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New Bis-Cresol-Bridged *bis*(1,4,7-Triazacyclononane) Ligand As Receptor for Metal Cations and Phosphate Anions

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The synthesis and characterization of a new *bis*([9]aneN$_3$) ligand (H_2L) containing two [9]aneN$_3$ macrocyclic moieties separated by a 2,2′-methylene-*bis*-cresol (cresol = 4-methyl-phenol) unit is reported. A potentiometric and ^{1}H NMR study in aqueous solution reveals that H_2L is in a zwitterionic form, and protonation of the cresolate oxygens occurs only with the formation of the highly charged $(H_5L)^{3+}$ and $(H_6L)^{4+}$ species at acidic pH values. The coordination properties of H_2L toward Cu(II), Zn(II), Cd(II), and Pb(II) were studied by means of potentiometric and UV spectrophotometric measurements. The ligand gives both mono- and binuclear complexes in aqueous solution. At acidic pH values the ligand forms stable binuclear $[M_2H_2L]^{4+}$ complexes, where each metal is coordinated by two amine groups of [9]aneN$_3$ and the deprotonated oxygen of the adjacent cresol unit; the remaining amine group is protonated. Deprotonation of the $[M_2H_2L]^{4+}$ species at alkaline pH values affords $[M_2L]^{2+}$ complexes, where all amine groups of the [9]aneN$_3$ moieties are involved in metal coordination. Binding of mono-, di- and triphosphate, and adenosine triphosphate (ATP) was studied by means of potentiometric, ^{1}H and ^{31}P NMR measurements and by molecular dynamics simulations. The receptor forms stable 1:1 adducts with di-, triphosphate, and ATP, while the interaction with monophosphate is too low to be detected. In the complexes both the [9]aneN$_3$ moieties act cooperatively in the substrate binding process. The stability of the adducts increases in the order diphosphate < triphosphate < ATP. This trend is explained in terms of increasing number of charge−charge interactions between the phosphate chains and the protonated [9]aneN$_3$ subunits and, in the case of ATP, of stacking interactions between the adenine and cresol units.

Introduction

The chemistry of polyazamacrocycles have attracted great attention because of their ability to interact with both metal cations[1–20] and anionic species.[21–37] Polyamine macrocycles, in fact, can easily protonate in aqueous solutions, affording polyammonium receptors, which can be used for anion coordination. One of the most investigated classes of azamacrocycles is constituted by two or more polyamine groups separated by different spacers, such as aliphatic chains or aromatic units. While the polyamine moieties act as

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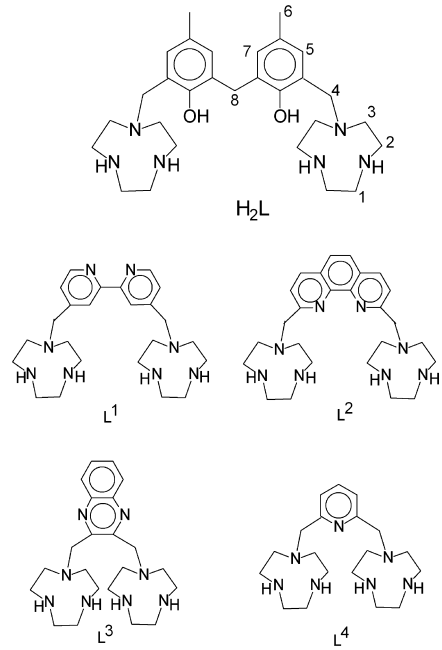
binding sites for metal cations or anionic groups, the spacers can modulate the distances between the binding moieties. An accurate tuning of both the binding features of the polyamine units and the structural characteristics of the spacers, in terms of flexibility, hydrophobicity, or presence of additional functional groups, has led to the achievement of receptors with specific functions, able not only to bind but also to find applications as models for catalytic metallobiosites[38] or as optical chemosensors,[21l,m,39–43] for metals or anions.

Among polyamine binding units, the tridendate macrocycle [9]aneN$_3$ is one of the most studied.[17,44–50] This ligand can form stable complexes with main groups and transition metals in aqueous solutions. In addition to sandwich complexes, a series of polynuclear complexes have also been prepared in the presence of suitable bridging ligands. In these species, the [9]aneN$_3$ macrocyclic framework serves as a face-capping group, allowing variation of the donor array at the remaining coordination site(s) of a coordinated metal ion, which can be used as anchoring point(s) for exogenous anionic or neutral substrates.

Scheme 1. Ligand Drawings



In this context, we have recently synthesized a series of ligands constituted by two [9]aneN$_3$ subunits linked by spacers with coordinating ability, such as dipyridine, phenanthroline, pyridine or quinoxaline (Scheme 1);[14b,51] it was found that their binding properties toward metal cations or small inorganic anions, such as chloride or bromide, are strongly influenced by the distance between the two [9]aneN$_3$ units, and, in the case of metal coordination, by the involvement of the heteroaromatic nitrogens in metal binding. For instance, while in the case of L,[1] L,[2] and L[4] the

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heteroaromatic units are involved in metal binding, the quinoxaline nitrogens of L^3 do not participate in metal coordination because of their poorer σ-donor properties. In anion coordination, the two [9]aneN$_3$ units of L^3 and L^4 define a cavity of appropriate dimensions to selectively host chloride and bromide, respectively. We have now decided to combine together the special binding features of these *bis*-macrocycles with those of phenol, a common binding unit in coordination chemistry; we have therefore synthesized ligand H_2L (Scheme 1), which contains two [9]aneN$_3$ units separated by a 2,2′-methylene-*bis*-cresol moiety. Although several polyamine ligands (including [9]aneN$_3$) containing phenol units have been prepared and their metal coordination properties investigated,[11c–f,52–57] less is known about dinucleating ligands containing diphenol spacers,[11c] and no study on their binding ability toward anions has been reported. With this in mind, we have investigated the coordination properties of H_2L toward Cu(II), Zn(II), Cd(II), and Pb(II), as well as toward phosphate inorganic anions and nucleotides.

Results and Discussion

Synthesis and Protonation of H_2L. Selective *N*-functionalization of [9]aneN$_3$ with only one or two pendant arms is a difficult task using a multistep approach. The most successful synthetic procedures for the monofunctionalization of [9]aneN$_3$ feature the use of appropriate protecting groups for two of the three nitrogen atoms of the macrocyclic ligand. For the synthesis of H_2L we successfully employed a synthetic route where only two amine nitrogens of the [9]aneN$_3$ starting material are *N*-protected with a ^{t}BuO$_2$C-(BOC) group.[58] Two equiv of the compound (BOC)$_2$-[9]aneN$_3$ containing only one (unprotected) secondary amine were reacted with one equiv of the appropriate aryl dihalide [2-(3-chloromethyl-2-hydroxy-5-methylbenzyl)-6-chloromethyl-4-methylphenol][59] to give, after removal of the protecting groups, the desired compound H_2L in reasonable good overall yield.

The protonation features of H_2L were studied by means of potentiometric, UV−vis, and ^{1}H NMR measurements.

Table 1. Protonation Constants (log *K*) of H_2L ($NaClO_4$ 0.1 M, 298.1 K)

equilibrium	log *K*
$H_2L + H^+ \rightleftharpoons (H_3L)^+$	10.81(5)
$(H_3L)^+ + H^+ \rightleftharpoons (H_4L)^{2+}$	10.06(4)
$(H_4L)^{2+} + H^+ \rightleftharpoons (H_5L)^{3+}$	7.26(4)
$(H_5L)^{3+} + H^+ \rightleftharpoons (H_6L)^{4+}$	6.04(6)
$(H_6L)^{4+} + H^+ \rightleftharpoons (H_7L)^{5+}$	3.0(1)

Table 1 collects the protonation constants of H_2L potentiometrically determined in 0.1 M $NaClO_4$ aqueous solutions at 298.1 K. The neutral H_2L species behaves as pentaprotic base in the pH range investigated (2.5−10.5). The equilibria relative to deprotonation of H_2L to give the anionic forms $(HL)^-$ and L^{2-}, which should be theoretically achieved in strong alkaline solutions, are not detected in our experimental conditions. The data in Table 1 clearly show that H_2L behaves as a rather strong base in the addition of the first two protons. The first two protonation constants are similar and higher than 10 log units. This behavior suggests that the first two protonation steps involve sites located far from each other, such as two secondary nitrogens one for each [9]aneN$_3$ unit, to minimize the electrostatic repulsion. A similar consideration can also explain the similar values of the third and fourth protonation constants observed. On the other hand, it is known that polyamine ligands with attached phenol groups can exist in a zwitterionic form[11c–f,52–57] and, in some cases, can contain ammonium groups and deproto-

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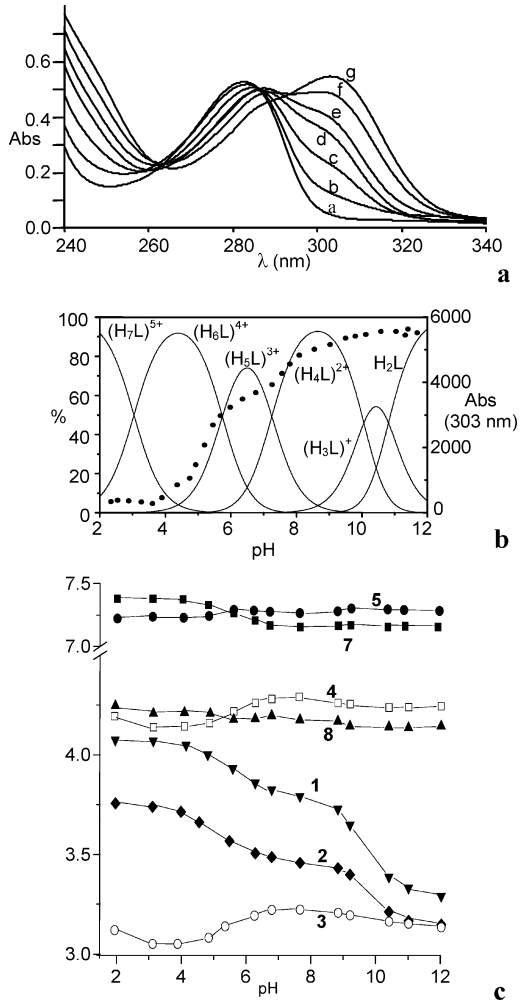

Figure 1. (a) UV spectra of H_2L at different pH values (a, pH 3.; b, pH 4.3; c, pH 4.6; d, pH 5.4; e, pH 6.8; f, pH 8.5; g, pH 10); (b) pH dependence of the ε value at 303 nm superimposed on the distribution diagram of the protonated species of the ligand; (c) pH dependence of the ^{1}H NMR signals of H_2L (See Scheme 1 for atom labeling; the chemical shift of the signal of the methyl group 6 does not vary significantly in the pH range investigated and it has been omitted for clarity).

nated phenol moieties even within overall positively charged species.[11c–f] From this point of view, the UV−vis absorption spectra of the ligand can give useful information on the role played by the cresol moieties in the acid−base behavior of H_2L. The cresol (4-methyl-phenol) unit, in fact, is characterized by an absorption band at 283 nm. Deprotonation of the -OH function gives rise to a new red-shifted absorption band at about 300 nm. The appearance of this band can be therefore used as diagnostic tool to detect deprotonation of the cresol unit in solution. Actually, in the case of H_2L a new band at 303 nm appears in aqueous solution above pH 4, and its absorption intensity increases as the pH increases from 4 to 10 (Figure 1a). In particular, the increase of the absorbance at 303 nm takes place with deprotonation of the $(H_6L)^{4+}$ species to give $(H_5L)^{3+}$ and $(H_4L)^{2+}$ (Figure 1b). This points out that the formation of $(H_5L)^{3+}$ and $(H_4L)^{2+}$ is accompanied by two successive deprotonation steps of the cresol moieties of the ligand. Therefore, the species $(H_5L)^{3+}$ and $(H_4L)^{2+}$, as well as those with lower protonation degree [$(H_3L)^+$ and H_2L], contains two cresolate functions, that is, they are in zwitterionic forms. Only in the highly charged species $(H_6L)^{4+}$ and $(H_7L)^{5+}$ are both phenolic moieties protonated.

To further elucidate the acid base behavior of H_2L, we collected ^{1}H NMR spectra in aqueous solutions at different pH values, and the pH dependence of the ^{1}H chemical shifts is reported in Figure 1c together with the distribution diagram of the protonated species of the ligand. The ^{1}H spectrum of the ligand at pH 11, where the H_2L species predominates in solution, displays six signals for the aliphatic protons and two for the aromatic ones, accounting for a C_{2v} time-averaged symmetry of the ligand, which is preserved over all the pH range investigated. As reported above, H_2L is in zwitterionic form, that is, the two protons are localized in the two [9]aneN_3 subunits. The NMR spectra of the H_2L species cannot give information on the localization of these protons within the cyclic moieties: each proton can be bound either to a secondary nitrogen or to the tertiary one of a macrocyclic unit. Figure 1c shows that the signals of the methylene groups 1 and 2, adjacent to the secondary nitrogens (Scheme 1), show the most relevant downfield shifts upon ligand protonation in the pH range 11−4. Minor shifts affect the other signals. In particular, the signals of 1 and 2 display remarkable downfield shifts between pH 11 and 9, where the first two protons bind to H_2L. This suggests that the formation of the species $(H_3L)^+$ and $(H_4L)^{2+}$ from H_2L is accompanied by protonation of the secondary nitrogens, as sketched in Figure 2a. Then, a second downfield shift of the resonances of 1 and 2 is observed between pH 7 and 4, where the species $(H_5L)^{3+}$ and $(H_6L)^{4+}$ are formed. Once again, this would indicate that the two further protons are bound to the secondary nitrogens. On the contrary, the UV data suggested that protonation of $(H_4L)^{2+}$ to form $(H_5L)^{3+}$ and $(H_6L)^{4+}$ is accompanied by protonation of the cresolate oxygens. This apparent contrast can be solved by considering that in H_2L, $(H_3L)^+$, and $(H_4L)^{2+}$ two protons are localized on the tertiary nitrogens. Most likely, protonation of $(H_4L)^{2+}$ to give $(H_5L)^{3+}$ and $(H_6L)^{4+}$ occurs on the deprotonated

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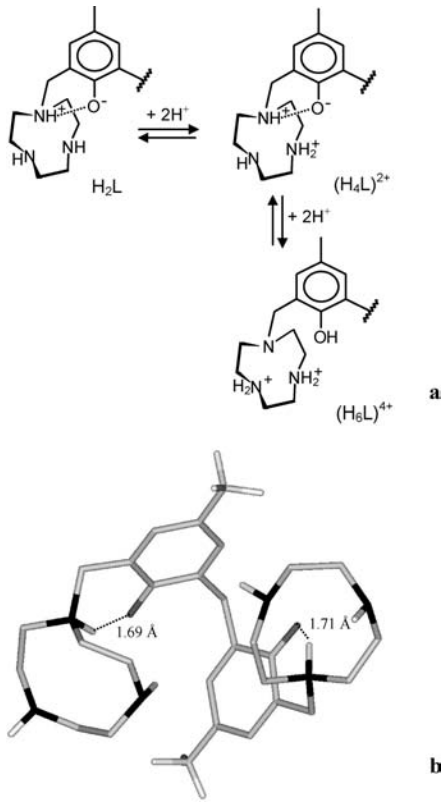

Figure 2. (a) Proposed distribution of protons in the different protonated species of the ligand; (b) representation of the lowest energy conformer of H_2L, displaying the $N-H^+\cdots O^-$ hydrogen bonds between the tertiary ammnonium groups and the adjacent cresolate oxygens.

hydroxyl function of cresolate and simultaneously leads to a rearrangement of the proton distribution within each [9]aneN_3 unit, for example, to a proton transfer from the tertiary nitrogens to the secondary ones (Figure 2a). The upfield shifts of the signals of the methylene groups 3 and 4 (Scheme 1), observed in the pH range 7−4, are in agreement with a deprotonation process involving the adjacent tertiary amine groups. Conformational searches, carried out at the semiempirical PM3 level,[60] on the two possible isomers of the H_2L zwitterion, that is, with the two protons localized on the secondary nitrogens or on the tertiary ones, confirms the hypotheses made on the basis of the ^{1}H NMR results. The lowest energy conformer found for the H_2L isomer containing two protonated tertiary nitrogens (Figure 2b) is more stable than the isomers with protonated secondary nitrogens of at least 9.72 kcal/mol because of the formation of stabilizing $N-H^+\cdots O^-$ hydrogen bonds between the tertiary ammonium groups and the adjacent deprotonated oxygen of the cresolate unit. Most likely, protonation of the cresolate oxygens in pH region 7−4 leads to disruption of these $N-H^+\cdots O^-$ hydrogen bonds, favoring the proton transfer process from the tertiary nitrogens to the secondary ones, as actually suggested by the ^{1}H NMR measurements.

Table 2. Stability Constants of the Cu(II), Zn(II), Cd(II), and Pb(II) Complexes with H_2L (I = 0.1 M, 298.1 K)

	log K			
equilibrium	Cu(II)	Zn(II)	Cd(II)	Pb(II)
$H_2L + M^{2+} \rightleftharpoons [MH_2L]^{2+}$	19.0(1)	14.6(1)	11.6(2)	12.1(1)
$[MH_2L]^{2+} + H^+ \rightleftharpoons [MH_3L]^{3+}$	7.5(1)	8.3(2)	9.7(1)	9.9(1)
$[MHL]^+ + H^+ \rightleftharpoons [MH_2L]^{2+}$	9.8(1)	10.7(1)		
$[ML] + H^+ \rightleftharpoons [MHL]^+$		9.9(2)		
$[MH_2L]^{2+} + M^{2+} \rightleftharpoons [M_2H_2L]^{4+}$	12.6(1)	8.9(1)	8.1(1)	8.5(1)
$[M_2HL]^{3+} + H^+ \rightleftharpoons [M_2H_2L]^{4+}$	4.14(5)	6.5(1)	8.2(1)	7.5(1)
$[M_2L]^{2+} + H^+ \rightleftharpoons [M_2HL]^{3+}$	8.8(1)	8.9(1)	9.481)	8.9(1)
$[M_2L]^{2+} + OH^- \rightleftharpoons [M_2L(OH)]^+$	2.6(2)	3.2(1)	2.7(1)	3.0(1)
$[M_2L(OH)]^+ + OH^- \rightleftharpoons [M_2L(OH)_2]$	2.4(2)	2.6(1)		

Metal Complexation. Metal Ion Complexation in Aqueous Solution. The molecular architecture of H_2L, constituted by two separated binding units, enables the ligand to form stable mono- and binuclear metal complexes in aqueous solution. The species formed in the presence of Cu(II), Zn(II), Cd(II), and Pb(II) and the corresponding stability constants are given in Table 2. H_2L generally displays a marked preference to give binuclear complexes which are largely present in aqueous solutions even when a metal to ligand 1:1 molar ratio is used (see Supporting Information, Figures S1 and S2). Although this behavior does

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not allow a spectroscopic characterization of the mononuclear complexes, the values of their stability constants can be informative about the coordination environment of the metal cations. Actually, the stability of the complexes $[MH_2L]^{2+}$ is remarkably higher than the corresponding complexes with [9]aneN$_3$ (for instance log K = 15.0 and 11.1 for the formation of the complexes $[Cu([9]aneN_3)]^{2+}$ and $[Zn([9]aneN_3)]^{2+}$ while log K = 19.0 and 14.6 for the formation of $[CuH_2L]^{2+}$ and $[ZnH_2L]^{2+}$, respectively),[61,62] suggesting that a higher number of ligand donors is bound to the metal center in the mononuclear complexes of H_2L. At the same time, the molecular architecture of H_2L rules out the possibility that both the cyclic [9]aneN$_3$ moieties participate in the coordination of a single metal cation. Most likely, the increased stability of the mononuclear complexes $[MH_2L]^{2+}$ with respect to $[M([9]aneN_3)]^{2+}$ is due to the involvement of a deprotonated cresolate oxygen in metal binding, together with the nitrogen donors from a [9]aneN$_3$ moiety. The two protons of the $[M_2H_2L]^{4+}$ complexes are reasonably located in the [9]aneN$_3$ unit not involved in metal coordination.

As shown in Figure 3b for the Zn(II) complexes, only binuclear complexes are generally present in aqueous solution containing ligand and metals in 1:2 molar ratios. In these conditions, only Pb(II) affords a mononuclear protonated complex $[PbH_3L]^{3+}$ in relevant percentage. The equilibrium constants for the addition of the second metal to the species $[MH_2L]^{2+}$ to afford the complexes $[M_2H_2L]^{4+}$ are high, especially for Cu(II), as generally observed for the formation of binuclear complexes where the metal cations occupy two separated binding compartments. Potentiometric measurements show that, for all the metals under investigation, the formation of these binuclear species takes place at slightly acidic pH values and is followed by a series of deprotonation processes to give $[M_2HL]^{3+}$, $[M_2L]^{2+}$, $[M_2L(OH)]^{+}$, and, in the case of Cu(II) and Zn(II), also $[M_2L(OH)_2]$ complexes. Once again, UV spectra recorded at different pH values can be used to elucidate the role of the cresol units in metal binding. As displayed in Figure 3a for Zn(II), the UV spectral features of solutions containing H_2L and Zn(II) in 1:2 molar ratio are remarkably affected by pH; in fact, the formation of a new band with a maximum at 298 nm is observed as the pH increases from 4 to 6. This effect can be reasonably due to deprotonation of the cresol moieties upon metal binding. Figure 3b shows that the absorbance measured at 298 nm increases with the formation in solution of the $[Zn_2H_2L]^{4+}$ complex; then, at higher pH values the absorbance remains almost constant. These data point out that the $[Zn_2H_2L]^{4+}$ contains two deprotonated cresol groups, that is, the ligand is in its zwitterionic form. Deprotonation of the cresol groups takes place in the presence of metal cations at remarkably lower pH values than in the case of the ligand alone, in agreement with a deprotonation process occurring upon binding of the Ar−OH function to the metal. These

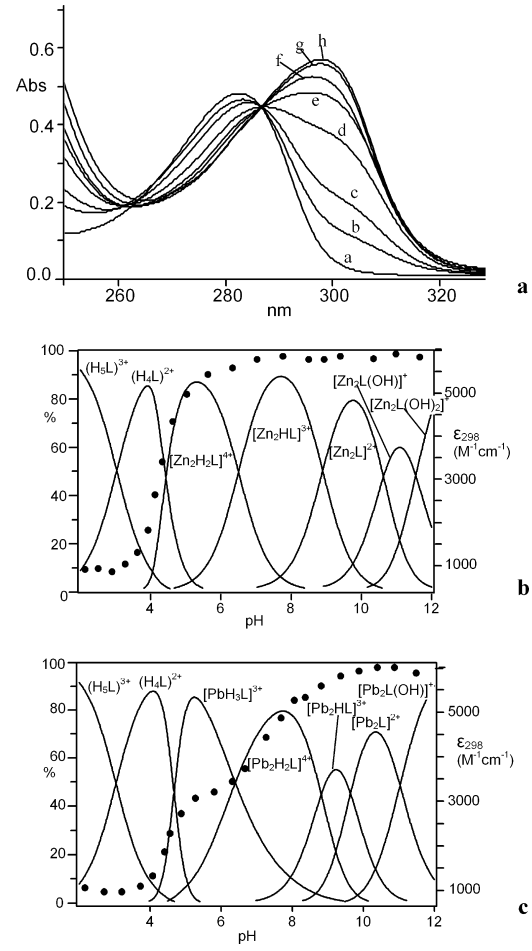


Figure 3. (a) UV spectra recorded on solutions containing H_2L and Zn(II) in 1:2 molar ratio at different pH values (a, pH = 3; b, pH = 3.4; c, pH = 4; d, pH 4.6; e, pH = 4.9; f, pH 6.5; g, pH = 6.8; h, pH 7.8); (b) pH dependence of the ε value at 298 nm for H_2L in the presence of 2 equiv of Zn(II), superimposed on the distribution diagram of the complexes; (c) pH dependence of the ε value at 298 nm for H_2L in the presence of 2 equiv of Pb(II), superimposed on the distribution diagram of the complexes.

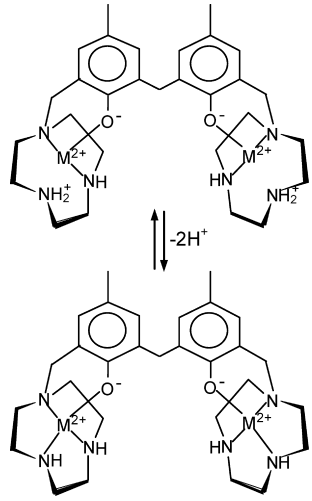
results indicate that in the $[Zn_2H_2L]^{4+}$ complex each metal is coordinated by a negatively charged oxygen and two amine groups of a [9]aneN$_3$ moiety, the third amine group being protonated. Similar increases of the absorbance as a consequence of the formation of the $[M_2H_2L]^{4+}$ complexes is also observed in the case of Cu(II) and Cd(II) (see Supporting Information, Figure S3). Interestingly, in the case of Pb(II), the formation of the mononuclear complex $[PbH_3L]^{3+}$ at slightly acidic pH values is accompanied by the appearance of the new band at about 300 nm (Figure 3c). This strongly suggests that in the $[PbH_3L]^{3+}$ complex one cresolate unit deprotonates upon metal binding, that is, the Pb(II) cation is coordinated by a single cyclic [9]aneN$_3$ unit and by the adjacent negatively charged oxygen of cresolate. As shown in Figure 3c, a further increase of the absorbance is then observed with the formation of the binuclear Pb(II) complexes because of deprotonation of the second cresol unit upon binding of the second metal.

For all the metals under investigation, the $[M_2H_2L]^{4+}$ complexes release acidic protons at slightly alkaline pH values, affording $[M_2HL]^{3+}$ and $[M_2L]^{2+}$ species; these deprotonation steps can be reasonably due to deprotonation

(61) log K = 9.5 and 10.8 for the equilibrium $M^{2+} + L \rightleftharpoons [ML]^{2+}$, with M = Cd and Pb, respectively, and L = [9]aneN$_3$; Yang, R.; Zompa, L. *Inorg. Chem.* **1976**, *15*, 1499–1502.

(62) log K = 15.0 and 11.1 for the equilibrium $M^{2+} + L \rightleftharpoons [ML]^{2+}$, with M = Cu and Zn, respectively, and L = [9]aneN$_3$; Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1978**, 1081–1085.

Scheme 2. Proposed Structures of the $[M_2H_2L]^{4+}$ and $[M_2L]^{2+}$ Complexes



of the ammonium groups. As sketched in Scheme 2, in the $[M_2L]^{2+}$ complexes the metal ions are likely to be coordinated by a N_3 set of donors and by the oxygen of a cresolate unit, as actually observed in the crystal structure of the Cu(II) complex (see below). Finally, deprotonation of metal-bound water molecules above pH 9 yields hydroxo complexes. The dinuclear complexes, however, do not show a high tendency to form $[M_2L(OH)_2]$ and/or $[M_2L(OH)_2]^{2+}$ species, the addition constants of a single hydroxide anion to the $[M_2L]^{2+}$ or $[M_2L(OH)]^{+}$ complexes being lower than 3.2 log units (Table 2). This suggests that the hydroxide anions are bound to a single metal cation. In fact, far higher addition constants of hydroxide to dimetal complexes, generally 5−7 log units, are found in the case of the assembly in aqueous solution of hydroxide-bridged dimetal cores.

Crystal Structure of $[Cu_2L(NO_3)_2]\cdot 2H_2O$. To ascertain whether the potentially 8-coordinate ligand H_2L would bind two metal ions into the two adjacent four coordinating compartments, we treated H_2L with 2 equiv of the different metals in MeOH at room temperature. Although solid dimetal complexes were isolated and characterized for all the four metal cations (see Supporting Information for experimental details), only in the case of Cu(II), green crystals suitable for X-ray diffraction analysis were obtained by partial removal of the solvent and diffusion of Et_2O vapor into the reaction mixture. The single crystal X-ray structure shows the formation of the binuclear complex $[Cu_2L(NO_3)_2]\cdot 2H_2O$ (Figure 4, Table 3) in which each metal ion is coordinated by three nitrogen atoms from a single [9]aneN_3 unit and the adjacent negatively charged oxygen from the cresolate moiety. An oxygen atom from a nitrate monodentate ligand completes the coordination sphere of each metal center. The two binding subunits of the binuclear complex are symmetry related by a C_2 axis passing through the methylene group linking the two aromatic rings. The intermetallic distance is 7.679(1) Å. In both four-dentate halves of the ligand, the Cu(II) centers are in distorted square-based pyramidal environments with basal planes defined by the N2 and N3 nitrogen atoms, the O1 phenolate oxygen, and the O3 atom belonging to the nitrate anion [max deviation from the mean

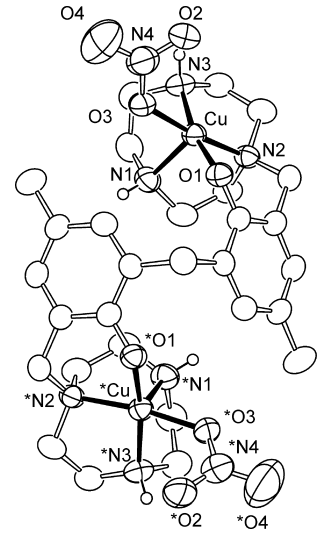


Figure 4. ORTEP (Oak Ridge Thermal Ellipsoid Plot) drawing of the $[Cu_2L(NO_3)_2]$ complex in $[Cu_2L(NO_3)_2]\cdot 2H_2O$. Displacement ellipsoids are drawn at 50% probability level.

Table 3. Selected Distances (Å) and Bond Angles (°) for the Metal Coordination Environment in $[Cu_2L(NO_3)_2]\cdot 2H_2O$

Cu−N1	2.262(4)	Cu−O1	1.925(3)
Cu−N2	2.065(4)	Cu−O3	1.974(3)
Cu−N3	2.045(4)		
N1−Cu−N2	82.4(2)	N2−Cu−O3	172.9(1)
N1−Cu−N3	81.4(2)	N1−Cu−O3	99.9(1)
N2−Cu−N3	84.1(2)	N3−Cu−O1	167.7(2)
N1−Cu−O1	110.1(1)	N3−Cu−O3	89.7(2)
N2−Cu−O1	93.0(1)	O1−Cu−O3	92.4(1)

basal plane 0.071(2) Å for N3] or by their symmetric equivalents *N2, *N3, *O1, and *O3 (starred atoms are related by the x, 0.5 − y, 0.5 − z symmetry operation). In each half the apical position is occupied by the secondary nitrogen atom N1 or its symmetric equivalent *N1. The copper atoms lie 0.138(2) Å above the basal planes, shifted toward the apical position; the Cu−N1 vector shows a slight deviation from perpendicularity with the basal N_2O_2 plane.

The crystal packing of $[Cu_2L(NO_3)_2]\cdot 2H_2O$ shows that the $[Cu_2L(NO_3)_2]$ complex can assume a right-handed or left-handed helical conformation, affording two different optical isomers. In particular, the crystal packing is formed by alternate columns formed respectively by complexes with right-handed and left-handed helical conformations (Figure 5). The complexes belonging to the same column interact through a hydrogen bond involving the *N3 (or N3) secondary nitrogen and the nitrate oxygen O2 of an adjacent complex unit. The right-handed and left-handed helices interact via edge to face π-stacking interactions between two cresolate units, the distance between the C5 carbon and the centroid of cresolate unit being 3 Å. The nitrate oxygen O2 and the cresolate oxygen *O1 (or O1) are also involved in hydrogen bonding interactions with the water molecule O5 (O5···O2 2.783(6) Å, O5···*O1 2.771(5) Å).

Anion Complexation. It was shown that ligand L^3 and L^4 in their protonated forms are featured by two [9]aneN_3 units kept at a close distance by the short and rigid quinoxaline or pyridine linkage, thus generating a cavity of optimal dimension for the encapsulation of small anions, such

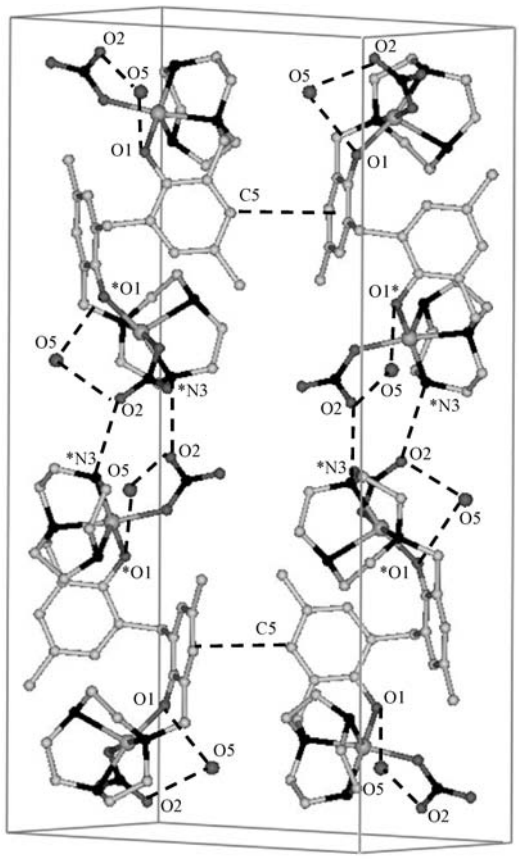


Figure 5. Crystal packing of the compound $[Cu_2L(NO_3)_2]\cdot 2H_2O$, displaying hydrogen bonding and π-stacking interactions between different complex units or involving external water molecules.

as bromide or chloride.[51] From this point of view, ligand H_2L presents a larger distance and a much more flexible structure with respect L^3 and L^4 and, therefore, it does not define a preorganized cavity to host anionic species. On the other hand, its particular protonation characteristics, for example, its tendency to exist in zwitterionic forms, enables the ligand to afford species with a high number of positively charged ammonium groups even at alkaline pH values. For instance, $(H_4L)^{2+}$, which contains four protonated nitrogens, is the predominant species in aqueous solutions from pH 7.5 to 10 (Figure 1). It is known that the ability to form stable adducts with anions depends not only on the overall charge of the polyammonium receptor but also on the number and disposition of the ammonium groups able to give salt bridges with the anionic function of the hosts. On the basis of these considerations, we decided to carry out a potentiometric and NMR (^1H, ^{31}P) study on the coordination of H_2L with inorganic phosphate anions (phosphate, di- and triphosphate) and nucleotide anions (ADP, ATP) in aqueous solutions. Unfortunately, the ligand forms very low solubility adducts with ADP, preventing any analysis of its complexation features by either potentiometry or NMR.

The stability constants of the complexes of the ligand with diphosphate, triphosphate and ATP, determined by means of potentiometric measurements are reported in Table 4, while the distribution diagram for the system H_2L/ATP is given in Figure 6 (distribution diagrams for the systems H_2L/diphosphate and H_2L/triphosphate are reported within the Supporting Information, Figure S4). Phosphate complexation was not detected in our experimental conditions.[63]

Different protonated species, from $(H_3L)^+$ to $(H_5L)^{3+}$, interact with the substrates to give stable 1:1 adducts (see Table 4 and Figure 6). Differently from most of polyammonium receptors, the strength of the interaction does not necessarily increase with the positive charge present on the ligand. In fact, the less charged species $(H_3L)^+$ and $(H_4L)^{2+}$, formed in the alkaline pH region, give the strongest interaction with all the anionic substrates. $(H_4L)^{2+}$ forms somewhat more stable adducts than $(H_3L)^+$, in agreement with the presence of a larger number of ammonium functions (four in $(H_4L)^{2+}$ vs three in $(H_3L)^+$) able to form charge−charge and hydrogen bonding interactions with the anionic phosphate groups of the substrates. Conversely, further protonation of the ligand to give the species $(H_5L)^{3+}$ and $(H_6L)^{4+}$ leads to a slight decrease of the addition constants of the anionic substrates. In fact, protonation of $(H_4L)^{2+}$ to give $(H_5L)^{3+}$ and $(H_6L)^{4+}$ takes place on the cresolate oxygen and therefore increases the overall positive charge of the ligand but does not change the number of ammonium group available for anion binding. At the same time, the species $(H_5L)^{3+}$ and $(H_6L)^{4+}$ are formed at acidic pH values, where the substrates are in their less negatively charged mono- or diprotonated forms [$(HA)^{3-}$ or $(H_2A)^{2-}$ with A = diphosphate or ATP or $(HA)^{4-}$ or $(H_2A)^{3-}$ with A = triphosphate). These observations would explain the observed lower stability of the complexes with $(H_5L)^{3+}$ and $(H_6L)^{4+}$. Finally, no interaction was found between the protonated receptor and the monocharged or uncharged form of the substrates [$(H_3A)^-$ and H_4A with A = diphosphate or ATP and $(H_4A)^{3-}$ or H_5A with A = triphosphate), which are present in solution only at very low pH values (<3).

Comparing the binding ability of H_2L toward the different anions, the stability of the adducts increases in the order diphosphate < triphosphate < ATP (Table 4). As reported above, no interaction with phosphate was detected by means of potentiometric measurements. An appropriate way to visualize selectivity in anion coordination is to consider a competitive system containing the ligand and the three anions in equimolecular concentrations and to calculate the overall percentages of the three complexed anions over a wide pH range.[64] A similar plot (Figure 7) clearly shows that the formation of ATP adducts prevails on di- and triphosphate complexation in a wide pH range. Similarly, triphosphate is preferentially complexed with respect to diphosphate. Selective binding of triphosphate with respect to diphosphate could be ascribed either to the higher negative charge of the triphosphate anions and to the presence of three phosphate moieties able to interact with the ammonium group of the ligand or to a better ability of the longer triphosphate chain to interact with both the protonated [9]aneN_3 moieties of the receptor. From this point of view, the lower charge and the

(63) No change in the pH titration curves was observed in the potentiometric titrations of H_2L in the presence of phosphate as compared to the titration curves in the absence of this anion, indicating that H_2L does not interact or interacts very weakly with this substrate.

(64) Bianchi, A.; Garcia-España, E. *J. Chem. Educ.* **1999**, *76*, 1727–1732.

Table 4. Stability Constants of the Anion Complexes with H_2L (I = 0.1 M, 298.1 K)

	log K			log K
reaction	A = P_2O_7	A =ATP	reaction	A = P_3O_{10}
$(H_3L)^+ + A^{4-} \rightleftharpoons (H_3LA)^{3-}$	5.16(5)	6.66(3)	$(H_3L)^+ + A^{5-} \rightleftharpoons (H_3LA)^{4-}$	5.52(4)
$(H_4L)^{2+} + A^{4-} \rightleftharpoons (H_4LA)^{2-}$	5.44(5)	7.19(3)	$(H_4L)^{2+} + A^{5-} \rightleftharpoons (H_4LA)^{3-}$	6.05(5)
$(H_4L)^{2+} + (HA)^{3-} \rightleftharpoons (H_5LA)^{-}$	4.85(5)	6.10(3)	$(H_4L)^{2+} + (HA)^{4-} \rightleftharpoons (H_5LA)^{2-}$	5.46(5)
$(H_5L)^{3+} + (HA)^{3-} \rightleftharpoons (H_6LA)$	4.41(6)	5.65(4)	$(H_5L)^{3+} + (HA)^{4-} \rightleftharpoons (H_6LA)^{-}$	5.10(4)
$(H_6L)^{4+} + (HA)^{3-} \rightleftharpoons (H_7LA)^{+}$		5.28(4)	$(H_5L)^{3+} + (H_2A)^{3-} \rightleftharpoons (H_7LA)$	5.11(5)
$(H_5L)^{3+} + (H_2A)^{2-} \rightleftharpoons (H_7LA)^{+}$	4.17(5)		$(H_6L)^{4+} + (H_2A)^{3-} \rightleftharpoons (H_8LA)^{+}$	3.39(5)

smaller dimension could prevent the monophosphate anion from achieving a detectable interaction with the receptor. On the other hand, preferential binding of ATP with respect to inorganic anions cannot be interpreted only in terms of electrostatic and hydrogen bonding interactions with the protonated amine group of the receptor, since ATP contains a triphosphate chain similar to that of triphosphate and a lower negative charge. To get further information on the interaction mode of the different substrates, ^{1}H and ^{31}P NMR measurements were also performed. As shown in Figure 8, addition of diphosphate, triphosphate, or ATP to solutions of the receptor at pH 8.5 [at this pH value the ligand is mainly in its $(H_4L)^{2+}$ form] leads to a marked downfield shift of the ^{1}H signals of the methylene groups 1 and 2, adjacent to the secondary amine groups (see Scheme 1 for atom labeling). The chemical shift changes linearly generally up to 0.8:1 substrate to receptor molar ratio, to achieve a constant value for molar ratios greater than 1.2 in agreement with the formation of an adduct with a 1:1 receptor to anion

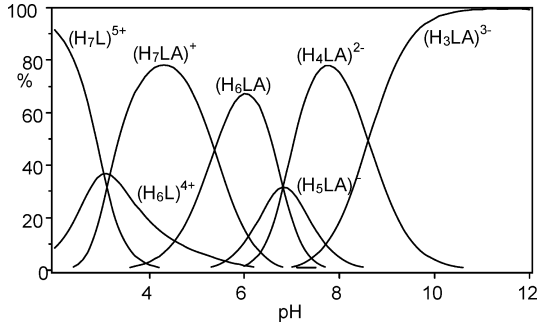


Figure 6. Distribution diagrams of the complexes for a system containing ATP (indicated as A for clarity) and H_2L in 1:1 molar ratio ([ATP] = [H_2L] = 1 × 10^{-3} M; 298 K, I = 0.1 M).

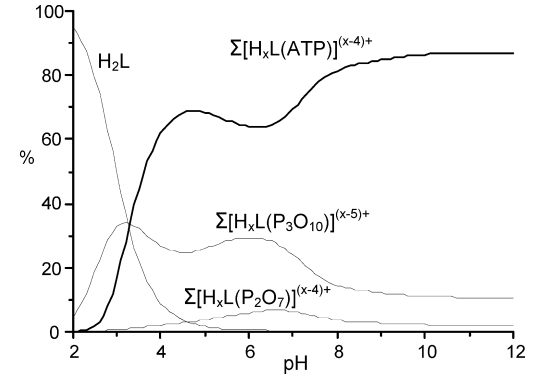


Figure 7. Plots of the overall percentages of the complexes with, di-, triphosphate and ATP [$\Sigma(H_xLA)^y$ = [H_3LA] + [H_4LA] + [H_5LA] + [H_6LA] + [H_7LA] + [H_8LA], A = $(P_2O_7)^{4-}$, $(P_3O_{10})^{5-}$, or $(ATP)^{4-}$] as a function of pH in a competitive system containing H_2L and $(P_2O_7)^{4-}$, $(P_3O_{10})^{5-}$, or $(ATP)^{4-}$ in equimolecular ratio ([H_2L] = [$(P_2O_7)^{4-}$] = [$(P_3O_{10})^{5-}$] = [$(ATP)^{4-}$] = 1 × 10^{-3} M).

stoichiometry. Minor shifts are observed for the signals due to H3 and H4, adjacent to the tertiary nitrogens. These shifts are reasonably due to formation of salt bridges between the protonated nitrogens and the anionic phosphate chains of the substrates; this interaction seems to involve mainly the secondary amine groups of the [9]aneN_3 moieties while the protonated tertiary amine groups would be engaged at a lesser extent in substrate binding. The observed downfield shifts are more marked in the case of ATP and triphosphate with respect to diphosphate, thus suggesting that these anions form stronger salt bridges with the ammonium groups of the receptor. No significant shift of the signals was instead found in the presence, even in large excess (5 equiv), of mono-

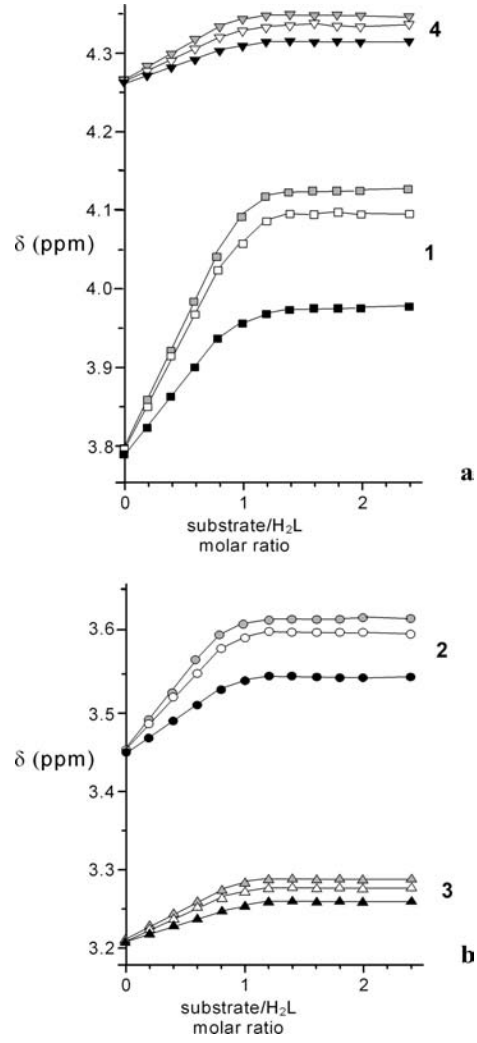


Figure 8. Chemical shift of the signals of aliphatic protons 1 and 4 (a) and 2 and 3 (b) of H_2L in the presence of increasing amounts of diphosphate (black filled symbols), triphosphate (gray filled symbols), and ATP (white filled symbols, aqueous solution at pH 8.5 and 298 K).

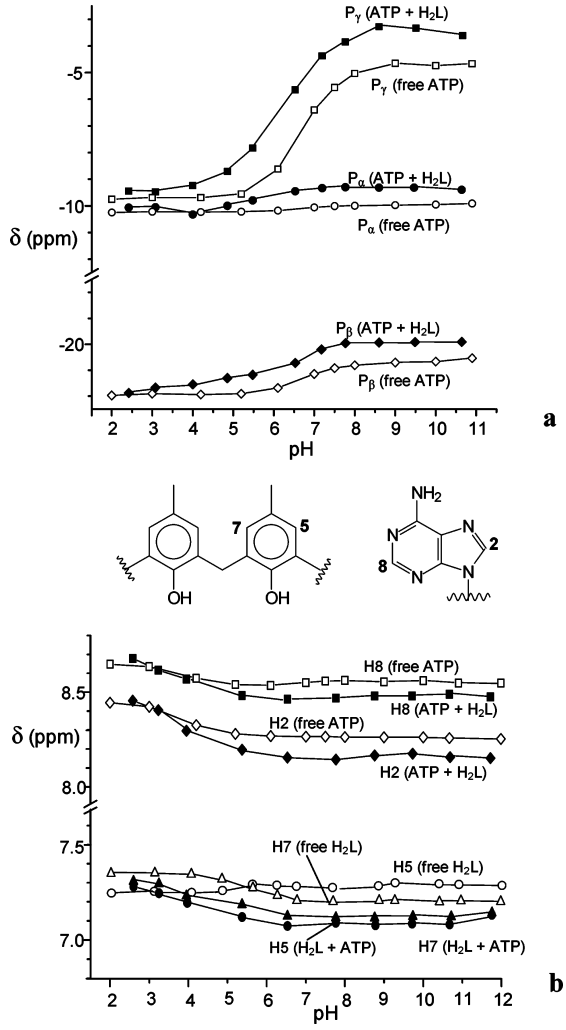


Figure 9. (a) pH dependence of the chemical shifts of the ATP ^{31}P NMR signals in the presence of 1 equiv of H_2L; (b) pH dependence of the chemical shifts of the ^{1}H NMR signals of the aromatic protons of ATP (H8, H2), H_2L (H5, H7), and for a system containing ATP and H_2L in 1:1 molar ratio.

phosphate, in agreement with the absence of detectable interactions observed in the potentiometric measurements. The formation of the complexes with di-, triphosphate, and ATP also affects the ^{31}P signals of the substrates, which are generally downfield shifted upon complexation.[65]

The chemical shifts of the ^{31}P NMR signals of the three substrates, and, in the case of ATP, the ^{1}H signal of the adenine are also strictly pH dependent in the presence of the receptor (see Figure 9 for the ATP/H_2L system). In particular, the difference of chemical shift between the signals of free ATP and the signals of complexed ATP is larger from alkaline to slightly acidic pH values, where complexation takes place to a larger extent, and decreases below pH 4, where ATP is mainly present in solution in its H_4ATP or $(H_3ATP)^-$ forms, which do not interact with the receptor (Table 4 and Figure 6). As often found in ATP complexation by polyammonium receptors, the signal of the terminal P_γ phosphate shows the largest downfield shift upon complexation (Figure 9a), suggesting a stronger involvement of this anionic group in charge−charge and hydrogen bonding interactions with the ammonium functions of the receptor. The ^{1}H NMR spectra of the system at different pH values provide unambiguous evidence for the involvement of an adenine moiety in π-stacking interactions with the aromatic unit of the receptor. Upfield displacements are in fact observed for the resonances of the adenine protons H2 and H8, as well as for the signals of cresol moieties H5 and H7 (Scheme 1, Figure 9b), the H5 signal being the most shifted. Clearly, the presence of stabilizing π-stacking interactions between adenine and aromatic subunits of H_2L would account for the observed higher stability of the ATP complexes with respect to that of inorganic anions.

To further elucidate the binding mode of H_2L we carried out a molecular modeling study by using the empirical forcefield AMBER[66] on the complexes formed by the different anionic substrates with the receptor in its $(H_4L)^{2+}$ form, which is prevalent in neutral and slightly alkaline solutions. The lowest energy conformers of the $(H_4L)^{2+}/(P_2O_7)^{4-}$, $(H_4L)^{2+}/(P_3O_{10})^{5-}$, and $(H_4L)^{2+}/(ATP)^{4-}$ complexes are reported in Figure 10. In all cases, the ligand assumes a "clamp-like" conformation, the "jaws" being defined by the two cresol-[9]aneN_3 subunits linked by the bridging methylene group. In consequence of this receptor structure, both

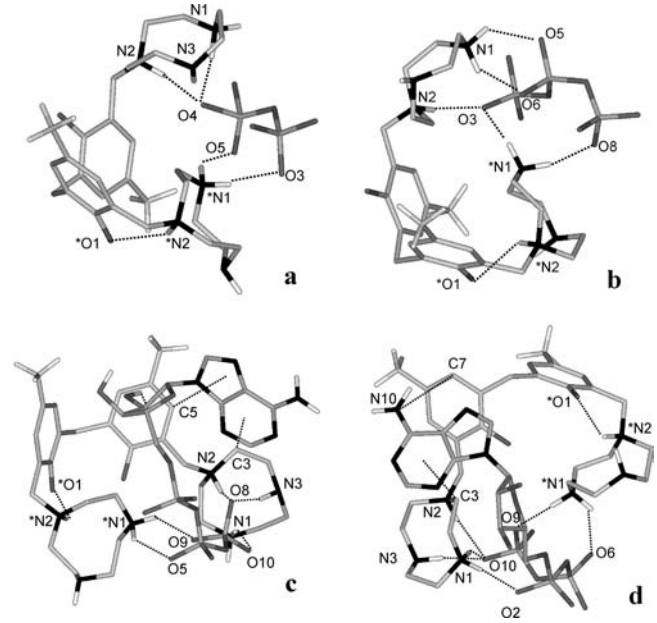


Figure 10. Lowest energy conformers for the adducts (a) $(H_4L)^{2+}/(P_2O_7)^{4-}$ (Hydrogen bonding distances: N2−H···O4, 1.82 Å; N1−H···O4, 1.80 Å; *N1−H···O5, 2.13 Å; *N1−H···O3, 2.01 Å; *N2−H···*O1, 2.21 Å), (b) $(H_4L)^{2+}/(P_3O_{10})^{5-}$ (Hydrogen bonding distances: N2−H ···O3, 1.93 Å; N1−H···O5, 2.12 Å; N1−H···O6, 1.93 Å; *N1−H···O3, 2.07 Å; *N1−H···O8, 1.87 Å; *N2−H···*O1, 2.21 Å), (c) $(H_4L)^{2+}/ATP^{4-}$ in the A family (Hydrogen bonding distances: N2−H···O8, 2.09 Å; N1−H···O10, 1.86 Å; N3−H···O8, 2.21 Å; *N1−H···O9, 2.00 Å; *N1−H···O5, 1.99 Å; *N2−H···*O1, 2.22 Å) and (d) $(H_4L)^{2+}/ATP^{4-}$ in the B family (Hydrogen bonding distances: N2−H···O10, 2.26 Å; N1−H···O10, 1.99; N3−H···O10, 1.84 Å; N1−H···O2, 1.83 Å; *N1−H···O9 1.80 Å; *N1−H···O6, 2.28 Å; *N2−H···*O1, 2.22 Å).

(65) For instance, at pH 8.5 the signal of diphosphate is 1.3 ppm downfield shifted in the presence of 1 equiv of H_2L, the P_β and P_α resonances of triphosphate are 2.1 and 1.0 ppm downfield shifted, while the P_γ, P_β and P_α of ATP are downfield shifted of 1.9, 1.0, and 0.8 ppm in the presence of 1 equiv of H_2L.

(66) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230–252.

the two protonated [9]aneN$_3$ units act as chelating units for the phosphate chain of the substrates. In all the complexes, only three of four ammonium groups participate in substrate binding; one of the protonated tertiary amine function, in fact, is involved in a strong hydrogen bonding interaction with a cresolate oxygen and is not involved in hydrogen bonding with the phosphate chain of the substrates. This result is in accord with the lower interaction of the tertiary nitrogens with the phosphate chains deduced from the ^{1}H NMR experiments (Figure 8).

In the case of $(P_2O_7)^{4-}$, one [9]aneN$_3$ moiety interacts with both the phosphate groups via hydrogen bond contacts involving only the protonated secondary nitrogen *N1 (Figure 10a) while the second macrocyclic unit simultaneously binds to a single phosphate group by using both the protonated secondary and tertiary nitrogens N1 and N2. Similarly, in the $(H_4L)^{2+}/(P_3O_{10})^{5-}$ adduct both the [9]aneN$_3$ units interact with the phosphate chains by using the three protonated nitrogens N1, N2, and *N1 (Figure 10b). In this case, however, all phosphate groups are used to bind to the receptor to give an overall higher number of salt bridges than those found for the $(P_2O_7)^{4-}/(H_4L)^{2+}$ adduct, in agreement with the higher stability of the adducts with triphosphate observed in solution. In the case of the ATP complexes, the different conformers can be grouped in two equally populated families, which mainly differ in the interaction mode of the nucleobase with the receptor (herein indicated with A and B, see Figures 10c,d for the lowest energy conformers). While in the A family the five-membered ring of adenine gives rise to an edge to face π-stacking interaction with one cresol ring (Figure 10c), in the B family of conformers the adenine−cresol interaction is featured by a $NH_2\cdots\pi$ contact between the NH_2 group of adenine and a cresol unit (Figure 10d). In both families, the C3 methylene group of a [9]aneN$_3$ unit gives a C−H$\cdots\pi$ interaction with the six-membered aromatic ring of adenine. As in the case of di- and triphosphate, in the A and B families the two [9]aneN$_3$ units are simultaneously involved in the binding process to the phosphate chain of ATP. As previously suggested on the basis of the different ^{31}P NMR shifts observed upon coordination for the signals of the P_α, P_β, and P_γ groups, the terminal phosphate P_γ of ATP gives the strongest interaction with the ammonium groups of the receptor, forming a network of hydrogen bond contacts with both the macrocyclic moieties. P_β gives rise to a single interaction in both families of conformers while P_α interacts with an ammonium group only in the second family.

As discussed above, the penta- and hexaprotonated forms of the ligand, $(H_5L)^{3+}$ and $(H_6L)^{4+}$ differ from $(H_4L)^{2+}$ for the presence of one or two protonated −OH functions within the *bis*-cresol moiety. To ascertain whether the OH groups of *bis*-cresol are involved in substrate binding, we carried out molecular modeling (MM) calculations on the adduct between $(H_6L)^{4+}$ and $(HATP)^{3-}$, which forms, among the different substrates, the most stable complex with $(H_6L)^{4+}$.

In the $(H_6L)^{4+}/(HATP)^{3-}$ adduct the receptor assumes a "clamp-like" conformation similar to that found in the adducts with $(H_4L)^{2+}$, the two protonated [9]aneN$_3$ units being cooperatively used to interact with the anionic phosphate chain (Supporting Information, Figure S5). As in the case of $(H_4L)^{2+}$, the different conformers can be grouped in two families, which mainly differ in the role of the nucleobase in substrate binding. In fact, only in the most populated family of conformers (A family in Supporting Information, Figure S5, population percentage 60%) adenine interacts with the receptor through a π-stacking interaction with one cresolic unit. The two −OH functions of the *bis*-cresol moiety do not interact with the nucleotide anion. Conversely, in both families the two −OH groups are associated together through an O$\cdots$H−O intramolecular hydrogen bond. Furthermore, in the less populated (40%) B family one hydroxyl function also interacts via hydrogen bonding with an ammonium group of a [9]aneN$_3$ moiety.

Concluding Remarks. The tendency of the ligand H_2L to maintain a zwitterionic form even in its polyprotonated species, such as $(H_3L)^+$ and $(H_4L)^{2+}$, strongly affects its binding features toward both metal cations and phosphate anions. In metal coordination the neutral ligand in its zwitterionic form, H_2L, gives stable binuclear complexes of the type $[M_2H_2L]^{4+}$. Only two of three amine groups of each [9]aneN$_3$ subunit are coordinated, together with the deprotonate oxygen of the adjacent cresolate moiety, while the remaining amine function of the macrocycle is protonated. Deprotonation of these complexes occurs only at alkaline pH values and is accompanied by a change of the coordination sphere of the metal, which is now bound to all nitrogen of a [9]aneN$_3$ subunit. While in metal coordination each macrocyclic unit acts as an independent binding site, in phosphate anion binding both [9]aneN$_3$ may act cooperatively in the binding process, thanks to the clamp-like conformation assumed by the receptor. The most stable complexes are unusually formed at alkaline pH values, where the receptor is still in its zwitterionic form, but contain up to four charged ammonium groups as potential binding sites for the anionic phosphate chains. In the case of ATP, weak forces (edge to face π-stacking, C−H$\cdots\pi$ and N−H$\cdots\pi$ interactions) are also at work leading to a higher stability of the complexes.

Experimental Section

All melting points are uncorrected. Microanalytical data were obtained using a Fison EA CHNS-O instrument operating at 1000 °C. Electrospray Ionization (ESI) mass spectra were recorded with a mass spectrometry Hewlett-Packard model 5989. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR300 or a Varian VXR400 spectrometer. Toluene was dried over molecular sieves under nitrogen. Other solvents and starting materials were purchased from commercial sources where available. 1,4,7-Triazacyclononane-1,4-dicarboxylic acid, di-*tert*-butyl ester [(BOC)$_2$-[9]aneN$_3$],[58] and 2-(3-chloromethyl-2-hydroxy-5-methylbenzyl)-6-chloromethyl-4-methylphenol[59] were prepared according to the reported procedures.

Synthesis of (BOC)$_4$-L. To a solution of 1,4,7-triazacyclononane-1,4-dicarboxylic acid di-*tert*-butyl ester [(BOC)$_2$-[9]aneN$_3$] (1.1 g, 3.3 mmol) in toluene (20 mL) was added KOH (0.27 g, 4.8 mmol) at room temperature under nitrogen. To this mixture was added 2-(3-chloromethyl-2-hydroxy-5-methylbenzyl)-6-chloromethyl-4-methylphenol (0.46 g, 1.4 mmol) in toluene (10 mL) within 10

min. The mixture was stirred at 70 °C for 6 h and at room temperature for 24 h, filtered, dried over Na_2SO_4, and concentrated under reduced pressure to give a yellow solid (1.1 g, 85% yield). Mp 108 °C. Elem. Anal. found. (calcd for $C_{49}H_{78}N_6O_{10}$): C, 64.5 (64.6); H, 8.7 (8.6); N, 8.9 (9.2) %. ^{1}H NMR (300 MHz, $CDCl_3$), δ 1.45 (s, 36H, C(CH_3)$_3$), 2.12 (s, 6H, ArCH_3), 2.65−2.88 (m, 24H, [9]aneN_3 ring), 3.59 (s, 4H, ArCH_2N), 3.82 (s, 2H, ArCH_2Ar), 6.62 (s, 2H), 6.91 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 20.5 (ArCH_3), 28.5 (C(CH_3)$_3$), 30.7 (ArCH_2Ar), 48.9, 49.3, 50.2, 52.8 ([9]aneN_3 ring), 60.4 (ArCH_2N), 79.9, 80.1 (C(CH$_3$)$_3$), 125.2, 127.2, 128.1, 128.9, 130.3, 155.4 (aromatic carbons), 153.0 (C═O).

Synthesis of H_2L. To a solution of (BOC)$_4$-L (1.41 g, 1.54 mmol) in CH_2Cl_2 (10 mL) was added 50 v/v % CF_3CO_2H/CH_2Cl_2 (10 mL), and the resulting solution was stirred at 0 °C under nitrogen for 30 min and at room temperature for 1 h, then concentrated under reduced pressure. Et_2O was added to the residue obtained. The solvent was removed under reduced pressure and the residue taken up in water, the pH value was adjusted to 9−10 by adding 1 M NaOH, and the product extracted into $CHCl_3$ (4 × 20 mL). The organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow solid (0.65 g, 82% yield). Mp. 161 °C. Elem. Anal. found. (calcd for $C_{29}H_{46}N_6O_2$): C, 66.5 (68.2); H, 8.5 (9.1); N, 15.5 (16.5) %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.16 (s, 6H, ArCH_3), 2.55−2.94 (m, 24H, [9]aneN_3 ring), 3.63 (s, 4H, ArCH_2N), 3.76 (s, 2H, ArCH_2Ar), 6.60 (s, 2H), 6.92 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4 (ArCH_3), 29.6 (ArCH_2Ar), 46.6, 47.2, 52.6 ([9]aneN_3 ring), 59.1 (ArCH_2N), 125.1, 125.4, 128.8, 130.1, 130.2, 155.6 (aromatic carbons). MS (ESI): m/z 128 $[C_6H_{14}N_3]^+$, m/z 252 $[C_{17}H_{18}O_2]^+$.

Synthesis of $[Cu_2L(NO_3)_2]\cdot 2H_2O$. $Cu(NO_3)_2\cdot 2.5H_2O$ (0.025 g, 0.078 mmol) in MeOH was added to a solution of H_2L (0.020 g, 0.039 mmol) in MeOH (10 mL). The solution was stirred at room temperature under N_2 for 3 h. Green crystals were obtained by diffusion of Et_2O vapor into the reaction mixture after partial removal of the solvent (0.024 g, yield 76%). Elem. Anal. found (calcd for $C_{29}H_{48}Cu_2N_8O_{10}$): C, 43.4 (43.8); H, 5.8 (6.1); N, 13.9 (14.1).

Potentiometric Measurements. Equilibrium constants for protonation and complexation reactions were determined by means of potentiometric measurements (pH = −log [H$^+$]) at 298.1 ± 0.1 K, in the pH range 2.5−11, by using the equipment that has been already described.[67] In the study of metal complexation, the ionic medium used was $NaClO_4$ 0.1 M while the analysis of anion complexation was carried out in NMe_4NO_3 0.1 M because of the low solubility of the anion complexes in $NaClO_4$. ^{1}H NMR spectra recorded on 2 × 10^{-3} M solutions of H_2L at pH 7.5 (TRIS buffer) in the absence or in the presence of increasing amount of NMe_4NO_3 (up to 0.1 M) did not show significant changes of the chemical shifts of the signals of H_2L, indicating that the interaction of nitrate with the ligand is absent or negligible.

The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by the Gran's method.[68] This allow one to determine the standard potential E_0, and the ionic product of water (pK_w = 13.73 ± 0.01 in 0.1 M $NaClO_4$, pK_w = 13.83 ± 0.01 in NMe_4NO_3). A 1 × 10^{-3} M ligand concentration was generally employed in the potentiometric measurements. In the study on metal complexation, the metal to ligand molar ratio was varied from 0.5:1 to 2:1, while in the measurements with anions, the anion to ligand molar ratio was varied from 0.5:1 to 5:1. At least three measurements (about 100 experimental points each one) were performed for each system. The computer program HYPERQUAD[69] was used to calculate the stability constants of metal complexes from emf data. The titration curves for each system were treated either as a single set or as separated entities without significant variations in the values of the protonation or complexation constants.

Table 5. Crystallographic Data and Refinement Details for $[Cu_2L(NO_3)_2]\cdot 2H_2O$

formula	$C_{29}H_{48}Cu_2N_8O_{10}$
Fw/g mol^{-1}	795.84
cystal system	orthorhombic
space group	*Pnna*
a/Å	8.0523(1)
b/Å	28.3800(4)
c/Å	16.1372(2)
V/Å^3	3687.74(8)
Z	4
λ/Å	1.5418
μ (Cu-kα)/mm^{-1}	1.953
Dc/g cm^{-1}	1.433
T/K	298
final R indices (for 3606 reflection with $I > 2\sigma(I)$)[a]	$R1$ = 0.0677, $wR2$ = 0.2081
final R indices (all data)[a]	$R1$ = 0.0848, $wR2$ = 0.2358
no. of refined parameters	223

[a] $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma\, wF_o^4]^{1/2}$.

Crystal Structure Determination of $[Cu_2L(NO_3)_2]\cdot 2H_2O$. Prismatic green crystals of $[Cu_2L(NO_3)_2]\cdot 2H_2O$ suitable for X-ray diffraction analysis were used for data collection at room temperature. A summary of the crystal data is given in Table 5. The structure was solved by means of direct methods of the SIR2004 program,[70] and refined by SHELX97.[71] Anisotropic displacement parameters were used for all non hydrogen atoms. Hydrogen atoms belonging to the macrocyclic molecules were introduced in calculated positions and isotropically refined in agreement with the linked atoms. No hydrogen atoms were introduced in the calculation for the solvent water molecules. A high anisotropic displacement parameter was found for the O4 nitrate oxygen, possibly because of movement of the nitrate group normal to its plane. A restrain for the N4−O4 bond distance has been used.

^{1}H NMR Measurements. ^{1}H and ^{31}P NMR spectra in D_2O solution at different pH values were recorded at 298 K on a Varian 300 MHz instrument. Peak positions are reported relative to HOD at 4.75 ppm. Small amounts of 0.1 M $DClO_4$ or NaOD solutions were added to a 5 × 10^{-3} M solution of the ligand to adjust the pD. The pH was calculated from the measured pD value by using the relationship pH = pD − 0.40.[72] Deuterated TRIS buffer (50 mM) was used in the ^{31}P and ^{1}H NMR titrations carried out at pH 8.5.

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UV Spectrophotometric Measurements. All aqueous solutions were prepared in 0.1 M $NaClO_4$. The pH value was measured on a Metrohm 713 pH meter. Absorption spectra were recorded on a Perkin-Elmer Lambda 25 spectrophotometer.

Molecular Modeling. MM investigation on the ligand in its zwitterionic form as well as on the adducts formed by $(H_4L)^{2+}$ with $(ATP)^{4-}$, $(P_2O_7)^{4-}$ and $(P_3O_{10})^{5-}$ and by $(H_6L)^{4+}$ with $(HATP)^{3-}$ has been performed by means of an empirical force field method (AMBER3),[66] as implemented in the HyperChem 7.51 package,[73] using an implicit simulation of the aqueous environment ($\varepsilon = 4r$) and atomic charges evaluated at the semiempirical level (PM3).[60] The acidic protons in $(H_4L)^{2+}$ were localized on the tertiary nitrogen and on a secondary one of each [9]aneN_3 unit, as determined by the ^{1}H NMR measurements. The potential energy surface of all the systems has been explored by means of simulated annealing (T = 600 K, equilibration time = 5 ps, run time = 10 ps and cooling time = 15 ps, time step = 1.0 fs). For each studied system, 240 conformations have been sampled and those featured by an energy falling in the range of 5 kcal/mol from the minimum have been manually clustered.

The selected conformations of the H_2L species in its two different distributions of the acidic protons were further minimized using a semiempirical level of theory (PM3).[60]

Supporting Information Available: Experimental details for the synthesis of the dinuclear complexes with Zn(II), Cd(II), and Pb(II). Distribution diagrams of the complexes calculated with 1:1 and 1:2 ligand to metal molar ratios (Figures S1 and S2). pH dependence of the ε value at 298 nm for H_2L in the presence of 2 equiv of Cu(II) and Cd(II) (Figure S3). Distribution diagrams of the complexes with inorganic phosphate anions (Figure S4). Lowest energy conformers for the adducts between $(H_6L)^{4+}$ and $(HATP)^{3-}$ (PDF). Cartesian coordinates of the lowest energy complexes with di-, triphosphate and ATP (as pdb files). CIF file for the crystal structure of $[Cu_2L(NO_3)_2]\cdot 2H_2O$. This material is available free of charge via the Internet at http://pubs.acs.org.

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