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From Flexible to Constrained Tris(tetraamine) Ligands: Synthesis, Acid–Base Properties, and Structural Effect on the Coordination Process with Nucleotides

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This work presents the synthesis and characterization of **L2** and **L3**, which formed with the previously described ligand **L1** a family of three tris(tetraamine) ligands based on a mesitylenyl spacer. The trimeric ligands differ from one to the other by the nature of their tetraamine moieties: triethylenetetraamine (**L1**), cyclen (**L2**), and constrained cyclen (**L3**). The impact of the rigidification of the structure from **L1** to **L3** on the acid–base properties is investigated, and the behavior of the ligands towards triphosphate (TriP), adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) anion receptors is explored by potentiometric investigations. To characterize and understand the driving forces implicated in the supramolecular assemblies, results are supported by ^{1}H and ^{31}P NMR measurements performed over a wide pH range. ATP appears as the most complexed anion and particular attention is given to comparison with its inorganic triphosphate analogue to highlight the contribution of π-stacking interactions. Results unambiguously show the various coordination scheme induced by the structure of the ligands.

Introduction

Anions have a great relevance from a biological point of view since a large number of cofactors and substrates involved in biology are of anionic nature.[1] Anion recognition came up as a privileged topic with the growing importance of supramolecular chemistry these last decades. Adenosine 5′-triphosphate (ATP) is a ubiquitous substrate for many biological reactions and generally regarded as an intracellular energy donor.[2] In consequence, studies focusing on the recognition of nucleotides and especially ATP has increased over the last years.[3] Generally, the binding forces are mainly based on electrostatic interactions, and the association constants may possibly be enhanced by providing additional binding elements as hydrogen-bonding or π-stacking interactions. Among the various hosts presented in the literature,[4] polyamines and especially protonated polyamines are of special interest for anionic guest complexation.[5] For charged synthetic polyamine studies, it is particularly important to determine the actual degree of protonation at a given pH and the distribution of the pH centers. Polycyclic or polylinear tetraamine ligands presenting an aromatic spacer were established in our previous investigations as relevant hosts for inorganic phosphate anions and nucleotides such as ATP. Ditopic linear octaamines proved to be highly protonated and flexible receptors in neutral medium[6] and cyclen-based bismacrocycles proved to be rigid but more selective ligands for binding of ATP or polyphosphates.[7] Furthermore, polytopic proton-sponge ligands, based on cross-bridged cyclens, were presented as constrained but efficient receptors. In continuation of these works focusing on the design of innovative anions receptors,[6,7] we present here the synthesis of tritopic tetraamine ligands with a mesitylenyl spacer followed by an acid–base study and their interaction with polyphosphate nucleotides by NMR and potentiometric investigations. The trimeric derivatives described here consist of three parent ligands that differ from one another by the structure of their tetraamine moieties: triethylenetetraamine for **L1**, cyclen for **L2**, and reinforced cyclen for **L3** (Scheme 1). The linear, cyclic, or constrained nature of the tetraamine moiety confers to the ligands different structures and consequently different acid–base properties, leading to various interactions with anionic substrates. The aim of the study is then to appreciate the influence, from flexible to rigid trimeric structures, on the coordination process. The binding interactions are studied with three nucleotides, namely, AMP (adenosine monophosphate), ADP (adenosine diphosphate), and ATP (adenosine triphosphate), and one inorganic anion: triphosphate ($P_3O_{10}^{5-}$).

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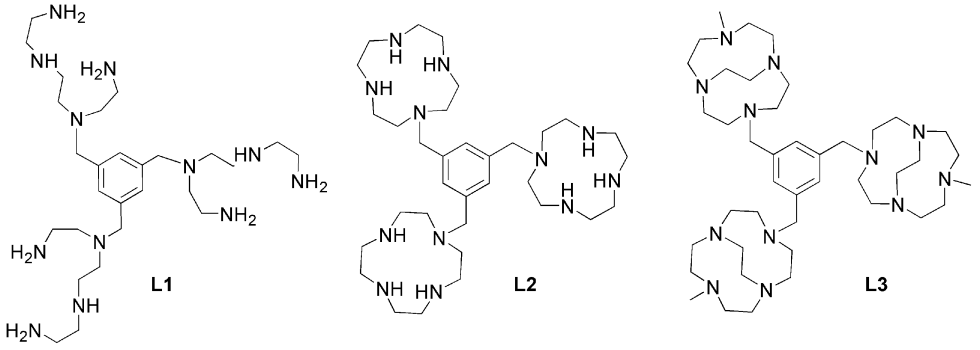
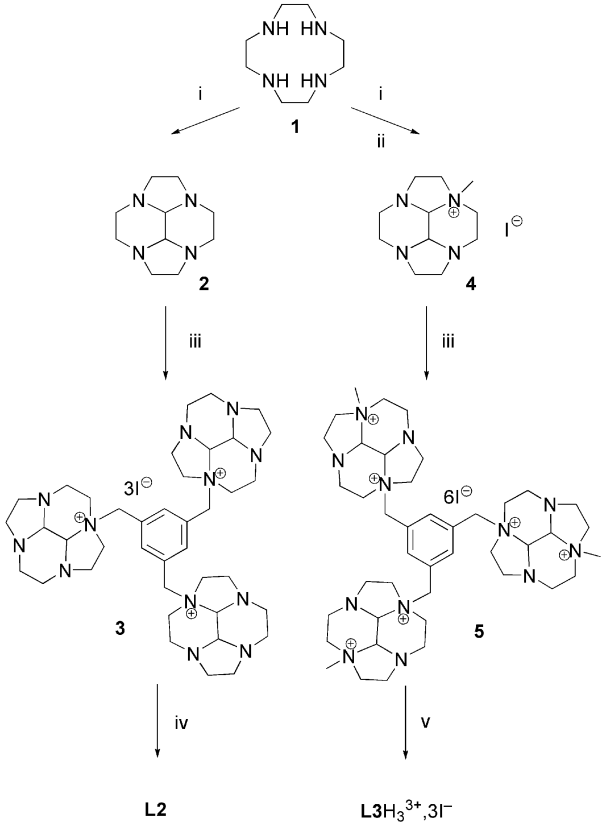


Scheme 1. From flexible to constrained tris(tetraamine) ligands.

Results and Discussion

Synthesis

The three azaligands were obtained by following the bisaminal methodology (see Scheme 2 for **L2** and **L3** and ref.[8] for **L1**), which presents the advantage of higher yields by following similar routes to the focused polyazaligands (polylinear,[8] polycyclic,[9,10] and constrained ligands[11]) through the selective *N*-alkylation of linear or cyclic tetraamines. Synthesis of **L1** was described previously.[8] Macrocyclic bisaminal **2** is cleanly obtained from cyclen **1** and leads easily to **4** by reaction with iodomethane (1 equiv.) following a previously described procedure.[9] Reaction of tris-electrophile 1,3,5-tris(iodomethyl)benzene (1 equiv.) with **2** and **4** leads to tris-salts **3** and **5**, respectively. Ligand **L2** is then obtained after treatment of **3** with hydrazine monohydrate, and **L3** is obtained by cleavage of the bisaminal bridges with $NaBH_4$ in absolute ethanol,[12] respectively, in 82 and 62% overall yield. Ligand **L3** was isolated as its LH_3^{3+} form, which is consistent with previous works presenting constrained ligands as proton sponges.[13]



Scheme 2. Synthesis of **L2** and **L3**. Reagents and conditions: (i) Glyoxal, MeOH; (ii) MeI, THF; (iii) 1,3,5-tris(iodomethyl)-benzene (1/3 equiv.), CH_3CN; (iv) NH_2NH_2,H_2O; (v) $NaBH_4$, EtOH (abs.).

Ligand Protonation

The protonation equilibria of the three ligands were investigated in aqueous solution at 25 °C by potentiometric pH (–log[H^+]) measurements. The logarithms of the stepwise protonation constants for the three studied ligands, obtained from mathematical treatment of their respective potentiometric titration data, are presented in Table 1.

Table 1. Logarithm of protonation constants of tritopic ligands [H_2O; I = 0.1 M (NaCl); T = 25 °C].

Equilibrium[a]	**L1**[b]	**L2**[b]	**L3**[b]
L + H = LH	10.55 (3)	11.00 (1)	–
LH + H = LH_2	9.85 (2)	9.80 (1)	–
LH_2 + H = LH_3	9.38 (2)	9.21 (1)	–
LH_3 + H = LH_4	8.71 (3)	8.30 (1)	4.60 (1)
LH_4 + H = LH_5	8.26 (1)	8.20 (1)	4.26 (1)
LH_5 + H = LH_6	6.63 (2)	6.92 (2)	3.23 (2)
LH_6 + H = LH_7	5.93 (2)	3.00 (2)	
LH_7 + H = LH_8	4.30 (2)		
LH_8 + H = LH_9	4.25 (2)		
$\Delta_1 = \log K_{011} - \log K_{012}$	0.70	1.20	–
$\Delta_2 = \log K_{012} - \log K_{013}$	0.47	0.59	–

[a] Charges are omitted for clarity. [b] Values in parentheses are the standard deviations in the last significant digit.

The three ligands differ from one another in the nature of their 12 amine functions. Ligand **L1** presents 6 primary, 3 secondary, and 3 ternary amines, whereas **L2** possesses 9 secondary and 3 ternary amines; **L3** presents 12 ternary amines.

In the investigated pH range (2–12), for the 3 ligands, the full set of the 12 protonation constants could not be determined. In spite of its acyclic nature, only nine protonation constants were determined for **L1**. Six correspond to

strong to moderate bases and three to moderate bases; the three last constants lower than 2 were not determined. In contrast, **L2** exhibits only six nitrogen atoms that behave as strong to moderate bases, and one which behaves as a weak base; the other protonation constants were not detected. Trismacrocyclic ligand **L3** behaves as a very strong base (proton sponge) in the three first protonation steps; in fact, deprotonation of LH_3^{3+} is not detectable by potentiometric titration (and ^1H NMR experiments as shown below). The three protonation constants behave as moderate to weak bases.

Observing the stepwise protonations in terms of minimization of electrostatic repulsions between positive charges in protonated species, the first six protons occupy alternate positions separated either by a nonprotonated amino group or by the linker. The first set of three basicity constants concerns the protonation of each tetraamine core, whereas the second set corresponds to the introduction of a supplementary proton on each polyaza subunit, and so on.

Ligand **L1** presents high protonated species, a consequence of its flexible structure, which allows full protonation of the two tetraamine pendant arms without important repulsion charge effects. The Δ_1 and Δ_2 values ($\Delta_1 = \log K_{011} - \log K_{012} = 0.70$, $\Delta_2 = \log K_{012} - \log K_{013} = 0.47$) indicate that the tetraamine moieties of the ligand are rather independent. The cyclic structure of each tetraamine of **L2** leads to fewer protonated species. Moreover, the value $\Delta_1 = 1.20$ indicates that the three cyclen moieties are relatively dependent, a consequence of the rigidity of the whole ligand skeleton. However, the weak value of $\Delta_2 = 0.59$ indicates the independence and easy protonation of the third cyclen core towards the others. The proton sponge behavior of **L3** generates an entirely different protonation scheme. The fourth, fifth, and sixth protonation constants are only observed at very low pH ($\log K_{014} = 4.60$, $\log K_{015} = 4.26$, $\log K_{016} = 3.23$) due to the presence and the central localization (see below) of the three first protons in each reinforced macrocycle. This uncommon behavior is ascribed to the constrained architecture of the bridged cyclen cores.[11–13]

Figure 1 depicts the distribution diagrams of the protonated species of **L1**, **L2**, and **L3**. The diagrams are notably different. Concerning firstly triscyclen **L2**, one can note the remarkably large ($LH_6^{6+} + LH_7^{7+}$) existence domain species that predominate at pH 2–7. Comparison with its open chain analogue **L1** is interesting: in sharp contrast, the zone of the ($LH_6^{6+} + LH_7^{7+}$) forms is considerably reduced and the highly protonated form LH_9^{9+} largely predominates in the acidic pH area. By increasing the pH, the lower protonated species of **L1** and **L2** progressively appear, and at pH 12, the fully deprotonated ligands become the main species. Concerning **L3**, one can note the remarkable predominance of LH_3^{3+} at pH 5–12.

To throw more light on the protonation sequence of the three ligands, ^1H NMR measurements were carried out in aqueous solutions over a wide pH range. The proton attribution was elucidated with the help of HMQC, HMBC, and COSY NMR sequences. Figure 2 illustrates the pH dependence of the ^1H NMR signals.

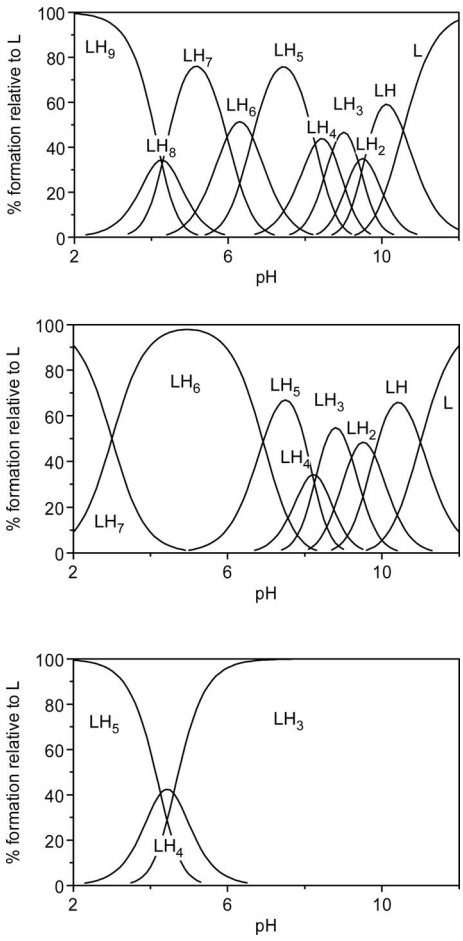


Figure 1. Species distribution diagrams for **L1** (top), **L2** (middle), and **L3** (bottom) as a function of pH.

Depending on the structure of the ligand, the ^1H NMR spectra of **L1**, **L2**, and **L3** at neutral pH in D_2O (300.13 MHz, 298 K) display five or seven signals in the aliphatic area (H_1 to H_7); they also present resonances in the aromatic area near 7.3 ppm attributed to the aromatic Ha proton.

The time-averaged C_{3v} symmetry due to a fast proton exchange process in aqueous solution is kept across the whole investigated pH range for both ligands **L1** and **L3**. One can however think that steric hindrance occurs from the open-chain skeleton to the cyclic one. Moreover, between pH 2 and 5, **L2** NMR investigation shows two subspectra (Supporting Information, Figure S1) due to a slower exchange process for the LH_7^{7+} species. This behavior can be linked to the rigidity of the structure induced by the high protonation state of each macrocyclic unit in acidic medium. For clarity, time averaged shifts have been selected for Figure 2 in the concerned pH region.

The analysis of the pH dependence of the ^1H NMR resonances of **L1** is quite complicated due to the number of signals in the 2.8–3.6 ppm area. From basic to acidic medium, the H5 and H7 protons firstly present a downfield shift relating the preferential protonation of the more distant amines N3 and N4. The second step consists in the

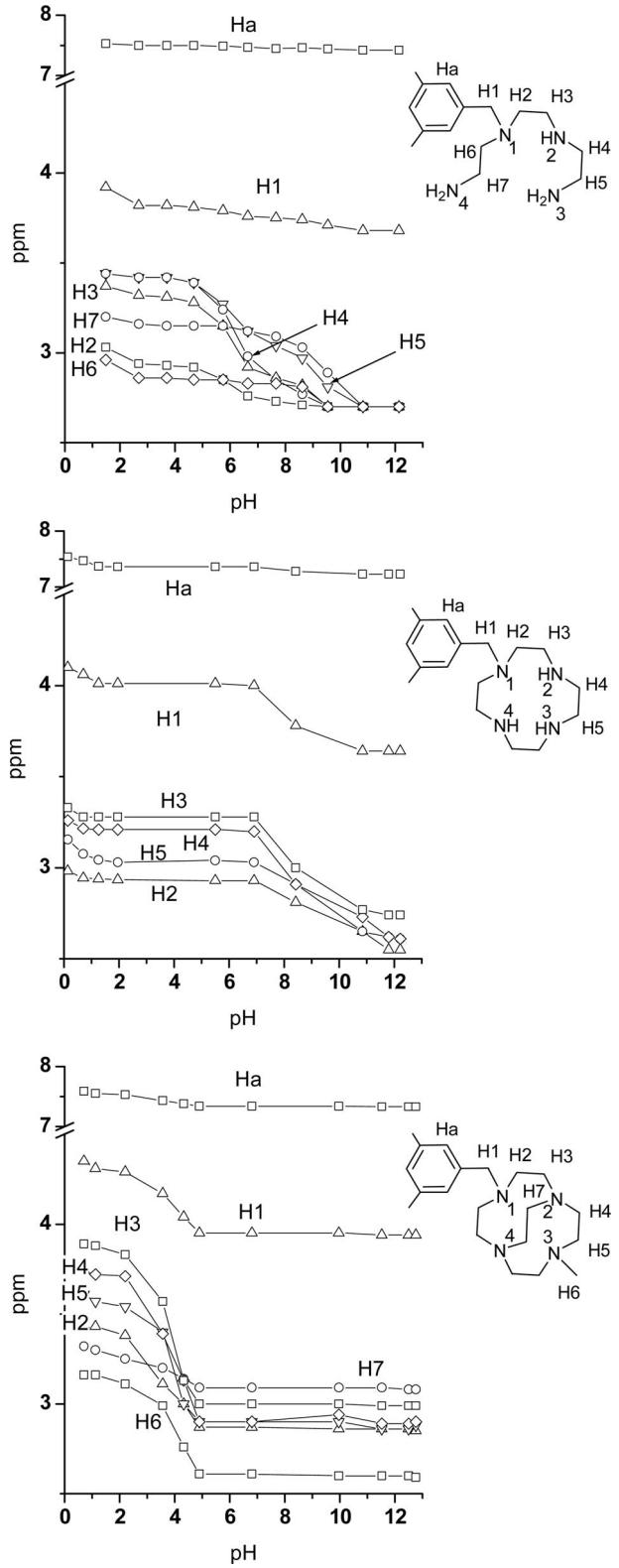


Figure 2. pH dependence of the ^{1}H NMR signals of **L1**, **L2**, and **L3** (300.13 MHz, D_2O, 25 °C; [L] = 0.02 M). Caution: scale is different in the 7–8 ppm area.

downfield shift of H3 and H4, revealing the protonation of the secondary amine N2, followed in very acidic medium by the slight downfield in H2 and H6 induced by the protonation of the ternary amine N1.

The pH dependence of the ^{1}H NMR resonances of **L2** also allows us to deduce its protonation pattern. At pH 12–7, where the first six protons bind to the ligand, a remarkably higher downfield shift is observed for the signals of H3 and H4. Minor shifts are observed for the remaining proton signals. This indicates that the first six protonations take place on the N2 and N4 secondary amines. Therefore, in LH_6^{6+} species, which is prevalent in aqueous solution at pH 2–6, each macrocyclic unit contains two protonated nitrogen atoms, separated from one another by an uncharged nitrogen atom (one secondary and one ternary amine), thus achieving an optimal minimization of the electrostatic repulsions between charged ammonium groups. Binding of the seventh and higher protons below pH 4 is followed by the downfield shift in the resonances of the H5 and H2 protons in the ^{1}H NMR spectra. These spectral features indicate that the last protonation steps occur on N3 then on N1 amino groups.

Ligand **L3** shows a somewhat different behavior. As shown in Figure 2, the presence of three protons in the structure leads to unusually high chemical shifts in the ^{1}H NMR resonances of the H1–7 protons, dealing with a central place of the proton in each constrained cycle. The analysis of the whole spectra may indeed suggest the localization of the three acidic protons in the LH_3^{3+} species. The methylene groups H7 adjacent to N2 and N4 give rise to two signals with high chemical shifts for a CH_2 group adjacent to the ternary nitrogen atoms. This suggests that the acidic proton is shared between the N2 and N4 nitrogen atoms. This clearly highlights the strong stability of the LH_3^{3+} species and the fact that the following protonations take place in quite acid medium; this confirmed the potentiometric data. The ^{1}H NMR spectra of the ligand in concentrated NaOH, at pH 12 or at pH 5, are equal, indicating that the LH_3^{3+} species is present from pH 5 to basic medium and could not be deprotonated even in strongly alkaline solution, that is, **L3** behaves as a triple proton sponge. Finally, bindings of the fourth, fifth, and sixth protons occur, producing once again general downfield shifts of the H1–7 protons.

One can finally note that the aromatic Ha resonances follow very different pathways depending on the nature of the tetraamine moieties. For **L1**, no real variation can be detected in the whole pH range, whereas for **L2** and **L3**, the Ha protons are sensitive to the protonation happening in acidic medium certainly as a consequence of the nitrogen N1 protonation.

The most interesting demonstration is that from a flexible to a constrained parent tritopic structure, the acid–base properties of the ligands are deeply different. Ligand **L1** presents more protonated species than **L2** and is the most protonated at neutral pH. The reinforced and more rigid analogue **L3** is poorly charged around pH 7 but presents a very stable LH_3^{3+} form. In addition, this necessary preliminary study clearly allows, for each ligand, the identification of the ammonium sites at a given pH and then the potential anchoring points involved in the following host–guest interaction.

Phosphate Anions Binding

Potentiometric and NMR investigations were performed to evaluate and characterize the binding interactions between the ligands and the anions. The potentiometric data of a solution containing equimolar amounts of ligand and anion are resolved, giving the $\log K_{alh}$ values for the species present in solution. Computer analysis furnished the overall equilibrium constants β_{alh} of the complexes formed between the anion (A) and the ligand (L), according to Equation (1) (charges omitted for simplicity). The knowledge of the number i of protons bound to the bis(tetraamine) ligand (L) in the general $A_aL_lH_h$ complex allows the complexation reaction in Equation (2) to be written, according to the actual protonation state (charges omitted) and to calculate $\log K_{alh}$.

$$aA + lL + hH \rightleftharpoons A_aL_lH_h\beta_{alh} \quad (1)$$

$$AH_{(h-i)} + LH_i \rightleftharpoons ALH_hK_{alh} \quad (2)$$

Protonation constants of the nucleotides, measured according to our experimental conditions and in good agreement with those of the literature, are reported in the Supporting Information (Table S1). Species distribution diagrams for substrates AMP, ADP, ATP, and triphosphate, deduced from their protonation constants, are presented in the Supporting Information (Figure S2). The logarithm of the stability constants, $\log K_{alh}$, of ligands **L1**, **L2**, and **L3** with AMP, ADP, ATP, and triphosphate anions is presented in Table 2. The protonation constants of the anionic guests provide several sets of ternary species: in each case, the most probable equilibrium is kept. The overall stability constants ($\log \beta$) of the substrates adduct with the three ligands are proposed in the Supporting Information (Table S2).

Data analyses under our experimental conditions reveal that only complexes with a 1:1 anion/ligand stoichiometry were found in solution. The species distribution diagrams as a function of pH for the six anion–ligand systems were carried out and are presented in the Supporting Information (Figure S3a–c). By comparing the binding ability of the three ligands towards the various substrates, the stability of the adducts increases in the order AMP < ADP = triphosphate < ATP. This order was already previously obtained for similar ligands.[7d,11c] For **L1**, the stability constants are quite significant from $\log K_{111}$ to $\log K_{114}$, around $\log K$ = 3–4 considering the weak protonation degree of the systems. The interaction process involving **L2** and **L3** required more protonated sites.

The comparison of the $\log K_{alh}$ values at a given and equivalent degree of protonation, between the different ligands is, in this study, useless, considering their high difference of protonation state at a given pH value. For that reason, studying the pH dependence of the logarithms of the conditional constants for the different systems is more relevant. Special attention is directed to triphosphate and ATP anions in Figure 3. Plots of the conditional constants versus pH for the different systems show that both substrates form relatively stables complexes with the three receptors in a wide pH range with various maxima depending on the nature of the ligand.

Table 2. Logarithm complexation constants, $\log K_{alh}$, for ligands **L1**, **L2**, and **L3** with anions [H_2O; I = 0.1 M (NaCl); T = 25 °C].

L1[a]	AMP[b]	ADP[b]	ATP[b]	TriP[b]
A+LH = ALH	2.34 (1)	2.46 (1)	3.16 (2)	2.72 (3)
$A+LH_2 = ALH_2$	2.60 (1)	2.69 (1)	3.40 (3)	3.04 (2)
$A+LH_3 = ALH_3$	2.81 (1)	3.21 (1)	3.60 (3)	3.45 (2)
$A+LH_4 = ALH_4$	3.06 (1)	3.51 (1)	4.03 (3)	3.91 (4)
$A+LH_5 = ALH_5$	3.76 (1)	4.26 (1)	4.76 (5)	4.72 (2)
$A+LH_6 = ALH_6$	4.20 (2)	4.73 (2)		
$AH+LH_5 = ALH_6$			6.05 (4)	5.26 (3)
$AH+LH_6 = ALH_7$	4.30 (2)	4.67 (2)	6.51 (4)	6.01 (4)
$AH+LH_7 = ALH_8$	4.47 (2)	5.20 (2)	6.43 (4)	5.77 (5)
$AH+LH_8 = ALH_9$	3.95 (3)	4.67 (3)	6.53 (5)	5.44 (4)
$AH+ LH_9 = ALH_{10}$	3.34 (3)	4.55 (3)		
$AH_2+LH_8 = ALH_{10}$	3.87 (3)	4.69 (3)	5.53 (5)	3.13 (4)
L2	AMP	ADP	ATP	TriP
$A+LH_2 = ALH_2$			3.41 (1)	2.91 (1)
$A+LH_3 = ALH_3$	2.48 (1)		3.60 (1)	3.01 (1)
$A+LH_4 = ALH_4$	3.10 (1)	3.05 (1)	4.47 (1)	3.64 (2)
$A+LH_5 = ALH_5$	3.09 (1)	3.37 (3)	4.77 (2)	4.10 (2)
$A+LH_6 = ALH_6$	3.94 (2)	4.13 (3)	5.91 (2)	
$AH+LH_5 = ALH_6$				4.20 (2)
$AH+LH_6 = ALH_7$	3.53 (2)	4.48 (3)	5.88 (2)	5.00 (2)
$AH_2+LH_6 = ALH_8$	4.31 (2)	5.31 (3)	6.50 (1)	5.40 (2)
$AH_2+LH_7 = ALH_9$	4.24 (3)	5.59 (3)	6.96 (1)	5.59(2)
$AH_3+LH_7 = ALH_{10}$			7.01 (1)	
L3	AMP	ADP	ATP	TriP
$A + LH_3 = ALH_3$	2.36 (1)	2.99 (1)	3.02 (1)	2.77 (1)
$AH + LH_3 = ALH_4$	2.74 (1)	3.29 (1)	3.24 (1)	2.86 (1)
$AH + LH_4 = ALH_5$	3.66 (1)	4.46 (1)	4.63 (1)	3.54 (1)
$AH_2 + LH_4 = ALH_6$	3.72 (2)	4.61 (4)	4.73 (2)	3.98 (2)
$AH_2 + LH_5 = ALH_7$	4.04 (3)	4.68 (3)	4.68 (2)	4.01 (2)
$AH_2 + LH_6 = ALH_8$	4.51 (3)	5.31 (3)	5.29 (3)	5.00 (3)
$AH_3 + LH_8 = ALH_9$			6.07 (3)	5.41 (3)

[a] Charges are omitted for clarity. [b] Values in parentheses are the standard deviations in the last significant digit.

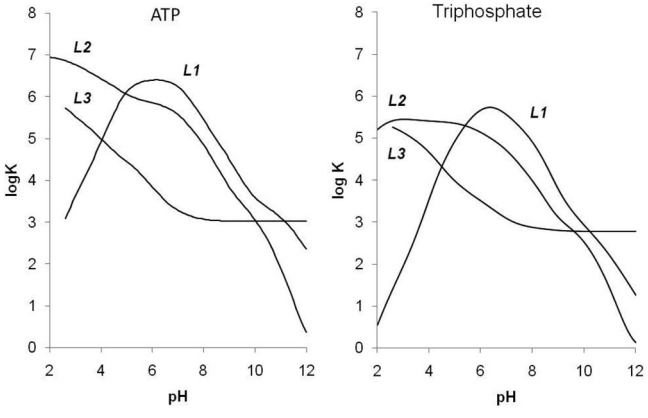


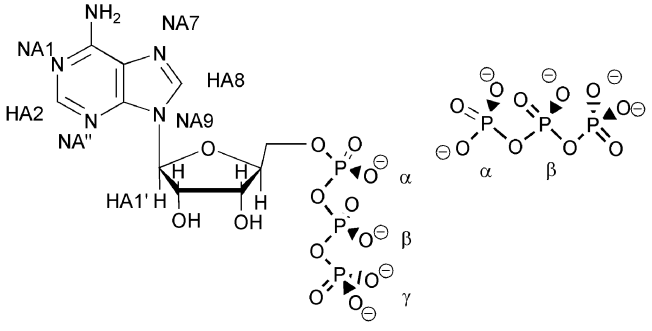
Figure 3. Logarithms of the conditional constant versus pH for the three systems **L1**, **L2**, **L3** – ATP, triphosphate.

Concerning **L1**, the stability constants increase from alkaline to slightly acidic pH values (substrates are in their higher charged forms and ligands in an increasing number of ammonium sites) with a maximum between pH 6 and 7

for both substrates and decrease at higher pH values. This marked decrease can be attributed to the presence in solution of highly or fully protonated forms of substrates, implying repulsions between the highly charged tetraamine moieties. For **L2** and **L3**, $\log K_{alh}$ increases from alkaline medium to attain a maximum in very acidic medium in spite of the lack of negative charge on the substrates. This behavior, which was observed in previous work[7,8] for polycyclic ligands, can be reasonably attributed to the lesser protonation states of both trimeric ligands and to the rigidity induced by the cyclic nature of the tetraamine moieties. The situation involving a N^+–H···OH interaction,[5d] effective in acidic solution can be invoked here.

These data can be expressed in terms of the percent of formation of the adducts. Comparison between the amounts of the ternary species formed (Supporting Information, Figure S4a–c) confirm the affinity of these ligands for the anionic substrates, but each one at different pH: pH 4 < **L1** < pH 8 (ATP 97.83%, triphosphate 95.30%), pH 2 < **L2** < pH 7 (ATP 98.94%, triphosphate 94.07%), and pH 2 < **L3** < pH 4 (ATP 95.78%, triphosphate 92.8%).

As explained in numerous works investigating ATP binding (Scheme 3), the differences observed in the host–guest interactions can be only partially explained in terms of electrostatic and hydrogen-bonding interactions. In addition to the electrostatic interaction between the phosphate chain and the polyammonium ligands, other effects, even as minor as π-stacking interactions between the heteroaromatic linker and the adenine moiety of the nucleotides, have to be considered. Consequently, in light of the better results obtained with ATP, we investigated the structural features of the complexes with ATP by comparing the behavior of the three ligands with its inorganic analogue $P_3O_{10}^{5-}$ by using NMR measurements. In this way, the anion interaction was been followed by recording ^{1}H and ^{31}P NMR spectra of solutions containing an equimolar amount of receptors and substrates in a wide pH range.



Scheme 3. ATP and triphosphate anions and labeled atoms.

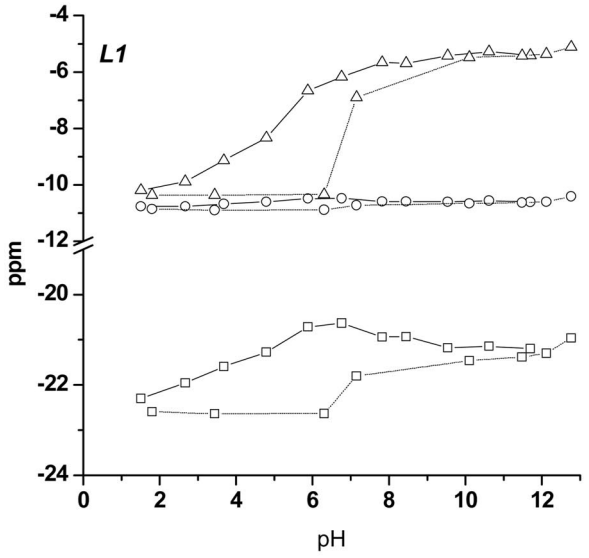
Figures 4 and 5 show the pH dependence of the ^{31}P NMR chemical shifts of the phosphate groups of ATP and triphosphate in a 1:1 molar ratio with ligands **L1**, **L2**, and **L3** at different pH values together with those of the free anions. The variations in the ^{31}P NMR chemical shifts clearly observable in the plots of the different diagrams evidence interactions between the phosphate chains and the polyammonium receptors. However, differences between the behaviors of the three ligands for a given substrate clearly emphasize various coordination modes depending on their organic or inorganic nature.

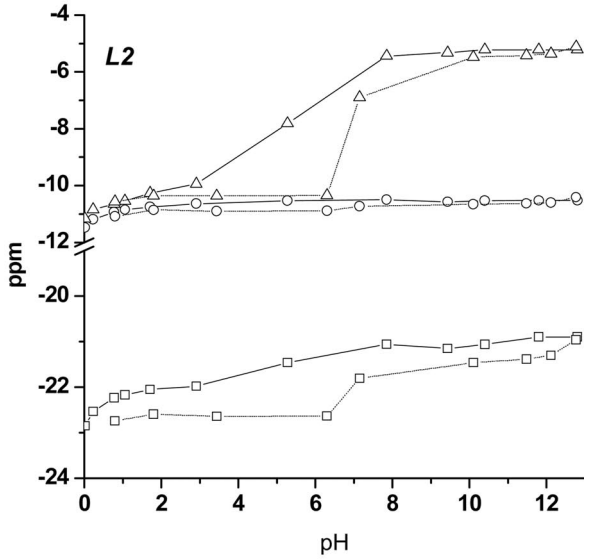
In the case of complexation of ATP, Figure 4 shows a downfield shift upon complexation of the signals of the phosphate groups in the order Pγ > Pβ > Pα. This sequence is comparable to previous results[6a,7d] and suggests that in the case of ATP binding, the ligands principally bind the phosphate chain by interaction with the two terminal phosphate groups of the nucleotide. Moreover, the NMR shifts are higher for **L1** than for **L2** (especially for Pβ), whereas the phosphate groups are less influenced by the presence of **L3**. The downfield shifts slightly persist in alkaline medium for **L1** and **L2** but are mainly significant at pH 2–9.

In conclusion, for ATP complexation, variations in chemical shifts are pH dependent, being higher at pH 3–9. This is in accordance with the potentiometric results of the same systems, which showed that a large amount of receptor–substrate adducts are formed from slight alkaline to acidic pH values, that is, in the pH region where the highly protonated species of the receptor and the anionic species are simultaneously present in solution.

Concerning the case of triphosphate (Figure 5), which obviously presents only two different phosphate groups Pα and Pβ, both are clearly influenced by the presence of the receptor in solution since the resonances of Pα and Pβ shift downfield over a wide pH range, indicating that in the adducts with triphosphate, the polyammonium receptors interact equally with Pα and Pβ. However, some significant differences can be pointed out depending on the pH domain. Ligands **L1** and **L2** present quite similar and classical diagrams with highest chemical shifts situated around pH 5–6 and decreasing ones when going to the acidic and alkaline media. Ligand **L3** shows an entirely different behavior with chemical shifts of both the Pα and Pβ signals being higher from basic to acidic medium.

The ^{31}P NMR spectroscopic titrations clearly confirm that electrostatic interactions and hydrogen bonds between the ammonium sites of the ligands and the polyphosphate chains of the four anionic substrates govern the host–guest complexation process. Results definitely show important differences in the formation of the adducts with ATP or with triphosphate, highlighting the active role played by the organic moiety of the nucleotides. In the aim to clarify this point, ^{1}H NMR spectra were carried out on a solution containing an equimolar amount of the substrates and the ligands with ATP or triphosphate and **L1**–**3**. Concerning the shifts relating to the tetraamine moieties, results are quite similar for both anions. Plots involving ATP are presented in Figure 6 and have been compared to Figure 2 (see Figure S5 in the Supporting Information for a comparison in a same Figure). In addition, the ^{1}H NMR investigations permit the localization of the ammonium sites involved in the complexation. From alkaline to acidic medium, the progressive and simultaneous shifts of the protons of **L1**, espe-





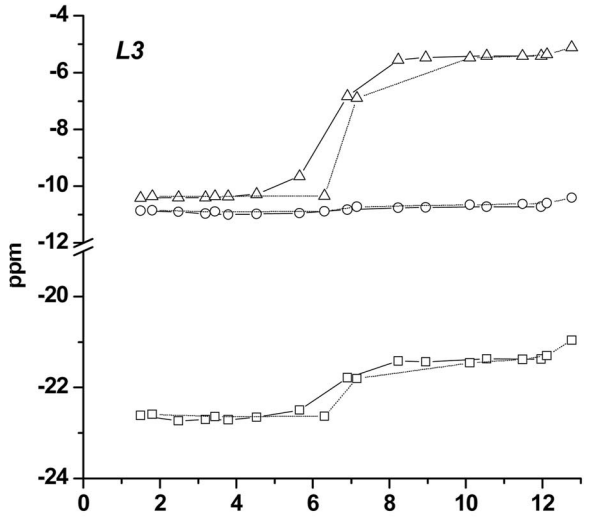
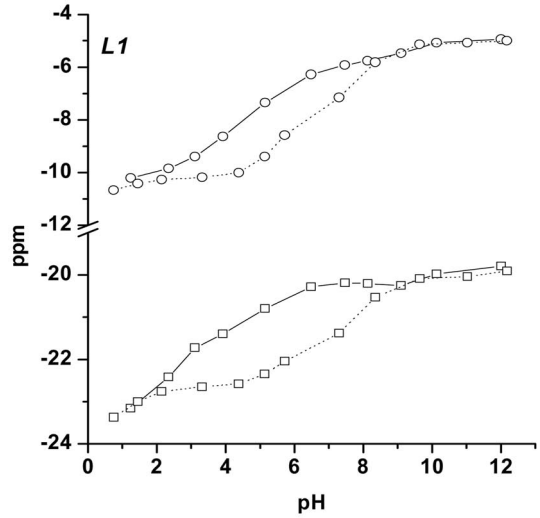
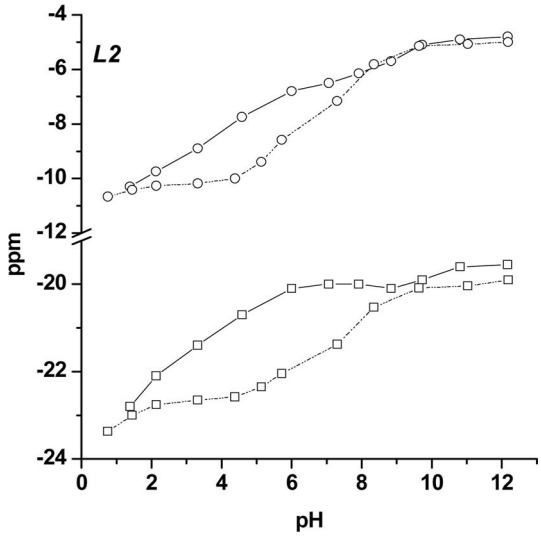


Figure 4. ^{31}P NMR chemical shifts of the ATP phosphorus atoms at various pH values (121.49 MHz, D_2O, 25 °C; [A] = [L] = 0.02 M). Free (dotted lines) or in presence of the ligand (solid lines); α ○, β □, γ △.





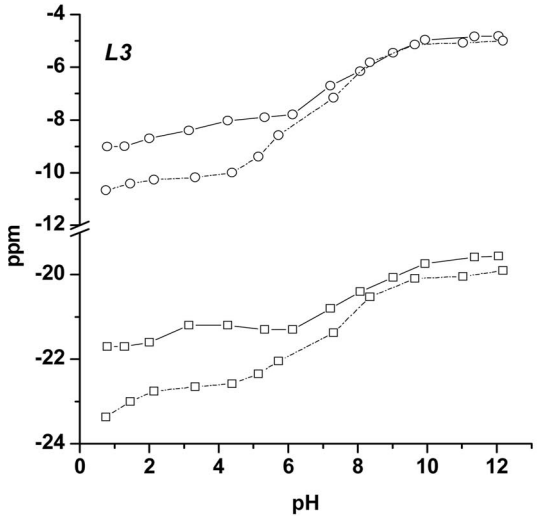


Figure 5. ^{31}P NMR chemical shifts of the triphosphate phosphorus atoms at various pH values (121.49 MHz, D_2O, 25 °C; [A] = [L] = 0.02 M). Free (dotted lines) or in presence of the ligand (solid lines); α ○, β □.

cially H3, H4, H5, and H7 however indicate that the coordination responsibility is shared by all nitrogen atoms N2, N3, and N4. Moreover, the downfield shifts of H1, H2, and H6 at pH 2–5 indicate the additional participation of N1 in this domain. The spectrum analysis is easier with **L2**, which shows downfield shifts of the H3 and H4 signals, indicating that N2, and therefore N4, are more implicated in the binding system of the complex in the area $3 < pH < 12$. The downfield shift of the signal of H5 (protonation of N3),

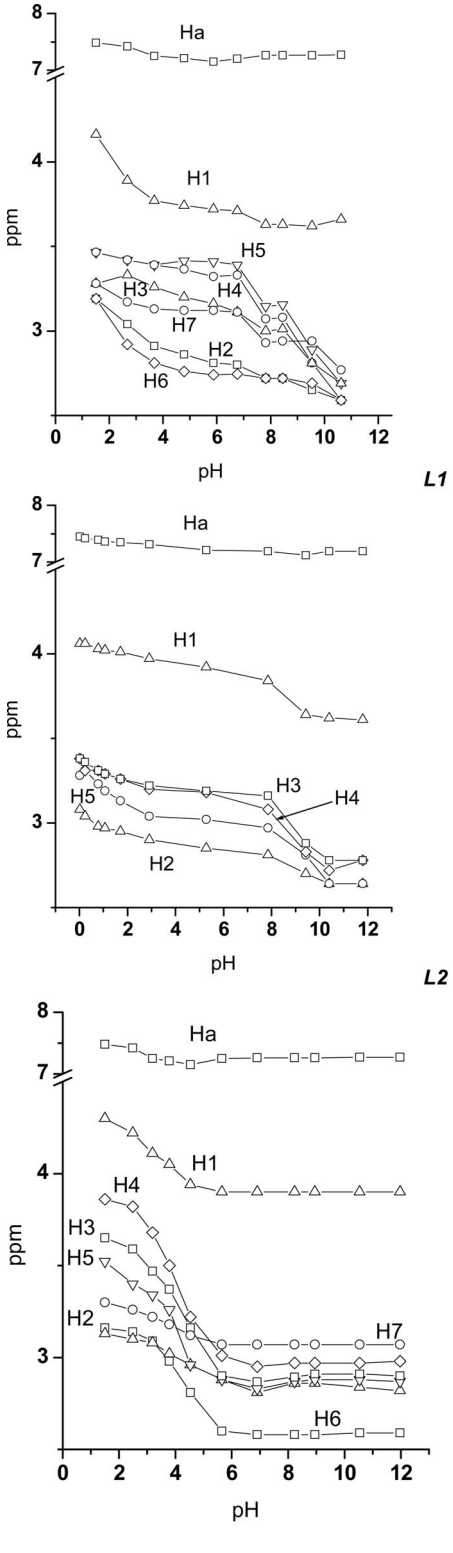


Figure 6. Experimental ^{1}H NMR chemical shifts for the protons of the trismacrocycles in the presence of ATP (300.13 MHz, D_2O, 25 °C; [A] = [L] = 0.02 M). Caution: scale is different in the 7–8 ppm area.

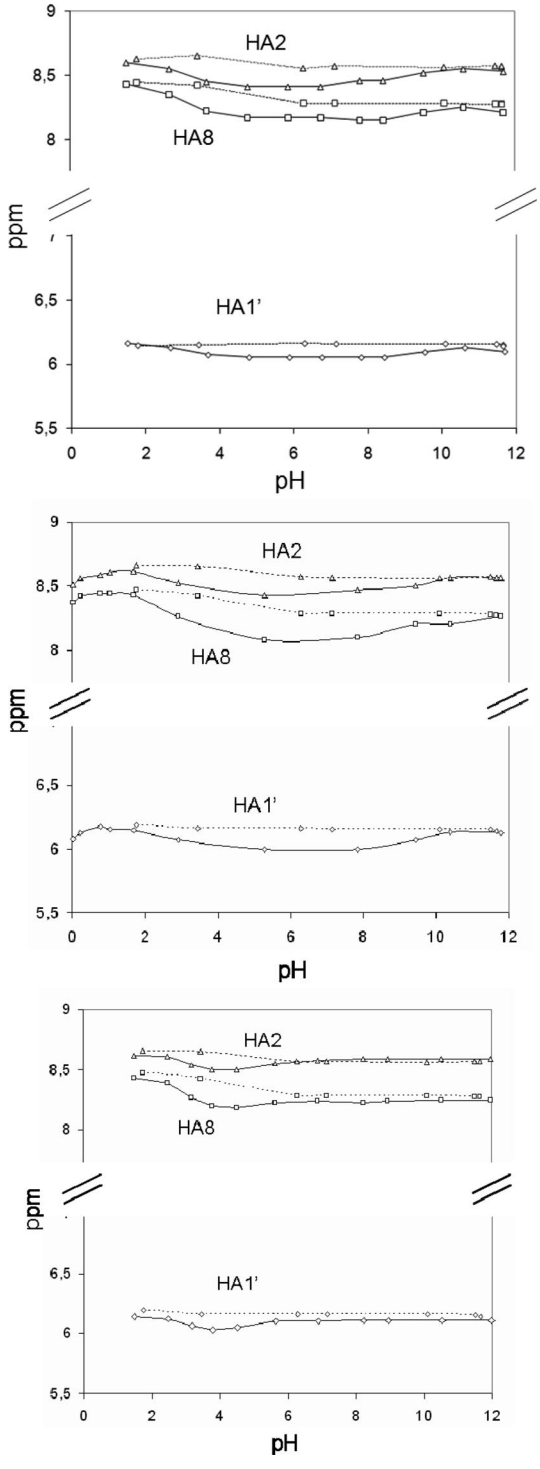


Figure 7. Experimental ^{1}H NMR chemical shifts for the HA1′, HA2, and HA8 protons of free ATP (dotted lines) and ATP in the presence of ligands (solid lines) (300.13 MHz, D_2O, 25 °C; [A] = [L] = 0.02 M).

which occurs when pH < 3, clearly indicate that in acidic medium the complexation is reinforced by the participation of N3. Results obtained for **L3**, especially in acidic medium below pH 5, are remarkable. If between pH 5 and 12 the weak host–guest interaction leads to no apparent shifts in the ^{1}H NMR signals, strong downfield shifts are simultaneously observed for the H3, H4, H5, and H6 signals as well as for H1. This indicates that all the nitrogen atoms contribute to the coordination scheme in acid medium.

Concerning the aromatic proton Ha, results are obviously different since plots presenting experimental ^{1}H NMR shifts of the free ligands and in presence of ATP or triphosphate (Supporting Information, Figure S6a–c) only present significant displacements in the case of adducts with ATP. The observed upfield shifts can already be interpreted by interaction between the aromatic centers of the receptors and the nucleotides. ^{1}H NMR spectra can also provide evidence for the contribution of π-staking interaction in the stabilization of ternary species. The pH dependence of the signals of the adenine protons HA2 and HA8 and the anomeric proton HA1′ of the nucleotide for ATP free and in presence of the ligands is displayed in Figure 7. The significant upfield displacements observed for these three protons are consistent with the participation of interactions between the aromatic spacer and the adenine moiety in the stabilization of the adducts. This behavior exists at pH 2–10 for **L1** and **L2** but is limited for **L3** to lesser shifts and to a smaller pH range (2–5), which corroborates the fact that the organic part of the anion is less involved in the coordination scheme than for **L1** and **L2**. This correlates that the potentiometric data are quite similar between triphosphate and ATP in the case of adducts formed with **L3**.

Potentiometric and ^{31}P and ^{1}H NMR titrations clearly highlight that the three ligands are efficient receptors for nucleotides and inorganic phosphate anions. Nevertheless, the differences in the structures of **L1**, **L2**, and **L3** clearly lead to various potentiometric results in very acidic or alkaline media, which come from different complexation modes. For the different ligands, the coordination sites N1, N2, N3, and N4 are not involved in the same pH domain in the complexation process and the influence of π-stacking interactions is not comparable, leading obviously to different coordination schemes at a given pH value.

Conclusions

Obtained in high overall yields following the easy to run bisaminal methodology, the three tris(tetraamine) ligands proposed here can be involved in host–guest investigations to assess their acid–base properties and their ability to interact with triphosphate and nucleotides. The synthetic routes used in this paper give alternative access to already known triscyclen[14] **L2** without fastidious protection–deprotection steps and give rise to a simple access to the new tris-reinforced-cyclen **L3**.

The protonation study clearly underlines the influence of the structure of the tetraamine moiety on the acid–base properties of the trimeric ligands. The tetraamine open chain leads to the more basic receptor **L1**, whereas the cyclic structure present in **L2** constitutes an evident drawback in terms of repulsions between charged nitrogen atoms leading to less protonated species at a given pH. The constrained structure of the cyclen moieties present in **L3** leads to an attractive compound that behaves as a triple proton sponge. In aqueous solution, **L3** generates LH_3^{3+} species that cannot be deprotonated even in very basic medium.

The three ligands show significant interaction properties, especially with ATP, and their differences mainly give rise to comparable stepwise stability constants but in different pH domains. Spectroscopic investigations clearly highlight that the formation of the adducts is significantly different for each receptor, showing that the lack of protonated sites from **L1** to **L3** is partially compensated by the rigidification of the structures. On one hand, the cyclic nature of **L2** and **L3** leads to high interactions in acidic medium. On the other hand, the ability of their open chain analogue **L1** to form highly protonated species implies strong charges repulsions in acidic medium leading to weak adducts between pH 2 and 6.

Spectroscopic titrations also allow the complexation of triphosphate and ATP to be compared. Both lateral and central phosphate groups of the triphosphate interact with the ligands, whereas only the terminal and central ones are implicated in nucleotide recognition. Moreover, the presence of the adenine part necessarily permits the contribution of the π-stacking interactions, which induces a different coordination scheme stabilizing the ternary species; this contribution is higher in the case of **L2**.

Taking into account that this host–guest study is performed at 25 °C and that the interactions between anionic substrates and ammonium receptors could be temperature dependant, especially in the case of such ligands presenting so different rigidity, results are subject to variations, for example, in physiological samples. Nevertheless, this study clearly demonstrates that with nucleotides such as ATP the contribution of three important factors that have the ability to form ammonium sites, the rigidity of the structure and the possibility to form π-stacking interactions is different depending on the nature of the trimeric ligands and leads however to significant interactions with the targeted substrates.

Experimental Section

Materials: Reagents were purchased from Acros Organics and from Aldrich Chemical Co. Cyclen and triethylenetetraamine were purchased from Chematech (Dijon, France). Elemental analyses were performed at the Service de Microanalyse, CNRS, 91198 Gif sur Yvette, France. NMR and mass spectrometry were investigated at the "services communs" of the University of Brest. ^{1}H and ^{13}C NMR spectra were recorded with an Avance 500 Bruker (500 MHz), Avance 400 Bruker (400 MHz), or AMX-3 300 Bruker

(300 MHz) spectrometer by using MeOH as internal reference. 2D ^1H–^1H homonuclear and ^1H–^{13}C heteronuclear correlations and homonuclear decoupling experiments permitted the full assignment of the ^1H and ^{13}C NMR signals. Mass spectrometry analyses were performed on an Autoflex MALDI TOF III LRF200 CID.

L1: Synthesized according to ref.[8]

Cyclen–Glyoxal 2: Synthesized according to ref.[9] starting from cyclen **1**.

Compound 3: A solution of 1,3,5-tris(iodomethyl)benzene (4.27 g, 8.58 mmol) dissolved in dry acetonitrile (35 mL) was added dropwise to a stirred solution of cyclen–glyoxal **2** (5 g, 25.75 mmol) in dry acetonitrile (40 mL). The mixture was stirred at room temperature for two weeks. The precipitate was collected by filtration, washed with diethyl ether, and dried in vacuo to give **3** as a white powder (95% yield). ^{13}C NMR (100.62 MHz, D_2O, 298 K): δ = 46.5, 50.5, 51.1, 51.2, 54.2, 59.8, 62.8, 64.4 (αCH_2), 74.4, 86.5 (CHaminal), 133.2 (CHAr), 141.4 (CAr) ppm. MS (MALDI-TOF, DHB, H_2O): m/z = 1081.25 $[M + H]^+$. $C_{39}H_{63}I_3N_{12}{\cdot}2H_2O$ (1116.75): calcd. C 41.95, H 6.05, N 15.05; found C 42.01, H 6.12, N 15.31.

Compound L2: Tris-salt **3** was heated at refluxed in hydrazine monohydrate as described previously[9b] to give **L2** as a white powder (90% yield). ^1H NMR (400.13 MHz, $CDCl_3$, 298 K): δ = 2.59 (t, 4 H, αCH_2, H2), 2.64 (t, 4 H, αCH_2, H4), 2.69 (t, 4 H, αCH_2, H5), 2.80 (t, 4 H, αCH_2, H3), 3.72 (s, 2 H, αCH_2Ph, H1), 7.26 (s, 1 H, CHAr, Ha) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$; 298 K): δ = 45.1, 46.6, 47.2, 51.0 (αCH_2), 59.0 (αCH_2Ph), 129.4 (CHAr), 138.9 (CAr) ppm. MS (MALDI-TOF, MeOH, dithranol): m/z = 632.61 $[M + H]^+$. $C_{33}H_{67}N_{12}$ (631.98): calcd. C 62.72, H 10.69, N 26.60; found C 63.01, H 10.89, N 10.37.

Compound 4: A solution of iodomethane (3.65 g; 25.75 mmol) in dry tetrahydrofuran (20 mL) was added dropwise to a stirred solution of cyclen–glyoxal **2** (5 g; 25.75 mmol) in dry tetrahydrofuran (40 mL). The mixture was stirred at room temperature for 2 d. The precipitate was collected by filtration, washed with diethyl ether, and dried in vacuo to give **4** as a white powder (90% yield). ^{13}C NMR (100.62 MHz, D_2O, 298 K): δ = 49.9, 50.5 (2 C), 50.7, 51.0, 51.2, 54.1, 63.9, 68.3 (αCH_2), 74.4, 86.3 (CHaminal) ppm. MS (MALDI-TOF, DHB, H_2O): m/z = 335.12 $[M + H]^+$. $C_{11}H_{21}IN_4{\cdot}H_2O$ (354.23): calcd. C 37.30, H 6.54, N 15.82; found C 37.55, H 6.76, N 16.01.

Compound 5: A solution of 1,3,5-tris(iodomethyl)benzene (2.48 g; 15.1 mmol) dissolved in dry acetonitrile (35 mL) was added dropwise to a stirred solution of **4** (5 g; 14.96 mmol) in dry acetonitrile (40 mL). The mixture was stirred at room temperature for two weeks. The precipitate was collected by filtration, washed with diethyl ether, and dried in vacuo to give **5** as a white powder (88% yield). ^{13}C NMR (100.62 MHz, D_2O, 298 K): δ = 45.6, 45.9, 49.2, 49.3, 49.7, 57.7, 62.0, 62.4, 64.2 (αCH_2), 80.7, 81.4 (CHaminal), 132.8 (CHAr), 141.6 (CAr) ppm. MS (MALDI-TOF, H_2O, DHB): m/z = 1507.11 $[M + H]^+$. $C_{42}H_{72}I_6N_{12}{\cdot}3H_2O$ (1560.57): calcd. C 33.32, H 5.04, N 10.77; found C 33.01, H 5.12, N 11.01.

Compound $L3H_3^{3+}$,3I$^-$: A large excess of $NaBH_4$ (24 equiv.) was added in small portions over 1 h to a stirred solution of polyammonium salt **5** in absolute ethanol (3 g in 200 mL). The mixture was allowed to stir at room temperature for one week. After cooling to 0 °C, HI (2 M) was added to pH ≈ 3–4, and the resulting mixture was evaporated to dryness. The resulting white solid was then dissolved in a small quantity of water and potassium hydroxide pellets were added until a basic medium was obtained. The aqueous phase was then evaporated to dryness, and the residue was extracted with chloroform (3 × 70 mL). The combined organic phase was finally evaporated to give $\mathbf{L3}H_3^{3+}$,3I$^-$ (78% yield). ^1H NMR (400.13 MHz, D_2O, 298 K): δ = 2.55 (s, 3 H, Me, H6), 2.77 (t, 4 H, αCH_2, H2), 2.83 (t, 4 H, αCH_2, H5), 2.88 (t, 4 H, αCH_2, H4), 2.94 (t, 4 H, αCH_2, H3), 3.02 (t, 4 H, αCH_2, H7), 3.81 (s, 2 H, αCH_2Ph, H1), 7.29 (s, 1 H, CHAr, Ha) ppm. ^{13}C NMR (75.47 MHz, D_2O, 298 K): δ = 45.2, 49.9, 53.6, 55.1, 56.9, 57.5 (αCH_2), 60.5 (αCH_2-Ph), 132.4 (CHAr), 141.6 (CAr) ppm. MS (MALDI-TOF, DHB, H_2O): m/z = 751.64, $[M + H]^+$. $C_{42}H_{78}N_{12}{\cdot}3HI{\cdot}2H_2O$ (1170.92): C, 43.08; H, 7.32; N, 14.35; I, 32.51; found C, 43.31; H, 7.02; N, 14.44; I, 32.80.

Ligands Used for Analysis: Chlorohydrated derivatives of **L1**, **L2**, and **L3** were obtained by adding concentrated HCl to an ethanolic solution of the ligand at 0 °C. The resulting white precipitate was filtered, washed with absolute ethanol, and dissolved in a solution of 6 N HCl. After 2 h at 80 °C, the solvent was evaporated to give a white powder that was dried under vacuum at 80 °C for 24 h.

Potentiometric Titrations: Potentiometric measurements were performed in a jacketed cell thermostatted at 25.0 °C, kept under inert atmosphere of purified argon, using an automatic titrator (Metrohm, DMS Titrino 716) connected to a microcomputer. The free hydrogen concentrations were measured with a glass–Ag/AgCl combined electrode (Metrohm) filled with 0.1 M NaCl. The electrode was calibrated to read –log[H^+], designated as pH, by titration of a small quantity of diluted HCl by standardized NaOH at 0.02 M ionic strength and 25.0 °C (and determining the equivalent point by the Gran's method) followed by adjustment of the pH meter so as to minimize the calculated pH versus observed values. A logK_w for the system, defined in terms of log([H^+][OH^-]), was found to be –13.78 at the ionic strength employed and was maintained fixed during refinements.[15] Potentiometric measurements of solutions containing equimolecular amounts of azaligands and the appropriate anion were made at about 1 mM concentration and ionic strength I = 0.10 M (NaCl). Each titration utilized at least 10 points per neutralization of a hydrogen ion equivalent and titrations were repeated until reaching a satisfactory agreement. A minimum of three sets of data was used in each case to calculate the overall stability constants and their standard deviations. The standard deviations obtained for the different stability constants are reported in Tables 1 and 2. The range of accurate pH measurements was considered to be 2–12. Equilibrium constants and species distribution diagrams were calculated by using the program HYPERQUAD 2003.[16] The stability constants K_{alh} were noted with respect to ternary species $A_aL_lH_h$ where a, l, and h are the stoichiometric number of A: anion, L: ligand, and H: proton, respectively.

NMR Measurements: ^1H and ^{31}P NMR spectra in D_2O solutions at different pH values (adjusted with NaOD or DCl solutions) were recorded at 298 K with Bruker spectrometers. In the ^1H NMR spectra, the reported peak positions are relative to HOD at δ = 4.79 ppm. ^1H–^1H and ^1H–^{13}C 2D correlation experiments were performed to assign the signals. Small amounts of 0.01 M NaOD or DCl solutions were added to a solution of the chlorohydrated ligand to adjust the pD. The pH was calculated from the measured pD values with the following relationship: pH = pD – 0.40.[17]

Supporting Information (see footnote on the first page of this article): The pH dependence of the of **L2**; logarithm recognition constants, logK_{alh}, for the ligands with anions; species distribution diagrams ligands/anions as a function of pH; overall percentages of complexed ALH_h species with ligands, as a function of pH; ^1H NMR chemical shifts of aromatic Ha protons.

Acknowledgments

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