77 [s, 1 H, H(2)] ppm. HR MS : m/z = [MH]$^+$ 155.23. $C_7H_8ClNO_3$ (189.60): calcd. C 44.34, H 4.25, N 7.39, Cl 18.70; found C 44.41, H 4.03, N 7.32, Cl 18.43. A 97.86% purity was obtained from potentiometric titrations.

1M4H3P: CH_3I (4.25 mL, d = 2.280 g·cm^{-3}, 68.26 mmol) was added to a 50-mL DMF solution containing 4H3P (1.89 g, 13.87 mmol). The mixture was heated to 100 °C for 2 h. The volatiles were then evaporated and the solid residue was re-crystallized from ethanol yielding a crystalline white product (2.10 g). Yield 99%; m.p. 248–249 °C. IR (KBr): $\tilde{\nu}$ = 3050, 1720, 1655 cm^{-1}. ^{1}H NMR ([D$_6$]DMSO): δ = 3.73 (s, 3 H, CH_3), 6.75 [d, $^3J_{5,6}$ = 7.43 Hz, 1 H, H(5)], 8.06 [dd, $^3J_{5,6}$ = 7.43 and $^4J_{2,6}$ = 2.2 Hz, 1 H, H(6)], 8.69 [d, $^4J_{2,6}$ = 2.2 Hz, 1 H, H(2)], 16.36 (br. s, 1 H, COOH) ppm. HR MS: m/z = [MH]$^+$ 154.11. $C_7H_7NO_3$ (153.14): calcd. C 54.9, H 4.61, N 9.15; found C 54.24, H 4.71, N 8.89. A 99.33% purity was obtained from potentiometric titrations.

Solution Equilibria

The experimental apparatus, reagents and measurement methods were almost the same as reported previously.[27] In the following, a summary will be given; details are reported only when different from those already described.

Apparatus: All potentiometric measurements were performed with a Radiometer ABU93 Triburette apparatus. UV spectra were recorded with a Perkin–Elmer Lambda 25 spectrophotometer, ^{1}H NMR spectra with Bruker DRX-400 and DRX-600 spectrometers operating at 400.13 and 600.09 MHz, respectively.

Reagents: All analyte concentrations were expressed in the molality scale (mol/kg of water). For potentiometric and UV measurements, working solutions of HCl (0.2 m), NaOH (0.2 m) and $AlCl_3$ (0.1 m, containing 0.3 m HCl) were prepared and standardised. 1M3H4P and 1M4H3P were used as synthesized to prepare respectively 0.14 m and 0.15 m working solutions. The ionic strength of all the solutions was adjusted to 0.6 m (0.594 M) (Na)Cl. Solutions for ^{1}H NMR measurements were prepared by dissolving in D_2O (Aldrich, 99.9 atoms% D), or in H_2O, weighed amounts of ligand, $AlCl_3$ (Fluka, ≥ 99%) and NaCl.

Potentiometric Titrations: Potentiometric titrations carried out in this work are summarized in Table 5. Temperature was 25.0 ± 0.1 °C. Duplicate potentiometric measurements were executed by using two different glass electrodes (Radiometer pHG201 and BDH 309/1015/02) and a Ag/AgCl/0.6 m NaCl reference electrode[28,29] with a J-shaped junction. Its acidic liquid junction potential has been determined previously[30] and it can be represented by the equation[29] $E_j = -S\cdot\log(1 + J\cdot[H^+])$, with J = 3.69 ± 0.08.

Table 5. Potentiometric titrations (titrant: NaOH).

Solutions		Conditions $[Al]_0/10^{-3}$ m	$[L]_0/10^{-3}$ m	$[L]_0/[Al]_0$	pH
1.	1M3H4P	–	0.47–3.78	–	2.5–11.0
	1M4H3P		1.29–4.14		2.6–11.0
2.	Al + 1M3H4P	0.36–1.03	1.16–3.76	1, 2, 3, 4, 8	2.2–9.0
	Al + 1M4H3P	0.32–1.10	1.27–4.12		

As a check of the accuracy of the entire experimental system, the pK_w value for water in (Na)Cl 0.6 m was computed from HCl + NaOH titrations. The values obtained from two different series of experiments (pK_w = 13.719 ± 0.002 and pK_w = 13.710 ± 0.002) compare well with the literature value[31] in NaCl 0.6 M at 25 °C (13.727 ± 0.001).

All stability constants were calculated with the computer program PITMAP.[32] The hydroxo metal species reported by Öhman[13] have been considered and the corresponding log β values kept fixed during the elaboration of the data.

After the addition of the metal and one of the two ligands to an acidic solution (pH < 3), the measured electromotive force drifted and reached a constant value only after ca. 1 hour, suggesting a low complex formation rate. The kinetics of the aluminium–ligand reaction became "normal" (equilibration after ca. 1 min) at pH values above 3. These findings are in agreement with previous results with other HPs.[9,14,33] Experimental details regarding the handling of the slow kinetics during the titrations are reported.[14]

UV Measurements: UV measurements (Table 6) were carried out for solutions containing the ligand alone, and for solutions containing the ligand and the metal ion, at various pH values (cell path 0.1 cm). When the pH of the solution was below 2, it was computed from the stoichiometric concentration of HCl, because the $[H^+]$ modifications produced by the other species were negligible under these conditions. In other cases the pH was measured with the same electrodes and procedures as for potentiometric titrations. The pK_{a1} for both ligands and the log β values for some aluminium–ligand complexes were computed by the program PITMAP.

Table 6. UV measurements.

Solutions	Conditions $[Al]_0/10^{-3}$ m	$[L]_0/10^{-3}$ m	pH	λ (nm) for calcd.
1M3H4P	–	0.98	0.25–2.00	217; 249; 324; 346
1M4H3P	–	1.01	0.23–2.55	252; 290
Al + 1M3H4P	0.29	0.92	2.05–6.74	229; 303; 338
Al + 1M4H3P	0.30	0.93	1.80–7.16	256; 266; 298

NMR Measurements: ^{1}H NMR spectra were obtained at 298 K. The chemical shift values are given in δ units with reference to internal Me_4Si. Suitable integral values for the proton spectra were obtained by a pre-scan delay of 10 s. The assignments of the proton resonances were performed by standard chemical shift correlations, COSY, TOCSY and NOESY experiments. ^{1}H spectra were collected for both 1M4H3P and 1M3H4P at various pD values in D_2O solutions containing only the free ligand or the ligand and the metal. For Al+1M3H4P, additional spectra were obtained in H_2O (Table 7). For D_2O solutions, the pH was measured with a glass electrode BDH 309/1025/02 previously calibrated in buffered solutions at pH = 4 and pH = 7, and 0.41 pH units were added to the pH meter readings to correct for isotopic and solvent effects,[34] i.e. pD rather than pH values were measured. For the H_2O solutions, the pH was measured as described above for potentiometric titrations. Heavy water, necessary for the instrumental lock, was in a capillary concentric with the NMR tube.

n-Octanol/Water Distribution and Chelation Efficacy

Materials: NaCl, KCl, $NaHCO_3$, H_3BO_3 and *n*-octanol were obtained from EM, Fisher, or Sigma; Al borate from Pfaltz and Bauer (Waterbury, CT) and an Al atomic absorption standard from Sigma. Polypropylene test tubes (10 mL) with polyethylene caps were purchased from VWR.

Methods: The distribution coefficients ($D_{o/w}$) of the ligands were determined as their concentration in the octanol phase/their concentration in the aqueous phase at equilibrium with a Shimadzu UV-2501PC UV/Vis recording spectrophotometer. UV absorbance was measured at λ_{max}, determined from a wavelength scan of the ligand.

A method to assess the aluminium chelation efficiency in vitro was used.[35] An octanol/aqueous system containing 125 mM NaCl, 25 mM $NaHCO_3$, 4.5 mM KCl and 100 mM borate at pH 7.4 was established. Equal volumes (4 mL) of the aqueous phase and 1-octanol were placed in a screw-cap polypropylene tube. The octanol phase was saturated with the aqueous solution before use to avoid distribution of hydrophilic compounds into the aqueous component of the octanol phase which would produce a falsely elevated value for octanol distribution. The opposite (i.e., the saturation of the aqueous phase with octanol) was not needed for our hydrophilic compounds.

For each experiment, duplicate tubes were prepared to contain 1) only the octanol and the aqueous phase, 2) as 1) plus 7 to 8 mg of aluminum borate to provide an excess of undissolved Al, saturating the aqueous phase and octanol with Al, 3) as 1) plus the ligand introduced into the aqueous phase at $1 \cdot 10^{-3}$ M, and 4) as 3) plus 7 to 8 mg of aluminum borate. The tubes were agitated overnight at room temperature to establish equilibrium.

Aluminum Analysis: The tubes were centrifuged (3400 g, 10 min), the aqueous phase was filtered through a filter with a 0.22 μm nylon membrane and then each phase analyzed by electrothermal atomic absorption spectroscopy (Perkin–Elmer 4100ZL) to determine the Al concentration. Aluminum recovery from the filtered aqueous phase was found to be 101% ± 6% (mean ± standard deviation of 12 observations), showing no loss of Al due to the filter.

Chelator Efficiency: The amount of Al in each liquid phase of tubes with added Al but no ligand, minus the amount of Al in tubes without added Al or ligand represented Al solubility from aluminum borate. The increase in Al in each liquid phase of tubes with Al plus ligand, over that seen in the absence of the ligand, was taken as complexation of Al by the ligand. In addition to 1M3H4P and 1M4H3P, values for the hexadentate ligand desferrioxamine (DFO) and the bidentate ligand deferiprone were obtained as positive controls to confirm the ability of the method to predict Al complexation (values close to 100% were expected). For DFO, where at pH = 7.4 a 1:1 aluminium/ligand complex was anticipated, the chelator efficiency was calculated by

$$\frac{[\text{sum of the M Al concentration in each phase}]}{1 \times 10^{-3}\ \text{M}}$$

For deferiprone, 1M3H4P and 1M4H3P, where at pH = 7.4 a 1:3 aluminium/ligand complex was anticipated (see, for example Figure 2), the chelator efficiency was calculated by

$$\frac{3[\text{sum of the M Al concentration in each phase}]}{1 \times 10^{-3}\ \text{M}}$$

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Table 7. NMR measurements.

Solutions	Conditions $[Al]_0/10^{-3}$ m	$[L]_0/10^{-3}$ m	Solvent	pH/pD	Bidimensional spectra
1M3H4P	–	19	H_2O	2.1; 3.0; 4.0; 5.0; 6.1	–
1M3H4P	–	19	D_2O	1.9; 4.0; 7.4	–
1M4H3P	–	11	D_2O	1.1; 2.8; 7.4	–
Al + 1M3H4P	6	19	H_2O	2.1; 3.0; 4.0; 5.0; 6.1	–
Al + 1M3H4P	6	19	D_2O	1.9	COSY, TOCSY, NOESY
				4.0	–
				6.5	COSY, TOCSY, NOESY
Al + M4H3P	6	11	D_2O	1.1	–
		11		2.8	–
		20		6.4	–
		20		7.4	COSY

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