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Exploring the Binding Ability of Phenanthroline-Based Polyammonium Receptors for Anions: Hints for Design of Selective Chemosensors for Nucleotides

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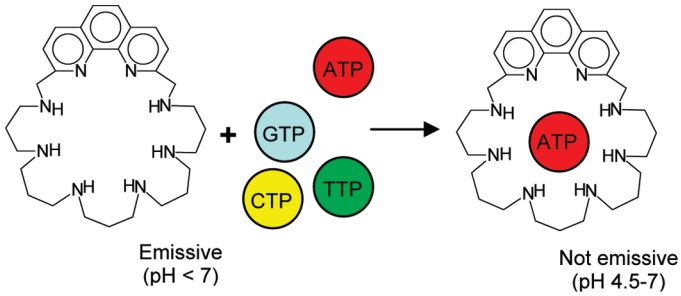

The synthesis of receptor 2,6,10,14,18-pentaaza[20]-21,34-phenanthrolinophane (L1), containing a pentaamine chain linking the 2,9 positions of a phenanthroline unit, is reported. The protonation features of L1 and of receptor 2,6,10,14,18,22-hexaaza[23]-24,37-phenanthrolinophane (L2) have been studied by means of potentiometric, ^{1}H NMR, and spectrofluorimetric measurements; this study points out that the fluorescent emission of both receptors depends on the protonation state of the polyamine chain. In fact, the receptors are emissive only at neutral or acidic pH values, where all the aliphatic amine groups are protonated. Potentiometric titrations show that L2 is able to bind selectively ATP over TTP, CTP, and GTP. This selectivity is lost in the case of L1. ^{1}H and ^{31}P NMR measurements and molecular mechanics calculations show that the phosphate chains of nucleotides give strong electrostatic and hydrogen-bonding interactions with the ammonium groups of the protonated receptors, while the nucleobases interact either via π-stacking with phenanthroline or via hydrogen bonding with the ammonium groups. Of note, MM calculations suggest that all nucleotides interact in an inclusive fashion. In fact, in all adducts the phosphate chain is enclosed within the receptor cavities. This structural feature is confirmed by the crystal structure of the $[(H_6L2)_2(TTP)_2(H_2O)_2]^{4+}$ adduct. Fluorescence emission measurements at different pH values show that L2 is also able to ratiometrically sense ATP in a narrow pH range, thanks to emission quenching due to a photoinduced electron transfer (PET) process from an amine group of the receptor to the excited phenanthroline.

Introduction

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 proteins able to selectively bind the appropriate nucleotide anion, thanks to the encapsulation of the anionic substrates within clefts or pockets with the appropriate dimensions and disposition of the binding sites to optimally host a selected nucleotide. It is now accepted that the binding process is regulated by different noncovalent interactions, such as charge−charge and charge−dipole interactions, hydrogen bonding, hydrophobic effects, and stacking interactions, that work cooperatively to stabilize the adducts.

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In this context, the design of synthetic receptors able to bind phosphate anions in aqueous solution represents one of the approaches to the analysis of the weak forces which regulate the recognition processes in biological systems.[2] Actually, several examples of nucleotide anion binding by synthetic receptors, mostly of polyammonium type, have been recently reported.[3−29] Similarly to natural systems, the formation of stable host−guest adducts requires the incorporation in the receptor of sites for multiple interactions with substrates. In fact, to achieve a better recognition of nucleotide anions, the receptor needs to contain not only positively charged ammonium groups able to interact with the anionic phosphate moiety but also binding sites able to interact via hydrogen bonding or π-stacking with the nucleobases or the sugar moieties.[2−11,13,19−21] Finally, the binding sites of the receptor need to be opportunely preorganized to optimally interact with the anionic substrate. From this point of view, encapsulation of the nucleotide, or of a portion of the nucleotide, in clefts or cavities of the receptor may strengthen the overall host−guest interaction affording particularly stable adducts or favoring recognition of a selected nucleotide anion. Actually, encapsulation of a determined subunits of nucleotides, e.g., the phosphate chain or the nucleobase, within the cavity of cyclic polyammonium receptors has been often proposed on the basis of molecular modeling results.[4,5]

The insertion of heteroaromatic subunits with fluorogenic characteristics within the receptor structure may represent an important "added value" to this class of receptors. In fact, in this case, the aromatic moiety can be used not only to bind the substrates via π-stacking and or hydrophobic interactions but also to signal their presence in solution, thanks to quantifiable changes of its emission properties.[2,12−29]

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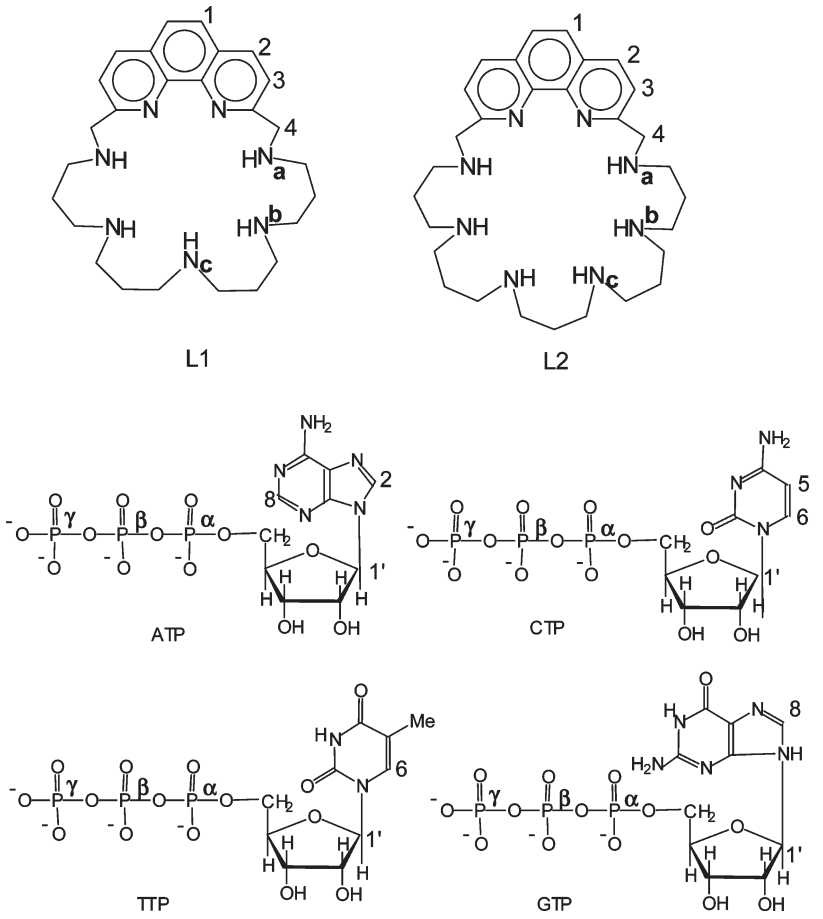
SCHEME 1. Drawings of Ligands and Nucleotides with Atom Labeling



In the course of our study on polyamine ligands containing heteroaromatic units as fluorescent receptors for both metal cations and anions,[8,30] we have synthesized receptors L1 and L2, which contain respectively a pentaamine and a hexaamine chain linking the 2,9 positions of 1,10-phenanthroline (Scheme 1). In principle, both ligands may give highly charged polyammonium cations in aqueous solutions, due to the high number of protonable amine groups gathered on the receptors. At the same time, the protonated species of these receptors are characterized by large and positively charged cavities, where the anionic phosphate chain of nucleotides could be enclosed. Finally, phenanthroline is characterized by an intense fluorescence emission at ca. 370 nm, which could be affected by substrate binding.

Therefore, we decided to analyze the ability of L1 and L2 to bind and sense nucleotide triphosphate anions, e.g., ATP, GTP, TTP, and CTP. A preliminary investigation on L2 showed that this receptor is able to bind and selectively sense at pH 6 ATP over GTP, TTP, and CTP,[31] although the four nucleotides present similar structures and possess similar binding sites. This result suggests that recognition processes can be controlled by slightly different structural parameters. At the same time, it cannot be neglected the fact that nucleotide recognition by polyamines in aqueous solutions can be strongly influenced by the protonation states of the host and guest species, i.e., by the pH of the medium.

With this in mind, we have now compared the binding features of L1 and L2, which possess an overall similar structure, toward nucleotide triphosphate, with the purpose to evaluate the structural parameters which determine nucleotide binding and sensing. At the same time, we have also carried out a study on the binding ability of the receptors toward nucleotides in a wide pH range, with the aim to draw useful indications for design of selective chemosensors for anions in aqueous solutions.

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(32) In the case of L2, the values of the first and second protonation constants, as well as those of the fifth and sixth ones, are very similar. This behavior has been already found in other cyclic and acyclic polyamine receptors, and in some cases, the constant for the addition of an acidic protons to a $[H_xL]^{x+}$ species, e.g., for the equilibrium $[H_xL]^{x+} + H^+ = [H_{x+1}L]^{(x+1)+}$, was found to be equal or even higher than the constant for the addition of a proton to the less protonated species $[H_{x-1}L]^{(x-1)+}$, e.g., relative to the equilibrium $[H_{x-1}L]^{(x-1)} + H^+ = [H_xL]^{x+}$ (see, for instance: (a) Micheloni, M.; Sabatini, A.; Paoletti, P. *J. Chem. Soc., Perkin Trans. 2* **1978**, 828–830. (b) Micheloni, M.; Paoletti, P.; Vacca, A. *J. Chem. Soc., Perkin Trans. 2* **1978**, 1978–1980. (c) Hosseini, M. W.; Lehn, J.-M. *J. Am. Chem. Soc.* **1982**, *104*, 3525–3526. (d) Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1986**, *69*, 587-603 and (e) Dietrich, B.; Hosseini, M. W.; Lehn, J.-M.; Session, R. B. *Helv. Chim. Acta* **1983**, *66*, 1262–1278. Other examples can be found in ref 33). This unexpected behavior is generally due either to the formation of a stabilizing intramolecular hydrogen-bonding network in the $[H_{x+1}L]^{(x+1)+}$ species upon protonation of the $[H_xL]^{x+}$ one, or to energetically expensive conformational changes of the receptor upon protonation of $[H_{x-1}L]^{(x-1)}$ to give $[H_xL]^{x+}$. Both these effects can energetically favor the $[H_xL]^{x+} + H^+ = [H_{x+1}L]^{(x+1)+}$ process with respect to the $[H_{x-1}L]^{(x-1)} + H^+ = [H_xL]^{x+}$ one, leading to similar values for the protonation constants of the $[H_{x-1}L]^{(x-1)}$ and $[H_xL]^{x+}$ species (see ref 33 for a more detailed discussion).

TABLE 1. Protonation Constants of L1 and L2 (0.1 M NMe_4Cl, 298. 1 K)

	log K	
reaction	L = L1	L = L2[a]
$L + H^+ = [HL]^+$	10.24(1)	10.14(1)
$[HL]^+ + H^+ = [H_2L]^{2+}$	9.87(1)	10.10(1)
$[H_2L]^{2+} + H^+ = [H_3L]^{3+}$	8.02(1)	8.59(1)
$[H_3L]^{3+} + H^+ = [H_4L]^{4+}$	7.24(1)	7.79(1)
$[H_4L]^{4+} + H^+ = [H_5L]^{5+}$	6.51(2)	6.98(1)
$[H_5L]^{5+} + H^+ = [H_6L]^{6+}$		6.90(1)

[a]From ref 31.

Results and Discussion

Protonation of the Receptors. Since the binding properties of polyammonium receptors are generally determined by the formation of protonated species in aqueous solutions and by the localization of the ammonium groups within the receptor structure, we preliminarily carried out a study on the basicity properties of L1 and L2 by coupling potentiometric, ^1H NMR, spectrophotometric, and fluorimetric measurements in aqueous solutions. The protonation constants of L2 have been previously communicated,[31] while the protonation equilibria of L1 have been potentiometrically determined in this study. The basicity constants of both ligands are reported in Table 1.

Receptors L1 and L2 can bind up to five and six acidic protons in the pH range investigated (2.5−10.5), respectively.[32] Their protonation constants are higher than those observed for the corresponding protonation equilibria in the phenanthroline-containing macrocycle 2,5,8,11,14-pentaaza[15]-16,29-phenanthrolinophane,[30e] which contains five amine groups separated by ethylenic chains, in agreement with the larger +I inductive effect exerted on amine groups by propylenic chains and with the higher flexibility of L1 and L2 which allow a better minimization of the electrostatic repulsion between positive charges in the polyprotonated forms of the receptors.[33] All protonation constants of L1 and L2 are also higher than those reported for 1,10-phenanthroline (log $K = 4.96$),[34] suggesting that the heteroaromatic nitrogens are not directly involved in the process of proton binding in aqueous solutions, at least in the pH range investigated. The analysis of the pH dependence of the ^1H NMR signals often give useful information on the localization of the acidic protons in polyammonium cations.[33] However, in the case of L1 and L2, the resonances of the hydrogen atoms of the propylenic chains cannot be safely attributed at all pH values, due to their similar chemical shifts. Conversely, the sharp singlet of the benzylic methylene group (4 in Scheme 1) at 4.69 (L1, pH 1.9) or 4.72 ppm (L2, pH 2), adjacent to the phenanthroline unit, is easily recognizable at all pH values in both receptors.

As shown in Figure 1 for L1, the chemical shift is not affected by pH in the pH range 11−8. A remarkable downfield shift is observed below pH 8, i.e., with the formation of the $[H_4L1]^{4+}$ and $[H_5L1]^{5+}$ species in solution, suggesting that the last two protonation steps of the receptor occur on the benzylic nitrogen atoms adjacent to phenanthroline N_a.

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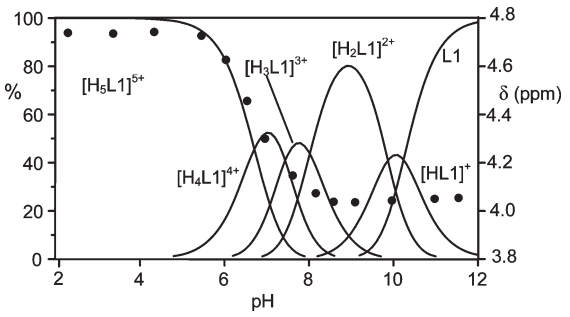

FIGURE 1. pH dependence of the ^1H NMR chemical shifts of the benzylic protons of L1 (●) and distribution diagrams of its protonated forms (298 K, 0.1 M NMe_4Cl).

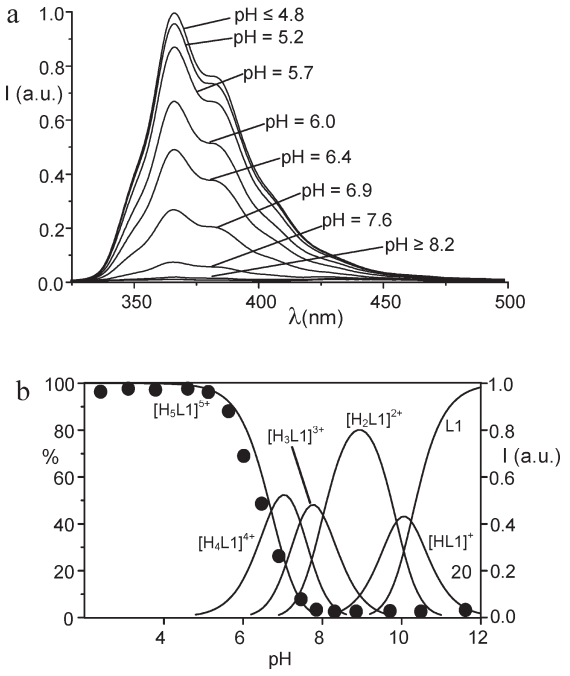

FIGURE 2. Emission spectra of L1 at selected pH values (a) and pH dependence of its emission intensity at 366 nm (298 K, λ_{exc} 270 nm, 0.1 M NMe_4Cl) (b).

A similar behavior is also found in the case of L2, where a marked upfield shift of this signal is observed upon the formation of the $[H_5L2]^{5+}$ and $[H_6L2]^{6+}$ protonated species (Supporting Information, Figure S2). The first three (L1) or four protonation steps (L2) take place on the central amine groups of the aliphatic chain. The lower proton affinity of the N_a nitrogen atoms, in comparison with the N_b and N_c ones, can be reasonably ascribed to the electron-withdrawing effect of the heteroaromatic unit on the adjacent N_a amine groups. Therefore, L1 and L2 present a preferential binding zone for protons, i. e., the central amine groups N_b and N_c of the aliphatic chain, located far from the heteroaromatic units. Of note, in both L1 and L2, the resonances of the heteroaromatic protons show only minor shifts in the pH range 2−12, supporting the hypothesis that phenanthroline is not directly involved in the process of proton binding.

This fact is substantially confirmed by the analysis of the UV spectra of L1 and L2 recorded in aqueous solutions at different pH values. In fact, the UV band of phenanthroline at 270 nm is

TABLE 2. Stepwise Stability Constants of the Adducts Formed by L1 and L2 with Nucleotides (0.1 M NMe_4Cl, 298.1 K)

	log K			
reaction	S^{4-} = ATP	S^{4-} = CTP	S^{4-} = TTP	S^{4-} = GTP
$[HL1]^{+} + S^{4-} = [HL1S]^{3-}$	2.61(3)		3.57(1)	
$[H_2L1]^{2+} + S^{4-} = [H_2L1S]^{2-}$	3.12(3)		3.80(1)	3.24(2)
$[H_3L1]^{3+} + S^{4-} = [H_3L1S]^{-}$	4.11(3)	3.01(4)	4.27(2)	4.07(2)
$[H_4L1]^{4+} + S^{4-} = [H_4L1S]$	5.66(3)	4.64(5)	5.70(2)	5.59(3)
$[H_3L1]^{3+} + [HS]^{3-} = [H_4L1S]$	5.67(4)		5.64(2)	5.30(3)
$[H_4L1]^{4+} + [HS]^{3-} = [H_5L1S]^{+}$	5.73(4)	6.01(5)	6.09(2)	5.93(3)
$[H_5L1]^{5+} + S^{4-} = [H_5L1S]^{+}$		6.13(5)		
$[H_5L1]^{5+} + [HS]^{3-} = [H_6L1S]^{2+}$	6.05(4)	5.29(5)	5.31(2)	5.48(3)
$[H_5L1]^{5+} + [H_2S]^{2-} = [H_7L1A]^{3+}$	5.30(4)	5.83(5)	4.29(2)	4.60(3)
$[H_5L1]^{5+} + [H_3S]^{-} = [H_8L1S]^{4+}$	5.64(4)			
$[H_2L2]^{2+} + S^{4-} = [H_2L2S]^{2-}$	4.29(2)[a]	3.16(2)[a]	3.03(3)[a]	2.87(3)[a]
$[H_3L2]^{3+} + S^{4-} = [H_3L2S]^{-}$	5.41(2)	4.38(2)	4.15(3)	4.47(3)
$[H_4L2]^{4+} + S^{4-} = [H_4L2S]$	7.08(2)	5.98(2)	5.24(3)	5.75(3)
$[H_4L2]^{4+} + [HS]^{3-} = [H_5L2S]^{+}$	8.86(3)	7.84(2)	7.11(3)	6.60(3)
$[H_5L2]^{5+} + S^{4-} = [H_5L2S]^{+}$	8.89(3)	7.27(2)	7.21(3)	6.93(3)
$[H_5L2]^{5+} + [HS]^{3-} = [H_6L2S]^{2+}$	9.68(3)	8.95(2)	7.67(4)	7.87(3)
$[H_6L2]^{6+} + S^{4-} = [H_6L2S]^{2+}$	9.91(3)	8.62(2)	7.98(3)	8.40(3)
$[H_6L2]^{6+} + [HS]^{3-} = [H_7L2S]^{3+}$	9.55(3)	8.58(4)	8.06(3)	7.16(3)
$[H_6L2]^{6+} + [H_2S]^{2-} = [H_8L2S]^{4+}$	9.17(3)	8.06(4)	3.57(1)	

[a]From ref 31.

affected only by a small redshift (ca. 4 nm) accompanied by a slight increase of the absorbance below pH 9.

These spectral changes can be attributed to hydrogen bonding with the ammonium groups of aliphatic chains rather than to direct involvement of the phenanthroline in proton binding. In fact, protonation of 1,10-phenanthroline[35] is accompanied by a far higher redshift (ca. 20 nm) of its absorption band. Conversely, the emission spectra of L1 and L2 are strongly pH-dependent. As shown in Figure 2 for L1, the receptor is not emissive in alkaline aqueous solutions and shows the typical emission of phenanthroline only at acidic pH values, i.e., upon protonation of the $[H_4L1]^{4+}$ species to give the $[H_5L1]^{5+}$ one. This effect can be attributed to the presence, in $[H_4L1]^{4+}$, of an unprotonated benzylic amine group (N_a in Scheme 1). In fact, as already observed in other phenanthroline-containing polyamine ligands,[30d,e] the lone pair of the benzylic nitrogens, located close to phenanthroline, can efficiently quench the fluorescence emission of the fluorophore through an electron transfer process. Protonation of both the benzylic amine groups below pH 7 makes their lone pairs no longer available for the PET process and gives rise to a consequent renewal of the fluorescence emission of phenanthroline. A similar behavior is found for L2, where only the hexaprototonated $[H_6L2]^{6+}$ species, which is prevalent is solution at in the acidic pH region, is emissive (see the Supporting Information, Figure S3).

Binding of Nucleotide Triphosphates in Aqueous Solutions. Since the binding properties of polyamine receptors toward anionic species in aqueous solution are often dependent on their protonation state and, therefore, on the pH of the solutions, we decided to investigate first their ability to interact with nucleotide triphosphate anions by means of potentiometric measurements. Both receptors form stable 1:1 adducts in aqueous solutions (Table 2). Although the formation of both 1:1 and 2:1 anion/receptor adducts has been observed in previously reported cases,[36] data analysis with the program

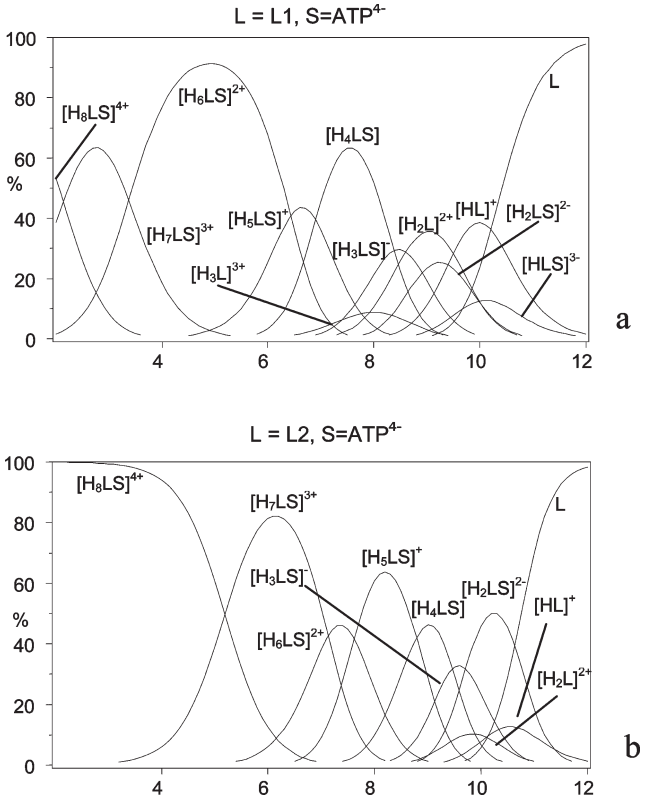

FIGURE 3. Distribution of the ATP adducts with L1 (a) and L2 (b) ([L1] = [L2] = [ATP] = $1 \cdot 10^{-3}$ M, 298 K, 0.1 M NMe_4Cl).

(35) (a) Armaroli, N.; De Cola, L.; Balzani, V.; Sauvage, J.-P.; Dietrich-Buchecker, C. O.; Kern, J. M. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 553–556. (b) Kern, J. M.; Sauvage, J.-P.; Weidmann, J. L.; Armaroli, N.; Flamigni, L.; Ceroni, P.; Balzani, V. *Inorg. Chem.* **1997**, *36*, 5329–5338. (c) Armaroli, N.; De Cola, L.; Balzani, V.; Sauvage, J.-P.; Dietrich- Buchecker, C. O.; Kern, J. M.; Bailal, A. *J. Chem. Soc., Dalton Trans.* **1993**, 3241–3247. (d) Armaroli, N.; Ceroni, P.; Balzani, V.; Kern, J. M.; Sauvage, J.-P.; Weidmann, J. L. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 4145–4150.

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

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

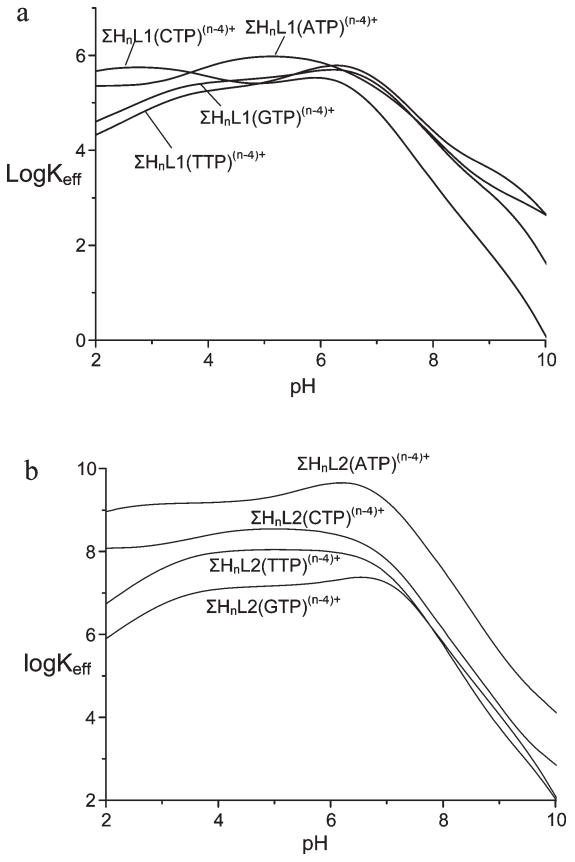

FIGURE 4. Plot of log K_{eff} as a function of pH for the L1 (a) and L2 (b) adducts with nucleotides ($K_{eff} = \Sigma H_hLS/(\Sigma H_{(h-i)}S \cdot \Sigma H_iL)$, with L = L1 or L2 and S = ATP, CTP, TTP or GTP).

HYPERQUAD[37] under our experimental conditions reveals only 1:1 stoichiometries for all species detected in our systems. The eventual formation of oligomeric adducts (with 2:2 stoichiometries, for instance) was also ruled out by performing potentiometric titrations in a wide range of concentrations of receptors and substrates (see the Experimental Section).

Analysis of the titration curves allows one to determine the species formed in solution and their overall formation constants β_{HLS} (S = ATP, CTP, TTP or GTP, L = L1 or L2), relative to equilibria of the type $L + S^{4-} + nH^+ = [H_nLS]^{(n-4)+}$ (see the Supporting Information, Table S1). Plots of the distribution curves of the species formed by L1 and L2 with the nucleotides (see Figure 3 for the ATP adducts with both receptors and the Supporting Information, Figure S4−S6, for the CTP, GTP, and TTP adducts) outline that complexation occurs in a wide pH range (for instance ATP is almost completely bound by both polyammonium receptors for pH values lower than 9), with the formation of a large number of 1:1 adducts with different protonation degrees.

The determination of the stepwise formation constants for the adducts between the protonated receptors and the anionic substrates, relative to equilibria of the type $H_{h-i}L + H_iS = [H_hLS]$, implies the knowledge of the localization of the acidic protons on receptor and substrates in the $[H_hLS]$ host−guest species. This task is made difficult by the presence in aqueous solution of a large number of overlapping

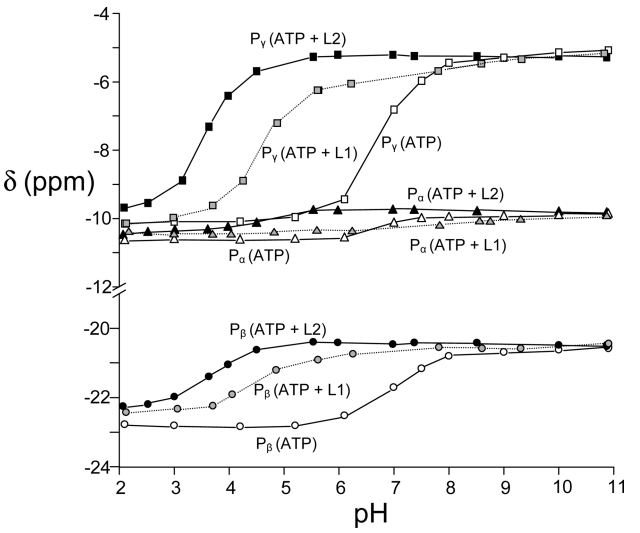

FIGURE 5. pH dependence of the ^{31}P NMR chemical shifts of the ATP signals in the absence and in the presence of 1 equiv of L1 and L2 ($[L1] = [L2] = [ATP] = 5 \times 10^{-3}$ M).

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 receptors. In these cases, in fact, different equilibria can be proposed for the formation of the same supramolecular adduct. For instance, the $[H_6L2(ATP)]^{2+}$ adduct could be formed either through the equilibrium $[H_5L2]^{5+} + [HATP]^{3-} = [H_6L2(ATP)]^{2+}$ or $[H_6L2]^{6+} + ATP^{4-} = [H_6L2(ATP)]^{2+}$, with calculated equilibrium constants of 9.68 and 9.91, respectively. The values of the stability constants of the adducts, calculated by using this approach are given in Table 2.

To overcome the presence of different equilibria present in solution at the same pH value, it can be useful to calculate effective stability constants.[38] For a given pH value, if the total amount of free substrate ($\Sigma H_{(h-i)}S$), free receptor (ΣH_iS) and adduct formed (ΣH_hLS) are known, one can define an effective stability constant by using the following equation:

$$K_{eff} = \Sigma H_hLS/(\Sigma H_{(h-i)}S \cdot \Sigma H_iL)$$

Plots of the pH dependence of the logarithms of the effective constants (Figure 4) point out that in all cases the stability of the adducts is strongly influenced by pH. In fact, a marked increase of the log K_{eff} values is generally observed from alkaline to slightly acidic pH values, with a maximum between pH 7 and 4; then the stability generally shows a slight decrease at lower pH values. In the alkaline and slightly acidic pH region, the substrates are in their less protonated and highly charged forms (S^{4-} or HS^{3-}), and therefore the observed increasing stability from pH 10 to slightly acidic pH values can be reasonably attributed to the increasing 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, the decrease of the log K_{eff} values at more acidic pH values can be reasonably attributed to the progressive formation in solution of highly

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TABLE 3. ^{31}P NMR Shifts (δ, ppm) of Nucleotides in Their Adducts with L1 and L2 and Corresponding Complexation-Induced ^{31}P NMR Chemical Shifts (CIS, ppm), Measured in D_2O Solution at pH 6, 298 K

	ATP			CTP			TTP			GTP		
	P_α	P_β	P_γ	P_α	P_β	P_γ	P_α	P_β	P_γ	P_α	P_β	P_γ
						Receptor L1						
δ (ppm)	−10.7	−21.8	−6.0	−10.7	−22.0	−7.7	−10.8	−22.6	−7.9	−11.0	−21.9	−8.6
CIS	0.2	2.2	3.0	0.2	0.7	2.9	0.0	0.5	2.6	0.1	0.5	2.0
						Receptor L2[a]						
δ (ppm)	−9.9	−20.3	−5.2	−10.5	−21.5	−6.5	−10.8	−22.2	−7.6	−10.9	−21.3	−7.4
CIS	1.0	2.7	4.8	0.4	1.2	3.8	0.1	0.9	2.9	0.2	1.1	3.2

[a] From ref 31.

protonated and less negatively charged forms of the substrates (H_2S^{2-} and H_3S^-) and to the consequent reduced electrostatic and hydrogen-bonding interactions between the two partners.

If the pH dependence of the conditional constants is similar for both receptors, significant differences are instead observed in the binding ability at a given pH value of the two receptors. In fact, L2 shows a remarkable higher binding affinity for each substrate than L1 and a marked selectivity for ATP over GTP, TTP, and CTP (Figure 4b) all over the pH range investigated. Conversely, a similar binding affinity for all four nucleotides features L1, which displays only a slight preference for ATP binding in the pH range 4−6. While the higher affinity of L2 toward nucleotide anions could be simply attributed to the higher positive charge gathered on the receptor at a given pH (for instance, at pH 6, L1 and L2 are present in solution as penta- and hexaprotonated species, $[H_5L1]^{5+}$ and $[H_6L2]^{6+}$), the selectivity displayed by L2 for ATP cannot be interpreted only in terms of charge−charge and hydrogen-bonding interactions between receptors and substrates. Therefore, we also analyzed the different host−guest systems by means of ^{1}H and ^{31}P NMR spectra recorded at various pH values.

Figure 5 reports the chemical shifts of the ^{31}P NMR signals of ATP in the presence and in the absence of L1 or L2 at different pH values. Similar plots have been also obtained for CTP, GTP, and TTP and are supplied within the Supporting Information (Figure S7), while Table 3 lists the CIS (complexation-induced shifts) of the different signals measured at pH 6, where the most stable adducts are generally formed. Binding of all substrates to L1 or L2 gives noticeable changes in the chemical shifts of the ^{31}P NMR signals of the triphosphate chain. In fact, marked upfield shifts are observed for the signals of the terminal P_γ phosphate group and, to a lesser extent, of the central P_β group. The signal of the P_α phosphate is generally only slightly affected by the presence of the receptors, and therefore, it is probably weakly involved in the stabilization of the adducts.

Considering nucleotide complexation by L2, Table 3 and Figures 5 and S7 show that the upfield shifts of the ^{31}P signals in the presence of the receptor decrease in the order ATP > CTP > TTP ≈ GTP, which is almost the same sequence found for the stability constants of the adducts determined by potentiometric measurements (Figure 4). At the same time, for a given nucleotide, the adducts with L2 display higher complexation induced upfield shifts than those observed for L1. At a first glance, these results seem to suggest that the stability of the adducts is mainly determined by charge−charge and hydrogen-bonding interactions involving the phosphate chain. On the other hand, analysis of the ^{1}H NMR spectra recorded at different pH values on D_2O solutions containing the nucleotides and L1 or L2 provides unambiguous evidence of the involvement of the nucleobases in the recognition processes. In fact, all nucleotides, in the presence of L1 and L2, display downfield shifts for the signals of the aromatic protons of nucleobases and of the anomeric proton H1′ of the sugar moiety.

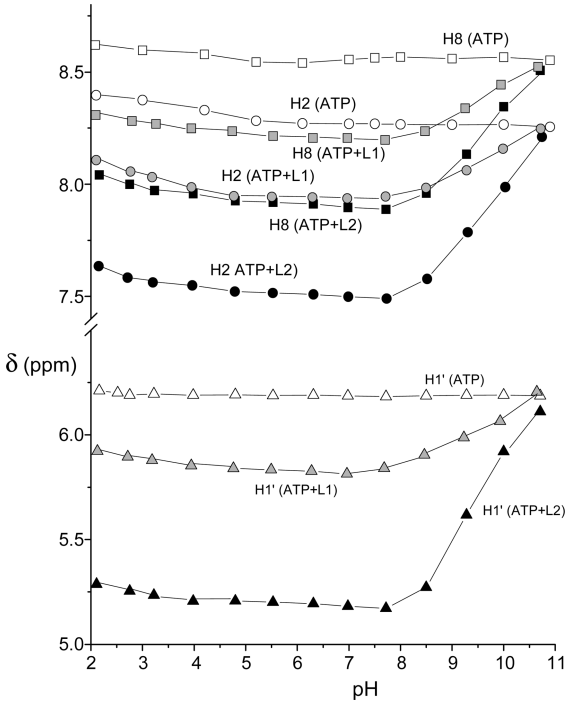


FIGURE 6. pH dependence of the ^{1}H NMR signals of the adenine protons H2 and H8 and of the ribose proton H1′ of ATP in the presence of 1 equiv of L1 and L2.

Plots of the chemical shifts of these signals of nucleotides in the presence and in the absence of L1 or L2 vs pH (see Figure 6 for ATP and Supporting Information, Figures S8, for the other nucleotides) show that the complexation-induced chemical shifts are higher at slightly acidic pH values and strongly decrease in the alkaline pH region. However, the observed complexation-induced upfield shifts of the heteroaromatic protons of phenanthroline and nucleobases are less affected by pH than the downfield shifts observed for

TABLE 4. ^{1}H NMR Chemical Shifts for Protons of Phenanthroline, for Aromatic Protons of Nucleobases, and for the Anomeric H1′ Proton of Ribose in the Adducts of L1 and L2 with ATP, CTP, GTP, and TTP and Corresponding Complexation-Induced Chemical Shifts (CIS, ppm), Measured in D_2O Solution at pH 6, 298 K

		H2	H1	H3		H8	H2	H1′
L1	δ	8.31	7.78	7.76	ATP	8.22	7.95	5.83
	CIS	−0.09	−0.11	−0.07		−0.33	−0.34	−0.38
L2[a]	δ	8.27	7.63	7.65	ATP	7.91	7.49	5.25
	CIS	−0.26	−0.32	−0.14		−0.60	−0.76	−0.96
		H2	H1	H3		H6	H5	H1′
L1	δ	8.32	7.82	7.65	CTP	7.75	6.11	5.88
	CIS	−0.08	−0.07	−0.08		−0.20	−0.11	−0.14
L2[a]	δ	8.39	7.73	7.72	CTP	7.67	6.02	5.79
	CIS	−0.14	−0.22	−0.08		−0.28	−0.20	−0.24
		H2	H1	H3		H6		H1′
L1	δ[a]	8.34	7.83	7.67	TTP	7.68		6.13
	CIS	−0.06	−0.06	−0.06		−0.10		−0.19
L2[a]	δ	8.35	7.75	7.71	TTP	7.43		5.90
	CIS	−0.18	−0.20	−0.09		−0.35		−0.42
		H2	H1	H3		H8		H1′
L1	δ[a]	8.28	7.78	7.63	GTP	8.08		5.75
	CIS	−0.11	−0.11	−0.10		−0.15		−0.10
L2[a]	δ	8.35	7.67	7.70	GTP	7.79		5.46
	CIS	−0.23	−0.28	−0.10		−0.44		−0.39

[a]From ref 31.

the ^{31}P signals of the phosphate chain. Whereas the latter are strongly reduced or, in the same cases, almost absent below pH 4−5 and at alkaline pH values, the former show only minor changes in a wide pH range, generally from pH 2 to 8, indicating that the interactions between phenanthroline and nucleobases are less sensible to the pH of the medium than the charge−charge interactions involving the phosphate moieties of nucleotides.

In the case of the larger receptor L2, the observed downfield shifts induced by complexation with the receptor (Table 4) decreases in the order ATP ≫ GTP > TTP > CTP accounting for a stronger interaction of adenine of ATP with the receptor. Of note, the ^{1}H NMR signals of the phenanthroline unit of L2 also exhibit relevant downfield shifts (see Table 4 and Figure 7b for ATP and Supporting Information, Figures S9−S11) in the presence of all nucleotides. The downfield shifts of the phenanthroline protons of L2 are larger in the case of ATP and decrease in the order ATP ≫ GTP > TTP ≈ CTP, a sequence similar to that observed for the protons of nucleobases in the presence of L2. These results may indicate that the phenanthroline unit of L2 and the nucleobases are coupled by of π-stacking interactions, which decrease on passing from ATP to CTP.

In the case of the smaller receptor L1, the formation of the host−guest adducts with all nucleotides induces smaller shifts of the ^{1}H resonances of both nucleobases and phenanthroline with respect to complexation with the larger host L2 (Table 4, Figures 6 and 7 and Supporting Information, Figures S8−S11). In particular, the shift of the phenanthroline signals of L1 in the presence of nucleotides are only slightly affected by complexation, suggesting that the heteroaromatic unit of this receptor is weakly involved in the stabilization of the adducts.

These NMR results suggest that the stability sequences observed for the host−guest adducts is not only determined by electrostatic interactions and hydrogen-bonding contacts between the ammonium groups of L1 or L2 and the triphosphate chains of nucleotides, but also by the interactions between the nucleobases and the two polyammonium hosts. Overall, the ATP adducts with L2, the most stable among all the systems investigated, display the strongest charge−charge interactions involving the phosphate chain and robust π-stacking interactions between adenine and phenanthroline. Conversely, the phenanthroline unit of the smaller receptor L1 is likely to play a minor role in the stabilization of the adducts, accounting for the lower stability of the adducts with this receptor.

To corroborate these results, we carried out a MM analysis on the adducts with L1 and L2, and we tried to obtain crystals suitable for X-ray analysis. Our attempts to crystallize the complexes were unfruitful, with the only exception of the TTP−L2 adduct, which was structurally characterized.

Molecular Modeling and Structure Analysis. The host−guest interactions between the two receptors and nucleotides were analyzed by means of molecular modeling studies by using an implicit simulation of aqueous solution (preliminary results on ATP and CTP complexation with L2 have been previously presented).[31] In particular, we focused our attention on the interaction between the $[H_5L1]^{5+}$ and $[H_6L2]^{6+}$ protonated receptors, which are the main species present in aqueous solution at neutral and slightly acidic pH values, and the tetraanionic forms of nucleotides. The most interesting finding is the encapsulation of the triphosphate chain of nucleotides within the macrocyclic cavity of L1 and L2. This structural feature is common to all sampled conformers of the four different anionic substrates. In all adducts, the two protonated receptors assume a similar conformation, folded along the axis connecting the two benzylic amine groups, while the nucleotides are characterized by a bent conformation which allows the simultaneous encapsulation of the phosphate chain within the cavity of the protonated guest and the interaction of the nucleobase either with the phenanthroline unit or with the polyammonium chain.

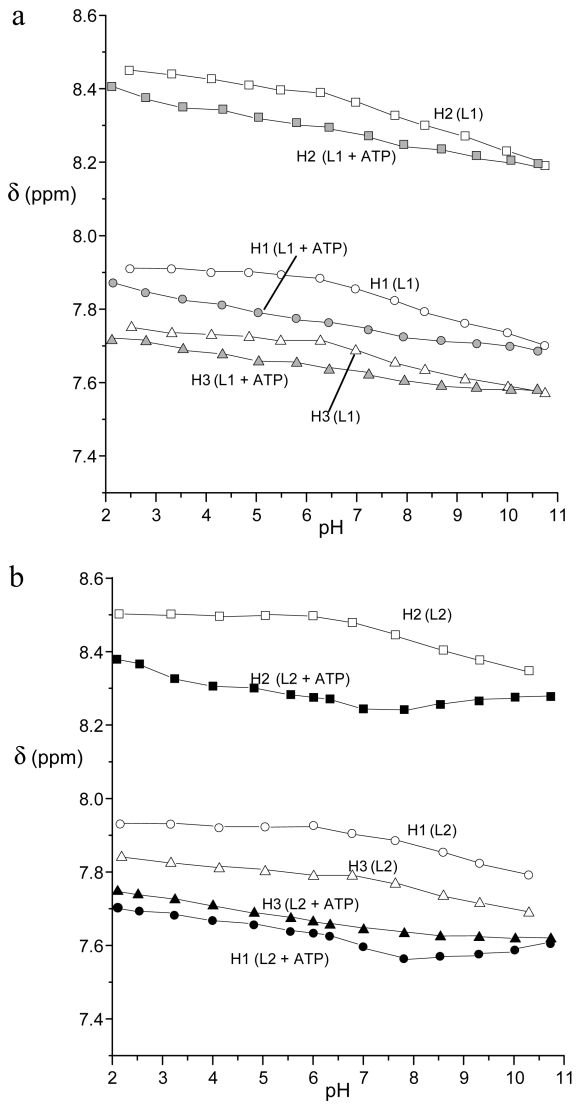


FIGURE 7. pH dependence of the signals of the phenanthroline protons of L1 (a) and L2 (b) in the absence and in the presence of 1 equiv of ATP.

In the case of the adducts with L2, the sampled conformers can be grouped in two different families, which differ in the interaction mode with the receptor. In the first family (Figure 8, A family) the nucleobases interact essentially via face to face π-stacking with the heteroaromatic unit of the receptor. In the case of ATP and GTP, the purine bases are stacked above the phenanthroline unit and lie on planes almost parallel to that of phenanthroline. In the adducts with CTP and TTP, the pyrimidine bases lie on planes slightly bent (24.6° and 7.6° for CTP and TTP, respectively) with respect to that phenanthroline and are less "overlapped" with this heteroaromatic unit.

The A family is the most populated in the case of ATP (70% of sampled conformers) and the less populated in the adducts with CTP (35%), TTP (30%) and GTP (40%), accounting for a higher tendency of ATP to form π-stacked adducts. In the second family, the most populated for CTP, TTP, and GTP, the nucleobases are still located close to the phenanthroline unit, but the interaction takes place essentially via hydrogen bonding with the protonated nitrogens of

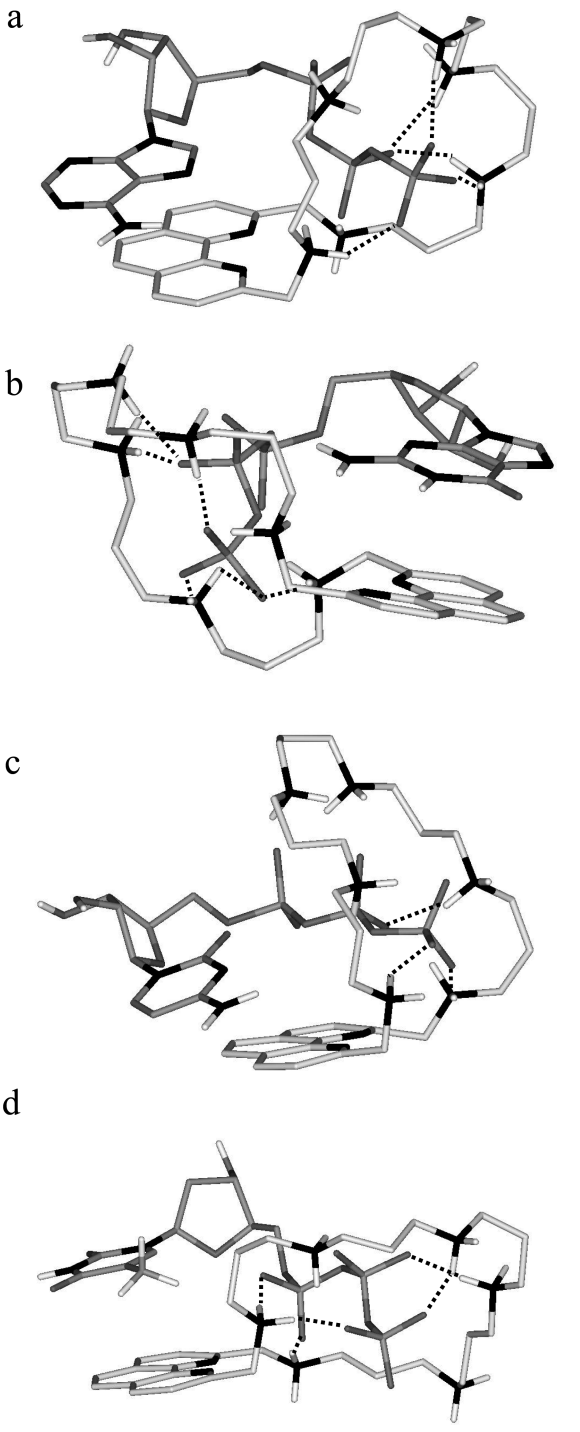


FIGURE 8. Lowest energy conformers of the adducts between $[H_6L2]^{6+}$ and ATP (a), GTP (b), CTP (c), and TTP (d) in the A families of conformers.

the receptor (Figure 9). In particular, the oxygen group O8 of the carbonyl function of GTP and TTP or the nitrogen atoms N7 and N3 of ATP and CTP are hydrogen bonded with one ammonium group of the aliphatic chain (see the Supporting Information, Figures S12−S14 for labeling).

In both families, encapsulation of the triphosphate unit within the receptor cavity enables the formation of a number of hydrogen-bonding contacts with the charged polyammonium chain (see the Supporting Information for a complete

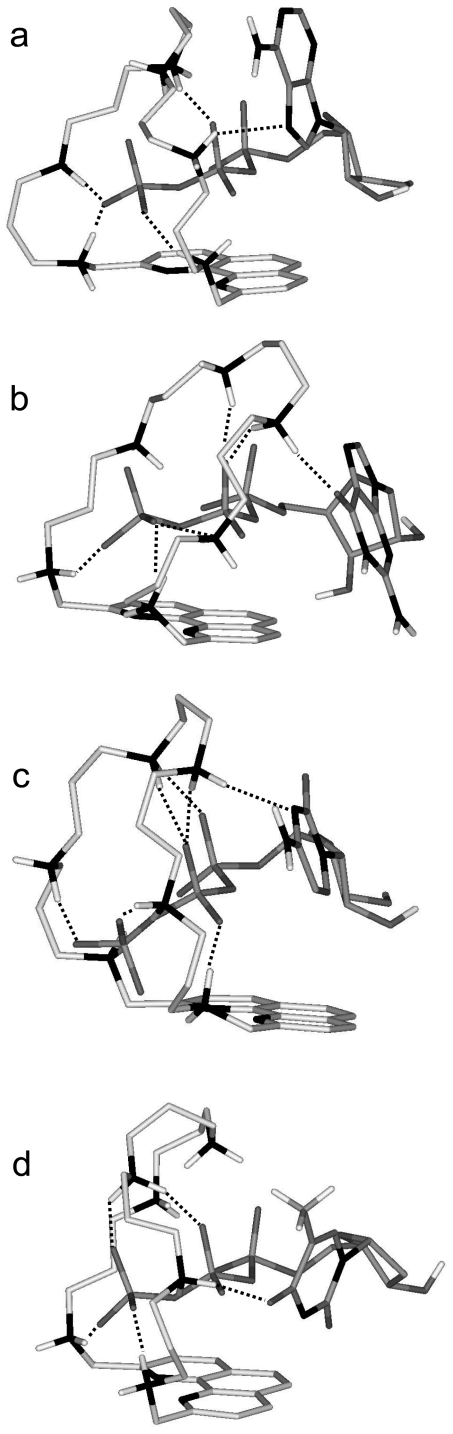


FIGURE 9. Lowest energy conformers of the adducts between $[H_6L2]^{6+}$ and ATP (a), GTP (b), CTP (c), and TTP (d) in the B families of conformers.

list of the hydrogen bonds, Tables S3−S5). The terminal and central phosphate groups are the most involved in the resulting hydrogen-bonding network, in keeping with the ^{31}P NMR results. Of note, among the different nucleotides, the triphosphate unit of ATP gives a robust hydrogen-bonding network with the polyammonium chain and, at the same time, exhibits the most pronounced π-stacking interaction between the nucleobase and phenanthroline. These results are in good agreement with the largest shifts observed for the ^{31}P and ^{1}H NMR signals of ATP in the presence of the receptor and can account for the higher stability found for the ATP adducts with L2. It seems likely that the phosphate chain and the adenine unit of ATP play a more pronounced cooperative role in the binding process than the corresponding triphosphate units and nucleobases of the other nucleotides. If this can be ascribed, in the case of CTP and TTP, to the poorer tendency of cytosine and thymine to give π-stacking and hydrophobic interactions than adenine, due to their less extended aromatic structure, this consideration cannot be applied to GTP, since guanine and adenine generally display similar ability to give π-stacked complexes. On the other hand, it is known that steric effects can influence the stability of host−guest adducts. From this point of view, a tentative explanation for the higher stability displayed by the ATP adducts with respect to the GTP ones could reside in the mutual disposition of the nucleobase and the aliphatic chain of L2. In fact, to achieve simultaneously inclusion of the phosphate chain and face-to-face π-stacking, the receptor assumes a folded conformation, with the polyamine chain almost perpendicular to the phenanthroline plain. Therefore, to "overlap" phenanthroline, guanidine and adenine need to assume a position very close and almost perpendicular to the aliphatic chain of L2. The guanine structure is somewhat more hindered than adenine, due to the simultaneous presence of the two bulky C=O and $-NH_2$ groups. Differently from the $-NH_2$ group of ATP, in the guanine adduct with L2 these two groups are also oriented toward the aliphatic chain of L2 (Figure 8b). Steric hindrance between guanine and the aliphatic portion of L2 could justify the observed less tendency of GTP to give π-stacked adducts.

In the L1 adducts, the receptor and nucleotides assume bent conformations similar to those found in the case of L2, which enable the encapsulation of the triphosphate unit within the cavity of the receptor (Supporting Information, Figure S12) and the formation of a hydrogen bonding network with the ammonium groups of the receptor. The most significant difference with respect to the adducts with L2 can be observed in the interaction mode of the nucleobases with the receptor. In fact, most of the sampled conformers display a mutual disposition of the nucleobases and phenanthroline similar to that observed in the B families of the L2 adducts, featured by hydrogen bonding contacts between the nucleobases and the ammonium groups of the receptor. Actually, for all nucleotides, only a limited number of adducts ($< 15\%$) display face-to-face π-stacking pairing, suggesting that in L1 this kind of interaction plays a minor role in the stabilization of the adducts and, overall, that phenanthroline is weakly involved in the recognition process. This result, once again, is in agreement with the small complexation induced shift observed for the ^{1}H NMR signals of the phenanthroline unit of L1.

The different interaction mode of the nucleobases in the L1 adducts can be related to the smaller dimension of this receptor. In fact, in the case of the larger receptor L2, the nucleotides assume a bent conformation, which allows the simultaneous encapsulation of the phosphate chain within the receptor cavity and the formation of π-stacking interactions with phenanthroline. In the case of L1, the triphosphate chain is still fitted within the receptor cavity, in a fashion similar to that observed in the adducts with L2. If encapsulat-

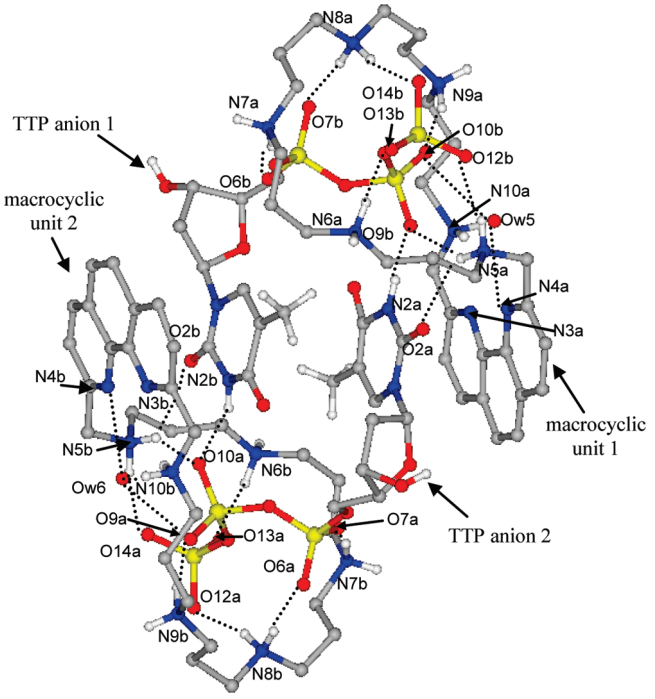


FIGURE 10. Crystal structure of the $[(H_6L2)_2TTP_2(H_2O)_2]^{4+}$ cation. Hydrogen bond distances (Å): N2a···O9b 2.75(1), N2b···O10a 2.76(1), N6a···O13b 2.63(2), N9b···O9a 2.78 (3), N8a···O7b 2.77(2), N8a···O14b 2.73 (2), N5a···O2a 2.96(1), N5a···O9b 2.78(2), N5a···O12b 2.82(2), N7a···O6b 2.72(3), N9a···O10b 2.61(3), N5b···O2b 3.04(2), N5b···O10a 2.69 (2), N5b···O14a 2.75(3), N6b···O13a 2.70(2), N10a···Ow5 2.79(3), N8b···O12a 2.83(3), N8b···O6a 2.86(2), N7b···O7a 2.72(3), N10b···Ow6 2.76(3), N4a···Ow5 2.89(2), Ow5···O10b 2.94(2), N4b···Ow6 2.94(2), Ow6···O9a 2.87(2).

ion of the phosphate chains enables the formation of a stabilizing network of hydrogen-bonding interactions, the smaller dimension of L1 does not allow to the nucleobases to assume simultaneously a spatial disposition "overlapped" to the phenanthroline unit of the receptor, preventing the formation of face-to-face π-stacking interactions.

To obtain further information on the structural features of the adducts, we attempted to get crystals suitable for X-ray analysis from aqueous solutions containing receptors and nucleotides in 1:1 molar ratio. In the case of the system TTP-L2 we obtained crystals of compound $[(H_6L2)_2(TTP)_2(H_2O)_2]Br_4 \cdot 7H_2O$, which was structurally characterized. The crystal structure is constituted by a dimeric adduct $[(H_6L2)_2(TTP)_2(H_2O)_2]^{4+}$ (Figure 10), four bromide anions and seven crystallization water molecules. The $[(H_6L2)_2(TTP)_2(H_2O)_2]^{4+}$ adduct, featured by a non crystallographic inversion symmetry, is formed by two protonated L2 receptors, two TTP anions, and two water molecules, held together by several hydrogen-bonding contacts and π-stacking interactions. As shown in Figure 10, each protonated receptor is characterized by a conformation folded along the axis linking the benzylic nitrogen atoms, in a manner similar to that observed in the calculated conformers of the $[(H_6L2)(TTP)]^{2+}$ adduct. The two receptors assume a head-to-tail cyclic disposition and define a three-dimensional cavity, where two TTP anions are hosted. The triphosphate chain of each TTP anion is encapsulated within the macrocyclic cavity of one protonated receptor to give a network of charge-assisted hydrogen-bonding interactions with the ammonium groups, while its pyrimidine base is sandwiched between the phenanthroline unit of the second receptor and the thymine group of the other TTP anion. This leads to the formation of a face-to-face π-stacked array of two thymidine and two phenanthroline units, with interplanar distances of 3.3 Å between phenanthroline and thymine planes and of 3.6 Å between the two thymine units.

Of note, each phosphate chain is fitted inside a macrocyclic cavity affording hydrogen-bonding interactions between all phosphate groups and the protonated amine groups. Indirect hydrogen bonds contacts, i.e., mediated by a bridging water molecule, are also observed between the P_β phosphate and both phenanthroline nitrogens (N4a or N4b) and a single benzylic ammonium group (N10a or N10b). Finally, the thymine carbonyl group O2a or O2b interacts via hydrogen bonding with a protonated nitrogen of the receptor, while the thymine nitrogen atom N2a or N2b is hydrogen bonded to the P_β phosphate of a different TTP unit. The interaction modes observed in this crystal structure, such as the inclusion of the phosphate chain within the cavity of L2 and the π-pairing between phenanthroline and thymine, resemble those observed in the calculated conformers for the nucleotides/L2 adducts.

Conversely, the formation of a dimeric assembly, not found in aqueous solution, may reflect the scarce tendency of thymine to give π-stacking interaction with the phenanthroline of the receptor in the 1:1 adducts. As a consequence, in the solid state the phosphate chain of each TTP unit is enclosed in the cavity of one of the polyammonium receptor, while the corresponding thymine moiety prefers to interact via π-stacking with the phenanthroline of a second protonated receptor and via hydrogen bonding and π-stacking with a second TTP anion.

Although nucleotide complexation by synthetic polyammonium receptors has been widely investigated, only a single crystal structure of this type of adducts has been previously reported.[8a] At the same time, no crystal structure displaying an inclusive binding mode of the anionic moiety of nucleotides has been previously shown, although this binding feature has been often proposed to justify the formation of stable adducts between nucleotides and cyclic polyammonium receptors.[4,5]

The resolution of this crystal structure allowed us to perform a molecular modeling analysis of the adduct between $[H_6L2]^{6+}$ and TTP in its tetraanionic form, using as starting coordinates those obtained from the X-ray analysis. In $[(H_6L2)_2TTP_2(H_2O)_2]^{4+}$, each polyammonium receptor interacts simultaneously with one TTP anion via inclusion within its cavity of the triphosphate chain and with a second TTP anion essentially via π-stacking. Therefore, we performed two different MD simulations by using either the coordinates of the polyammonium receptor and of the TTP anion interacting together in the inclusive fashion (indicated as "macrocyclic unit 1" and "TTP anion 1" in Figure 10), or the coordinates of the macrocycle and of TTP interacting together mainly *via* π-stacking ("macrocyclic unit 1" and "TTP anion 2" in Figure 10). In both cases, simulated annealing leads to two different families of conformers with structural characteristics almost equal to those previously achieved using starting coordinates obtained by manual docking (see Supporting Information, Tables S6 and S7

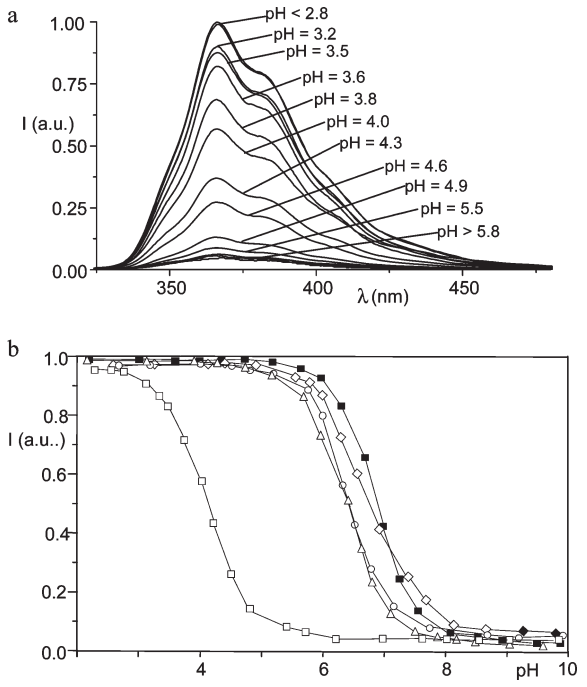


FIGURE 11. Emission spectra of L2 at selected pH values (a) and pH-dependence of its emission intensity at 366 nm in the absence (-■-) and in the presence of 1 equiv of ATP (-□-), CTP (-△-), GTP (-○-) and TTP (-◇-) ([L2] = [ATP] = [CTP] = [GTP] = [TTP] = 2.5×10^{-5} M, 298 K, λ_{exc} 270 nm, 0.1 M NMe_4Cl) (b).

and Figure S16). In fact, both families display the triphosphate anion enclosed within the macrocyclic cavity, while the nucleobase interacts either via π-stacking (A family) with phenanthroline or via hydrogen bonding between one carbonyl oxygen thymine and ammonium group of the receptor (B family). This second interaction mode is the preferred one by TTP (the 68% of sampled conformers belong to the B families), as already found by means of the manual docking procedure. Although these results do not add new information on binding mode of the receptor, they give confidence to the overall minimization procedure used in the absence of crystallographic data as starting coordinates.

Fluorescence Sensing of ATP. As discussed above, both receptors exhibit an ON-OFF pH dependence of their fluorescence emission. In fact, they are not emissive in the alkaline pH region and show the typical emission band of phenanthroline only below pH 7−8 (Figure 2 and Supporting Information, Figure S3). Analogous pH profiles are also observed for the adducts of L1 with the four nucleotides. Conversely, ATP gives significant changes in the pH dependence of the emission spectra of the larger receptor L2 (Figure 11), while the pH dependence of the spectra is only slightly affected by the presence of the other nucleotides. In fact, the phenanthroline unit of L2 becomes emissive at remarkably more acidic pH values in the presence of ATP (below pH 4.5), while only minor displacements of the pH profile toward acidic pH values are observed in the case of CTP, GTP, and TTP.

In consequence, in the pH range 4.5−7 L2 is emissive in the presence of CTP, TTP and GTP, while is quenched in the presence of ATP (Figure 11b). Therefore, L2 is not only able of selectively bind ATP over the other nucleotides, but also to selectively sense ATP in a narrow pH range (pH 4.5−7), thanks to a complexation induced quenching of fluorescence.

In principle, the quenching of the fluorescent emission of L2 in the presence of ATP in the pH range 4.5−7 could occur through an electron-transfer (PET) process involving either the adenine moiety or a not protonated amine group of the receptor. In fact, the electron-rich adenine unit may give rise to a PET process to excited phenanthroline, a typical aromatic electron-rich moiety. This mechanism has been already proposed for similar quenching effects upon ATP binding to fluorogenic polyammonium cations.[3b] This hypothesis is supported by MM calculations and ^{1}H NMR measurements, which clearly show that, among the four nucleotides, adenine of ATP gives the strongest π-stacking interactions with phenanthroline of L2, and assumes a spatial position very close to that of phenanthroline, which could favor the PET process. On the other hand, Figure 6 shows that the interaction between adenine and phenanthroline is not much affected by pH and it is present also at pH values lower than 4.5, i.e., in the pH region where the ATP adducts are emissive.

However, among the adducts with L2, ATP gives not only the more robust π-stacked pairing, but also the strongest charge−charge interactions between its triphosphate unit and the polyammonium chain of the receptor. As already observed in the absence of nucleotides at pH > 7, the quenched status of phenanthroline is due to the presence of a not protonated amine group. L2 becomes emissive below pH 7, where all amine groups are protonated, and therefore, their lone pairs are not available to quench the excited fluorophore through a PET process. The fact that L2 in the presence of ATP is not emissive in the pH range 4.5−7 may be related to the hydrogen-bonding and charge interactions between the anionic phosphate moiety and the polyammonium chain of the protonated receptor, which could lead to an intracomplex proton transfer from one ammonium group to a phosphate, making an amine lone pair available for the quenching process in this pH range. This mechanism has been already proposed to explain the quenching of the emission of other polyammonium receptors in the presence of phosphate anions.[13] Of note, Figure 5 shows that the downfield shift observed for the signals of the phosphate chain in the presence of ATP is maximum at slightly acidic pH values, where the L2−ATP adduct is quenched, but decreases remarkably below pH 4.5, where protonation of the triphosphate moiety of the nucleotide reduces its charge−charge interactions with the polyammonium groups. This may prevent the process of proton transfer from the polyammonium chain, renewing the fluorescence emission of phenanthroline. This observation suggests that the quenching of the emission at slightly acidic pH vales is probably due to a PET process involving the lone pair of an amine group, which deprotonates upon a proton-transfer process induced by the strong electrostatic and hydrogen-bonding interactions present in this pH range.

L2 is also able to ratiometrically detect ATP.[31] In fact, addition of increasing amounts of ATP to a solution of L2 in the pH range 5−7 leads to linear decrease of the fluorescence emission of the receptor and the fluorescence of L2 is completely quenched in the presence of 1 equiv of ATP. The other triphosphate nucleotides produce only small

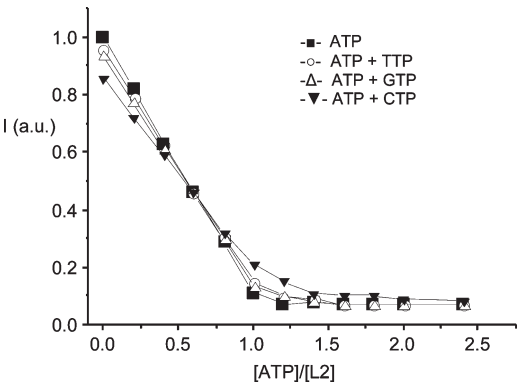


FIGURE 12. Fluorescence intensity of L2 at 366 nm in the presence of increasing amounts of ATP in the absence (-■-) and in the presence of 1 eqiv of TTP (-△-), GTP (-○-) and CTP (-▼-). (λ_{exc} 270 nm, NMe_4Cl 0.1 M, 298.1 K, pH = 6, [L] = 2.5×10^{-5}).

decrease of the fluorescence emission (at maximum 18% for CTP). Of note, ratiometrically sensing of ATP is scarcely affected by the presence in solution of 1 equiv of the other nucleotides. In fact, a competition experiment, carried out by adding increasing amounts of ATP to a solution buffered at pH 6 containing 1 equiv of TTP or GTP, shows a linear decrease of the fluorescence emission of the fluorophore very similar to that observed in the absence of these nucleotides. In the presence of 1 equiv of CTP, the most efficient competing agent for ATP, the fluorescence emission of L2 linearly decreases up to a 1:0.8 L2/ATP molar ratio and achieves a constant value for L2/ATP molar ratios greater than 1.4 (Figure 12).

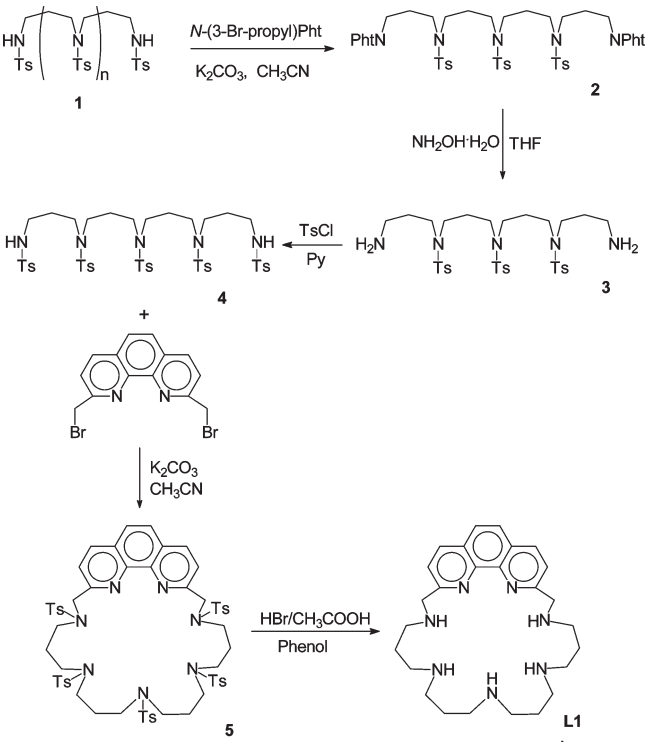
These results indicate not only that the sensing ability for ATP is scarcely affected by the presence of the other nucleotides, but also confirms, at least qualitatively, that the binding ability of L2 toward nucleotides decreases in the order ATP $\gg$ CTP > TTP $\cong$ GTP, in keeping with the results obtained by means of potentiometric measurements.

Concluding Remarks

The comparison of the binding properties of L1 and L2 shows that slight differences in the receptor and/or nucleotide structures can determine the binding mode as well as the fluorescence emission of the adducts. The host−guest adducts formed by the highly protonated forms of L1 and L2 present similar inclusive coordination modes of the phosphate chain within the receptor cavity, which allow the formation of an hydrogen-bonding network between the ammonium groups of L1 and L2 and the anionic phosphate groups of nucleotides. In both the adducts with L1 and L2, all nucleotides assume folded conformations, which allow the simultaneous encapsulation of their triphosphate chain and the interaction of the nucleobases with the receptor. However, only receptor L2 is larger enough to allow the π-pairing of nucleobases with phenanthroline. In fact, despite the bent conformation assumed by nucleotides, the nucleobases cannot achieve an appropriate disposition to give π-stacking with the phenanthroline of L1, due to the somewhat smaller dimension of this receptor. The adducts with L2 are consequently more stable than those with L1.

To achieve selective recognition of a given substrate, the receptor binding sites need to be simultaneously involved in strong interactions with the guest species. Despite the similar structure of nucleotide triphosphates, ATP gives the most stable adducts with L2, thanks to a pronounced cooperative role played in the binding process by the phosphate chain and the adenine unit, which give rise respectively to a robust hydrogen-bonding network with the polyammonium chain of L2 and to strong π-stacking interactions with phenanthroline. This binding mode justifies not only the higher stability of the ATP-L2 adducts, but also the quenching of emission of phenanthroline, which takes place via a PET process from the receptor to the excited phenanthroline. Analysis of pH-dependence of the fluorescence emission shows that quenching of phenanthroline is induced by ATP binding only in a narrow pH range, between pH 4.5 and 7, that is the same pH region where the phosphate moiety of ATP gives the strongest interactions with the ammonium chain of L2. This suggests that the quenching effect is likely to be related to a proton-transfer process from an ammonium group to the anionic phosphate chain.

SCHEME 2. Synthetic Procedure To Achieve L1



Experimental Details

Synthesis of Receptors. The procedure for the synthesis of receptors L1 is depicted in Scheme 2. L2,[31] 2,9-bis-(bromomethyl)-1,10-phenanthroline,[39] and tosylated amine 1,5,9-tritosyl-1,5,9-tetraazanonane (**1**)[40] were prepared as previously described. The reaction of pertosylated polyamine **4** with 2,9-bis(bromomethyl)-1,10-phenanthroline in dry acetonitrile at reflux and in presence of K_2CO_3 afforded macrocycle **4** in moderate yield. Deprotection of the nitrogen atoms was achieved by treatment of **4** with HBr in acetic acid and in

(39) Chandler, C. J.; Deady, L. W.; Reiss, J. A. *J. Hetercycl. Chem.* **1981**, *18*, 599–601.

(40) (a) Bencini, A.; Burguete, M. I.; Garcia-España, E.; Luis, S. V.; Miravet, J. F.; Soriano, C. *J. Org. Chem.* **1993**, *58*, 4749–4753. (b) Aguilar, J.; Diaz, P.; Escarti, F.; Garcia-España, E.; Gil, L.; Soriano, C.; Verdejo, B. *Inorg. Chim. Acta* **2002**, *339*, 307–316.

presence of phenol. Deprotected macrocyclic receptor L1 was obtained in good yield as its hydrobromide salt.

1,17-Phthalimido-5,9,13-tritosyl-5,9,13-triazaheptadecane (2). Tosylated amine **1** (15.8 g, 26.6 mmol) and K_2CO_3 (29.7 g, 215 mmol) were suspended in refluxing dry acetonitrile (150 mL). To this mixture was added dropwise a solution of *N*-(3-bromopropyl)phthalimide (14.7 g, 55 mmol) in dry acetonitrile (150 mL). After the addition was complete, the suspension was refluxed for 48 h and then filtered off. The solvent was evaporated, and the residue suspended in refluxing ethanol to give **2** as a white solid. Yield: 23.2 g, (90%). Mp: 265−267 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.83−7.81 (m, 4H), 7.72−7.64 (m, 10H), 7.34−7.28 (m, 6H), 3.69 (t, J = 7.1 Hz, 4H), 3.23−3.13 (m, 12H), 2.43 (s, 9H), 1.97−1.87 (m, 8H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 168.1, 143.2, 136.0, 133.9, 132.0, 129.7, 127.1, 123.1, 46.8, 46.6, 35.6, 28.6, 27.8, 21.5. Anal. Calcd for $C_{49}H_{53}N_5O_{10}S_3$: C, 60.79; H, 5.52; N, 7.23. Found: C, 60.9; H, 5.6; N, 7.3.

5,9,13-Tritosyl-1,5,9,13,17-pentaazaheptadecane (3). A mixture of **2** (3.48 g, 3.6 mmol) and hydrazine hydrate (85% in water, 8 mL) in THF (400 mL) was refluxed overnight and then cooled to room temperature and the resulting solid filtered off. The filtrate was concentrated to dryness, affording the deprotected amine **3** as a white solid. Yield: 2.51 g (98%). Mp: 150−152 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.67−7.53 (m, 6H), 7.23 (m, 6H), 3.12−3.03 (m, 12H), 2.64 (t, J = 6.6 Hz, 4H), 2.34 (s, 9H), 1.83−1.75 (m, 4H), 1.62−1.54 (m, 4H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 143.3, 143.1, 135.9, 135.7, 129.6, 129.5, 127.0, 126.7, 46.8, 46.5, 38.9, 32.1, 28.7, 21.4. Anal. Calcd for $C_{33}H_{49}N_5O_6S_3$: C, 55.98; H, 6.98; N, 9.89. Found: C, 56.1; H, 6.9; N, 9.7.

1,5,9,13,17-Pentatosyl-1,5,9,13,17-pentaazaheptadecane (4). To a stirred solution of tosyl chloride (6.67 g, 35 mmol) in pyridine (55 mL) at 0 °C was added dropwise a solution of the amine **3** (8.5 g, 12 mmol) in 50 mL of pyridine. After the addition was complete, the red solution was stirred at room temperature overnight and then poured in a water−ice mixture: an orange, gummy solid was obtained; it was ground and stirred in water until it turned into a pale pink powder, which was filtered and dried in vacuo at 40 °C overnight. The product **4** was recrystallized from hot $CHCl_3$ by addition of cold EtOH and obtained as a white solid (10.3 g, 85% yield). Mp: 203−205 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.84−7.73 (m, 12H), 7.43−7.35 (m, 12H), 3.24−3.12 (m, 16H), 3.09−3.06 (m, 4H), 2.53−2.51 (m, 18H), 2.02−1.88 (m, 8H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 143.5, 143.4, 143.2, 136.8, 135.4, 129.8, 129.6, 127.1, 127.0, 47.5, 47.2, 46.7, 40.2, 29.4, 29.1, 21.5. Anal. Calcd for $C_{47}H_{61}N_5O_{10}S_5$: C, 55.54; H, 6.05; N, 6.89. Found: C, 55.7; H, 6.2; N, 6.6.

2,6,10,14,18-Pentatosyl-2,6,10,14,18-pentaaza[19](2,9)cyclo-1,10-phenanthrolinophane (5). To a refluxing, stirred suspension of the tosylated polyamine **4** (2.54 g, 2.5 mmol) and K_2CO_3 (3.45 g, 25 mmol) in dry acetonitrile (250 mL) and under nitrogen atmosphere was added a suspension of 2,9-bis-(bromomethyl)-1,10-phenanthroline (1.0 g, 2.75 mmol) in dry acetonitrile (250 mL) during 4 h. At the end of the addition, the mixture was stirred and refluxed for additional 2 h and then cooled to room temperature. The suspension was filtered on Celite and washed with acetonitrile, and the filtrate was evaporated to obtain a solid, which was purified by column chromatography on neutral aluminum oxide, using a mixture of PE/AcOEt 1:1.5 as the eluant. The fractions containing **6** (R_f = 0.5) were evaporated to dryness; **5** was obtained as a pale brown solid (1.08 g, 35% yield). Mp: 195−197 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.29 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.1 Hz, 2H), 7.70−7.64 (m, 6H), 7.62−7.56 (m, 8H), 7.34−7.26 (m, 8H), 4.78 (s, 4H), 3.24 (t, J = 6.9 Hz, 4H), 2.99 (m, 4H), 2.66 (m, 4H), 2.55 (s, 9H), 2.41 (s, 6H), 1.84 (m, 4H), 165 (m, 4H). ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 157.7, 143.2, 143.4, 143.0, 137.5, 135.9, 135.4, 129.6, 127.9, 127.1, 77.5, 55.3, 48.1, 46.7, 46.4, 46.3, 28.3, 28.1, 28.0, 21.2. Anal. Calcd for $C_{61}H_{69}N_7O_{10}S_5$: C, 60.03; H, 5.70; N, 8.03. Found: C, 59.9; H, 5.9; N, 7.9.

2,6,10,14,18-Pentaaza[19](2,9)cyclo-1,10-phenanthrolinophane Hexahydrobromide (L1·6HBr). The tosylated macrocycle **5** (1.23 g, 1.0 mmol) and phenol (9.4 g, 100 mmol) were dissolved in HBr/AcOH 33% (100 mL); the reaction mixture was stirred and refluxed for 20 h until a white solid formed. The reaction mixture was cooled to room temperature, 100 mL of dichloromethane was added to complete the precipitation, and the mixture was stirred for an additional 1 h. The solid was filtered and washed with dichloromethane to remove the residual phenol. Receptor L1, as hydrobromide salt, was then recrystallized from a mixture of EtOH/water 3:1 (v/v), filtered/ and dried at 40 °C overnight in the presence of KOH (559 mg, 55% yield). Mp: 200 °C dec. ^{1}H NMR (D_2O, 300 MHz, pH = 1.9) δ (ppm): 8.46 (d, J = 8.4 Hz, 2H), 7.92 (s, 2H), 7.75 (d, J = 8.1 Hz, 2H), 4.68 (s, 4H), 3.29 (m, 4H), 3.15 (m, 12H), 2.19 (m, 4H), 2.03 (t, J = 7.0 Hz, 4H). ^{13}C NMR (D_2O, 75.4 MHz, pH = 1.9) δ (ppm): 151.4, 144.6, 139.4, 129.2, 127.1, 123.6, 52.5, 45.2, 45.95, 44.86, 42.6, 23.0, 22.4. ESI-MS (m/z) 450.33 (100) ($[M + H]^+$), 226.2 (58) $(M + 2H)^{2+}$. Anal. Calcd for $C_{26}H_{39}N_7 \cdot 6HBr$: C, 33.39; H, 4.85; N, 10.49. Found: C, 33.1; H, 4.8; N, 10.2.

Synthesis of the $[(H_6L2)_2(TTP)_2(H_2O)_2]Br_4 \cdot 7H_2O$ Adduct. L2·6HBr (10 mg, 0.01 mmol) and thymidine triphosphate monosodium salt (4.8 mg, 0.01 mmol) were dissolved in water, and the pH of the solution was raised to 6 with 0.1 M NaOH. The solution was then kept under an acetone atmosphere until the formation of colorless crystals suitable for X-ray analysis. Yield: 7.0 mg (56.9%) Anal. Calcd for $C_{78}H_{148}Br_4N_{20}O_{37}P_6$: C, 38.13; H, 6.06; N, 11.37. Found: C, 38.3; H, 6.2; N, 11.2.

Potentiometric Measurements. Equilibrium constants for protonation and complexation reactions with L were determined by pH-metric measurements at 298.1 ± 0.1 K in 0.1 M NMe_4Cl, by using equipment and procedures which have been already described.[8b,31] In the experiments to determine the stability of the adducts with L1 and L2, the receptor concentrations were varied from 5×10^{-4} to 5×10^{-3} M, while the concentration of nucleotides was varied in the range 4×10^{-4}−9×10^{-3} M. At least three measurements (about 100 data points each one) were performed for each system in the pH range 2.5−10.5, and the relevant emf data were treated by means of the computer program HYPERQUAD.[37] Full details are given within the Supporting Information.

Spectrophotometric and Spectrofluorimetric Measurements. Absorption and fluorescence emission spectra were recorded as previously described.[30] In the measurements carried out at different pH values, HCl and NaOH were used to adjust the pH values which were measured on a pH meter. TRIS buffer (1 mm) was used in the titrations performed at pH 6.0. In the competition experiments, successive readings of the emission intensity were carried out after each addition of CTP, TTP, or GTP to the ATP solution to ensure that the equilibrium was reached.

NMR Spectroscopy. ^{1}H (300 MHz) and ^{13}C (75 MHz) in $CDCl_3$ and D_2O solutions at different pH values were recorded at 298 K on a 300 MHz spectrometer. To adjust the pD, small amounts of 0.01 m NaOD and DCl were added to the solutions. The pH was calculated by the measured pD values by using the following formula: pH = pD − 0.40.[41] 2D correlation experiments were performed to assign the ^{1}H NMR signals. Complexation-induced ^{31}P and ^{1}H NMR chemical shifts (CIS, ppm) were measured as the difference $\delta_{OBS} - \delta_{nucleotide}$ where δ_{OBS} is the chemical shift of a signal measured in D_2O solutions containing receptor and substrate in a 1:1 molar ratio (both 5×10^{-3} M, in these conditions all adducts are completely formed in solution

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

and no amounts of uncomplexed receptor or substrate is present in solution), and $\delta_{nucleotide}$ is the chemical shift of the corresponding signal of the not complexed receptor or substrate.

Crystal Structure Analyses. Data for the X-ray structural analysis of $[(H_6L2)_2TTP_2(H_2O)_2]Br_4 \cdot 7H_2O$ were collected on an Oxford Diffraction Xcalibur3 diffractometer equipped with CCD area detector and graphite-monochromated Mo Kα radiation (λ = 0.71069 Å, T = 150 K). Data collection was performed using ω scan with the CrysAlis CCD program.[42] Data reduction was carried out with the CrysAlis Red program,[43] and an empirical absorption correction was applied using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.[43]

Crystals of the compound belong to the triclinic family ($C_{78}H_{148}Br_4N_{20}O_{37}P_6$, M_w = 2463.62, $P1$, a = 13.1824(8) Å, b = 14.2832(9) Å, c = 17.092(1) Å, α = 91.782(5)°, β = 109.531(5)°, γ = 115.968(6)°; V = 2666.3(3) Å^3; Z = 1; ρ_{calc} = 1.534 mg/m^3; μ = 1.689 mm^{-1}; $F(000)$ = 1282; θ_{max} = 24.71). Structures were solved by direct methods (SIR2004)[44] and refined against F^2 by using SHELXL-97,[45] with non-hydrogen non-carbon atoms anisotropic. Carbon atoms were refined isotropically in order to avoid a too low data/parameters ratio. Hydrogen atoms linked to carbon or nitrogen atoms were introduced in calculated position and refined in riding mode. Hydrogen atoms belonging to the solvent water molecules were not localized in the final ΔF map and not introduced in the calculation. All six-membered aromatic rings were restrained to idealized hexagons. Due to low intensity of data at high resolution, only data up to senθ/λ = 0.59 were used during refinement. Agreement factors at the end of refinement were R1 = 0.0777, wR2 = 0.1686 (4847 reflections with $I > 2\sigma(I)$ − 846 parameters refined); R1 = 0.1769, wR2 = 0.2180 (all data). The Flack parameter at the end of refinement was −0.019(16).

(42) *CrysAlis CCD*, Oxford Diffraction Ltd., Version 1.171.32.5, 2008.
(43) *CrysAlis RED*, Oxford Diffraction Ltd., Version 1.171.32.5, 2008.
(44) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
(45) Sheldrick, G. M., *SHELX-97*, Göttingen, 1997.

Computational Details. MD calculations were performed by using the AMBER3 forcefield, as implemented in the Hyperchem 7.51 package,[46] by using the procedure and method which has been already described.[8b,31] Starting conformations of the $[H_6L2(S)]^{2+}$ or $[H_5L1(S)]^{+}$ adducts (S = ATP^{4-}, GTP^{4-}, CTP^{4-}, TTP^{4-}), obtained by manual docking of the minimized conformer of the receptor to the substrate (minimum distance between the atoms of receptor and substrate > 5 Å) and, in the case of the $[H_6L2(TTP)]^{2+}$ complex, derived from the X-ray crystal structure, were freely minimized. Full details are given within the Supporting Information.

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Supporting Information Available: Full details for potentiometric measurements and molecular mechanics calculations; overall stability constants of the adducts; list of files containing the atomic coordinates of the minimized conformers; hydrogen-bonding distances in the low energy conformers of the adducts of $[H_5L1]^{5+}$ and $[H_6L1]^{6+}$ with nucleotides; titration data and fit curves for potentiometric titrations performed to determine the protonation constants of L2; pH dependence of the chemical shifts of the benzylic protons and of the fluorescence emission at 365 nm of L2; distribution diagrams of the adducts of L1 and L2 with CTP, TTP, and GTP; pH dependence of the ^{31}P NMR and ^{1}H NMR chemical shifts of the phosphate chain and of the nucleobases of CTP, TTP, and GTP in the absence and in the presence of L1 and L2; pH dependence of the ^{1}H NMR chemical shifts of the phenanthroline protons of L1 and L2 in the absence and in the presence of CTP, TTP, and GTP; labeled lowest energy conformers of the adducts of $[H_5L1]^{5+}$ and $[H_6L1]^{6+}$ with nucleotides; ORTEP drawing of the $[(H_6L2)_2(TTP)_2(H_2O)_2]^{4+}$ cation; ^{1}H and ^{13}C NMR spectra of compounds **2**−**5** and of **L1**; atomic coordinates of the calculated low energy conformers in MDL_Mol format. This material is available free of charge via the Internet at http://pubs.acs.org.

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