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Exploring the Binding Ability of Polyammonium Hosts for Anionic Substrates: Selective Size-Dependent Recognition of Different Phosphate Anions by Bis-macrocyclic Receptors

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Supporting Information

ABSTRACT: Binding of mono-, di-, and triphosphate, adenosine diphosphate (ADP), and adenosine triphosphatase (ATP) with receptors L1−L3, composed of two [9]aneN$_3$ units separated by a 2,9-dimethylene-1,10-phenanthroline (L1), a 2,6-dimethylenepyridine (L2), or a 2,3-dimethylenequinoxaline (L3) spacer, has been studied by means of potentiometric titrations, ^{1}H and ^{31}P NMR measurements in aqueous solutions, and molecular modeling calculations. In the case of inorganic phosphates, the binding properties of the receptors appear to be determined by their geometrical features, in particular the distance between the two [9]aneN$_3$ units imposed by the spacer separating the two macrocyclic units. While L1 is able to selectively bind triphosphate over di- and monophosphate, L3 selectively coordinates the smaller monophosphate anion. Finally, L2 shows preferential binding of diphosphate. ^{1}H and ^{31}P NMR measurements show that the complexes are essentially stabilized by charge−charge and hydrogen-bonding interactions between the anion and the protonated amine groups of the macrocyclic subunits of the receptors. Molecular dynamics simulations suggest that the larger distance between the two macrocyclic units of L1 allows this receptor to form a larger number of hydrogen-bonding contacts with triphosphate, justifying its selectivity toward this anion. Conversely, in the case of L3, the two facing [9]aneN3 units give rise to a cleft of appropriate dimensions where the small monophosphate anion can be conveniently hosted. Considering nucleotide coordination, L1 is a better receptor for ATP and ADP than L2, thanks to the higher ability of phenanthroline to establish stabilizing π stacking and hydrophobic interactions with the adenine units of the guests.

INTRODUCTION

Inorganic phosphate anions and nucleotides are ubiquitously present in biological systems and play crucial roles in many cellular functions, such as transport across membranes, DNA synthesis, cell signaling, and energy- or electron-transfer processes.[1] These functions are generally regulated by recognition processes involving metal-free proteins able to selectively bind the appropriate anion, thanks to encapsulation of the anionic substrate within clefts or pockets with the appropriate dimensions and disposition of the binding sites. It is now accepted that the binding process is regulated by different noncovalent interactions, such as charge−charge and charge−dipole interactions, hydrogen-bonding interactions, hydrophobic effects, and stacking interactions, that work cooperatively in stabilizing the adducts and determining the binding selectivity.

In this context, the design of synthetic receptors able to bind phosphate anions in aqueous solution represents the main approach to the analysis of the weak forces that regulate the recognition processes in biological systems.[2] Actually, several examples of phosphate anion binding by synthetic receptors, mostly of the polyammonium type, have been recently reported.[2−33] However, recognition of phosphate anions still remains a difficult task, in particular in aqueous solution, because of solvation of these substrates that strongly competes with the process of complex formation and their propensity to undergo pH-dependent protonation processes in water. Similar to natural systems, the selective formation of stable host−guest adducts requires a rational incorporation in the abiotic receptor of different sites for multiple noncovalent interactions with substrates topologically oriented to achieve the best possible host−guest "complementarity" under specific pH conditions.[2−17] From this point of view, encapsulation of inorganic phosphate anions or of the phosphate moiety of nucleotides within clefts or cavities of the receptor, geometrically configured to fit the stereochemical requirements and size of the anionic group, is expected to strengthen the overall host−guest interaction affording particularly stable adducts or favoring recognition of selected substrates.[2−6,10,14,33] The

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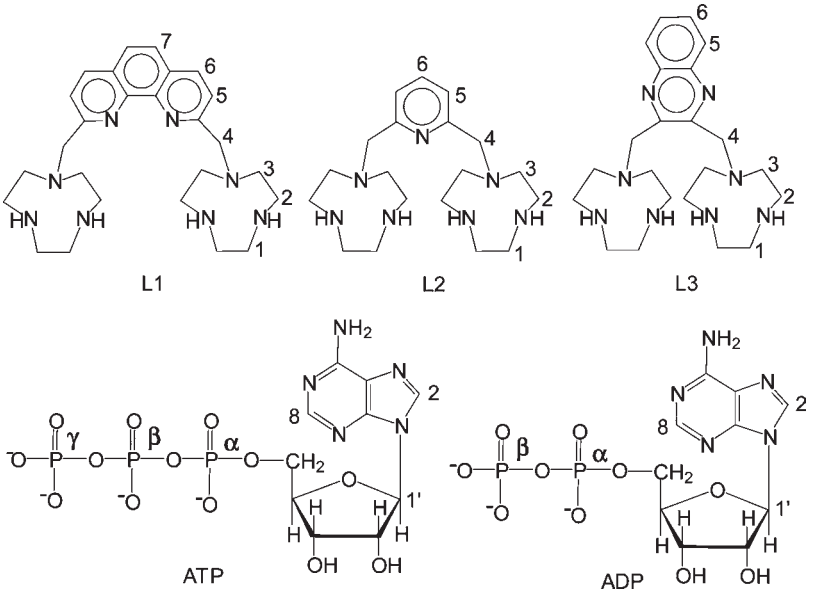
Scheme 1. Drawings of Receptors, ATP^{4-}, and ADP^{3-} with the Atom Numbering Used in the NMR Experiments



fundamental idea is that a three-dimensional inclusion of an anionic guest within the intramolecular cleft of a hollow receptor molecule would increase the selectivity and strength of the host−guest interaction following the principle at the base of supramolecular chemistry, of a geometrical size/cavity fitting and binding complementarity of the two partners.

In the course of our study on polyamines containing heteroaromatic units as receptors for metal cations and anions,[8,9,32−34] we recently reported the synthesis and metal binding properties of a series of ditopic receptors featuring two $[9]aneN_3$ macrocycles linked by different heteroaromatic rigid spacers, including quinoxaline, pyridine, and 1,10-phenanthroline (Scheme 1).[9,32]

Ligands L1−L3 can easily give charged polyammonium cations in aqueous solutions because of the relatively high number of protonable amine groups gathered on the macrocyclic subunits. At the same time, the two $[9]aneN_3$ units can face each other at a distance that depends on the heteroaromatic spacer and on their degree of protonation, thus creating *pseudo*cavities of varying dimensions, where the anionic phosphate chain of nucleotides or inorganic phosphate species could be encapsulated. The different dimensions of these potential binding clefts, which mainly depend on the distance between the $[9]aneN_3$ moieties, could favor a selective binding pattern toward inorganic/organic phosphate anions of different size. At the same time, these receptors also present heteroaromatic units, which could give π-stacking interactions with aromatic sections of organic phosphates, such as nucleotides. With this in mind, we decided to compare the binding features of L1−L3 toward mono-, di-, and triphosphate and adenosine 5'-monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphatase (ATP), with the purpose of evaluating their selectivity in phosphate anion recognition in connection with their structural topological features, in particular the different distances between the two $[9]aneN_3$ units determined by rigid spacers. For this reason, we did not consider in this study the analogues of L1−L3 having a 2,2′-bipyridine spacer.[32] In this case, in fact, the possible rotation of the two pyridine moieties along the 1,1′ axis may allow the receptor to assume conformations remarkably different from those of L1−L3, in particular that with the two $[9]aneN_3$ units in a trans disposition. Of note, $[9]aneN_3$ is a classic chelating agent in coordination chemistry, capable of forming stable complexes with a variety of transition-metal and post-transition-metal cations. As a matter of fact, previous studies have shown that ligands L1−L3 can give stable dinuclear complexes with transition metals, including copper(II) and zinc(II), where each metal is hosted by a single $[9]aneN_3$ unit.[32] Therefore, the present study may be useful in acquiring preliminary information for a subsequent analysis of the binding ability of the dinuclear metal complexes with L1−L3 toward phosphate anions. In principle, also these complexes could be capable of selectively binding anionic species having the appropriate dimensions to bridge the two coordinated metal cations.

Examples of polyammonium receptors for selective phosphate anion binding featuring two cyclic or acyclic polyamine moieties constrained to stay face to face at a fixed distance by a rigid spacer(s) are limited in the literature.[12a,b,20] Earlier, Vance and Czarnik reported on selective recognition and fluorescence sensing in an aqueous solution of diphosphate by a receptor constituted by two protonated tren moieties linked by an anthracene moiety.[20b] More recently, Tripier and Handel showed that pyridine-based receptors containing two facing cyclen units can selectively recognize triphosphate,[12a,b] probably as a consequence of insertion of the anion within the cleft delimited by the two polyamine subunits. In none of the cases reported in the literature though has a clear attempt been made to reach size-dependent selectivity in the host−guest recognition of each of the three inorganic phosphates with a homologous series of receptors through a systematic tuning of the necessary geometrical complementarity between the host and the anionic guest, keeping unchanged the polyammonium units devoted to establishing charge−charge and hydrogen-bonding interactions but changing their distances. From this point of view, the ditopic receptors L1−L3 offer this opportunity because the two $[9]aneN_3$ units, which can be protonated to a degree sufficient to establish strong electrostatic interactions with the substrates, are disposed at distances significantly different from each other,

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thus generating *pseudo*cavities of significantly different dimensions that could be preorganized for an auspiciously selective size-dependent encapsulation of each inorganic phosphate anion considered. Furthermore, L1−L3 offer the additional opportunity to carry out a systematic investigation on the effect of the extension of the aromatic linker on the binding properties toward nucleotides via π-stacking interactions with the aromatic sections of these organic phosphates. We have also analyzed the pH dependence of the anion-binding ability of these receptors, with the aim of drawing useful indications for the design of selective new anion binders in aqueous solutions based on polyamine systems.

EXPERIMENTAL SECTION

Potentiometric Measurements. All of the pH metric measurements (pH = −log [H$^+$]) were carried out in degassed 0.1 mol dm^{-3} NMe_4NO_3 aqueous solution at 298.1 K by using equipment and procedures that have already been described.[9] An electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NMe_4OH solutions and determining the equivalent point by Gran's method,[35] which allows one to determine the standard potential $E°$ and the ionic product of water [pK_w = 13.83(1) at 298.1 K in 0.1 mol dm^{-3} NMe_4NO_3]. In the experiments to determine the stability of the adducts with L1−L3, the receptor concentrations were varied from 5.0×10^{-4} to 5.0×10^{-3} M, while the concentrations of the substrates were varied in the range 4.0×10^{-4}−9.0×10^{-3} M. At least three measurements (about 100 data points each) were performed for each system in the pH range 2.5−10.5, and the relevant electromotive force data were treated by means of the computer program *HYPERQUAD*.[36] The titration curves for each system were treated either as a single set or as separated entities without significant variation in the values of the protonation or complex formation constants. Data treatment ruled out all proposed models that considered stoichiometries different from 1:1 for the adducts.

NMR Spectroscopy. ^{1}H NMR spectra (400 MHz) in D_2O solution were recorded at 298 K on a 400 MHz Bruker Avance III spectrometer. In experiments carried out at different pH values, small amounts of 0.01 M NaOD and DCl were added to the solution to adjust the pD. The measurements at pH 7.0 were performed in D_2O solution using a deuterated Tris buffer. The pH was calculated from the measured pD values by using the following formula: pH = pD − 0.40.[37] Complexation-induced ^{31}P and ^{1}H NMR chemical shifts (CISs, ppm) were measured as the difference $\delta_{OBS} - \delta_{anion}$, where δ_{OBS} is the chemical shift of a signal determined in D_2O solutions for the complete formation of complexes (generally using a 2-fold excess of the receptor for determination of the CIS of substrates or a 2-fold excess of substrates for the CIS of receptors) and δ_{anion} is the chemical shift of the corresponding signal of the noncomplexed receptor or substrate.

Computational Details. Molecular dynamics calculations were performed by using the *AMBER3* force field,[38] as implemented in the *Hyperchem 7.51* package.[39] Atomic charges for receptors and substrates were calculated at the PM3 semiempirical level.[40] Starting conformations of the $(H_2L)^{2+}$/A adducts (L = L1, L2, or L3; A = HPO_4^{3-}, $P_2O_7^{4-}$, $P_3O_{10}^{5-}$, ADP^{3-}, or ATP^{4-}), obtained by the manual docking of the minimized conformer of the receptor to the substrate (minimum distance between the atoms of the receptor and substrate > 5 Å) were freely minimized. The Polak−Ribiere (coniugate gradient) algorithm was used in the minimization procedures to a root-mean-square energy gradient of less than 0.001 kcal mol^{-1} Å^{-1}. The potential energy surfaces of both adducts were explored by means of simulated annealing (running temperature = 600 K, equilibration, running, and cooling time = 10 ps, and time step = 1.0 fs). For each adduct, 80 conformations were sampled. A distance-dependent dielectric factor ($\varepsilon = 4R_{ij}$) was used.

Table 1. Formation Constants (log *K*) of the Adducts Formed by Mono-, Di-, and Triphosphate with Receptors L1−L3 (0.1 M NMe_4NO_3, 298 K)

	log *K*		
equilibrium	L1	L2	L3
$(HL)^+ + (HPO_4)^{2-} \rightleftharpoons [H_2LPO_4]^-$		3.27(4)	3.57(5)
$(H_2L)^{2+} + (HPO_4)^{2-} \rightleftharpoons [H_3LPO_4]$		3.92(5)	4.70(5)
$(HL)^+ + (H_2PO_4)^- \rightleftharpoons [H_3LPO_4]$		3.72 (5)	
$(H_2L)^{2+} + (H_2PO_4)^- \rightleftharpoons [H_4LPO_4]^+$		3.99(4)	4.77(4)
$(H_3L)^{3+} + (H_2PO_4)^- \rightleftharpoons [H_5LPO_4]^{2+}$		4.45(6)	4.90(3)
$(H_4L)^{4+} + (H_2PO_4)^- \rightleftharpoons [H_6LPO_4]^{3+}$		4.90(6)	5.20(5)
$(H_2L)^{2+} + P_2O_7^{4-} \rightleftharpoons [H_2LP_2O_7]^{2-}$	2.55(7)	3.93(7)	4.0(1)
$(H_2L)^{2+} + (HP_2O_7)^{3-} \rightleftharpoons [H_3LP_2O_7]^-$	3.82(2)	3.73(9)	3.6(1)
$(H_2L)^{2+} + (H_2P_2O_7)^{2-} \rightleftharpoons [H_4LP_2O_7]$	4.02(2)	5.02(8)	3.5(1)
$(H_3L)^{3+} + (HP_2O_7)^{3-} \rightleftharpoons [H_4LP_2O_7]$			3.4(1)
$(H_3L)^{3+} + (H_2P_2O_7)^{2-} \rightleftharpoons [H_5LP_2O_7]^+$	4.41(2)	4.7(1)	3.7(1)
$(H_3L)^{3+} + (H_3P_2O_7)^- \rightleftharpoons [H_6LP_2O_7]^{2+}$	4.48(3)	4.00(8)	
$(H_4L)^{4+} + (H_2P_2O_7)^{2-} \rightleftharpoons [H_6LP_2O_7]^{2+}$	4.55(3)		
$(H_2L)^{2+} + P_3O_{10}^{5-} \rightleftharpoons [H_2LP_3O_{10}]^{3-}$	5.4(1)	4.4(1)	3.74(5)
$(H_2L)^{2+} + (HP_3O_{10})^{4-} \rightleftharpoons [H_3LP_3O_{10}]^{2-}$	5.29(5)	4.1(1)	3.52(6)
$(H_2L)^{2+} + (H_2P_3O_{10})^{3-} \rightleftharpoons [H_4LP_3O_{10}]^-$	4.97(6)	4.0(1)	3.40(5)
$(H_3L)^{3+} + (HP_3O_{10})^{4-} \rightleftharpoons [H_4LP_3O_{10}]^-$	5.02(6)		
$(H_3L)^{3+} + (H_2P_3O_{10})^{3-} \rightleftharpoons [H_5LP_3O_{10}]$	6.2(1)	4.5(2)	4.03(5)
$(H_3L)^{3+} + (H_3P_3O_{10})^{2-} \rightleftharpoons [H_6LP_3O_{10}]^+$	6.05(6)		3.93(4)
$(H_4L)^{4+} + (H_2P_3O_{10})^{3-} \rightleftharpoons [H_6LP_3O_{10}]^+$		5.1(2)	

RESULTS AND DISCUSSION

Binding of Inorganic Phosphate Anions in Aqueous Solution. Because the binding properties of polyamine receptors toward anionic species in aqueous solution are often dependent on their protonation state and, therefore, on the solution pH, we decided to investigate first the ability of L1−L3 to interact with the anionic substrates by means of potentiometric measurements. The protonation constants of the three receptors were previously determined in the course of a study on zinc(II) coordination. It was found that these receptors bind up to four (L2 and L3) or six (L1) acidic protons in aqueous solution. In the resulting polyammonium cations, the acidic protons tend to be equally distributed on the two [9]aneN_3 moieties, with the four secondary amine groups being the preferred protonation sites, in keeping with the generally observed higher basicity of the secondary amine groups with respect to the tertiary ones.[41] In fact, the first four protonation steps occur on the secondary nitrogen atoms, while the tertiary amine groups protonate only in the fifth and sixth protonation steps of L1. The heteroaromatic nitrogen atoms do not protonate in aqueous solution, at least in the pH range investigated (2.5−11), as was expected considering the scarce basicity of the heteroaromatic nitrogen atoms of pyridine, quinoxaline, and phenanthroline.[41] The three receptors in their protonated forms give stable 1:1 adducts in aqueous solution with all inorganic phosphate anions under investigation (Table 1), the only exception being L1, which does not give any detectable interactions with monophosphate. Although the formation of both 1:1 and 2:1 anion-to-receptor adducts has been observed in previously reported cases of polyamine-based hosts,[42] analysis of the titration curves with the program *HYPERQUAD*[36] under our experimental conditions reveals only 1:1 stoichiometries for all species detected in our systems.

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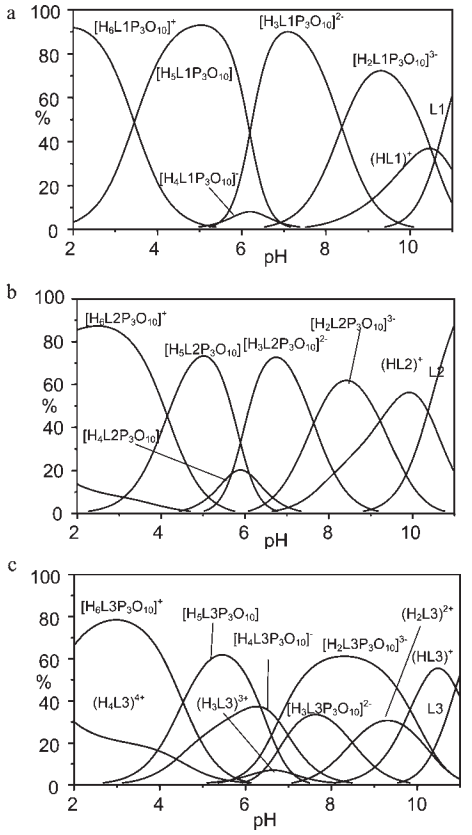

Figure 1. Distribution diagrams of the triphosphate adducts with L1 (a), L2 (b), and L3 (c) ($[L1] = [L2] = [L3] = [P_3O_{10}^{5-}] = 1.0 \times 10^{-3}$ M, 298 K, $I = 0.1$ M).

Analysis of the titration curves allows one to determine the species formed in solution and their overall formation constants β_{HLS} (S = PO_4^{3-}, $P_2O_7^{4-}$, or $P_3O_{10}^{5-}$; L = L1, L2, or L3), relative to equilibria of the type $L + S^{x-} + nH^+ \rightleftharpoons [H_nLS]^{(n-x)+}$, with $x = 3$, 4, or 5 (see the Supporting Information, Table S1). Plots of the distribution curves of the species formed by L1−L3 with the anions (see Figure 1 for the $P_3O_{10}^{5-}$ adducts with all receptors and Figures S1 and S2 in the Supporting Information for the PO_4^{3-} and $P_2O_7^{4-}$ adducts) outline that complexation occurs in a wide pH range, with the formation of a large number of 1:1 adducts having different protonation degrees.

Determination of the stepwise formation constants for the adducts between the protonated receptors and the anionic substrates, relative to equilibria of the type $(H_{n-i}L)^{(n-i)+} + (H_iS)^{(i-x)-} \rightleftharpoons [H_nLS]^{(n-x)+}$, implies knowledge of the localization of acidic protons on both the receptor and substrate in the $[H_nLS]^{(n-x)+}$ host−guest species. This task is made difficult in this case by the presence in an aqueous solution of a large number of overlapping equilibria in the same pH range and by the values of the protonation constants of the substrates, which are quite similar, in some cases, to the protonation constants of the receptors. In these cases, in fact, different equilibria can be proposed for the formation of the same supramolecular adduct. For instance, the $[H_3L2PO_4]$ adduct could be formed via either the equilibrium $(H_2L2)^{2+} + (HPO_4)^{2-} \rightleftharpoons [H_3L2PO_4]$ or $(HL)^+ + (H_2PO_4)^- \rightleftharpoons [H_3L2PO_4]$, with calculated stepwise equilibrium constants of 3.92 and 3.72 log units, respectively. The values of the stability constants of the adducts calculated by using this approach are given in Table 1.

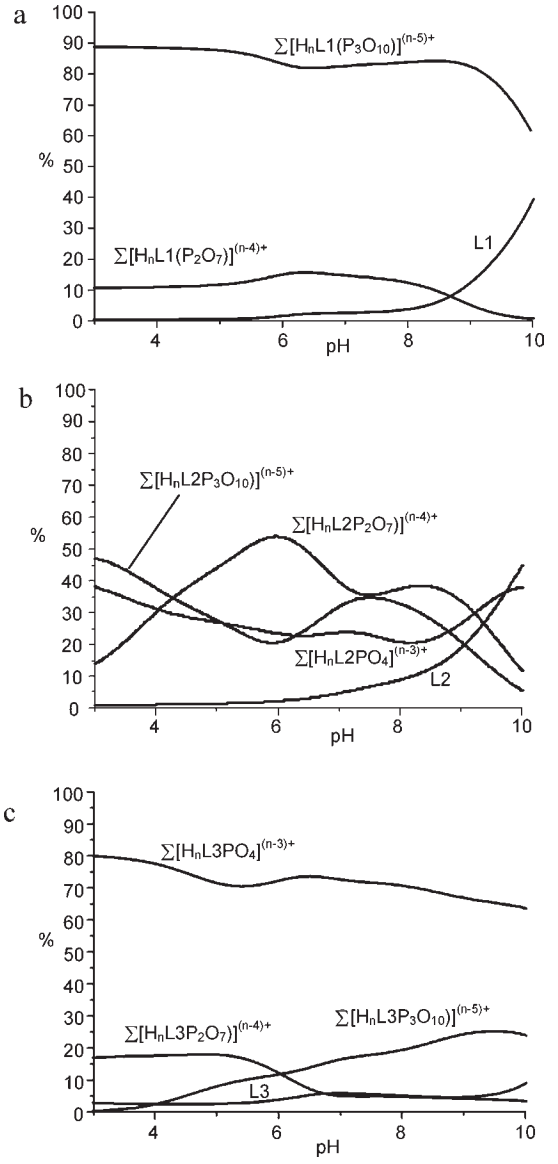

Figure 2. Overall percentages of L1 (a), L2 (b), and L3 (b) complexed species with mono-, di-, and triphosphate as a function of the pH in competitive systems containing L1, L2, or L3 and mono-, di-, and triphosphate in equimolecular ratios ($[L] = [PO_4^{3-}] = [P_2O_7^{4-}] = [P_3O_{10}^{5-}] = 1.0 \times 10^{-3}$ M; L = L1, L2, or L3).

Nevertheless, the data in Table 1 outline some general trends commonly observed in anion coordination by polyammonium receptors. In most cases, for a given anion−receptor system, the stability of the adducts increases with the number of protonated ammonium functions gathered on the receptor, which enhances the receptor ability to give electrostatic and hydrogen-bonding interactions with the anionic substrates. Conversely, for a given protonated species, protonation of the anions and a consequent decrease of their negative charge often reduce the stability of the host−guest adducts.[2] However, some exceptions to this rule can be found in Table 1. For instance, $(H_2P_2O_7)^{2-}$ forms a more stable complex with $(H_2L2)^{2+}$ than $(HP_2O_7)^{3-}$ [log K = 5.02(8) vs 3.73(9)], and $(H_2P_3O_{10})^{3-}$ shows a higher binding constant with $(H_3L1)^{3+}$ than $(HP_3O_{10})^{4-}$ [log K = 6.2(1) vs 5.02(6)].

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A further level of complexity in the interpretation of the data in Table 1 is represented by the different acid−base characteristics of the various substrates and receptors, which makes it difficult to compare the binding ability of the receptors at a given pH value and to evaluate eventual selectivity patterns.

This problem can be conveniently overcome by considering a competitive system containing a single receptor and the three anionic substrates in equimolecular concentrations and calculating the overall percentages of the different complexed anions over a wide pH range.[43] Plots of the percentages versus the pH produce species distribution diagrams from which the binding ability of the receptor can be interpreted in terms of selectivity. Figure 2 displays the plots obtained for competitive systems containing respectively L1, L2, or L3 and the three phosphate anions in equimolecular concentrations.

The selective binding of triphosphate by L1 is clearly outlined by the plot in Figure 2a, which shows that the formation of triphosphate adducts with L1 prevails over a wide pH range with respect to diphosphate complexation. As was previously anticipated, no interaction was detected between L1 and monophosphate by potentiometric measurements. Conversely, monophosphate is selectively bound by L3 over di- and triphosphate over the entire pH range investigated by potentiometric titrations (Figure 2c). Finally, in the case of L2, formation of the complexes with diphosphate prevails over the pH range 4.5−9 (Figure 2b). However, preferential binding of this anion over mono- and triphosphate is remarkably less marked than the selective recognition of monophosphate observed in the case of L3 and of triphosphate in the case of L1.

At a given pH value, the negative charge gathered on these anionic substrates increases in the order monophosphate < diphosphate < triphosphate. At the same time, triphosphate possesses a larger number of phosphate groups as potential sites for hydrogen bonding. Therefore, selective binding of triphosphate by L1 could be simply attributed to a stronger charge−charge interaction with the polyammonium receptor as well as to the formation of a higher number of hydrogen-bonding contacts. However, these considerations cannot apply to the referential binding of di- and monophosphate by L2 and L3, respectively. Overall, the plots in Figure 2 strongly suggest that the selectivity properties displayed by L1−L3 also depend on the geometrical/topological features of the receptors, in particular on the distance between the two protonated [9]aneN$_3$ units. This case study may represent a classic example of dimensional recognition in anion binding. In fact, while L3, featuring two macrocyclic units separated by a short *o*-xylyl spacer, selectively binds monophosphate, L1 shows a marked preference for the larger triphosphate anion, thanks to the larger distance between the two [9]aneN$_3$ units. Finally, the 2,5-dimethylenpyridine bridge of L2 has an length intermediate between those of the phenanthroline and o-xylyl spacers of L1 and L3, and this receptor preferentially interacts with diphosphate.

From this point of view, a previous study[9] showed that the insertion of a 2,2′-methylenedicresol linker between two [9]aneN$_3$ units affords a receptor (L4) capable of forming remarkably stable complexes with both di- and triphosphate, with a preference for triphosphate binding, as was actually observed in the case of L1. Actually, the L4 protonated species give complexes with di- and triphosphate with similar or slightly higher stability than that observed for the corresponding complexes with L1 in the same protonation degree. However, it was shown that the binding characteristics of L4 are strongly affected by its zwitterionic nature in aqueous solution. For instance, the H_2L4^{2+} protonated species contains two deprotonated cresol units and four ammonium groups, equally distributed between the two [9]aneN$_3$ units, which cooperate in phosphate anion binding. In fact, the flexibility imparted by the 2,2′-methylenedicresol unit allows the receptor to adopt a conformation that allows both macrocyclic units to participate in substrate binding, through the formation of a network of hydrogen-bonding contacts with the substrates, in particular with $P_3O_{10}^{5-}$. Conversely, L1 probably contains a preformed cleft well suited to host the triphosphate anion, but its diprotonated form possesses only two ammonium groups available for anion binding. Although the different arrangements of the charges in $(H_2L1)^{2+}$ and $(H_2L4)^{2+}$ make a straight comparison between the binding ability of the two receptors difficult, we can speculate that, in the $(H_2L4)^{2+}$ complexes, the formation of a larger number of hydrogen-bonding contacts than in the corresponding complexes with $(H_2L1)^{2+}$ is compensated for by the energetically expensive process of ligand rearrangement to wrap around the anions.

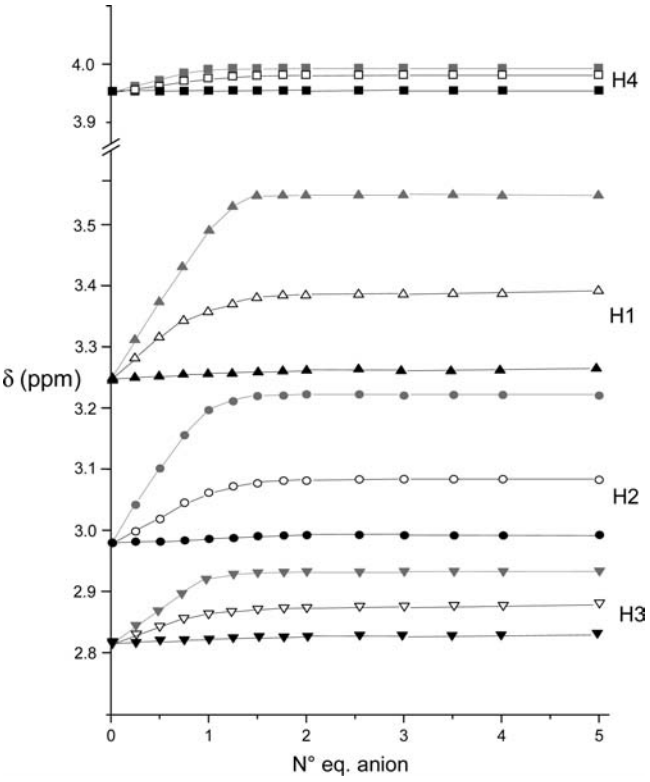

Figure 3. ^{1}H NMR chemical shifts of the signals of the aliphatic protons of L1 in the presence of increasing amounts of monophosphate (black symbols), diphosphate (white symbols), and triphosphate (gray symbols) ([L1] = 1.0×10^{-2} M, 298 K, pH 7.0).

Considering L2, it can be of interest to compare its binding ability with those of the receptor, recently reported by Handel and Tripier,[12] containing the same 2,6-dimethylpyridinyl spacer linking two 1,4,7,10-tetraazacyclododecane (cyclen) moieties. The latter displays preferential binding of triphosphate over diphosphate.[12a] This could suggest that the selectivity properties of these bis-macrocyclic receptors are determined not only by the dimension of the spacer but also by the structural features of the macrocyclic units. Of interest, it was found that the pyridine nitrogen atom, when protonated at acidic pH values, can interact

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Table 2. ^{1}H NMR Chemical Shifts (ppm) for the Aliphatic Protons of L1−L3 in Their Adducts with Mono-, Di-, and Triphosphate and Corresponding CISs (ppm) Measured in D_2O Solution at pH 7.0 and 298 K[a]

		L1		
		H1	H2	H3
monophosphate	δ	3.26	2.99	2.82
	CIS	0.01	0.01	0.00
disphosphate	δ	3.39	3.08	2.87
	CIS	0.14	0.10	0.05
triphosphate	δ	3.58	3.23	2.93
	CIS	0.33	0.26	0.11
		L2		
		H1	H2	H3
monophosphate	δ	3.38	3.11	2.86
	CIS	0.13	0.13	0.04
disphosphate	δ	3.46	3.18	2.89
	CIS	0.21	0.19	0.07
triphosphate	δ	3.41	3.12	2.88
	CIS	0.16	0.14	0.06
		L3		
		H1	H2	H3
monophosphate	δ	3.40	3.13	2.81
	CIS	0.20	0.18	0.05
disphosphate	δ	3.35	3.08	2.80
	CIS	0.15	0.13	0.04
triphosphate	δ	3.34	3.06	2.79
	CIS	0.14	0.11	0.03

[a] The signals for the hydrogen atoms belonging to the methylene group 4 of L1−L3 (Scheme) do not shift significantly upon complexation (CIS values ≤ 0.03 ppm) and are not reported.

with the triphosphate anion. In the case of L2, protonation of pyridine is not observed. Most likely, the high positive charge density gathered on the small 1,4,7-triazacyclonane moieties in the highly protonated forms of L2 prevents protonation of the adjacent heteroaromatic nitrogen atom, which would lead to increased electrostatic repulsions. As a consequence, pyridine does not protonate and cannot be involved in triphosphate binding.

To further elucidate the binding modes of receptors L1−L3, we recorded both ^{1}H and ^{31}P NMR spectra either on aqueous solutions at neutral pH containing a single receptor and a substrate in different molar ratios or on solutions containing the receptor and the substrate in an equimolecular molar ratio at different pH values. All ligands display a C_{2v} time-averaged symmetry in the pH range 2−12, which is preserved in the presence of the substrates. The ^{1}H NMR chemical shifts of the aliphatic signals of L1 in the presence of increasing amounts of tri-, di-, and monophosphate are reported in Figure 3. The addition of triphosphate to an aqueous solution of L1 gives rise to a downfield shift of the ^{1}H NMR signals of the [9]aneN$_3$ units, which increases almost linearly up to a 1:1 anion-to-receptor molar ratio (R). The chemical shifts of the signals then achieve constant values for R > 1.25. Of note, the observed downfield

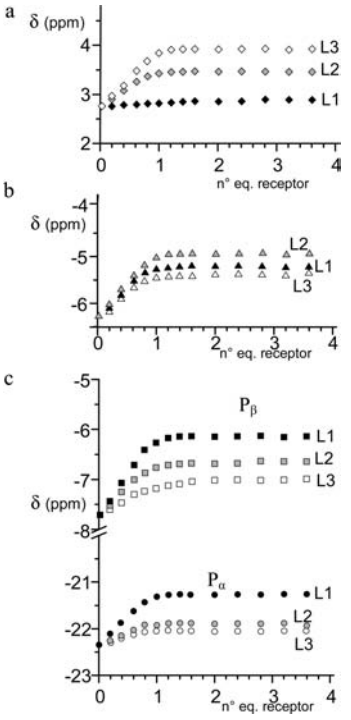

Figure 4. ^{31}P NMR chemical shifts of the signals of monophosphate (a), diphosphate (b) and triphosphate (c) in the presence of increasing amounts of L1 (black symbols), L2 (gray symbols), and L3 (white symbols). P_α and P_β indicate the central and terminal phosphate groups of triphosphate, respectively ([monophosphate] = [diphoshate] = [triphosphate] = 1.0×10^{-2} M, 298 K, pH 7.0).

shifts are remarkably larger for the signals of the hydrogen atoms H1 and H2 belonging to the methylene groups (0.33 and 0.26 ppm, respectively, in the presence of an excess of triphosphate), adjacent to the secondary amine groups (Scheme 1). Smaller shifts are observed in the case of the resonances of H3 (0.11 ppm) and H4 (<0.03 ppm). Finally, the resonances of the aromatic protons (not shown) are almost not affected by complexation.

A similar behavior is also observed in the presence of diphosphate. In this case, however, a linear increase of the shifts is observed up to R = 0.75 and the signals achieve a constant value with R > 1.5 (under these conditions, the signals of H1 and H2 are downfield-shifted of 0.14 and 0.10 ppm, respectively). As in the case of triphosphate, the signals of the hydrogen atoms H3 and H4 are less affected by substrate complexation. Finally, no significant shift is observed upon the addition of monophosphate. These experimental observations support the results from potentiometric measurements, i.e., the formation of 1:1 adducts between L1 and the anionic substrates, and, overall, the stronger interaction occurring between L1 and triphosphate with respect to diphosphate and the absence of any detectable interaction with monophosphate. Similar plots have also been obtained upon the addition of different phosphate anionic substrates to solutions of L2 and L3 and are reported in the Supporting Information (Figures S3 and S4), while Table 2 summarizes the CIS values determined for the complete formation of complexes with receptors L1−L3.

Different from L1, in the case of L3, the downfield shifts of the ^{1}H NMR resonances increase in the order triphosphate < diphosphate < monophosphate (Table 2), suggesting that the electrostatic and hydrogen-bonding interactions between the

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Table 3. ^{31}P NMR Chemical Shifts (ppm) for the Phosphate Signals of Mono-, Di-, and Triphosphate in Their Adducts with L1−L3 and Corresponding CISs (ppm) Measured in D_2O Solution at pH 7.0 and 298 K

	monophosphate	diphosphate	triphosphate (P_α)	triphosphate (P_β)
			L1	
δ	2.76	−5.39	−21.26	−6.12
CIS	<0.1	0.93	1.12	1.56
			L2	
δ	3.49	−4.95	−21.90	−6.60
CIS	0.73	1.37	0.50	1.08
			L3	
δ	3.92	−5.48	−22.13	−7.08
CIS	1.16	0.84	0.27	0.60

two partners in the supramolecular complexes become stronger on passing from the larger triphosphate anion to the smaller monophosphate substrate, in good agreement with the stability trend determined by means of potentiometric measurements (Figure 2b). Finally, in the case of L2, diphosphate gives rise to the largest downfield shift of the aliphatic ^{1}H NMR signals of the receptor (Table 2).

Of note, in all cases, the largest downfield shifts are observed for the hydrogen atoms H1 and H2 belonging to the methylene groups adjacent to the secondary nitrogen atoms of the receptors. On the other hand, all titrations have been carried out at neutral pH, where the receptors are in their diprotonated form $(H_2L)^{2+}$ and contain two singly protonated [9]aneN$_3$ units, with the acidic protons being localized on secondary amine groups. Therefore, the highest CIS values measured for the methylene groups 1 and 2 strongly suggest that the receptor−substrate interaction is mainly due to electrostatic and hydrogen-bonding contacts between the protonated secondary amine groups and the anionic substrates.

^{31}P NMR titrations, carried out by the addition of receptors to neutral solutions of the substrates buffered at pH 7.0 and monitoring of the ^{31}P NMR chemical shift of the phosphate anions, give confidence to the hypothesis made on the basis of the ^{1}H NMR spectra. As shown in Figure 4a−c, the addition of receptors to solutions of the anionic substrates produces a progressive downfield shift of the ^{31}P NMR chemical shifts of the phosphate anions, which increases linearly, up to 0.6:1 or 0.8:1 receptor-to-substrate molar ratios (R′), to achieve a constant value in the presence of a slight excess of the receptors, in keeping with the formation of stable 1:1 complexes.

Once again, no relevant shift is observed upon the addition of L1 to solutions of monophosphate. Figure 4a clearly shows that the ^{31}P NMR signal of monophosphate undergoes the largest downfield shift upon binding to the smaller receptor L3. Conversely, among the three receptors, L1 induces the largest shift of both ^{31}P NMR resonances of triphosphate. The downfield shifts experienced by the ^{31}P NMR signals of the three substrates upon complexation by each receptor are compared in Table 3.

As was already observed for the ^{1}H NMR signals, both the ^{31}P NMR resonances of triphosphate display larger displacements upon complexation with L1 than the single signal of diphosphate, indicating, once again, that the larger triphosphate anion forms stronger electrostatic and hydrogen-bonding contacts with the [9]aneN$_3$ macrocycles of this receptor. Of note, the signal of the terminal phosphate group P_β of triphosphate shows a larger ^{31}P NMR displacement upon complexation than the central phosphate group P_α, suggesting that the former is reasonably involved at a larger extent in charge−charge and hydrogen-bonding interactions. Different from L1, complexation with L3 produces downfield shifts of the ^{31}P NMR resonances, which increase in the order triphosphate < diphosphate < monophosphate, the same trend as that observed for the CIS values of the ^{1}H NMR signals of the L3 hydrogen atoms H1, H2, and H3 (Scheme 1) in the presence of different substrates, and corresponds, at least qualitatively, to the selectivity pattern displayed by this receptor. Finally, complexation with L2 produces somewhat larger displacements of the ^{31}P NMR signals of diphosphate with respect to those of mono- and triphosphate, as expected considering that L2 preferentially binds diphosphate over mono- and triphosphate.

The results outlined above strongly suggest that the formation of complexes between L1−L3 and inorganic phosphates is mainly determined by electrostatic interactions and hydrogen bonding between the ammonium groups of the receptors and the negatively charged phosphate groups modulated by the dimensions of the clefts formed by the two facing [9]aneN$_3$ units.

These interactions depend on the charges gathered on the receptor and substrate, e.g., by their protonation state, and, therefore, are expected to be strongly pH-dependent. For this reason, we also recorded ^{1}H and ^{31}P NMR spectra on solutions containing the receptor and substrate in an equimolecular ratio at different pH values. As shown in Figure 5 for the system L1/triphosphate, both the ^{31}P NMR signals of the substrates and the ^{1}H NMR signals of the receptors shift downfield upon complex formation in a wide pH range. However, the downfield shifts of the ^{31}P and ^{1}H NMR signals are generally larger in the pH range 4−8 and decrease at higher or lower pH values. In fact, in the alkaline and slightly acidic pH regions, the substrates are in their less protonated and highly charged forms (S^{x-}, $HS^{(x-1)-}$, or $H_2S^{(x-2)-}$), respectively, while the receptors are in the protonated forms $(H_2L)^{2+}$ and $(H_3L)^{3+}$, thus enhancing the receptor ability to give electrostatic and hydrogen-bonding interactions with the anionic substrates. These interactions are clearly reduced by the formation of highly protonated and less negatively charged forms of the substrates at more acidic pH values or of less charged forms of the receptors at strongly alkaline pH values, justifying the observed decrease of the CISs of both ^{1}H and ^{31}P NMR resonances. All of the host−guest adducts analyzed in the course of this study displayed a similar pH dependence of the ^{31}P and ^{1}H NMR CISs (see the Supporting Information, Figures S5−S11).

To further elucidate the binding mode of L1−L3 and to better understand their size-dependent selectivity in the recognition of tri-, di-, and monophosphate, respectively, we carried out a molecular modeling study by using the empirical force-field *AMBER*[38] on complexes formed by different anionic substrates with receptors in their $(H_2L)^{2+}$ form (L = L1, L2, or L3). All diprotonated receptors $(H_2L)^{2+}$, in fact, form stable complexes in solution with $(HPO_4)^{2-}$, $P_2O_7^{4-}$, and $P_3O_{10}^{5-}$ [with the only exception being L1, which appears unable to bind $(HPO_4)^{2-}$], to give stable $[(H_2L)(HPO_4)]$, $[(H_2L)(P_2O_7)]^{2-}$, and $[(H_2L)(P_3O_{10})]^{3-}$ adducts, respectively, where two acidic protons can confidently be localized on the receptors because of the far higher constant for protonation of $(HL)^+$ to give $(H_2L)^{2+}$ with respect to that for protonation of $(HPO_4)^{2-}$, $P_2O_7^{4-}$, or $P_3O_{10}^{5-}$. The lowest-energy conformers of the adducts formed by $(H_2L1)^{2+}$ and $(H_2L3)^{2+}$ with the three anions $(HPO_4)^{2-}$, $P_2O_7^{4-}$, and $P_3O_{10}^{5-}$ are reported in

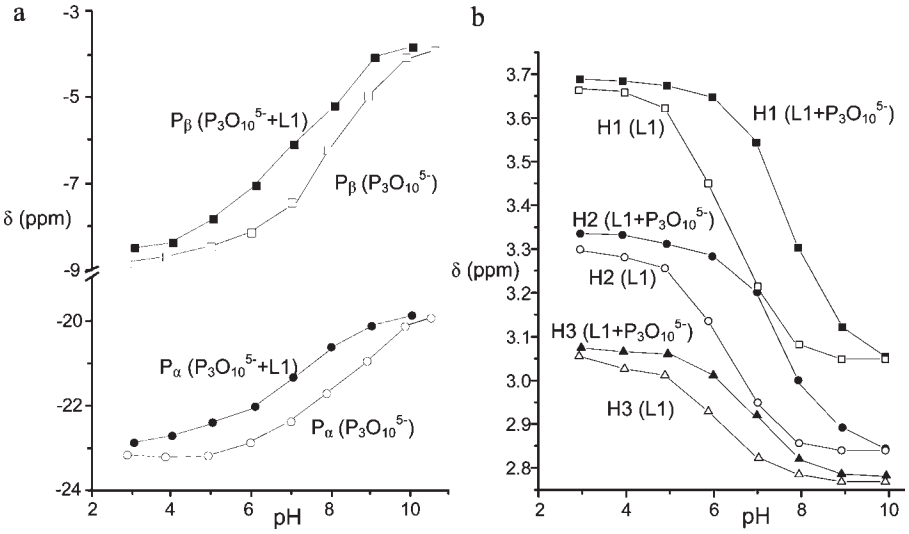

Figure 5. pH dependences of the ^{31}P NMR of the signals of triphosphate (P_α and P_β indicate the central and terminal phosphate groups of triphosphate, respectively) in the absence (white symbols) and in the presence (black symbols) of 1 equiv of L1 (a) and of the ^{1}H NMR signals H1, H2, and H3 of L1 in the absence (white symbols) and in the presence (black symbols) of 1 equiv of triphosphate (b) ([L1] = [triphosphate] = 1.0×10^{-2} M, 298 K).

Figures 6 and 7, respectively, while those formed by L2 are given in the Supporting Information (Figure S12).

In all cases, the receptors assume a "clamp-like" conformation, with the "jaws" being defined by the two monoprotonated [9]aneN$_3$ subunits. As a consequence of this receptor structure, both protonated [9]aneN$_3$ units may act as chelating units for the phosphate substrates. This is the case of the $P_3O_{10}^{5-}/(H_2L1)^{2+}$ and $P_2O_7^{4-}/(H_2L1)^{2+}$ adducts, where the anionic substrates interact with both [9]aneN$_3$ units via the formation of hydrogen-bonding contacts, mainly involving the protonated ammonium groups of $(H_2L1)^{2+}$. However, the smaller diphosphate anion shows a lower number of hydrogen-bonding interactions than $P_3O_{10}^{5-}$ at somewhat higher $NH^+\cdots{}^-O$ distances (Table S2 in the Supporting Information). Interestingly, the triphosphate anion interacts via hydrogen bonding with receptor L1 via the terminal P_β phosphate groups, while the central P_α moiety does not give hydrogen-bonding contacts with the receptor, in good agreement with the lower interaction of the P_α group deduced from the ^{31}P NMR experiments in aqueous solution (Figure 4c). Finally, the molecular modeling study of $(HPO_4)^{2-}/(H_2L1)^{2+}$ leads to a lowest-energy conformer, showing the monophosphate anion that interacts with only one protonated [9]aneN$_3$ unit via a single hydrogen bond. Most likely, this weak interaction mode affords complexes with low stability in solution, formed in amounts too low to be detected by potentiometric or NMR measurements. The lowest-energy conformers formed by $(H_2L3)^{2+}$ with the three anions, sketched in Figure 7, highlight different binding modes among the three substrates. In fact, in the $(HPO_4)^{2-}/(H_2L3)^{2+}$ adduct, the phosphate anion is almost encapsulated within the cleft formed between the two [9]aneN$_3$ units, affording a network of hydrogen-bonding interactions with both the protonated nitrogen atoms and a nonprotonated amine group (Figure 7a). In the case of di- and triphosphate, the anions assume a more "external" position with respect the *pseudo*cavity formed by the macrocyclic subunits, which allow them, however, to interact with both [9]aneN$_3$ units. From this point of view, the $P_2O_7^{4-}/(H_2L3)^{2+}$ conformers can be grouped in two families (see Figure 7b,c for the lowest-energy conformer of each family), which differ in the mutual disposition of the two [9]aneN$_3$ units and the diphosphate anion. While in the most populated A family (65% of the sampled conformers), $P_2O_7^{4-}$ is located "below" the two facing [9]aneN$_3$ units (Figure 7b), far from the quinoxaline unit, in the less populated B family, the anion is located "above" the two [9]aneN$_3$ units, close to the heteroaromatic spacer (Figure 7c). In the case of diphosphate, despite the larger number of phosphate groups available for hydrogen bonding, the number of hydrogen-bonding contacts with the receptor is lower than (A family) or at most equal to (B family) the corresponding adduct with $(HPO_4)^{2-}$. At the same time, while monophosphate is almost shielded from the solvent because of its inclusion within the receptor cleft, the larger di- and triphosphate anions appear to be more exposed to external solvent molecules. The latter observation may suggest also a larger desolvation upon complexation of the small $(HPO_4)^{2-}$ and a consequent higher entropic stabilization of the monophosphate adduct with L3.

In the case of $(H_2L2)^{2+}$, the low-energy conformer of the adduct with monophosphate shows the $(HPO_4)^{2-}$ anion located between the two [9]aneN$_3$ units (see the Supporting Information, Table S3 and Figure S12). Different from the adduct with L3, the anionic substrate mainly interacts via hydrogen bonding with a single protonated macrocyclic moiety. Di- and triphosphate show similar coordination motifs in their adducts with $(H_2L2)^{2+}$. In fact, $P_2O_7^{4-}$ uses both of its phosphate groups to form hydrogen-bonding contacts with the two [9]aneN$_3$ moieties of the receptor. Similarly, both terminal phosphate P_β moieties are employed by $P_3O_{10}^{5-}$ to form a hydrogen-bonding network mainly involving the ammonium function of each macrocyclic moiety. This similar binding mode displayed by $(H_2L2)^{2+}$ toward di- and triphosphate reflects the lower selectivity in the phosphate anion binding of L2 deduced from the potentiometric measurements.

Binding of ATP and ADP in Aqueous Solution. Receptors L1−L3 possess a further potential binding moiety for organic phosphate anions, such as nucleotides, i.e., the aromatic spacer linking the two triamine units, which may interact with aromatic moieties of the guest anions via hydrophobic and π-stacking interactions. For this reason, we decided to investigate the

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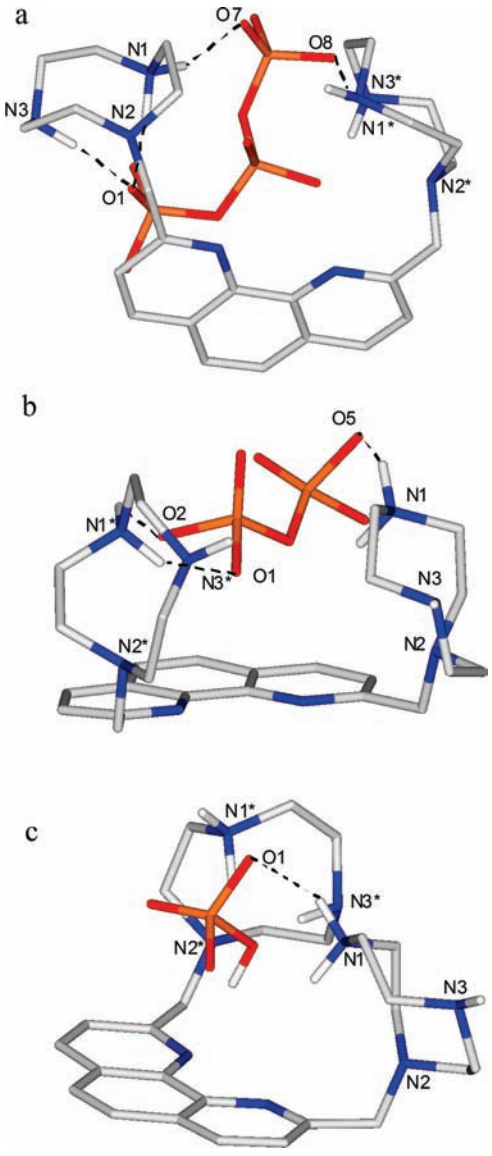

Figure 6. Lowest-energy conformers of the adducts between $(H_2L1)^{2+}$ and $P_3O_{10}^{5-}$ (a), $P_2O_7^{4-}$ (b), and $(HPO_4)^{2-}$ (c). Hydrogen-bonding distances are listed in the Supporting Information (Table S2).

binding ability of L1−L3 toward ADP and ATP (acronysms ATP, ADP, and AMP with omitted charges will be used throughout this section when referring to nucleotides independently of their protonation degree). These nucleotides are among the most targeted anionic substrates in anion coordination chemistry because of their biological relevance and, at the same time, feature a tri- and diphosphate chain connected through the sugar moiety to adenine, a nucleobase well-known for its ability to give π-stacked complexes. As in the case of inorganic phosphates, we first analyzed the binding properties of the present receptors by means of potentiometric measurements. Unfortunately, the low solubility of the complexes of L3 prevented the study of ATP and ADP binding in aqueous solution. Analysis of the AMP coordination, which could be of interest in the context of the present work, was also prevented by the low solubility of its adducts with the three receptors.

Table 4 reports the stepwise formation constants of the complexes obtained by using the same approach as that described in the case of inorganic phosphates, while the cumulative formation constants and the distribution diagrams of the adducts formed by L1 and L2 with ATP and ADP are supplied in the Supporting Information (Table S5 and Figure S13). As in the case of inorganic phosphates, the data in Table 4 point out that, for a given substrate/receptor system, the stability of the complexes is often determined by the protonation state of the two partners, in agreement with a host−guest interaction mainly due to the formation of strong $NH^+\cdots{}^-O$ contacts.

As in the case of inorganic phosphate complexation, selectivity plots displaying the overall percentage of complexed species in competitive systems can be a useful tool in comparing the binding abilities of L1 and L2 toward the two nucleotides. Parts a and b of Figure 8 report similar plots for competitive systems containing both inorganic and nucleotide phosphate anions and L1 or L2 respectively. At a first glance, these selectivity plots show selective binding of ATP and ADP over their inorganic counterparts, triphosphate and diphosphate, respectively. This feature, often observed in anion coordination by polyammonium receptors, is generally attributed to involvement of the adenine moiety of ATP or ADP in the overall host−guest interaction. Similarly, the higher affinity for ATP over ADP, outlined in Figure 8a,b, is often found in polyammonium receptors and is generally attributed to the higher negative charge of ATP with respect to ADP at a given pH value.

In the present case, however, L1 displays a more marked preference for ATP coordination over ADP than L2. For instance at pH 6, the percentages of ATP and ADP complexed by L1 are ca. 75% and 10%, respectively, while the percentages of ATP and ADP coordinated to L2 are 45% and 15%. This may suggest that in L1 the geometrical arrangement of the protonated [9]aneN$_3$ moieties is better suited to optimally interact with the triphosphate chain of ATP than with the diphosphate unit of ADP, in agreement with the results obtained from the study of inorganic mono-, di-, and triphosphate binding. More interestingly, the comparison between the binding abilities of L1 and L2 toward ATP (Figure 8c) and ADP (Figure 8d) points out that L1 is overall a better nucleotide binder than L2. In fact, preferential formation of the L1/ATP and L1/ADP adducts over the corresponding L2 complexes occurs to a large extent over the entire pH range investigated. This result may be due to the presence in L1 of a large heteroaromatic unit, 1,10-phenanthroline, capable of giving stronger π-stacking and hydrophobic interactions with adenine of nucleotides than the pyridine moiety of L2. It seems likely that, in nucleotide binding, the binding features of L1 and L2 toward ATP or ADP are determined not only by the spatial disposition of the binding site for the phosphate chain (the [9]aneN$_3$ units) but also by the electronic and structural characteristics of the heteroaromatic moieties. These suggestions are substantially corroborated by the ^{1}H and ^{31}P NMR spectra on the different host−guest systems, both recorded at neutral pH values with different receptor/substrate molar ratios or with a 1:1 molar ratio at different pH values.

The addition of L1 or L2 to a solution of ATP or ADP produces a linear downfield shift of the phosphate signals of nucleotides up to a 0.7−0.8:1 receptor-to-substrate molar ratio (R'). Then, the shift of the signals achieves constant values for $R' >$ 1.2−1.4 (Figure S14 in the Supporting Information) This result confirms the formation of 1:1 complexes in aqueous solution. As is generally observed in nucleotide recognition by polyammonium macrocycles,[2e] the CIS of the resonances increases in the order $P_\alpha \ll P_\beta < P_\gamma$ in the case of ATP and $P_\alpha \ll P_\beta$ in the case of ADP,

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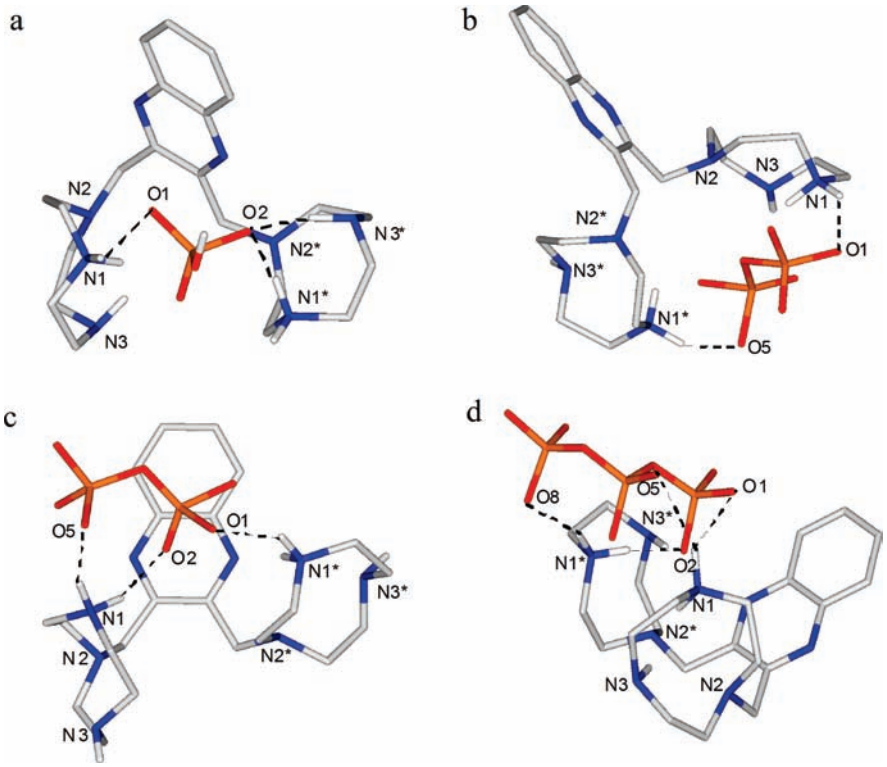

Figure 7. Lowest-energy conformers of the adducts between $(H_2L3)^{2+}$ and $(HPO_4)^{2-}$ (a), $P_2O_7^{4-}$ in its A (b) and B (c) families of conformers, and $P_3O_{10}^{5-}$ (d). Hydrogen-bonding distances are listed in the Supporting Information (Table S4).

Table 4. Formation Constants (log *K*) of the Adducts Formed by ATP and ADP with Receptors L1 and L2 (0.1 M NMe_4NO_3, 298 K)

	log *K*	
equilibrium	L1	L2
$(HL)^{+} + ATP^{4-} \rightleftharpoons [HLATP]^{3-}$	5.02(8)	4.40(4)
$(H_2L)^{2+} + ATP^{4-} \rightleftharpoons [H_2LATP]^{2-}$	6.20(5)	5.32(9)
$(H_2L)^{2+} + (HATP)^{3-} \rightleftharpoons [H_3LATP]^{-}$	5.82(7)	4.98(9)
$(H_3L)^{3+} + (HATP)^{3-} \rightleftharpoons [H_4LATP]$	6.95(7)	5.87(9)
$(H_3L)^{3+} + (H_2ATP)^{2-} \rightleftharpoons [H_5LATP]^{+}$	7.90(7)	6.11(9)
$(H_3L)^{3+} + (H_3ATP)^{-} \rightleftharpoons [H_6LATP]^{2+}$	7.30(7)	6.0(1)
$(H_4L)^{4+} + (H_2ATP)^{2-} \rightleftharpoons [H_6LATP]^{2+}$	7.32(7)	5.7(1)
$(H_2L)^{2+} + ADP^{3-} \rightleftharpoons [H_2LADP]^{-}$	4.9(1)	4.7(1)
$(H_2L)^{2+} + (HADP)^{2-} \rightleftharpoons [H_3LADP]$	4.9(1)	4.45(4)
$(H_3L)^{3+} + (HADP)^{2-} \rightleftharpoons [H_4LADP]^{+}$	5.91(7)	5.51(6)
$(H_3L)^{3+} + (H_2ADP)^{-} \rightleftharpoons [H_5LADP]^{2+}$	5.44(9)	4.95(6)
$(H_4L)^{4+} + (HADP)^{2-} \rightleftharpoons [H_5LADP]^{2+}$	5.71(8)	4.93 (7)
$(H_4L)^{4+} + (H_2ADP)^{-} \rightleftharpoons [H_6LADP]^{3+}$	6.44(8)	5.90(7)

as summarized in Table 5. The chemical shifts of the P_α groups in ATP and ADP are almost not affected by complexation, probably because of the steric hindrance of the adenosine moiety, which can hamper interaction of this phosphate moiety with the polyammonium binding sites of the receptor. Furthermore, the shifts of the signals of the terminal P_β groups of ADP are lower than those observed for the P_γ resonances of ATP, in keeping with the preferential binding of ATP over ADP displayed by both ligands. More interestingly, the data in Table 5 point out that for a given phosphate group of ATP or ADP the downfield shifts observed upon complexation with L1 are similar or just slightly higher than those measured for the corresponding adducts with L2 (for instance, at pH 7.0, the CIS values for the P_γ and P_β phosphate groups of ATP are 1.50 and 0.65 for the L1/ATP complex and 1.44 and 0.63 for the L2/ATP adduct). This observation may suggest that the phosphate chain of ATP (or ADP) displays a similar binding motif in its interaction with the two receptors.

Both the ^{31}P and ^{1}H NMR chemical shifts of the signals in the complexes are also strongly pH-dependent, as reported in Figures 9 and 10 for the ^{1}H NMR resonances of the systems L1/ATP and L2/ATP (see the Supporting Information, Figures S15−S18, for the pH dependences of the ^{31}P NMR signals of both nucleotides and of the ^{1}H NMR resonances of ADP in the presence of L1 and L2).

As in the case of inorganic phosphates, the shifts induced by complexation are larger at neutral or slightly acidic pH values, where highly charged species of both receptors and ATP are simultaneously present in solution. Furthermore, the ^{1}H NMR signals of the H1, H2 and, at a lesser extent, H3 methylene groups of L1 and L2 experience relevant downfield shifts upon complex formation (Figure 9), in agreement with the formation of charge−charge and hydrogen-bonding interactions between the phosphate chain of ATP and the protonated [9]aneN$_3$ moieties of the receptors. Of note, the pH dependence and the shift induced by complexation at a given pH for the methylene groups of L1 and L2 are similar for the L1/ATP and L2/ATP complexes. For instance, at pH 7.0, the CIS values for the H1 and H2 methylene groups are 0.23 and 0.19 ppm in the L1/ATP complex and 0.26 and 0.17 ppm in the L2/ATP complex, as reported in Table 6. Analogously, the ADP complexes with

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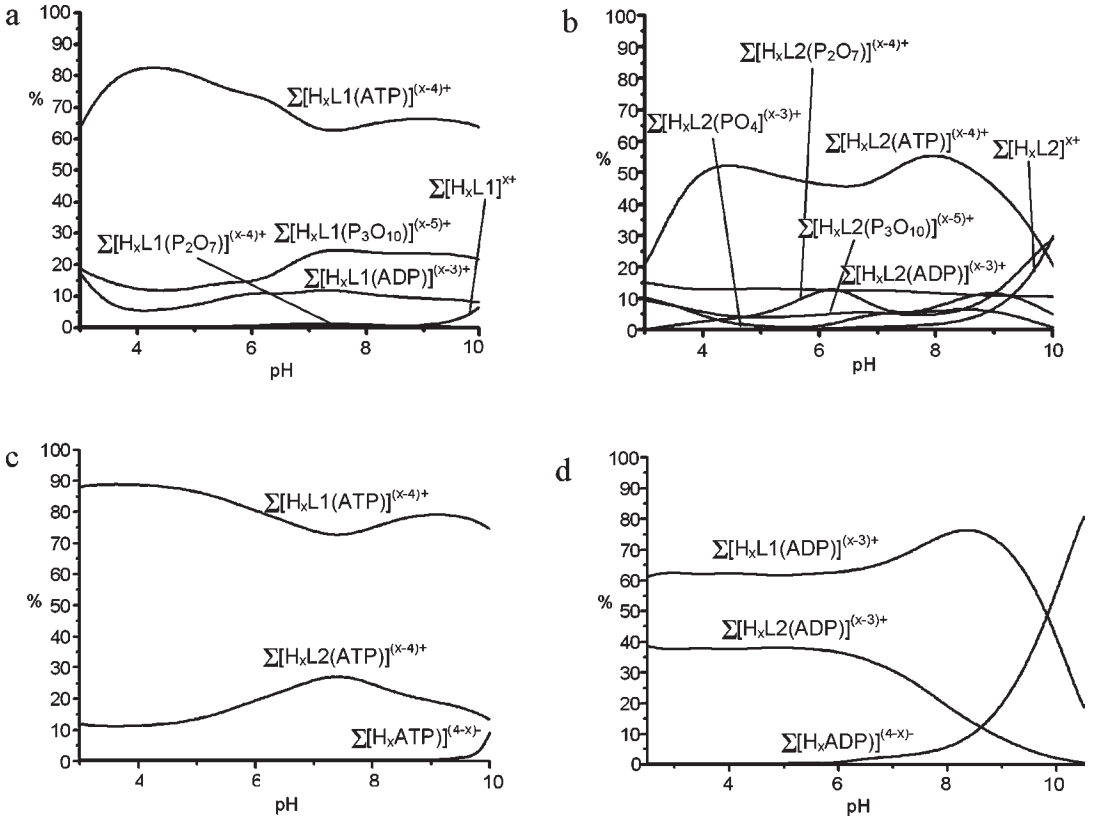

Figure 8. Overall percentages of L1 (a) and L2 (b) complexed species with mono-, di-, and triphosphate, ADP, and ATP as a function of the pH in competitive systems containing L1 or L2 and mono-, di-, and triphosphate, ADP, and ATP in an equimolecular ratio and overall percentages of the ATP (c) and ADP (d) complexed species with L1 and L2 as a function of the pH in competitive systems containing ATP or ADP and L1 and L2 in an equimolecular ratio ($[L1] = [L2] = [PO_4^{3-}] = [P_2O_7^{4-}] = [P_3O_{10}^{5-}] = [ADP^{3-}] = [ATP^{4-}] = 1.0 \times 10^{-3}$ M; L = L1 or L2).

Table 5. ^{31}P NMR Chemical Shifts (ppm) for the Phosphate Signals of ATP and ADP in Their Adducts with L1 and L2 and Corresponding CISs (ppm) Measured in D_2O Solution at pH 7.0 and 298 K

	ATP			ADP	
	P_α	P_β	P_γ	P_α	P_β
			L1		
δ	−9.98	−21.08	−5.34	−9.93	−5.63
CIS	0.12	0.65	1.50	0.05	1.35
			L2		
δ	−9.99	−21.10	−5.41	−9.83	−5.56
CIS	0.11	0.63	1.44	0.15	1.42

L1 and L2 are characterized by similar downfield shifts of the signals of the methylene groups belonging to the [9]aneN$_3$ units (Figure S17 in the Supporting Information). These ^{1}H NMR data confirm that the overall interaction mode of the phosphate chain of ATP (or ADP) with the aliphatic macrocyclic moieties is analogous in both complexes with L1 and L2.

While in the case of inorganic phosphate anions, the signals of the aromatic protons of L1 and L2 are essentially not affected by the binding process, in the case of ATP and ADP, the resonances of the phenanthroline and pyridine hydrogen atoms (see Figures 9 and S17 in the Supporting Information for ATP and ADP complexation, respectively) as well as those of adenine (see Figures 10 and S18 in the Supporting Information for ATP and ADP, respectively) are upfield-shifted upon complex formation. These data unambiguously indicate that π-stacking and/or hydrophobic interactions between adenine and the heteroaromatic units of the receptors also participate in the process of complex formation.[9,14] Of note, the resonances of the adenine protons as well as those of the heteroaromatic units of the receptors in the ATP and ADP complexes with L1 display remarkably larger complexation-induced upfield shifts than those in the corresponding adducts with L2 (see Figures 9 and 10 and Table 6).

For instance, at pH 7.0, the H8 and H2 signals of adenine are upfield-shifted of 0.46 and 0.43 ppm in the ATP/L1 complexes, while the upfield shift is only 0.12 ppm for both the H8 and H2 resonances in the ATP/L2 adducts. This accounts for the presence of tighter π-stacking interactions in the adducts with L1, thanks to the presence of phenanthroline, a planar and extended heteroaromatic unit capable of giving stronger π pairing with adenine than the smaller pyridine unit of L2.

This result can justify the observed better binding ability toward ADP and ATP of L1 with respect to L2. While in the case of inorganic phosphate anions the recognition properties of L1 and L2 are mainly determined by charge−charge and hydrogen-bonding interactions between the [9]aneN$_3$ units and the phosphate groups and they are modulated by an appropriate spatial disposition of the polyammonium units, in the case of nucleotides, the formation of stronger π-stacking interactions can account for the higher binding ability displayed by L1 over L2.

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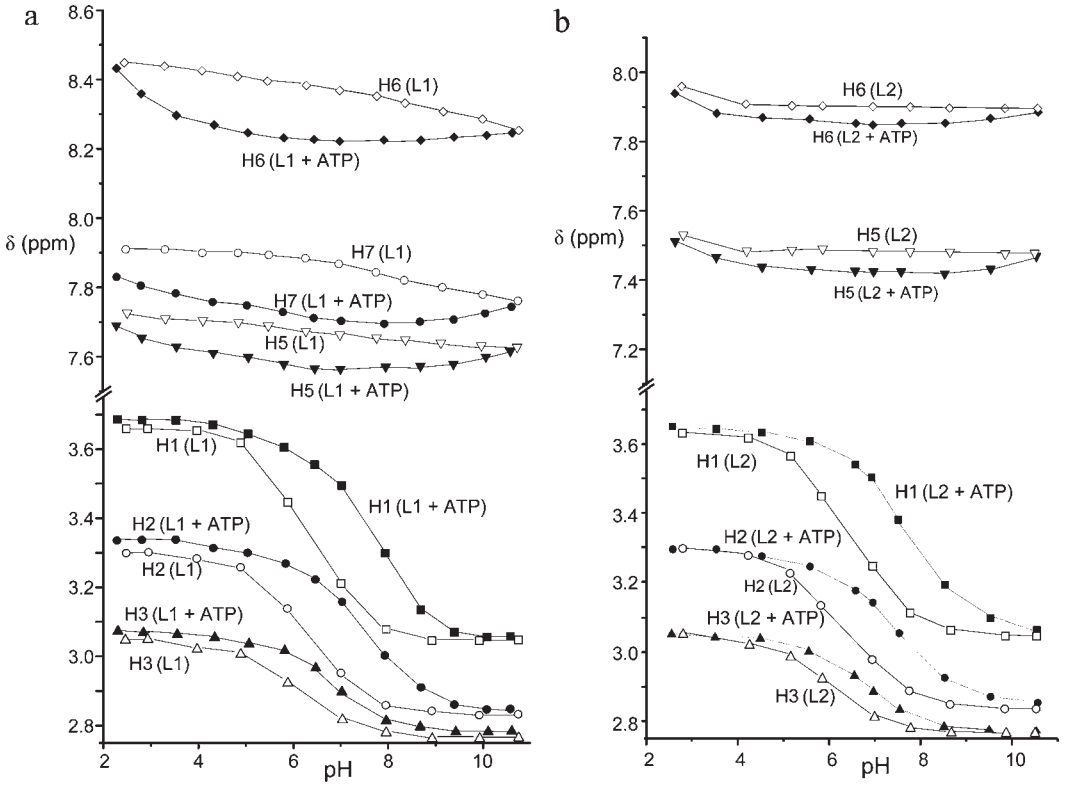

Figure 9. pH dependences of the ^{1}H NMR signals of L1 (a) and L2 (b) in the absence (white symbols) and in the presence (black symbols) of 1 equiv of ATP ($[L1] = [L2] = [ATP^{4-}] = 1.0 \times 10^{-2}$ M, 298 K). The pH dependence of the signal of H4 is almost not affected by the presence of the anion, and it is not reported.

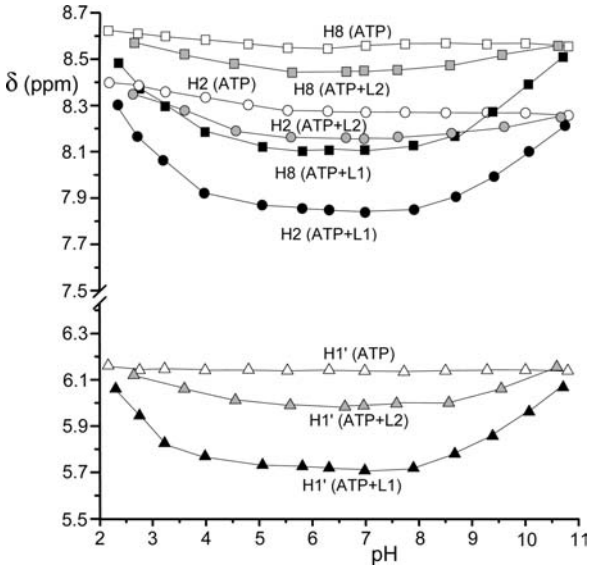

Figure 10. pH dependences of the ^{1}H NMR signals of the adenine protons H2 and H8 and of the ribose proton H1′ of ATP in the absence (white symbols) and in the presence of 1 equiv of L1 (black symbols) and L2 (gray symbols).

Table 6. ^{1}H NMR Chemical Shifts (ppm) for the Aliphatic Protons of L1 and L2 (H1, H2, and H3), for the Phenanthroline Hydrogen Atoms (H5, H6, and H7) of L1 and the Pyridine Hydrogen Atoms (H5 and H6) of L2, and for the Aromatic Protons of Adenine (H2 and H8) and the Anomeric H1′ Proton of Ribose of ATP and ADP, in the Adducts of ATP and ADP with L1 or L2 and Corresponding (ppm) Measured in D_2O Solution at pH 7 and 298 K

		H1	H2	H3	H5	H6	H7		H8	H2	H1′
Adducts with ATP											
L1	δ	3.48	3.17	2.90	7.56	8.21	7.72	ATP	8.11	7.84	5.72
	CIS	0.23	0.19	0.08	−0.11	−0.17	−0.16		−0.46	−0.43	−0.42
L2	δ	3.51	3.14	2.92	7.44	7.85		ATP	8.45	8.15	5.99
	CIS	0.26	0.17	0.10	−0.05	−0.05			−0.12	−0.12	−0.15
Adducts with ADP											
L1	δ	3.42	3.09	2.88	7.57	8.24	7.73	ADP	8.08	7.84	5.70
	CIS	0.16	0.11	0.05	−0.10	−0.14	−0.15		−0.40	−0.38	−0.40
L2	δ	3.42	3.09	2.88	7.42	7.85		ADP	8.38	8.13	5.99
	CIS	0.17	0.12	0.06	−0.07	−0.05			−0.10	−0.09	−0.11

These suggestions are substantially confirmed by the molecular modeling analysis carried out on the adducts formed by $(H_2L1)^{2+}$ and $(H_2L2)^{2+}$ with ATP^{4-} and ADP^{3-}. Figure 11 shows the lowest-energy conformers for the complexes formed by $(H_2L1)^{2+}$ and $(H_2L2)^{2+}$ and ATP^{4-} (the lowest-energy conformers of the ADP complexes are reported in the Supporting Information, Figure S19). The different conformers of the ATP^{4-} complexes with two diprotonated receptors can be grouped into two families (herein indicated as A and B), which mainly differ in the interaction mode of the nucleobase with the receptor. While in the A family adenine gives rise to a face-to-face π-stacking interaction with phenanthroline or pyridine

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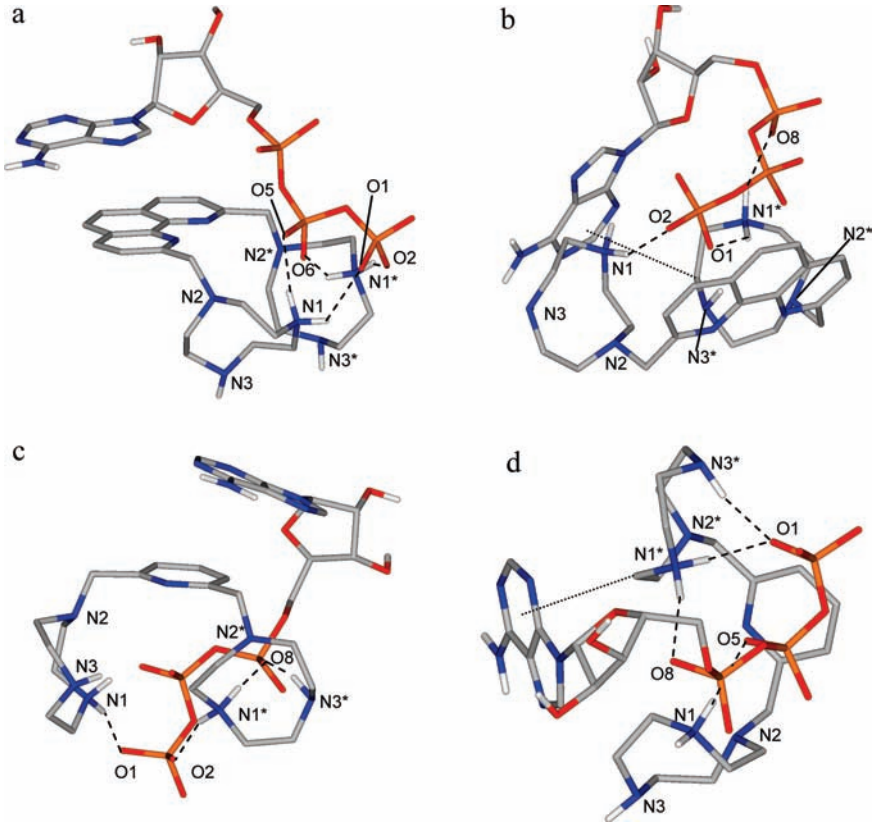

Figure 11. Lowest-energy conformers of the adducts between $(H_2L1)^{2+}$ and ATP^{4-} in the A (a) and B (b) families of conformers and of the adducts between $(H_2L2)^{2+}$ and ATP^{4-} in the A (c) and B (d) families of conformers. Hydrogen-bonding distances are listed in the Supporting Information, Tables S6 and S7).

(see Figure 11a,c), in the B family of conformers, the adenine–receptor interaction is featured by C–H···π contacts between the ammonium or methylene group of one of the [9]aneN$_3$ units (see Figure 11b,d) and the six-membered aromatic ring of adenine. For both receptors, the A family is the most populated (70% and 65% of the sampled conformers of the $(H_2L1)^{2+}$/ATP^{4-} and $(H_2L2)^{2+}$/ATP^{4-} adducts, respectively). As in the case of inorganic di- and triphosphate, in both A and B families of the $(H_2L1)^{2+}$/ATP^{4-} and $(H_2L2)^{2+}$/ATP^{4-} complexes, the two [9]aneN$_3$ units are simultaneously involved in the binding process to the phosphate chain of ATP^{4-}. However, the phosphate chain is enclosed within the cleft delimited by the two protonated macrocycles and the heteroaromatic spacer at a less extent than inorganic phosphates, probably because of the presence of the sterically hindering adenosine moiety. Nevertheless, in both adducts with $(H_2L1)^{2+}$ and $(H_2L2)^{2+}$, the triphosphate chain of ATP^{4-} gives rise to a dense network of stabilizing P–O$^-$···$^+$H$_2$N hydrogen bonds with the receptors. As was 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^{4-} gives the strongest interaction with the ammonium groups of the receptor, forming several hydrogen bonds with both macrocyclic moieties. The phosphate groups P_β and, in particular, P_α generally give rise to a lower number of hydrogen-bonding contacts, in most cases with a single [9]aneN$_3$ moiety.

If the overall interaction between the triphosphate chain of ATP^{4-} and the [9]aneN$_3$ units is comparable in the $(H_2L1)^{2+}$/ATP^{4-} and $(H_2L2)^{2+}$/ATP^{4-} adducts, significant differences are observable in the π pairing between adenine and the heteroaromatic units observed in the A family of the two adducts. In fact, in the $(H_2L1)^{2+}$/ATP^{4-} conformers, the adenine unit is stacked above the phenanthroline unit and lies on planes almost parallel to that of phenanthroline. In the lowest-energy conformer (Figure 11a), the five-membered ring of adenine is superimposed to the central aromatic ring of phenanthroline, with the distance between the centroids being 3.43 Å. Conversely, in the $(H_2L2)^{2+}$/ATP^{4-} conformers, adenine lies on planes somewhat bent (24.6° in the case of the lowest-energy conformer in Figure 11c) with respect to pyridine and is less "overlapped" with this heteroaromatic unit. Once again, these results are in good agree ment with the largest shifts observed for the ^{1}H NMR signals of the adenine of ATP in the presence of L1.

The $(H_2L1)^{2+}$/ADP^{3-} and $(H_2L2)^{2+}$/ADP^{3-} modeled adducts display similar structural features, with the sampled conformers being grouped into two families, featured respectively by π-stacking interactions between adenine and phenanthroline or pyridine (A family) or by C–H···π contacts between the nucleobase and one [9]aneN$_3$ unit (B family) (see the Supporting Information, Figure S19). In both complexes with $(H_2L1)^{2+}$ and $(H_2L2)^{2+}$, the terminal P_β phosphate and, to a lesser extent, the P_α phosphate afford several hydrogen-bonding contacts with both [9]aneN$_3$ units. The hydrogen-bonding network, however, is less "dense" than that in the ATP^{4-} complexes with L1 and L2, in keeping with the observed lower stability of the ADP adducts with respect to the ATP ones. More interestingly, the L1 adducts in the A family still feature the adenine unit stacked above the heteroaromatic unit of the receptor, lying on a plane almost parallel to

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that of phenanthroline, while the L2 complexes belonging to the A family display a remarkable lower "overlapping" between the two heteroaromatic units.

CONCLUDING REMARKS

This study represents a case of recognition of different types of phosphate anions by bis([9]aneN$_3$) polyammonium receptors capitalizing on their structural features, i.e., the geometrical disposition of the polyammonium binding sites for the anionic phosphate chain and the characteristics of the heteroaromatic spacers between the [9]aneN$_3$ units as recognition sites for adenine. In the case of inorganic phosphate anions, the binding process is essentially driven by charge−charge and hydrogen-bonding interactions and, therefore, the selectivity properties of a receptor depend, almost exclusively, on the spatial arrangement of the ammonium groups. In the case of L1, the two [9]aneN$_3$ units, separated by the larger phenanthroline moiety, may simultaneously act as a chelating unit for the longer phosphate chain of triphosphate to form a tight "chelate" complex. This structural arrangement is partially lost in the case of diphosphate, while the smaller monophosphate anion can interact with only a single [9]aneN$_3$ unit and, actually, it is not bound by this receptor in aqueous solution. Conversely, in L3, the two macrocyclic units are at a short distance from one another, generating a cleft where only a monophosphate can be conveniently hosted.

While in inorganic phosphate recognition the heteroaromatic moieties act as simple spacers between the two [9]aneN$_3$ chelating units, defining the dimensions of the host cleft, in the case of ATP and ADP, they can play an "active" role in the binding process, thanks to their ability to give π-stacking interactions with the nucleobase. Actually, L1 and L2 can bind ATP or ADP via the simultaneous interaction of both [9]aneN$_3$ units with the anionic phosphate chain of these substrates. For a given nucleotide, this interaction mode is similar for the two receptors. Different from inorganic phosphate binding, the different recognition properties toward nucleotides displayed by L1 and L2 are mainly determined by the ability of the spacer to form π-stacked assemblies with adenine. As a consequence, L1, which contains the most extended heteroaromatic unit, is a better nucleotide binder than L2.

Finally, taking advantage from the ability of L1−L3 to form stable dinuclear complexes, it can be of interest, in future studies, to analyze the effect of the distance between the macrocyclic units on the recognition ability for phosphate anions of dimetal complexes with L1−L3 or similar ligands featuring two triaza moieties linked by different longer rigid spacers.

ASSOCIATED CONTENT

Supporting Information. Cumulative formation constants of the adducts between L1−L3 and inorganic phosphates (Table S1); hydrogen-bonding distances in the calculated lowest-energy conformers of the adducts between $(H_2L1)^{2+}$, $(H_2L2)^{2+}$, and $(H_2L3)^{2+}$ and inorganic phosphates (Tables S2−S4) and between $(H_2L1)^{2+}$, $(H_2L2)^{2+}$ and ATP and ADP (Tables S6 and S7); cumulative formation constants of the adducts between L1 and L2 and ATP and ADP (Table S5); distribution diagrams of the complexes with monophosphate, diphosphate, ATP, and ADP (Figures S1, S2, and S13); ^{1}H NMR chemical shifts of L2 and L3 in the presence of increasing amounts of mono-, di-, and triphosphate (Figures S3 and S4); ^{31}P NMR chemical shifts of ADP and ATP in the presence of increasing amounts of L1 and L2 (Figure S14); pH dependence of the ^{31}P NMR signals of substrates and ^{1}H NMR signals of the aliphatic protons of receptors in the complexes between L1 and diphosphate, ADP, and ATP, L2 and mono-, di-, triphosphate, ADP, and ATP, and L3 and mono-, di-, and triphosphate (Figures S5−S11, S15, and S16); pH dependence of the ^{1}H NMR signals of the aromatic protons in the ADP adducts with L1 and L2 (Figures S17 and S18); calculated lowest-energy conformers of the L2 complexes with inorganic phosphates and ADP and of the L1 adduct with ADP (Figures S12 and S19). This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) (a) Hirsch, A. K. H.; Fischer, F. R.; Diederich, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 338–352.(b) Dugas, H. *Bioorganic Chemistry: a Chemical Approach to Enzyme Action*; Springer: New York, 1996. (c) Davidson, A. M. L.; Dassa, E.; Orelle, C.; Chen, J. *Microbiol. Mol. Biol. Rev.* **2008**, *72*, 317–364. (d) Alton, G. R.; Lunney, E. A. *Expert Opin. Drug Discovery* **2008**, *3*, 595–605. (e) Lewis, J. A.; Lebois, E. P.; Lindsley, C. W. *Curr. Opin. Chem. Biol.* **2008**, *12*, 269–280. (f) Matte, A.; Delbaere, L. T. J. *Handb. Proteins* **2007**, *1*, 114–118. (g) Turk, B. E. *Curr. Opin. Chem. Biol.* **2008**, *12*, 4–10. (h) Hollenstein, K.; Dawson, R. J. P.; Locher, K. P. *Curr. Opin. Struct. Biol.* **2007**, *17*, 412–418. (i) Morrow, J. M.; Amyes, T. L.; Richard, J. P. *Acc. Chem. Res.* **2008**, *41*, 539–548. (j) Rye, C. S.; Baell, J. B. *Curr. Med. Chem.* **2005**, *12*, 3127–3141.

(2) Selected reviews: (a) Fabbrizzi, L.; Licchelli, M.; Rabaioli, G.; Taglietti, A. *Coord. Chem. Rev.* **2000**, *205*, 85–108. (b) Amendola, V.; Bonizzoni, M.; Esteban-Gomez, D.; Fabbrizzi, L.; Licchelli, M.; Sancenon, F.; Taglietti, A. *Coord. Chem. Rev.* **2006**, *250*, 1451–1470. (c) Garcia España, E.; Diaz, P.; Llinares, J. M.; Bianchi, A. *Coord. Chem. Rev.* **2006**, *250*, 2952–2980. (d) Kubik, S. *Chem. Soc. Rev.* **2010**, *39*, 3648–3663. (e) Bazzicalupi, C.; Bencini, A.; Lippolis, V. *Chem. Soc. Rev.* **2010**, *39*, 3709–3728. (f) Gale, P. A. *Chem. Soc. Rev.* **2010**, *39*, 3746–3771. (g) Kim, S. K.; Sessler, J. L. *Chem. Soc. Rev.* **2010**, *39*, 3784–3809. (h) Schmidtchen, F. P. *Chem. Soc. Rev.* **2010**, *39*, 3916–3935. (i) Kang, S. O.; Llinares, J. M.; Day, V. W.; Bowman-James, K. *Chem. Soc. Rev.* **2010**, *39*, 3980–4003.(j) Bianchi, A., Garcia-España, E., Bowman-James, K., Eds. *Supramolecular Chemistry of Anions*; Wiley-VCH: New York, 1997. (k) Katayev, E. A.; Ustynyuk, Y. A.; Sessler, J. L. *Coord. Chem. Rev.* **2006**, *250*, 3004–3037. (l) Caltagirone, C.; Gale, P. A. *Chem. Soc. Rev.* **2009**, *38*, 520–563. (m) Kim, S. K.; Lee, D. H.; Hong, J.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23–31. (n) Kang, S. O.; Hossain, M. A.; Bowman-James, K. *Coord. Chem. Rev.* **2006**, *250*, 3038–3052. (o) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094–3117. (p) Lankshear, M. D.; Beer, P. D. *Coord. Chem. Rev.* **2006**, *250*, 3142–3160. (q) Gimeno, N.; Vilar, R. *Coord. Chem. Rev.* **2006**, *250*, 3161–3189. (r) Gamez, P.; Mooibroek, T. J.; Teat, S. J.; Reedijk, J. *Acc. Chem. Res.* **2007**, *40*, 435–444. (s) Anzenbacher, P.; Nishiyabu, R.; Palacios, M. A. *Coord. Chem. Rev.* **2006**, *250*, 2929–2938. (t) Mateus, P.; Bernier, N.; Delgado, R. *Coord. Chem. Rev.* **2010**, 1726–1743.

(3) (a) Hossain, M. A.; Liljegren, J. A.; Powell, D.; Bowman-James, K. *Inorg. Chem.* **2004**, *43*, 3751–3755. (b) Hossain, M. A.; Morehouse, P.; Powell, D.; Bowman-James, K. *Inorg. Chem.* **2005**, *44*, 2143–2149.

(4) (a) Anda, C.; Llobet, A.; Salvado, V.; Martell, A. E.; Motekaitis, R. J. *Inorg. Chem.* **2000**, *39*, 3000–3008. (b) Anda, C.; Llobet, A.; Salvado, V.; Reibenspies, J.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **2000**, *39*, 2986–2999. (c) Anda, C.; Llobet, A.; Martell, A. E.; Donnadieu, B.; Parella, T. *Inorg. Chem.* **2003**, *42*, 8545–8550.

(5) (a) Dhaenens, M.; Lehn, J.-M.; Vigneron, J.-P. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1379–1381. (b) Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. *Supramol. Chem.* **1995**, *5*, 139–147. (c) Baudoin, O.; Gonnet, F.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Tabet, J.-C.; Lehn, J.-M. *Chem.—Eur. J.* **1999**, *5*, 2762–2771.

(6) Schneider, H.-J.; Blatter, T.; Palm, B.; Pfingstag, U.; Rüdiger, V.; Theis, I. *J. Am. Chem. Soc.* **1992**, *114*, 7704–7708.

(7) Furuta, H.; Magda, D.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 978–985.

(8) Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Faggi, E.; Giorgi, C.; Santarelli, S.; Valtancoli, B. *J. Am. Chem. Soc.* **2008**, *130*, 2440–2441.

(9) Arturoni, E.; Bazzicalupi, C.; Bencini, A.; Caltagirone, C.; Danesi, A.; Giorgi, C.; Garau, A.; Lippolis, V.; Valtancoli, B. *Inorg. Chem.* **2008**, *47*, 6551–6563.

(10) (a) Mateus, P.; Delgado, R.; Brandão, P.; Félix, V. *J. Org. Chem.* **2009**, *74*, 8638–8646. (b) Mateus, P.; Delgado, R.; Brandão, P.; Carvalho, S.; Félix, V. *Org. Biomol. Chem.* **2009**, *7*, 4661–4673.

(11) Vickers, M. S.; Martindale, K. S.; Beer, P. D. *J. Mater. Chem.* **2005**, *15*, 2784–2790.

(12) (a) Develay, S.; Tripier, R.; Le Baccon, M.; Patinec, V.; Serratrice, G.; Handel, H. *Dalton Trans.* **2005**, 3016–3024. (b) Delepine, A. S.; Tripier, R.; Handel, H. *Org. Biomol. Chem.* **2008**, *6*, 1743–1750. (c) Develay, S.; Tripier, R.; Le Baccon, M.; Patinec, V.; Serratrice, G.; Handel, H. *Dalton Trans.* **2006**, 3418–3426. (d) Develay, S.; Tripier, R.; Bernier, N.; Le Baccon, M.; Patinec, V.; Serratrice, G.; Handel, H. *Dalton Trans.* **2007**, 1038–1046.

(13) Ambrosi, G.; Formica, M.; Fusi, V.; Giorgi, L.; Guerri, A.; Macedi, E.; Micheloni, M.; Paoli, P.; Pontellini, R.; Rossi, P. *Inorg. Chem.* **2009**, *48*, 5901–5912.

(14) (a) Albenda, M. T.; Bernardo, M. A.; Garcia-España, E.; Godino-Salido, M. L.; Luis, S. V.; Melo, M. J.; Pina, F.; Soriano, C. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2545–2549. (b) Albenda, M. T.; Frías, J. C.; García-España, E.; Luis, S. V. *Org. Biomol. Chem.* **2004**, *2*, 816–822. (c) Aucejo, R.; Diaz, P.; Garcia-España, E.; Alarcon, J.; Delgado-Pinar, E.; Torres, F.; Soriano, C.; Guillem, C. M. *New J. Chem.* **2007**, *31*, 44–51. (d) Burguete, M. I.; García-España, E.; López-Diago, L.; Luis, S. V.; Miravet, J.; Sroczynski, D. *Org. Biomol. Chem.* **2007**, *5*, 1935–1944.

(15) Hosseini, M. W.; Blaker, A. J.; Lehn, J. M. *J. Am. Chem. Soc.* **1990**, *112*, 3896–3904.

(16) Casasus, R.; Climent, E.; Marcos, M. D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Amoros, P.; Cano, J.; Ruiz, E. *J. Am. Chem. Soc.* **2008**, *130*, 1903–1917.

(17) Sakamoto, T.; Ojida, A.; Hamachi, H. I. *Chem. Commun.* **2009**, 141–142.

(18) Zapata, F.; Caballero, A.; Espinosa, A.; Tarraga, A.; Molina, P. *J. Org. Chem.* **2008**, *73*, 4034–4040.

(19) Kwon, J. Y.; Singh, N. J.; Kim, H. N.; Kim, S. K.; Kim, K. S.; Yoon, J. *J. Am. Chem. Soc.* **2004**, *126*, 8892–8893.

(20) (a) Huston, M. E.; Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1989**, *111*, 8735–8736. (b) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397–9398.

(21) Kejik, Z.; Zaruba, K.; Michalik, D.; Sebek, J.; Dian, J.; Pataridis, S.; Volka, K.; Kral, V. *Chem. Commun.* **2006**, 1533–1535.

(22) Zyryanov, G. V.; Palacios, M. A.; Anzenbacher, P. *Angew. Chem., Int. Ed.* **2007**, *46*, 7849–7856.

(23) (a) Jose, D. A.; Mishra, S.; Ghosh, A.; Shrivastav, A.; Mishra, S. K.; Das, A. *Org. Lett.* **2007**, *9*, 1979–1982. (b) Ghosh, A.; Shrivastav, A.; Jose, D. A.; Mishra, S. K.; Chandrakanth, C. K.; Mishra, S.; Das, A. *Anal. Chem.* **2008**, *80*, 5312–5319.

(24) Khatua, S.; Choi, S. H.; Lee, J.; Kim, K.; Do, Y.; Churchill, D. G. *Inorg. Chem.* **2009**, *48*, 2993–2999.

(25) Costero, A. M.; Sanchis, J.; Gil, S.; Sanz, V.; Williams, J. A. G. *J. Mat. Chem.* **2005**, *15*, 2848–2853.

(26) Wang, H.; Chan, W. H. *Org. Biomol. Chem.* **2008**, *6*, 162–168.

(27) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S. J.; Yoon, J. *J. Am. Chem. Soc.* **2007**, *129*, 3828–3829.

(28) Lee, D. H.; Kim, S. Y.; Hong, J. I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777–4780.

(29) Nelissen, H. F. M.; Smith, D. K. *Chem. Commun.* **2007**, 3039–3041.

(30) Warden, A. C.; Warren, M.; Hearn, M. T. W.; Spiccia, L. *Inorg. Chem.* **2004**, *43*, 6936–6943.

(31) Yang, L.-Z.; Li, Y.; Jiang, L.; Feng, X.-L.; Lu, T.-B. *Cryst EngComm* **2009**, *11*, 2375–2378.

(32) (a) Bazzicalupi, C.; Bencini, A.; Faggi, E.; Garau, A.; Giorgi, C.; Lippolis, V.; Perra, A.; Valtancoli, B. *Dalton Trans.* **2006**, 1409–1418. (b) Arca, M.; Bencini, A.; Berni, E.; Caltagirone, C.; Devillanova, F. A.; Isaia, F.; Garau, A.; Giorgi, C.; Lippolis, V.; Perra, A.; Tei, L.; Valtancoli, B. *Inorg. Chem.* **2003**, *42*, 6929–6939.

(33) (a) Bazzicalupi, C.; Bencini, A.; Biagini, S.; Faggi, E.; Meini, S.; Giorgi, C.; Spepi, A.; Valtancoli, B. *J. Org. Chem.* **2009**, *74*, 7349–7363. (b) Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Danesi, A.; Giorgi, C.; Lodeiro, C.; Pina, F.; Santarelli, S.; Valtancoli, B. *Chem. Commun.* **2005**, 2630–2632. (c) Bazzicalupi, C.; Biagini, S.; Bencini, A.; Faggi, E.; Giorgi, C.; Matera, I.; Valtancoli, B. *Chem. Commun.* **2006**, 4087–4089. (d) Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Borri, C.; Danesi, A.; Garcia-España, E.; Giorgi, C.; Valtancoli, B. *J. Org. Chem.* **2008**, *73*, 8286–8295.

(34) (a) Arca, M.; Blake, A. J.; Casabò, J.; Demartin, F.; Devillanova, F. A.; Garau, A.; Isaia, F.; Lippolis, V.; Kivekas, R.; Muns, V.; Schröder, M.; Verani, G. *Dalton Trans.* **2001**, 1180–1188. (b) Aragoni, M. C.; Arca, M.; Demartin, F.; Devillanova, F. A.; Isaia, F.; Garau, A.; Lippolis, V.; Jalali, F.; Papke, U.; Shamsipur, M.; Tei, L.; Yari, A.; Verani, G. *Inorg. Chem.* **2002**, *41*, 6623–6632. (c) Aragoni, M. C.; Arca, M.; Bencini, A.; Biagini, S.; Blake, A. J.; Caltagirone, C.; Demartin, F.; De Filippo, G.; Devillanova, F. A.; Garau, A.; Gloe, K.; Isaia, F.; Lippolis, V.; Valtancoli, B.; Wenzel, M. *Inorg. Chem.* **2008**, *47*, 8391–8404. (d) Blake, A. J.; Devillanova, F. A.; Garau, A.; Harrison, A.; Isaia, F.; Lippolis, V.; Tiwary, S. K.; Schröder, M.; Verani, G.; Whittaker, G. *J. Chem. Soc., Dalton Trans.* **2002**, 4389–4394.

(35) (a) Gran, G. *Analyst* **1952**, *77*, 661–671. (b) Rossotti, F. J.; Rossotti, H. *J. Chem. Educ.* **1965**, *42*, 375–378.

(36) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739–1753.

(37) Covington, A. K.; Paabo, M.; Robinson, R. A.; Bates, R. G. *Anal. Chem.* **1968**, *40*, 700–709.

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

(39) *Hyperchem β1 release 7.51 for Windows MM System*; Hypercube, Inc.: Gainesville, FL, 2002.

(40) (a) Stewart, J. P. P. *J. Comput.-Aided Mol. Des.* **1989**, *10*, 209–220. (b) Stewart, J. P. P. *J. Comput.-Aided Mol. Des.* **1989**, *10*, 221–264.

(41) Bencini, A.; Bianchi, A.; Garcia-España, E.; Micheloni, M.; Ramirez, J. A. *Coord. Chem. Rev.* **1999**, *188*, 97–156.

(42) Dietrich, B.; Hosseini, M. W.; Lehn, J.-M.; Session, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 1282–1283.

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