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A New Tris(phosphonomethyl) Monoacetic Acid Cyclam Derivative: Synthesis, Acid-Base and Metal Complexation Studies

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A new cyclam-based ligand, [4,8,11-tris(phosphonomethyl)-1,4,8,11-tetraazacyclotetradec-1-yl]acetic acid (H_7te3p1a) was synthesised and characterised. The potentiometric and ^{31}P NMR spectroscopic titrations of the complexes in an aqueous solution at an ionic strength of 0.10 or 0.50 mol dm^{-3} in $[N(CH_3)_4]NO_3$, respectively, indicated that the ligand is highly basic. The stepwise protonations were dominated by extensive proton relocations and were influenced by the intramolecular hydrogen bonds. The thermodynamic stability constants for the complexes formed by H_7te3p1a in the presence of metal ions were determined by potentiometric titrations. The Cu^{2+} complexes exhibited very high stability, those of Zn^{2+}, Cd^{2+}, Pb^{2+}, La^{3+}, Sm^{3+}, Gd^{3+}, Ho^{3+} and Lu^{3+} presented high stability while that of Ca^{2+} had lower stability, as expected. The ligand and its complexes behaviour is similar to that of the tetrakis(phosphonomethyl)-cyclam derivative, H_8tetp. The NMR spectroscopic studies of the Zn^{2+} and Cd^{2+} complexes pointed to the involvement of the three phosphonate moieties in the coordination of the latter ion, while several isomer species were generally present in solution for the Zn^{2+} complex. A major isomer was stabilised in solution upon heating. In this isomer only one of the methylphosphonate arms was coordinated to the metal centre and this isomer probably adopts a distorted square pyramidal geometry with the macrocyclic ring in the type **I** conformation.

Introduction

Tetraaza macrocycles are efficient chelators of metal ions since they are able to form strong complexes with a variety of cations ranging from alkaline to post-transition metals. The addition of further donor atoms to the macrocyclic backbone in the form of pendant arms increases the coordination ability of the ligands and enables fine-tuning of the properties towards specific metal ions. Among these macrocyclic chelators, those based on cyclen (1,4,7,10-tetraazacyclododecane) and cyclam (1,4,8,11-tetraazacyclotetradecane) account for the vast majority of the studies.[1–3]

The main interest in these compounds lies in the possible medical applications of their metal complexes as contrast agents in magnetic resonance imaging (MRI) or as radiopharmaceuticals for diagnosis and therapy.[4–8] In order to be used in metal chelates, macrocyclic ligands must form strong and nonlabile complexes. In this respect, the desirable chemical properties are high thermodynamic stability and kinetic inertness, fast complexation and high solubility in physiological media, as well as a high hydrophilicity and a low charge on the complex.[5,8,9] A common strategy to improve the efficiency of radiopharmaceuticals is to attach the complex to a targeting biomolecule, such as a monoclonal antibody or its fragments, a small peptide or a receptor ligand, in the form of a bifunctional chelator (BFC). This BFC must contain a reactive group that can be used for the attachment of the complex to the biomolecule without disturbing the coordination sphere of the metal ion.[5,10–12]

Ligands based on the cyclam skeleton are interesting since they can suit the needs of very diverse ions, such as the transition metals and lanthanides. The tetraacetic (H_4teta) and tetrakis(phosphonomethyl) acid (H_8tetp) derivatives of cyclam have been studied for a long time (Scheme 1). A cyclam derivative containing one methylphosphonate and three acetate pendant arms, H_5te3a1p, has recently been studied and displayed a very good coordination ability towards the transition metal ions and also a good coordination ability towards the lanthanide(III) ions.[13,14] We have thus decided to develop a new ligand, H_7te3p1a, as an analogue of H_8tetp with an acetate arm replacing one of the methylphosphonate arms. This ligand can be considered to be a bifunctional chelator since a suitable chemical modification of the single carboxylic group can easily lead to bioconjugates. In this work, we report

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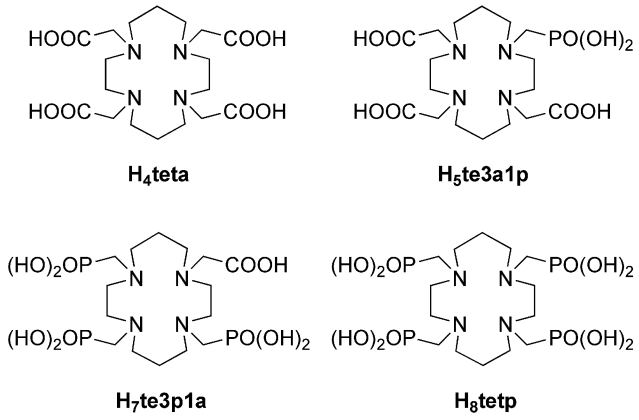
on the synthesis and detailed acid-base and complexation properties of the ligand as studied by potentiometry and multinuclear NMR spectroscopy.



Scheme 1. The structure of the cyclam ligands.

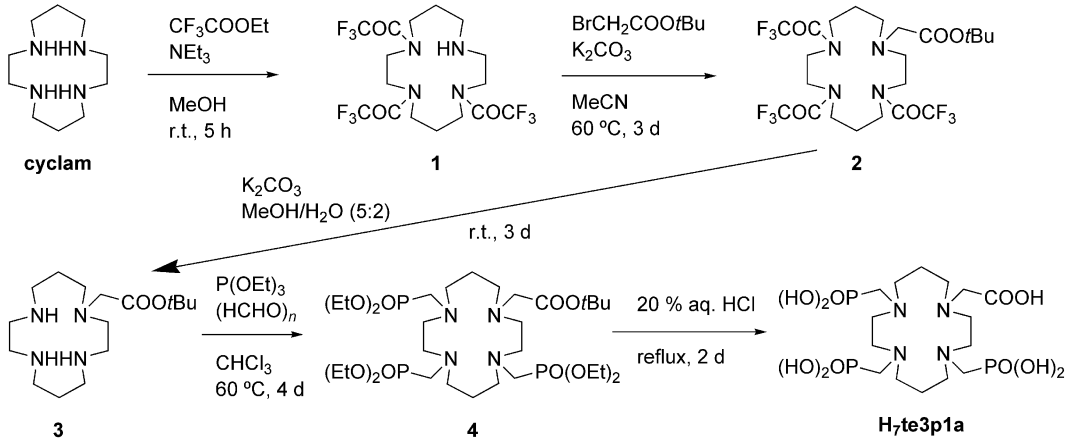
Results and Discussion

Synthesis of the Ligand

The synthesis of H_7te3p1a was achieved in five steps starting from cyclam (Scheme 2). The mono *N*-functionalisation of cyclam with a methylcarboxylic ester moiety was achieved from 1,4,8-tris(trifluoroacetyl)-1,4,8,11-tetraazacyclotetradecane (**1**), which was synthesised by a reported method for the selective triprotection of the secondary amines of cyclam.[15] Although a number of other macrocyclic polyamine protection methods have been reported in the literature,[16] this trifluoroacetyl protection proved to be particularly effective for our purpose because the relevant reaction of cyclam was extremely selective and high yielding. The subsequent *N*-alkylation step with *tert*-butyl bromoacetate proceeded under mild conditions in good yield. The removal of the trifluoroacetyl protection groups was easily achieved under very mild conditions in nearly quantitative yield. The methylphosphonic ester groups were appended by a Mannich-type reaction with paraformaldehyde and triethyl phosphite under anhydrous conditions, but formation of a byproduct hampered the yield and purity of ester **4**. Based on the mass spectra of the reaction mixture [*m*/*z* 626.7, formula $(C_{27}H_{57}N_4O_8P_2)^+$], the byproduct is probably a cyclam formaldehyde aminal with two appended methylphosphonate ester arms, one of which is bound to a quaternary nitrogen atom. It is known that formaldehyde can react with the two secondary amines that are separated by a propylene chain to form a six-membered aminal[17] with a structure that hinders the introduction of a fourth pendant arm. Efforts to minimise this side-reaction, by decreasing the reaction temperature and/or the excess amount of paraformaldehyde added, also resulted in a decrease in the yield of the desired ester **4**. The optimal conditions were found to be the slow addition of paraformaldehyde over the course of several days, which eventually decreased the amount of byproduct in the reaction mixture to 10 to 20%. A final hydrolysis of both the carboxylic and the phosphonic esters by heating **4** under reflux in azeotropic aqueous hydrochloric acid afforded the target ligand, together with the corresponding hydrolysed byproduct and a few other minor impurities. The product was purified by cationic exchange chromatography as described in the Exp. Sect.

Acid–Base Behaviour

The acid-base properties of H_7te3p1a were studied by both potentiometric and ^{31}P NMR spectrometric titrations in aqueous solution. The overall protonation constants of the ligand and the respective standard deviations, as obtained from fitting the experimental data, are collected in the Supporting Information (Table S1), together with the literature values for the related ligands, H_4teta, H_5te3a1p and H_8tetp. The calculated stepwise protonation constants for all of the ligands, as well as the cumulative value of their first five protonation constants, are presented in Table 1.[13,18,19]



Scheme 2. The synthetic pathway for H_7te3p1a.

Table 1. The stepwise protonation constants ($\log K_i^H$) of the ligands as determined by potentiometry; T = 298.2 K; I = 0.10 mol dm^{-3} in $[N(CH_3)_4]NO_3$.

Equilibrium quotient[a]	H_7te3p1a	H_4teta[b]	H_5te3a1p[c]	H_8tetp[b]
[HL]/[L][H]	24.88[d,e,f]	10.59	11.78[d]	25.28[d,e]
$[H_2L]/[LH][H]$		10.08	9.88	
$[H_3L]/[LH_2][H]$	8.08	4.15	6.34	8.85
$[H_4L]/[LH_3][H]$	6.60	3.29	3.85	7.68
$[H_5L]/[LH_4][H]$	5.54	1.84	2.63	6.23
$[H_6L]/[LH_5][H]$	2.83	–	2.14	5.33
$[H_7L]/[LH_6][H]$	4.33[e]	–	–	2.28
$[H_8L]/[LH_7][H]$		–	–	–
$[H_5L]/[L][H]^5$	45.10	29.95	34.48	48.04

[a] The charges of the equilibrium species are omitted for clarity. [b] Ref.[18,19] [c] Ref.[13] [d] Determined by ^{31}P NMR spectroscopy. [e] The constant ($\log\beta_2$) corresponds to a double protonation equilibrium ($[H_2L]/[L][H]^2$). [f] Converted from measurements at higher ionic strength; the $\log\beta_2$ obtained at I = 0.5 mol dm^{-3} was 24.17.

The ligand H_7te3p1a contains a total of eleven basic sites corresponding to the seven ionisable –OH groups of the pendant arms and the four tertiary amines. The potentiometric method used enabled accurate determination of the protonation constants corresponding to six of those basic sites. Polyaza macrocycles with appended methylphosphonate groups usually present very high values for the first protonation constant(s), which can hinder the accurate determination of their value(s) by potentiometry, as previously observed.[13,18,20–26] This trend has been explained by the effect of spreading the high electron density of the fully deprotonated phosphonate group to the nearest nitrogen atom,[3] or by the formation of strong intramolecular hydrogen bonds.[21]

In the case of H_7te3p1a, the first two protonations have very high values and only an overall constant that corresponds to this double protonation could be determined by the ^{31}P NMR spectroscopic titration in the alkaline region (pH 9–14); the measurement was performed in an aqueous solution and the ionic strength was maintained at 0.50 mol dm^{-3} with $[N(CH_3)_4]NO_3$. The spectra generally presented three resonances that correspond to the non-equivalent phosphorus atoms; for a detailed discussion, see the description of the entire NMR spectroscopic titration below (Figure 1). Determination of the constant was achieved using the two resonances that correspond to the phosphorus atoms bound to the amines that are being protonated (b and b′). The value thus obtained (24.17) was converted to one valid at an ionic strength of 0.10 mol dm^{-3} (see the details in the Exp. Sect.); this was later used as a fixed constant in the refinement of the remaining protonation constants. It was not possible to determine the protonation constant of $(Hte3p1a)^{6-}$ due to its low abundance in the equilibrium. This indicated that the values determined for the first two stepwise protonation constants should be very close, which can be explained by a protonation process that is highly concerted for the first two protons and must occur on two nitrogen atoms of the ring. The value obtained for the $\log\beta_2$ of H_7te3p1a (24.88) is almost as high as the corresponding value for H_8tetp (25.28) and much higher than those for H_5te3a1p (21.66) and H_4teta (20.68). This is in agreement with the previously mentioned influence of the phosphonomethyl pendants on the increased basicity of the macrocyclic amines.

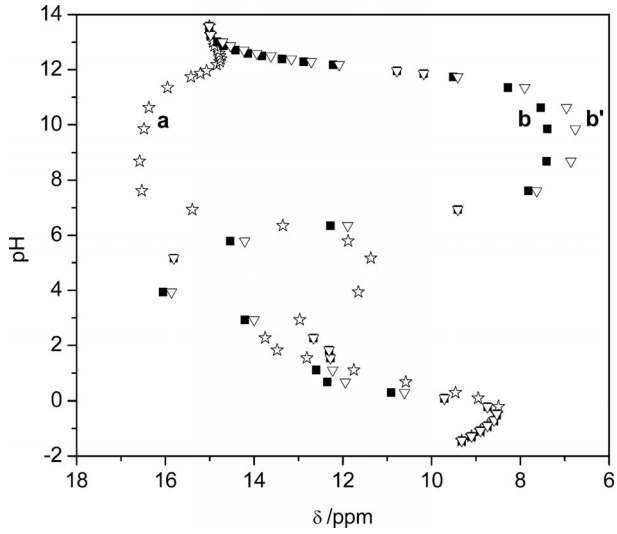
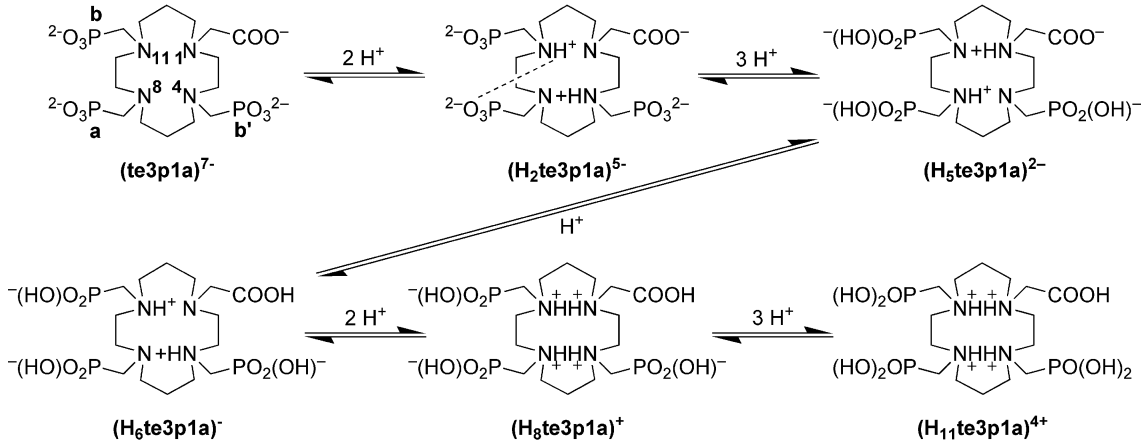


Figure 1. The ^{31}P NMR spectroscopic titration of H_7te3p1a in water at $I \approx 0.50$ mol dm^{-3} in $[N(CH_3)_4]NO_3$. The phosphorus resonance and the nitrogen atom labelling scheme adopted for the ligand can be seen in Scheme 3. In Figure S1 of the Supporting Information this image can be seen in colour.

The next three stepwise protonation constants, at intermediate pH values, were expected to correspond to the protonation of three aminomethylphosphonate groups. However, the value determined for the first of these constants (8.08) was much higher than the other two (6.60 and 5.54), which could be explained by the presence of intramolecular hydrogen bonds.[21] A similar difference can be seen between the fourth and fifth protonation constants for H_8tetp[18,19] where similar interactions are expected. The sixth constant was attributed to the protonation of the acetate group. Lastly, only a global constant could be determined for the two remaining protonations at low pH. It was not possible to obtain a separate constant for H_7te3p1a, which indicated that these last two protonations could be attributed to a concerted process analogous to the protonation of the first two nitrogen atoms. Similar concerted protonation phenomena have been previously observed for cyclam itself and for several of its derivatives.[18,19,21,23–25,27] Further protonations on the ligand could not be determined as they occur at an extremely low pH.

In order to assist with the assignment of the protonation sites for the various protonated ligand species, the protonation of H_7te3p1a was studied with an additional ^{31}P NMR spectroscopic titration in an aqueous solution over a pH range of –1 to 9. This titration was performed in conditions similar to those discussed in the previous paragraph, but without rigorous control of the ionic strength. Combination of the experimental data from the two titrations gave the pH dependence of the ^{31}P NMR spectroscopic chemical shifts throughout the entire pH range (Figure 1 and Figure S1 of Supporting Information in colour). The interpretation



Scheme 3. The proposed protonation sequence of H_7te3p1a, as well as the phosphorus resonances and the nitrogen atom labelling scheme.

of the ^{31}P NMR spectroscopic chemical shift changes is still mostly empirical as different classes of phosphorus compounds show diverse behaviour. However, a significant amount of data has been published with respect to the aminoalkylphosphonate groups in acyclic[28–31] and macrocyclic[13,20–22,25,26,32–35] compounds. In the ^{31}P NMR spectroscopic titration of H_7te3p1a, one of the resonances (δ_a) shows a different pH dependence from those of the other two signals (δ_b and $\delta_{b'}$). The H,P-HMQC correlation spectrum acquired at pH ca. 8.5 (see Figure S10 in the Supporting Information) enabled the assignment of the phosphorus resonances to the respective phosphonate groups. Thus, the δ_a resonance belongs to the phosphonate group bound to the N8 atom, the δ_b resonance belongs to the N11-bound phosphonate and the $\delta_{b'}$ resonance belongs to the N4-bound phosphonate pendant arm. On the basis of this assignment and the literature data for other macrocyclic aminoalkylphosphonate ligands, the protonation sequence was proposed and the protonation sites were tentatively assigned (Scheme 3).

Starting from the fully deprotonated $(te3p1a)^{7-}$ species at high pH, there is a momentary simultaneous upfield shift of all the resonances (pH > 12). This shows that protonation starts on the nitrogen atoms of the ring, as expected, but in a rather delocalised fashion that includes at least the three amines with the appended phosphonate groups. However, briefly afterwards, δ_a starts to shift downfield while δ_b and $\delta_{b'}$ continue to shift upfield concurrently (12 > pH > 10). These upfield shifts indicated that protonation occurs on the nitrogen atoms opposite to each other, N4 and N11, due to the electrostatic repulsion effect, to form a double protonated species $(H_2te3p1a)^{5-}$. This also indicated that protonation is simultaneous at these nitrogen atoms, as anticipated during the determination of the protonation constants. At the same time, the smaller and unexpected downfield shift of δ_a can only be explained by formation of a N–H···O hydrogen bond between a protonated amine and an oxygen atom of the phosphonate group labelled "a" (represented by a dashed line in Scheme 3). An interaction of this kind is more likely to occur with N11 than with N4 as it is one bond closer to the phosphonate group. If this interaction is strong enough to stabilise a particular ligand conformation in solution, as has been observed in similar compounds,[21,23] it will also affect the signal of the phosphonate group appended to N11. The smaller upfield shift of δ_b relative to $\delta_{b'}$ (indicative of a smaller protonation effect due to the hydrogen bonding) confirmed the assignment of resonance δ_b to the phosphonate group appended to N11.

The next three protonations should occur at the oxygen atoms of the three phosphonate groups to form the $(H_5te3p1a)^{2-}$ species and should lead to the dissociation of the hydrogen bond previously described. Indeed, all the phosphorus resonances changed sharply at the intermediate pH (9 > pH > 5). Resonances δ_b and $\delta_{b'}$ shifted strongly downfield but the effect was too extensive to be explained only by the protonation of the oxygen atoms of the phosphonate groups. Therefore, it must also reflect a simultaneous deprotonation of the corresponding amines at N4 and N11 and redistribution of the two ring protons to N1 and N8. This assumption is supported by the simultaneous upfield shift of δ_a to a lesser degree, which can be explained by the overall effect of the protonation of N8 and of one of the oxygen atoms of the appended phosphonate group. The subsequent protonation should take place at the oxygen atom of the acetate pendant arm bound to N1 to form the $(H_6te3p1a)^-$ species, and that should lead to another redistribution of the two ring protons back to N4 and N11. Indeed, the simultaneous upfield shift of δ_b and $\delta_{b'}$ and the downfield shift of δ_a (4 > pH > 2) is in agreement with the deprotonation at N1 and N8 and the concurrent protonation at N4 and N11. Protonation should then take place at the deprotonated amines N1 and N8 to form the $(H_8te3p1a)^+$ species. This was confirmed by the sharp upfield shift of δ_a and later, to a lesser degree, the upfield shift of δ_b and $\delta_{b'}$ (2 > pH > –0.5). However, a slight downfield shift is momentarily observed for one of the resonances (around pH 1). This could be indicative of either the deprotonation of an ammonium centre or the protonation of a second oxygen atom of a phosphonate group. Since the deprotonation of an ammonium is unlikely at this stage without further proton redistribution, the observation may be

explained by the transient formation of a N–H···O hydrogen bond between a deprotonated oxygen atom of one phosphonate group and the proton that is starting to protonate the nitrogen atom N8. This led to the tentative assignment of this resonance to δ_b because of the shorter distance between the corresponding phosphonate arm and N8 (see above).

Finally, the last protonations should occur simultaneously at the deprotonated oxygen atoms of all the phosphonate groups to eventually form the $(H_{11}te3p1a)^{4+}$ species, which was confirmed by the final downfield shift of all the combined resonances ($pH < -0.5$). The assignment of the protonation sites in the lower pH region was supported by the molecular structure of the zwitterionic form of H_8tetp found in the solid state wherein all the amines are protonated and each phosphonate group contains only one proton.[36]

The Thermodynamic Stability of the Metal Complexes

The complexation properties of H_7te3p1a with the ions Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, La^{3+}, Sm^{3+}, Gd^{3+}, Ho^{3+} and Lu^{3+} were studied by potentiometric titrations in an aqueous solution at 298.2 K and at an ionic strength of 0.10 mol dm^{-3} in $[N(CH_3)_4]NO_3$. The equilibria were attained quickly with Ca^{2+}, Cd^{2+} and Pb^{2+}, and relatively quickly with Cu^{2+}; hence, these systems could be studied by conventional "in-cell" titrations. The equilibria with Zn^{2+} and especially with the lanthanides(III), however, were attained slowly in the region of pH 7–10. For these systems, it was thus necessary to perform "out-of-cell" titrations in that pH region and the final equilibrium was attained after ca. 3 weeks of incubation at 298.2 K. Furthermore, the solubility of the heavier lanthanide(III) complexes was impaired due to the formation of insoluble species in the acidic pH region, which forced us to perform all titrations with less than 0.9 equiv. of the metal ion. In the calculation of the stability constants for most of the systems, data from the "out-of-cell" titrations were fitted together with the data from the fully stabilised parts of the conventional titrations. The calculated constants presented higher standard deviation values, notoriously in the case of the lanthanides(III), due to the lower precision of the "out-of-cell" measurements and the smaller number of data points in the decisive pH region. The values of the stability constants of H_7te3p1a and their respective standard deviations, as obtained from fitting the experimental data, are collected in the Supporting Information (Table S2), together with the literature values for the related ligands, H_4teta, H_5te3a1p and H_8tetp. The calculated stepwise constants for all of the ligands are presented in Table 2.[13,14,18,19,24,37–40]

Under the experimental conditions used, only mononuclear complexes were found for all of the metal ions. It is likely that the presence of excess metal ion could lead to the formation of dinuclear complexes, especially in the case of the lanthanides(III) and copper(II). The experimental limitations described above prevented us from using a

Table 2. The stepwise stability constants ($\log K_{MH_iL}$) for the complexes with selected divalent and lanthanide(III) metal ions as determined by potentiometry; T = 298.2 K; I = 0.10 mol dm^{-3} $[N(CH_3)_4]NO_3$.

Ion	Equilibrium quotient[a]	H_7te3p1a	H_4teta	H_5te3a1p	H_8tetp
Ca^{2+}	[ML]/[M][L]	7.47	8.4[b]	6.69[c]	19.33[d]
	[MHL]/[ML][H]	10.96	7.2[b]	8.73[c]	
	[MH_2L]/[MHL][H]	10.01	–	–	–
	[ML]/[MLOH][H]	–	–	10.24[c]	–
	[MLOH]/[$ML(OH)_2$][H]	–	–	11.74[c]	–
Cu^{2+}	[ML]/[M][L]	24.13	21.07[c]	21.58[c]	25.99[c]
	[MHL]/[ML][H]	7.61	3.46[c]	6.85[c]	8.09[c]
	[MH_2L]/[MHL][H]	6.80	2.35[c]	3.36[c]	6.93[c]
	[MH_3L]/[MH_2L][H]	5.89	–	–	5.89[c]
	[MH_4L]/[MH_3L][H]	–	–	–	5.03[c]
	[ML]/[MLOH][H]	10.34	–	11.28[c]	–
Zn^{2+}	[ML]/[M][L]	18.24	17.48[c]	18.16[c]	18.31[c]
	[MHL]/[ML][H]	7.74	4.16[c]	7.15[c]	9.13[c]
	[MH_2L]/[MHL][H]	7.04	3.37[c]	3.53[c]	7.19[c]
	[MH_3L]/[MH_2L][H]	5.73	–	–	6.66[c]
	[MH_4L]/[MH_3L][H]	–	–	–	5.50[c]
	[ML]/[MLOH][H]	10.13	10.80[c]	11.21[c]	11.09[c]
Cd^{2+}	[ML]/[M][L]	17.31	18.0[b]	17.90[c]	16.7[e]
	[MHL]/[ML][H]	8.75	4.04[b]	7.32[c]	–
	[MH_2L]/[MHL][H]	7.74	2.4[b]	3.59[c]	–
	[MH_3L]/[MH_2L][H]	5.92	–	–	–
	[ML]/[MLOH][H]	11.43	–	9.56[c]	–
Pb^{2+}	[ML]/[M][L]	15.37	14.3[b]	13.58[c]	15.5[e]
	[MHL]/[ML][H]	10.55	4.75[b]	8.08[c]	–
	[MH_2L]/[MHL][H]	7.13	4.25[b]	5.27[c]	–
	[MH_3L]/[MH_2L][H]	6.18	–	3.88[c]	–
	[MH_4L]/[MH_3L][H]	4.87	–	–	–
	[ML]/[MLOH][H]	11.74	–	9.86[c]	–
La^{3+}	[ML]/[M][L]	17.0	12.15[f]	12.07[g]	18.02[f]
	[MHL]/[ML][H]	9.7	–	–	9.27[f]
	[MH_2L]/[MHL][H]	8.0	–	–	8.65[f]
	[MH_3L]/[MH_2L][H]	6.9	–	4.12[g]	8.28[f]
	[MH_4L]/[MH_3L][H]	3.2	–	–	6.32[f]
	[MH_5L]/[MH_4L][H]	–	–	–	3.37[f]
	[ML]/[MLOH][H]	9.5	7.58[f]	10.9[g]	10.64[f]
Sm^{3+}	[ML]/[M][L]	18.4	14.15[f]	14.38[g]	19.11[f]
	[MHL]/[ML][H]	9.1	–	6.68[g]	9.63[f]
	[MH_2L]/[MHL][H]	8.0	–	6.44[g]	8.58[f]
	[MH_3L]/[MH_2L][H]	6.6	–	4.02[g]	7.77[f]
	[MH_4L]/[MH_3L][H]	3.3	–	–	6.15[f]
	[MH_5L]/[MH_4L][H]	–	–	–	3.35[f]
	[ML]/[MLOH][H]	9.3	7.37[f]	10.6[g]	8.78[f]
Gd^{3+}	[ML]/[M][L]	19.1	13.77[h]	14.62[g]	–
	[MHL]/[ML][H]	9.4	–	6.55[g]	–
	[MH_2L]/[MHL][H]	7.9	–	6.05[g]	–
	[MH_3L]/[MH_2L][H]	5.8	–	3.96[g]	–
	[MH_4L]/[MH_3L][H]	3.6	–	–	–
	[ML]/[MLOH][H]	10.9	–	10.3[g]	–
Ho^{3+}	[ML]/[M][L]	19.5	15.78[f]	15.21[g]	20.03[f]
	[MHL]/[ML][H]	9.0	–	6.57[g]	9.52[f]
	[MH_2L]/[MHL][H]	7.7	–	5.75[g]	9.25[f]
	[MH_3L]/[MH_2L][H]	6.1	–	3.98[g]	7.58[f]
	[MH_4L]/[MH_3L][H]	2.7	–	–	5.60[f]
	[MH_5L]/[MH_4L][H]	–	–	–	3.10[f]
	[ML]/[MLOH][H]	9.9	7.03[f]	10.5[g]	10.09[f]
Lu^{3+}	[ML]/[M][L]	19.9	15.31[i]	15.49[g]	–
	[MHL]/[ML][H]	8.7	–	6.68[g]	–
	[MH_2L]/[MHL][H]	8.5	–	5.68[g]	–
	[MH_3L]/[MH_2L][H]	7.1	–	3.70[g]	–
	[MH_4L]/[MH_3L][H]	3.8	–	–	–
	[ML]/[MLOH][H]	11.0	–	10.4[g]	–

[a] The charges of the equilibrium species are omitted for clarity. [b] Ref.[37] [c] Ref.[13] [d] The constant corresponding to the equilibrium quotient [MHL]/[M][L][H], ref.[24] [e] Ref.[38] [f] Ref.[18,19] [g] Ref.[14] [h] Ref.[39] [i] Ref.[40] with I = 0.20 mol dm^{-3} $NaNO_3$.

higher metal-to-ligand ratio to check the presence of such species. The value of their constants, however, should be quite low and should be similar to those determined for the transition metal complexes of H_4teta.[41] In addition, for most of the applications of this type of ligand, the metal complex has to be prepared with an excess of the ligand in order to guarantee that full chelation is achieved. Besides the fully deprotonated complex (ML), several protonated species (MHL to MH_4L) exist at an intermediate pH and a hydroxo complex (MLOH), probably corresponding to the deprotonation of a coordinated water molecule, could be found at alkaline pH.

Overall, and with the exception of calcium(II) and cadmium(II), the stability constant values (for the formation of the ML species) showed that H_7te3p1a forms more stable complexes than H_4teta and complexes that are almost as stable as those of H_8tetp. This could have been anticipated from the chemical structure of H_7te3p1a, which is more similar to that of H_8tetp than to that of H_4teta, and is in agreement with the assumption that the stability of this type of complex depends mainly on the basicity of the nitrogen atoms of the macrocycle.[3]

Interestingly, the stability of this calcium(II) complex is lower than that of the H_4teta complex as the complex species started to form in alkaline pH and its abundance was only significant after the complete deprotonation of the ligand. The formation constant values for the calcium(II) protonated species are lower than the first two protonation constants for the amine groups of H_7te3p1a (above 12) but are higher than the highest protonation constant for the phosphonate groups of the ligand (8.08). These values point to the deprotonation of the nitrogen atoms of the protonated complex species, which suggests that the metal ion is initially coordinated by the pendant arms only.

The stability constants for the copper(II) and zinc(II) complexes are very close to those for H_8tetp, especially for zinc(II), which has a very similar stability with all the ligands discussed. The formation constants for the MHL species of these complexes are slightly lower than the first protonation constant ascribed to the phosphonate group of the free ligand (8.08), which indicates that deprotonation of a noncoordinated phosphonate group occurred. The formation constants for the MH_2L and the MH_3L species, however, are slightly higher than those ascribed for the protonations of the other phosphonate groups of the free ligand (6.60 and 5.54, respectively). These unexpected protonation constants for the free ligand are lower, however, because of the existence of a hydrogen bond, as suggested above. Therefore, the higher constants found for the protonated complexes could be due to deprotonation of the noncoordinated phosphonate groups, which suggests that these complexes are formed initially by coordination of the ring amines, and only one or more of the pendant arms are involved in the deprotonation.

For the cadmium(II) and lead(II) complexes, the stability constants are close to those of the H_8tetp complexes, being higher and lower, respectively, and they fit the trend of the stability variation with the four ligands discussed. The formation constants for the MHL species of these complexes are slightly higher than the first protonation constant for the free ligand phosphonate groups (8.08). This indicates that a deprotonation of an amine group occurred, while the values of the formation constants for the more protonated species should correspond to deprotonations of the phosphonate groups.

The stability constants for the H_7te3p1a lanthanide(III) complexes increase along the series, which has already been observed for other tetraazamacrocyclic ligands with acetate and methylphosphonate pendant arms.[19] The values are lower than for those for the H_8tetp complexes but are much higher than those for the H_4teta complexes, which confirmed the importance of the overall basicity of the ligand. The formation constants for the MHL and MH_2L species are generally higher than those for the protonation of the phosphonate pendants of the free ligand. Thus, we assumed that the formation of these complexes occurred through the generally accepted mechanism for the complexation of lanthanide(III) ions by tetraazamacrocyclic ligands with pendant arms containing oxygen donor atoms. In this mechanism, the lanthanide(III) ions are initially captured by the pendant groups and only bind to the amines in the ring upon their deprotonation.[42] Given the high coordination demands of the lanthanide(III) ions, it is expected that all of the pendant arms, as well as the macrocyclic amines of the ligand, will be involved in the coordination of such ions.

The distribution diagrams of the representative species are presented in the Supporting Information (Figures S2 to S9). The distribution diagrams show that, at physiological pH (7.4), the copper(II), zinc(II) and cadmium(II) complexes exist as mixtures of deprotonated, mono- and diprotonated species, while the lead(II) and lanthanide(III) complexes exist mainly as mono- and diprotonated species. This corresponds to species bearing highly negative charges that are not very appropriate for biological use.

Assessment of the efficiency of the ligands in binding metal ions by a simple comparison of the stability constant values could be misleading as they depend to a large degree on the basicity of the ligand, as seen above. For a better comparison of the complexation behaviour of the various ligands, the pM values (–log[M]) for the H_7te3p1a complexes were calculated. These take into account the protonation constants and were calculated at pH 7.4 on the basis of the global set of stability constants describing each system. These values, which express the amount of free metal ion in solution at this pH, are presented in Table 3, together with the literature values for the other ligands.[13,14]

Several conclusions can be drawn from the calculated pM values. For the calcium(II) complexes, most of the metal ion is free in solution, which was expected since the complex species are only formed in significant quantities at a pH higher than 7.4. For the copper(II), zinc(II) and cadmium(II) complexes, H_7te3p1a seems to be less efficient than the other ligands in most cases, which is probably because there are more donor groups than the expected coordination number of these metal complexes. However, the copper(II) complex of H_7te3p1a has a pCu value much

Table 3. The calculated pM[a] values for the metal complexes at pH 7.4.

Ion	H_7te3p1a	H_4teta	H_5te3a1p	H_8tetp
Ca^{2+}	5.00	5.00[b]	5.01[b]	–[c]
Cu^{2+}	13.71	14.20[b]	14.79[b]	14.45[b]
Zn^{2+}	7.96	10.61[b]	11.46[b]	7.87[b]
Cd^{2+}	8.28	11.15[b]	11.26[b]	–[c]
Pb^{2+}	7.82	7.45[b]	7.45[b]	–[c]
La^{3+}	9.24	6.52[d]	5.67[d]	9.69[d]
Sm^{3+}	9.97	8.71[d]	7.56[d]	10.65[d]
Gd^{3+}	10.79	–[c]	7.78[d]	–
Ho^{3+}	10.69	10.67[d]	8.37[d]	11.99[d]
Lu^{3+}	11.60	–[c]	8.67[d]	–

[a] The values were calculated for 100% excess of the ligand and were based on the stability constants collected in Table S2 at a concentration of $c_M = 1.00 \times 10^{-5}$ mol dm^{-3}. [b] Ref.[13] [c] Not meaningful due to the incomplete set of constants available. [d] Ref.[14]

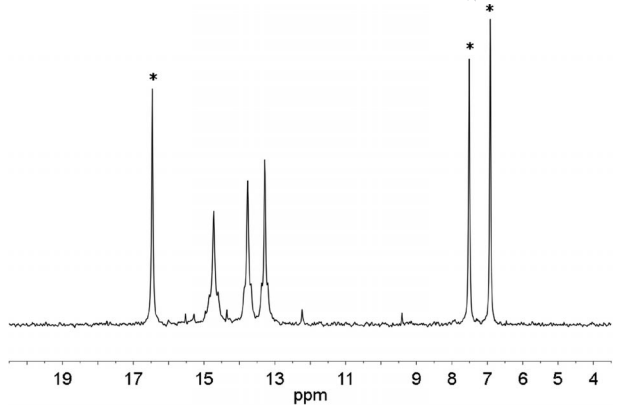


Figure 2. The $^{31}P\{^1H\}$ NMR spectrum of the Cd^{2+} complex of H_7te3p1a at pH 9.9, with excess ligand present (the ligand peaks are labelled with *).

closer to that of the other complexes, which is probably due to the plasticity of this metal ion that allows it to adopt a more distorted geometry. The pM values for the lead(II) and lanthanide(III) complexes, however, were closer to that of H_8tetp and higher than those for the other ligands, as shown by their stability constants. These conclusions indicate that the stability constant values alone must be analysed with care, and that any detailed comparison between ligands of different basicity must be made with regard to the pH range of interest. Ultimately, the pM values demonstrated that H_7te3p1a is a very efficient ligand for the complexation of all the metal ions studied, at physiological pH, except for calcium(II).

Spectroscopic Studies of the Zinc(II) and Cadmium(II) Complexes

In an attempt to understand the coordination modes of the metal complexes of H_7te3p1a, NMR spectroscopic studies of the Zn^{2+} (^1H, ^{13}C and ^{31}P) and the Cd^{2+} (^{31}P) complexes were carried out in an alkaline aqueous solution where the complexes are present mostly as the ML species.

The spectrum of the cadmium(II) complex that was acquired at pH 9.9 (Figure 2) shows three well-resolved resonances assigned to three nonequivalent phosphorus nuclei (at δ = 13.3, 13.8 and 14.7 ppm). All of the resonances are shifted relative to those of the free ligand. Additionally, all three resonances exhibit satellite signals ($^2J_{P,Cd}$ values are 20.2 Hz, 15.3 Hz and 13.8 Hz for the 14.7 ppm, 13.8 ppm and 13.3 ppm resonances, respectively) that arise from the coupling to the $^{111/113}$Cd isotopes. Therefore, only one conformational isomer of the complex was present in solution and it involved coordination of the metal ion to all three of the phosphonate groups. The coordination number of the metal ion should be seven if all of the nitrogen atoms and the three phosphonate pendant arms are coordinated. The most probable conformation of the cyclam skeleton for this coordination mode is the type **I** conformation;[43] the same one was also suggested as the most probable conformation for the zinc(II) complex (see below).

The spectra of the zinc(II) complex proved to be more complicated due to the possible presence of more than one isomer in solution. Throughout most of the alkaline pH range, the $^{31}P\{^1H\}$ spectra generally showed several broad and unresolved resonance signals together with some sharper ones. In order to elucidate the structure of the major species/isomer of the zinc(II) complex with H_7te3p1a in solution, a series of NMR spectroscopic measurements were performed on a sample prepared by prolonged heating of the metal ion/ligand mixture in order to ensure that the final complex isomer was present. The sample was prepared at a pH of ca. 9.5 since at this pH the major species was at maximum abundance (after heating the sample). The 1D ^1H, $^{13}C\{^1H\}$ and ^{31}P NMR spectra, together with the 2D H,H-COSY, H,H-NOESY, H,C-HMBC and H,P-HMQC correlations, were acquired and the ^{31}P T_1/T_2 relaxation times were determined.

The $^{31}P\{^1H\}$ NMR spectra exhibit three major signals, which have relative intensities of 1:1:1 and chemical shifts of 16.32, 16.86 and 17.09 ppm (Figure 3), together with some other minor signals, whose total intensity is ca. 15% and that probably correspond to other isomers present in the solution. This pattern did not change with additional heating of the solution. The relaxation time (Table 4) measurements indicated that the signals at δ_P = 16.32 and 16.86 ppm should correspond to phosphonate groups that have a similar chemical environment whereas the third signal exhibits significantly different values. This indicated that one of the phosphonate groups is coordinated to the central ion and the remaining two groups are not, or vice versa.

In the $^{13}C\{^1H\}$ NMR spectra, the carboxyl group signal appears at δ = 178.7 ppm compared to 179.8 ppm for the free ligand at pH 8.5, which indicates a noncoordinated acetate pendant arm. This is in agreement with the assumption that the carboxylate group is not coordinated in the presence of the more basic and fully deprotonated phosphonate pendant arms.

The ^1H NMR spectra shows a set of barely resolved signals, except for the protons of the $-CH_2-$ groups of the pendant arms that are discussed in the following text. The

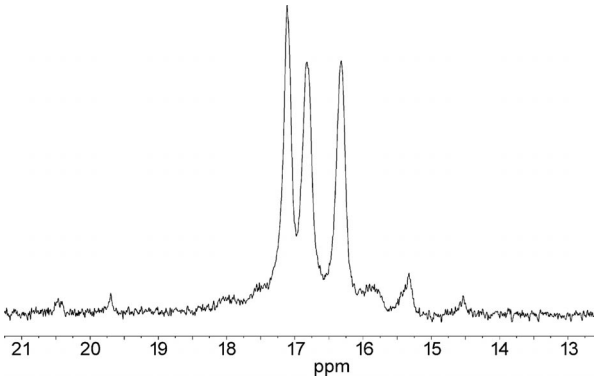


Figure 3. The $^{31}P\{^{1}H\}$ NMR spectrum of the H_7te3p1a zinc(II) complex at pH ≈ 9.5.

Table 4. The NMR relaxation times of the phosphorus atoms in the H_7te3p1a Zn^{2+} complex at pH ≈ 9.5.

Relaxation time	NMR chemical shift of the phosphorus atom (ppm)		
	16.32	16.86	17.09
T_1 [s]	0.66 ± 0.01	0.63 ± 0.04	0.77 ± 0.01
T_2 [ms]	23 ± 2	19 ± 4	48 ± 2

presence of an AB spin system for the $–CH_2COOH$ pendant arm (3.24 and 3.42 ppm, $J_{A,B}$ = 16.5 Hz; confirmed by the H,C-HMBC correlation spectra) and an ABX spin system for the $–CH_2PO_3H_2$ pendant arms (2.64 and 2.75 ppm, $J_{A,B} \approx J_{A,X} \approx J_{B,X} \approx$ 14 Hz; 2.60 and 2.74 ppm, $J_{A,B} \approx J_{A,X} \approx J_{B,X} \approx$ 14 Hz; 2.56 and 2.78 ppm, $J_{A,B} \approx J_{A,X} \approx J_{B,X} \approx$ 14 Hz, determined from the H,P-HMQC correlation spectrum, Figure S13 in the Supporting Information) indicated the coordination of all of the nitrogen atoms bearing the pendant arms. The proton signals for the acetate pendant arm show two sets of cross-peaks in the H,H-NOESY spectrum. The signal at δ = 3.04 ppm shows interaction with signals at 2.87, 2.78, 2.75 and 2.68 ppm, and the signal at 3.24 interacts with the signals at δ = 3.09, 2.78 and 2.75 ppm (Figure S12). The H,H-COSY spectrum indicates that the signals at δ = 2.87 and 2.68 ppm should be assigned to the protons of the propylenediamine fragment of the cyclam ring close to the acetate group and the signal at δ = 3.09 ppm belongs to one of the protons of the ethylenediamine fragment close to the acetate pendant arm. The signals at δ = 2.75 and 2.78 ppm are assigned to the protons of the $–CH_2PO_3^{2-}$ side arms: the former one to the phosphonate group at δ_P = 16.86 ppm and the latter one to that at δ_P = 17.09 ppm. Contacts of the acetate arm with two methylphosphonate groups were observed in the H,H-NOESY spectra, which suggested that only type **I** or **II** structures[43] can represent the complex in solution since in the remaining types, either contacts to only one pendant arm (type **III** and **IV**)[43] or no contact to any pendant arm (type **V**)[43] would be observed. The five possible spatial arrangements of the pendant arms together with the chelate ring conformations involving the macrocycle are shown in Figure 4. Based on the literature data, we can exclude the arrangement represented by the structure of type **II** as it is very rare[2] and conclude that type **I** represents the structure of the zinc(II) complex in solution.

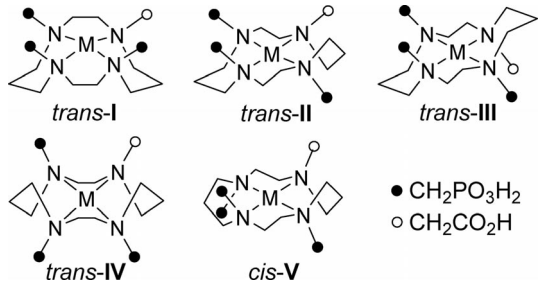


Figure 4. The isomers of the H_7te3a1p complexes.

Several coordination modes are still possible, however, because any of the four pendant arms might be coordinated. The species with three or four arms coordinated may be excluded since coordination numbers higher than six for zinc(II) complexes are very rare and thus only the isomers where one or two pendant arms are involved in the coordination are possible. As stated above, coordination of the acetate arm (bound to N1, see numbering in Scheme 3) was excluded on the basis of its ^{13}C NMR chemical shift; thus, only the phosphonate groups can be considered for coordination. The difference in the ^{31}P relaxation times of the phosphonate arms neighbouring the acetate group (δ = 16.86 and 17.09 ppm) indicates that one of them is coordinated whereas the other one is not (the values of the ^{31}P relaxation times for the free ligand are: T_{11} = 1.64 ± 0.03, T_{12} = 1.33 ± 0.03, T_{13} = 1.33 ± 0.01 s, and T_{21} = 39.1 ± 0.8, T_{22} = 45.5 ± 1.0, T_{23} = 50.8 ± 0.8 ms). We assumed that, for steric reasons, simultaneous coordination of the neighbouring phosphonates (either bound to N4/N8 or N8/N11) was highly improbable for the type **I** isomer. As the simultaneous coordination of the distant arms (bound to N4 and N11) is excluded by the difference in their relaxation times, we concluded that only one of the pendant arms is coordinated to the central zinc(II) atom. The modelling results indicated that the N4-bound phosphonate had to be coordinated as only then would the interactions that resulted in the observed NOE H,H cross-peaks be possible. If the N8-bound pendant arm was coordinated, the respective protons would be too far away from the acetate protons and would be shielded by the oxygen atom of the phosphonate group.

From the findings described above, it is evident that, under the conditions of the measurements, the macrocyclic framework is folded in the type **I** conformation and only one of the pendant arms, the N4-bound methylphosphonate one, is coordinated. Thus, it can be suggested that the coordination number of the central metal ion is five and the sixth possible coordination site remains unoccupied. The simulated structure is depicted in the Supporting Information (Figure S14). This is the most common conformation for the complexes of *N*,*N*′,*N*″,*N*‴-tetrasubstituted cyclam derivatives,[2,44] and their divalent metal complexes

presenting another ligand conformation, for example *trans*-**III**, are directly accessible only under special conditions.[44–46]

Conclusions

A new macrocyclic ligand that combines methylphosphonate and acetate pendant arms, H_7te3p1a, was synthesised from cyclam by a convenient triprotection methodology. The compound presented a very high overall basicity, which was between that of H_4teta and H_8tetp, and was closer to the latter ligand, as expected. The protonation of H_7te3p1a proceeds in a very dynamic sequence with translocation of the protons between several basic sites and with the protonated species stabilised by intramolecular hydrogen bonds, as demonstrated by ^{31}P NMR spectroscopy. The ligand forms complexes of high thermodynamic stability with the lanthanide(III) and zinc(II) ions, but the formation of these complexes is rather slow. A fast complexation was observed for the copper(II) and calcium(II) complexes. The copper(II) complex shows very high thermodynamic stability while that of calcium(II) exhibits a low stability. A greater selectivity for copper(II) than for zinc(II) and the lanthanides(III) was evident from the large difference in the values of their stability constants. The NMR spectra for the cadmium(II) and zinc(II) complexes, which present similar thermodynamic stability, indicated that while all three of the phosphonate arms are coordinated to cadmium(II), only one methylphosphonate group is coordinated to zinc(II) in the major isomer that was observed at a slightly alkaline pH, which thus supports the pentacoordination of the zinc(II) centre. It was shown that the acetate pendant arm was not coordinated to zinc(II) in the complex and should not be bound to the metal ion in the other divalent metal ion complexes either, except in the case of the lead(II) complex. Overall, the coordination ability of the new ligand is quite good compared to related ligands, and the advantage of H_7te3p1a over H_8tetp is that it forms less charged complexes and has better solubility in the case of the lanthanide(III) complexes.

Experimental Section

General: 1,4,8,11-Tetraazacyclotetradecane (cyclam)[47,48] and 1,4,8-tris(trifluoroacetyl)-1,4,8,11-tetraazacyclotetradecane[15] (**1**) were synthesised by published methods. Paraformaldehyde was filtered from aged solutions of formaldehyde and was dried in a desiccator with concentrated sulfuric acid. All the other chemicals were used as obtained from the commercial sources. The organic solvents were dried by standard methods.[49] Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminium sheets and detection was by ninhydrin spray, Dragendorff's reagent or by dipping in a 5% $CuSO_4$ aq. solution. The ^{1}H, ^{13}C{^{1}H}, ^{31}P and ^{31}P{^{1}H} NMR spectra were recorded with Varian VNMRS300 (299.940 MHz for ^{1}H), Varian INOVA^{UNITY} 400 (399.951 MHz for ^{1}H), Bruker Avance III 400 (400.135 MHz for ^{1}H) or Bruker Avance III 600 equipped with an Inverse TCI 5 mm H/C/N/D CryoProbe (600.174 MHz for ^{1}H) spectrometers, at a probe temperature of 298.2 K, unless otherwise noted. The chemical shifts (δ) are given in ppm and the coupling constants (J) in Hz. For the ^{1}H and ^{13}C NMR spectra, *tert*-butyl alcohol was used as the internal reference, while for the ^{31}P NMR spectra, H_3PO_4 was used as the external reference (inner-capillary method). The resonance assignments are based on peak integration, peak multiplicity and 2D correlation experiments. The electrospray ionisation mass spectra (ESI-MS) and the elemental analyses were carried out by the ITQB Analytical Services.

11-[(*tert*-Butyloxycarbonyl)methyl]-1,4,8-tris(trifluoroacetyl)-1,4,8,11-tetraazacyclotetradecane (2): Compound **1** (2.44 g, 5 mmol) was dissolved in acetonitrile (40 mL) and potassium carbonate (1.38 g, 10 mmol) was added. *Tert*-butyl bromoacetate (0.89 mL, 6 mmol) was then added in one portion and the mixture was heated to ca. 333 K under a nitrogen atmosphere for 4 d. The mixture was evaporated to dryness and then dissolved in chloroform (100 mL), the solids were filtered off and washed with chloroform (2 × 50 mL). The combined organic solutions were dried (anhydrous $MgSO_4$), filtered, evaporated to dryness and dried under vacuum to yield **2** in the form of a yellowish oil (2.6 g, 86%) that was judged pure by TLC (R_f = 0.6 in $CHCl_3$/EtOAc, 1:1). ESI-MS: m/z = 546.6 [M – *t*Bu + 2 H]$^+$, 602.6 [M + H]$^+$, 624.9 [M + Na]$^+$.

1-[(*tert*-Butoxycarbonyl)methyl]-1,4,8,11-tetraazacyclotetradecane (3): Compound **2** (2.47 g, 4.1 mmol) was suspended in a solution of potassium carbonate (400 mL, 10% w/v solution in methanol/water, 5:2). The mixture was vigorously stirred in a closed flask at room temperature for 3 d, during which time the solids completely dissolved. The solution was evaporated to dryness and then dissolved in dichloromethane (100 mL), the solids were filtered off and washed with dichloromethane (2 × 50 mL). The combined organic solutions were dried (anhydrous $MgSO_4$), filtered, evaporated to dryness and dried under vacuum to yield **3** in the form of a yellow oil (1.2 g, 93%) that was judged pure by TLC (R_f = 0.1 in EtOH/25% aq. NH_3, 10:1). ESI-MS: m/z = 258.8 [M – *t*Bu + 2 H]$^+$, 314.8 [M + H]$^+$.

11-[(*tert*-Butyloxycarbonyl)methyl]-1,4,8-tris[(diethoxyphosphoryl)-methyl]-1,4,8,11-tetraazacyclotetradecane (4): Compound **3** (1.10 g, 3.5 mmol) was suspended in triethyl phosphite (10.8 mL, 63 mmol) and a small volume (ca. 2 mL) of dry chloroform was added to the mixture to achieve complete dissolution. The solution was heated to ca. 333 K in a flask equipped with an anhydrous $CaCl_2$ drying tube, paraformaldehyde (0.63 g, 21 mmol) was then added in small portions over 48 h, and the mixture was heated for an additional 48 h. The resulting solution was filtered, evaporated to dryness and co-evaporated with toluene (4 × 50 mL) to remove the volatiles. The oil was dissolved in chloroform (50 mL) and the organic phase was extracted with water (2 × 25 mL) and then evaporated to dryness. The product was dissolved in minimal water/ethanol (ca. 5 mL, 1:1) and loaded onto a weak cationic exchange column (Amberlite CG-50, 20 × 2.5 cm, H^+ form), which had been conditioned with the water/ethanol (1:1) solvent system. The column was first eluted with the previous solvent (300 mL) and then with a 25% acetic acid solution in water/ethanol (1:1) (300 mL). After evaporation, the neutral fractions contained only a small amount of the non-ammonic byproducts, while the combined and vacuum dried acidic fractions yielded 2.5 g of a yellow oil containing **4**, which was contaminated with less than 20% of an aminal byproduct with only two (diethoxyphosphoryl)methyl pendant arms. This product was used directly in the next step without further purification. ESI-MS: m/z = 626.7 $(C_{27}H_{57}N_4O_8P_2)^+$ (byproduct), 764.5 [M + H]$^+$. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 24.56 (s, byproduct), 26.54 (s, 2P), 26.67 (s, 1P) ppm.

[4,8,11-Tris(phosphonomethyl)-1,4,8,11-tetraazacyclotetradec-1-yl]acetic Acid (H_7te3p1a): The reaction mixture containing **4** (2.40 g, ca. 2.5 mmol) was dissolved in aqueous HCl (20%, 50 mL) and the solution was heated under reflux for 48 h. After cooling, the solution was filtered, evaporated to dryness and co-evaporated with water (3 × 50 mL) to remove the excess acid. The resulting oil was dissolved in minimal water (ca. 5 mL), loaded onto a strong cationic exchange column (Dowex 50WX4, 15 × 3 cm, H^+ form) and eluted with water (300 mL) followed by a 5% aq. ammonia solution (300 mL). The water fractions contained only inorganic impurities, while the combined alkaline fractions contained the macrocyclic compounds and were evaporated to dryness and co-evaporated with water (3 × 100 mL) to remove the excess ammonia. The resulting oil was dissolved in water (ca. 5 mL), loaded onto a weak cationic exchange column (Amberlite CG-50, 20 × 2.5 cm, H^+ form) and eluted with water (500 mL) in fractions of ca. 50 mL. The fractions containing the desired compound were combined and the solution was treated with charcoal (1 g), filtered, concentrated to ca. 50 mL and added dropwise, with vigorous stirring, to anhydrous ethanol (250 mL). The white solid was filtered off, washed with ethanol (2 × 50 mL) and dried under vacuum to yield 1.2 g of H_7te3p1a·2H_2O (59% based on **3**). $C_{15}H_{37}N_4O_{12}P_3$ (576.41): calcd. C 31.26, H 6.82, N 9.72; found C 31.00, H 6.79, N 9.83. ESI-MS: *m*/*z* = 539.2 [M – H]$^-$. ^{1}H NMR (400 MHz, KOH in D_2O, pD = 6.4): δ_H = 1.95 (m, 2 H, CH_2-CH_2-CH_2), 2.01 (m, 2 H, CH_2-CH_2-CH_2), 2.89 (d, $^2J_{HP}$ = 11.3 Hz, 2 H, N-CH_2-P), 2.96 (d, $^2J_{HP}$ = 11.2 Hz, 4 H, N-CH_2-P), 3.01 (br. t, 2 H, N-CH_2-CH_2), 3.08 (br. t, 2 H, N-CH_2-CH_2), 3.15 (m, 4 H, N-CH_2-CH_2), 3.21 (m, 4 H, N-CH_2-CH_2), 3.30 (br. t, 2 H, N-CH_2-CH_2), 3.33 (br. t, 2 H, N-CH_2-CH_2), 3.40 (s, 4 H, N-CH_2-COOH) ppm. ^{13}C NMR (101 MHz, KOH in D_2O, pD = 6.4): δ = 23.0 (s, 1C, CH_2-*C*H_2-CH_2), 23.3 (s, 1C, CH_2-*C*H_2-CH_2), 50.0, 50.8, 50.9, 51.1, 51.4, 51.5, 51.8, 51.9, 52.0, 52.2, 52.3, 52.6, 53.5, 53.6 (N-*C*H_2-*C*H_2 and N-*C*H_2-P), 57.7 (s, 1C, N-*C*H_2-COOH), 177.0 (s, 1C, *C*OOH) ppm. ^{31}P NMR (162 MHz, KOH in D_2O, pD = 6.4): δ_P = 11.52 (br. s), 11.76 (br. s), 13.95 (br. s) ppm. For the complete NMR assignment of all the nuclei at pH 8.5, see the Supporting Information (Table S3).

Thermodynamic Stability Measurements

Preparation of the Reagents and Solutions: The purified water was obtained from a Millipore Milli-Q demineralisation system. A stock solution of H_7te3p1a was prepared at ca. 1.5 × 10^{-3} mol·dm^{-3}. [N(CH_3)$_4$]NO_3 was prepared by neutralisation of a commercial [N(CH_3)$_4$]OH solution with HNO_3. The metal ion solutions were prepared in water at 0.025 to 0.050 mol dm^{-3} from analytical grade nitrate salts of the metals [kept in excess HNO_3 in the case of the lanthanide(III) ions], and were standardised by titration with Na_2H_2edta.[50] Carbonate-free solutions of the titrant [N(CH_3)$_4$]OH were obtained at ca. 0.10 mol dm^{-3} by treating freshly prepared silver oxide with a solution of [N(CH_3)$_4$]I under a nitrogen atmosphere, were standardised by application of Gran's method[51] and were discarded as soon as the carbonate concentration reached ca. 1% of the total amount of the base. For the back titrations, a 0.100 mol dm^{-3} standard solution of HNO_3 was prepared from a commercial ampoule.

Potentiometric Measurements: The potentiometric setup used for conventional titrations has been previously described.[13] The measurements were carried out at 298.2 ± 0.1 K in solutions with an ionic strength that was maintained at 0.10 ± 0.01 mol dm^{-3} with [N(CH_3)$_4$]NO_3. Atmospheric CO_2 was excluded from the cell during the titrations by passing purified N_2 across the top of the experimental solutions. The [H^+] of the solutions was determined by measurement of the electromotive force of the cell, $E = E'^{\circ} + Q \times \log[H^+] + E_j$. The term pH is defined as $-\log[H^+]$. E'° and Q were determined by titration of a solution with a known H^+ concentration at the same ionic strength in the acidic pH region. The liquid-junction potential, E_j, was found to be negligible under the experimental conditions used. The value of $K_w = [H^+] \times [OH^-]$ was found to be equal to $10^{-13.80}$ mol^2 dm^{-6} by titrating a solution with a known H^+ concentration at the same ionic strength in the alkaline pH region, considering E'° and Q valid for the entire pH range. The measurements during the conventional titrations were carried out with ca. 0.04 mmol of the ligand in a total volume of ca. 30 mL, both in the absence of the metal ions and in the presence of each metal ion at ca. 0.9 equiv. relative to the ligand. For all metal ions, except calcium(II), a back titration was performed at the end of each direct titration in order to check if equilibrium had been attained throughout the pH range. Each titration curve typically consisted of 60–70 points in the 2.5 to 11.5 pH region and a minimum of two replicate titrations were performed for each system. In addition, the "out-of-cell" titrations were performed for the zinc(II) and lanthanide(III) ion systems and consisted of 10 to 12 points in the 7–10 pH region. Each sample was prepared in separate vials under the same conditions as for the conventional titrations but with a final volume 10 times smaller. After addition of the base, the vials were tightly closed and kept at 298.2 K for 20 d, at which time they had attained equilibrium and the electromotive force was then measured with a Metrohm 6.0210.100 combined pH electrode, which had been previously calibrated by an "in-cell" titration.

NMR Spectroscopic Measurements: In order to determine the two highest protonation constants of the ligand, the ^{31}P{^{1}H} NMR spectra of H_7te3p1a in water were recorded at 298.2 K at 16 pH points over the region of pH 9 to 14. A ligand solution was prepared at an ionic strength of 0.010 mol dm^{-3} and the titrant, a 25% (w/w) aqueous solution of [N(CH_3)$_4$]OH, was obtained from Aldrich and was standardised by titration with a 1.0 mol dm^{-3} HNO_3 solution. The titration was carried out in a closed titration cell and the titrant was added with a Crison microBU 2031 automatic burette. The pH was measured with an Orion 420A measuring instrument that was fitted with a Metrohm 6.0210.100 combined electrode, which had been calibrated with commercial buffer solutions with the standard pH (at 298.2 K) of 7.96 and 11.88. Atmospheric CO_2 was excluded from the cell during the titration by passing purified nitrogen across the top of the experimental solution. The measurement was carried out with 0.03 mmol of ligand in a total volume of ca. 5 mL and the ionic strength of the titration solution was kept at 0.50 mol dm^{-3} with [N(CH_3)$_4$]NO_3. Following each addition of titrant, the pH was measured after equilibration and a sample of the solution was placed in a 5 mm NMR tube, which had been adapted with an internal capillary tube containing D_2O and H_3PO_4 for locking and referencing purposes. After recording the ^{31}P{^{1}H} NMR spectra, the sample volume was returned to the titration cell.

Calculation of Equilibrium Constants: The calculation of the overall equilibrium constants $\beta_{M_mH_hL_l}$ (where $\beta_{M_mH_hL_l} = [M_mH_hL_l]/[M]^m \times [H]^h \times [L]^l$ and $\beta_{MH_{-1}L} = \beta_{ML(OH)} \times K_w$) was achieved by fitting the potentiometric data from the titrations with the HYPERQUAD program.[52] The spectroscopic data from the ^{31}P NMR titration in water were used to calculate the overall constant for the two highest protonations of H_7te3p1a at ionic strength of 0.50 mol dm^{-3} by fitting the data with the HYPNMR program.[53] The obtained value was then extrapolated to an ionic strength of 0.10 mol dm^{-3} by using the Davies equation,[54] and this adjusted value was used as a constant in the calculation of the remaining protonation constants for the ligand. The species distribution dia-

grams were plotted from the calculated constants with the HYSS program.[55] The difference, in log units, between the values for the protonated (or hydrolysed) and the non-protonated constants provided the stepwise (log *K*) protonation constants (where $K_{M_mH_hL_l} = [M_mH_hL_l]/[M_mH_{h-1}L_l] \times [H]$). The errors quoted are the standard deviations of the overall stability constants calculated by the fitting program from all the experimental data for each system.

Spectroscopic Measurements: A $^{31}P\{^1H\}$ NMR titration of H_7te3p1a in the region of pH –1 to 9 was performed in addition to that described in the previous thermodynamic stability measurements section (between pH 9–14). It was performed in a similar way except that $[N(CH_3)_4]OH$ and then conc. HNO_3 aq. solutions were used as the titrants, without rigorous control over the volume of the titrant that was added and the ionic strength. A solution of the H_7te3p1a Cd^{2+} complex was prepared in water at ca. 4×10^{-3} mol dm^{-3} of the ligand in a 1:2 metal to ligand ratio for the $^{31}P\{^1H\}$ NMR studies. The pH was adjusted to 9.9 by the addition of dilute $[N(CH_3)_4]OH$ and the solution was heated overnight at 353 K. An internal capillary tube containing D_2O and H_3PO_4 was used for locking and referencing purposes during the acquisition of the spectra. The solutions of the H_7te3p1a Zn^{2+} complex for the multinuclear NMR studies were prepared in D_2O. H_7te3p1a (30 mg) was dissolved in D_2O (0.5 mL) and the pD was adjusted to ca. 8 by means of a 2 mol dm^{-3} solution of KOH in D_2O. Zinc(II) chloride tetrahydrate (15 mg, 1.3 equiv.) was added to the solution and the pD was adjusted to ca. 9.5 (KOH in D_2O). The solution was transferred to a Teflon®-cap-sealed vial and heated at 353.2 K for 3 d (until no further change in the ^{31}P NMR pattern was observed).

Supporting Information (see footnote on the first page of this article): Table of the overall protonation and stability constants for the ligand H_7te3p1a and its metal complexes, a colour version of the ^{31}P NMR spectroscopic titration of H_7te3p1a, the species distribution diagrams for the free ligand and its complexes, the H,P-HMQC NMR spectrum as well as the signal assignments for the ^1H and ^{13}C NMR resonances of the free ligand, the ^1H, H,H-NOESY and H,P-HMQC NMR spectra of the H_7te3p1a zinc(II) complex and a representation of the suggested solution structure for the $[Zn(te3p1a)]^{5-}$ complex.

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