Phenanthroline-containing macrocycles as multifunctional receptors for nucleotide anions. A thermodynamic and NMR study †

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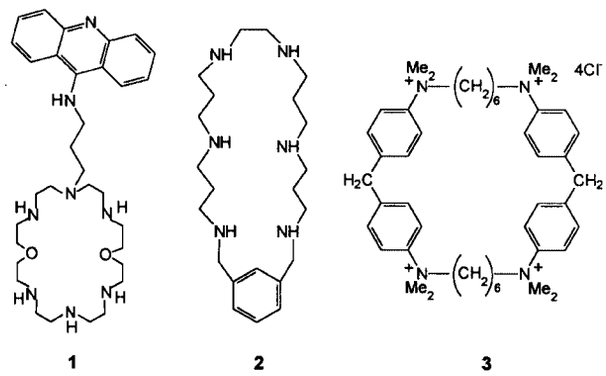
The synthesis of the phenanthroline-containing macrocycle 2,6,10,14-tetraaza[15](2,9)cyclo(1,10)phenanthrolinophane (**L1**) is reported. **L1** contains a tetraamine chain connecting the 2,9-positions of a phenanthroline unit. Protonation of **L1** has been studied by means of potentiometric and ^{1}H and ^{13}C NMR techniques, allowing the determination of the basicity constants and of the stepwise protonation sites. The protonation features of **L1** are compared with those of macrocycle 2,5,8,11-tetraaza[12](2,9)cyclophenanthrolinophane (**L2**), in which the amine groups are linked by ethylenic chains. Considering the $[H_4\mathbf{L1}]^{4+}$ and the $[H_4\mathbf{L2}]^{4+}$ species, the acidic protons are located on the aliphatic nitrogens, while phenanthroline is not involved in protonation. Binding of diphosphate, triphosphate, ATP and ADP has been studied by means of potentiometry and ^{1}H and ^{31}P NMR. Both **L1** and **L2** behave as multifunctional receptors for the nucleotide anions at neutral or slight acidic pHs, giving 1 : 1 complexes. Charge–charge and hydrogen bonding interactions take place between the polyphosphate chain of nucleotides and the polyammonium groups of **L1** and **L2**, while the adenine moiety gives charge–dipole interactions with the ammonium groups and π-stacking with the phenanthroline unit of the receptors. The high upfield displacements in the ^{1}H NMR spectra exhibited by the adenine protons upon complexation by **L1** suggest a partial inclusion of nucleotides inside the macrocyclic cavity.

The design of host molecules for recognizing anions, such as carboxylic acids or nucleotides, in aqueous solution is an important target according to the biological relevance of the studies in this solvent. This goal is not easily achieved as strong solvent–guest interactions can efficiently compete with the process of selective complexation. To this purpose, polyammonium macrocycles may behave as efficient receptors for polycharged anions in aqueous solution.[1–14] Protonated macrocyclic polyamines strongly bind to nucleotides *via* electrostatic interactions between the cationic binding sites (ammonium groups) of the receptor and the negatively charged polyphosphate chain. It is accepted, however, that selective coordination requires the incorporation of sites for multiple interactions with the substrates. To achieve a better recognition of nucleotides, receptors need to contain other binding sites capable of interactions with the sugar moiety[15–17] or the nucleic base,[2c,18–20] in addition to the anion binding sites. In particular, base-selective recognition is attainable either by hydrogen bonds to suitably constructed receptors or by stacking interactions with π-systems incorporated into the host molecule.

Earlier, Lehn and Hosseini reported one of the first synthetic ligands able to behave as a multifunctional receptor for nucleotides (**1** in Scheme 1).[2c] **1** contains two recognition sites, a macrocyclic polyammonium moiety, as anion binding site, and an acridine pendant arm for stacking interactions with the nucleobase. Recently, polyazacyclophanes, *i.e.* macrocycles containing phenylene subunits as an integral part of the cyclic framework, have been used as selective recognizers of nucleotides. In the case of ligands **2**[3] and **3**,[5c] as well as stacking interactions, the inclusion of nucleobases into the macrocyclic cavity is a peculiar structural motif of their adducts with nucleotides.

In this paper we report the synthesis of the new macrocyclic receptor **L1**, which contains a large heteroaromatic moiety, phenanthroline, as a non-pendant integral part of a polyamine macrocyclic structure. The insertion of phenanthroline may provide a further binding site for nucleotide anions. In fact, this large heteroaromatic moiety may offer an optimal binding site for the coordination of nucleotides, through stacking and hydrophobic interactions. Since anion coordination by polyamine ligands is strictly related to their basicity properties, we have analyzed the protonation features of **L1**. Protonation of **L1** gives $[H_n\mathbf{L1}]^{n+}$ cations, potential multifunctional receptors for nucleotides: they contain a polyammonium chain which may interact with the anionic polyphosphate chain of nucleotides and a large heteroaromatic unit as binding site for the nucleic bases. Furthermore, charge–dipole interactions between the polyammonium groups and the adenine ring may take place, as actually found for the adducts of **3** with nucleotides.[5a,c] Therefore, we have carried out a thermodynamic and ^{1}H and ^{31}P NMR study on the interaction of this receptor with ATP and ADP. For comparison, the binding properties of **L1** towards inorganic diphosphate and triphosphate have been also investigated. In order to analyze the effect of the dimensions of the macrocyclic cavity on nucleotide coordination, we have also studied the binding features of macrocycle **L2**.[21] Similarly to **L1**, **L2** contains a tetraamine unit linking the 2,9-phenanthroline positions; on the other hand this ligand possesses a smaller cavity than **L1**, due to the ethylenic chains connecting the aliphatic nitrogens.

† Supplementary data available: plots of the ^{1}H chemical shifts of the signals of selected aromatic protons of ligands and nucleotides against the nucleotide/ligand ratio (Fig. S1–S4) and plots of the ^{1}H chemical shifts of **L1** in the presence and in the absence of ATP and of ATP in the presence and in the absence of **L1** as a function of pH (Fig. S5). For direct electronic access see http://www.rsc.org/suppdata/p2/1999/1675, otherwise available from BLDSC (SUPPL. NO. 57587, pp. 5) or the RSC Library. See Instructions for Authors available *via* the RSC web page (http://www.rsc.org/authors).



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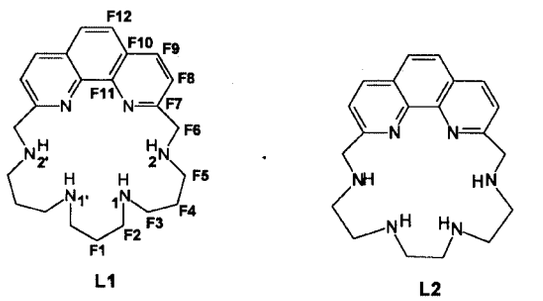
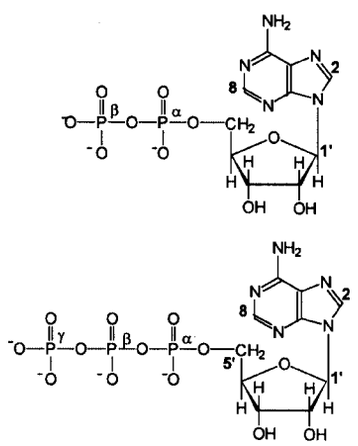


L1 L2



Scheme 1

Results and discussion

Protonation of L1

The protonation equilibria of **L1** have been studied in 0.1 mol dm^{-3} NMe_4Cl solution at 298.1 ± 0.1 K by means of potentiometric measurements. The protonation constants are reported in Table 1, together with those previously reported for **L2**.[21b] The distribution diagram for the species present in solution as a function of pH for the system **L1**/H^+ is shown in Fig. 1. Both **L1** and **L2** behave as tetraprotic bases in the pH range 3–11. Phenanthroline nitrogens are characterized by far lower basicity than aliphatic amine nitrogens: the first protonation constant of phenanthroline is 4.93 log units,[22] while the first protonation constant of secondary amines usually ranges between 9.5–11 log units.[23] Therefore, it is expected that at least the first protonation steps take place on the amine groups of the aliphatic chains. Indeed, UV spectra recorded on solutions containing **L2** at various pH values do not show significant variations in the pH range 2.5–11 (λ_{max} = 275 nm, ε = 29500 mol^{-1} dm^3 cm^{-1} at pH 5) indicating that the aromatic nitrogens are not involved in protonation. In the case of **L1**, the UV spectra of the ligand do not change significantly in the pH range 11–3.5 (λ_{max} = 273 nm, ε = 31200 mol^{-1} dm^3 cm^{-1} at pH 5), where the macrocycle binds up to four protons in aqueous solution (Fig. 1). On the other hand, a decrease of absorbance is observed at strongly acidic pHs (λ_{max} = 282 nm, ε = 25950 mol^{-1} dm^3 cm^{-1} at pH 1.5), suggesting that protonation of phenanthroline takes place only at very acidic pH values. These data suggest that the first four protonation steps of **L1** and **L2** take place on the four aliphatic amine groups. It is also to be noted that the basicity constants of **L1** are remarkably higher than those of **L2** in each step of protonation. This behavior can be ascribed to the larger +I inductive effect of the longer propylenic chains, which increases the basicity of each amine group, as well as to the longer distance between amine nitrogens, which allows a better minimization of the electrostatic repulsion between protonated ammonium groups in the $[H_n\mathbf{L1}]^{n+}$ polycharged cations with respect to the $[H_n\mathbf{L2}]^{n+}$ ones. This effect has been also observed in aliphatic diamines, such as *N*,*N*′-dimethyl-1,2-diaminoethane (log K_1 = 10.05 and log K_2 = 7.12)[24] and *N*,*N*′-dimethyl-1,3-diaminopropane (log K_1 = 10.80 and log K_2 = 9.1),[25] which contain two secondary amine groups linked respectively by an ethylenic and a propylenic chain.

To get further information on the protonation pattern of **L1** we have also analyzed the variations with pH of its ^1H and ^{13}C NMR spectra. All the assignments have been made on the basis of ^1H–^1H homonuclear and ^1H–^{13}C heteronuclear correlation experiments at the different pH values studied.

The ^{13}C spectrum of **L1** at pH 12.0, where the unprotonated amine predominates in solution, exhibits twelve peaks, six for the aliphatic carbons CF1–CF6 and six for the aromatic carbons CF7–CF12. The ^1H spectrum of **L1** at this pH shows two multiplets at 1.46 and 1.61 ppm (each integrating four protons and attributed to the hydrogen atoms HF1 and HF4 respectively), a multiplet at 2.42 ppm (eight protons, the hydrogens of HF2 and HF3), a triplet at 2.61 ppm (4 protons, HF5), a singlet at 3.95 ppm (4 protons, HF6), and a singlet and two doublets for the aromatic protons HF12, HF8 and HF9. These spectral features indicate a C_{2v} time averaged symmetry. This symmetry is preserved throughout all the pH range investigated.

Table 1 Protonation constants (log *K*) of **L1** and **L2** determined by means of potentiometric measurements in 0.1 mol dm^{-3} NMe_4Cl aqueous solution at 298.1 K

Reaction	Log *K*	
	L1	**L2**
$L + H^+ = HL^+$	10.48(1)[a]	9.72[b]
$HL^+ + H^+ = H_2L^{2+}$	9.06(2)	8.71
$H_2L^{2+} + H^+ = H_3L^{3+}$	7.39(3)	6.18
$H_3L^{3+} + H^+ = H_4L^{4+}$	6.08(3)	2.18

[a] Values in parentheses are standard deviations on the last significant figure. [b] From reference 21*b*.

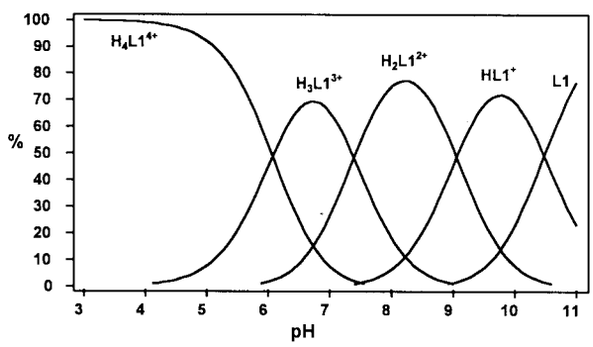


Fig. 1 Distribution diagram of the protonated species formed by **L1** as a function of pH (0.1 mol dm^{-3} NMe_4Cl, 298.1 K, [**L1**] = 1 × 10^{-3} mol dm^{-3}).

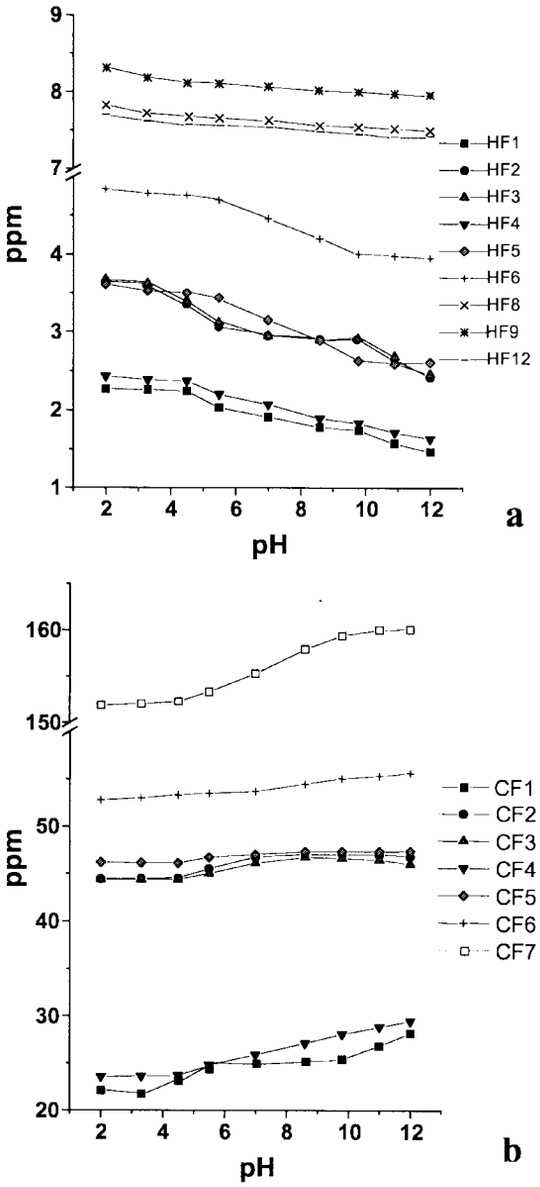


Fig. 2 Experimental ^{1}H chemical shifts (a) and ^{13}C NMR chemical shifts (b) of **L1** as a function of pH (the signals of the aromatic carbons CF8–CF12 have been omitted for clarity. Their chemical shifts do not significantly change in the pH range investigated).

Fig. 2a shows the ^{1}H NMR spectra recorded at various pH values and Fig. 2b reports the ^{13}C NMR chemical shifts of **L1** as a function of pH.

In the pH range 12–10, where the first proton binds to the ligand, the signals of the hydrogens HF2 and HF3, in the α-position with respect to N1, shift downfield; a slight downfield shift is also observed for the protons HF4 and HF1 of the central methylene of the propylenic chains, while the other signals do not appreciably shift. This suggests that the first protonation step involves the nitrogens N1 and N1′. This hypothesis is confirmed by the ^{13}C NMR spectra recorded in the same pH range, which show that the resonances of the carbon atoms CF1 and CF4, in the β-position with respect to N1, shift upfield (Fig. 2b), in good agreement with the β-shift reported for the protonation of polyamines.[26] The higher proton affinity of the nitrogen atoms N1 and N1′ in comparison with benzylic nitrogens N2 and N2′ can be ascribed to the inductive effect of the heteroaromatic moiety on the adjacent N2 and N2′ amine groups.

In the pH range 10–6 the macrocycle binds two further protons, giving the $[H_2\mathbf{L1}]^{2+}$ and $[H_3\mathbf{L1}]^{3+}$ species. The formation of these species dramatically affects the pattern of the ^{1}H and ^{13}C NMR spectra. Fig. 2a shows that the signals of HF5 and HF6 undergo a remarkable downfield shift. Considering the ^{13}C NMR spectra recorded in the same pH range, the resonances of CF4 and CF7, in the β-position with respect to N2, exhibit a marked upfield shift. These spectral features indicate that the second and third protonation steps take place on the benzylic nitrogens N2 and N2′. As can be seen in the distribution diagram (Fig. 1), **L1** is mainly in its tetraprotonated form at pH < 6. In the ^{1}H NMR spectrum recorded below pH 6, the signals of the hydrogens HF2 and HF3 shift downfield, suggesting that the nitrogens N1 and N1′ are involved in the fourth protonation step.

It is worth noting that the first four protonation steps involve only the nitrogens of the aliphatic chain. The ^{1}H and ^{13}C signals of the aromatic moiety (with the exception of CF7) do not vary significantly in the pH range 12–3. Only at strongly acidic pH values do the protons of the phenanthroline moiety show a downfield shift, probably due to the involvement of the aromatic nitrogens in the binding of the fifth proton.

Considering the ligand **L2**, the analysis of the ^{1}H and ^{13}C NMR spectra recorded at different pH values shows a similar protonation mechanism; in the $[H_4\mathbf{L2}]^{4+}$ species the four protons are located on the aliphatic amine groups, while phenanthroline is not protonated even at strongly acidic pHs (pH 2).

In conclusion, these protonation features make **L1** and **L2** promising receptors for nucleotides. They give polyprotonated forms at neutral pH. These polycharged species display a polyammonium chain, a potential binding site for the anionic phosphate moiety of nucleotides, and a phenanthroline unit, which may give π-stacking interactions with the nucleobase.

Phosphate anion binding

Binding of ATP, ADP, diphosphate and triphosphate by **L1** and **L2** has been studied by means of potentiometric measurements and, in the case of ATP and ADP, by ^{1}H and ^{31}P NMR measurements.

Protonation of the receptors gives charged species which enable **L1** and **L2** to form stable complexes with anionic forms of these nucleotides in aqueous solution. The formation of such species is strictly pH-dependent and, therefore, the relevant equilibria can be studied by pH-metric titrations. Table 2 collects the cumulative and stepwise equilibrium constants for the species formed by **L1** and **L2** with ATP, ADP, $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$.[27] The stability constants of the **L2** complexes with triphosphate cannot be determined, due to the low solubility of the adducts. Although the formation in some cases of both 1 : 1 and 2 : 1 anion–macrocycle complexes has been observed,[28] the data analysis with the program HYPERQUAD[29] under our experimental conditions revealed predominantly 1 : 1 stoichiometries for all the species detected. This is confirmed by Job plots of the ^{1}H NMR chemical shifts of the signals of selected aromatic protons of **L1** or **L2** and ATP or ADP against the nucleotide/ligand ratio, which show sharp breaks at ligand/nucleotide = 1.

By examining the different values of stability constants, several main features can be readily noticed. For all the studied anions, the interactions with the macrocycles start to be detectable for a minimum of protonation of two, except for the systems **L1**–ADP^{3-}, **L2**–$P_2O_7^{4-}$ and **L1**–$P_3O_{10}^{5-}$ for which at least three protons are required on the macrocycle to make the interaction detectable.

For a given macrocycle the strength of the interaction generally increases with its degree of protonation. For instance, the stability constants for the interaction of ATP^{4-} with **L1** and **L2** vary respectively from log $K = 2.57$ and 2.93 for the diprotonated macrocycles to log $K = 4.05$ and 5.26 for the

Table 2 Stability constants (log *K*) of the ADP, ATP, diphosphate and triphosphate adducts with **L1** and **L2**, determined by means of potentiometric measurements in 0.1 mol dm^{-3} NMe_4Cl at 298.1 K

Reaction	L1	L2	Reaction	L1	L2
	$A = ADP^{3-}$			$A = ATP^{4-}$	
$L + 2H^+ + A^{3-} = H_2LA^-$	—	20.8(1)[a]	$L + 2H^+ + A^{4-} = H_2LA^{2-}$	22.11(7)	21.36(6)
$L + 3H^+ + A^{3-} = H_3LA$	30.12(3)	28.62(5)	$L + 3H^+ + A^{4-} = H_3LA^-$	30.98(3)	29.87(4)
$L + 4H^+ + A^{3-} = H_4LA^+$	38.43(2)	33.49(6)	$L + 4H^+ + A^{4-} = H_4LA$	39.31(3)	34.31(5)
$L + 5H^+ + A^{3-} = H_5LA^{2+}$	43.65(2)	38.32(8)	$L + 5H^+ + A^{4-} = H_5LA^+$	45.34(4)	39.33(5)
$L + 6H^+ + A^{3-} = H_6LA^{3+}$	—	40.73(8)	$L + 6H^+ + A^{4-} = H_6LA^{2+}$	47.76(6)	—
$H_2L^{2+} + A^{3-} = H_2LA^-$	—	2.4(1)	$H_2L^{2+} + A^{4-} = H_2LA^{2-}$	2.57(7)	2.93(6)
$H_3L^{3+} + A^{3-} = H_3LA$	3.19(3)	4.01(5)	$H_3L^{3+} + A^{4-} = H_3LA^-$	4.05(3)	5.26(6)
$H_3L^{3+} + HA^{2-} = H_4LA^+$	3.78(3)	2.29(5)	$H_3L^{3+} + HA^{3-} = H_4LA$	5.20(3)	2.52(6)
$H_4L^{4+} + HA^{2-} = H_5LA^{2+}$	4.05(5)	4.94(6)	$H_4L^{4+} + HA^{3-} = H_5LA^+$	5.15(4)	6.36(4)
$H_4L^{4+} + H_2A^- = H_6LA^{3+}$	—	3.38(6)	$H_4L^{4+} + H_2A^{2-} = H_6LA^{2+}$	3.24(4)	—
Reaction	L1	L2	Reaction	L1	
	$A = P_2O_7^{4-}$			$A = P_3O_{10}^{5-}$	
$L + 2H^+ + A^{4-} = H_2LA^{2-}$	22.4(1)	—	$L + 3H^+ + A^{5-} = H_3LA^{2-}$	30.22(3)	
$L + 3H^+ + A^{4-} = H_3LA^-$	30.7(1)	30.2(1)	$L + 4H^+ + A^{5-} = H_4LA^-$	38.45(3)	
$L + 4H^+ + A^{4-} = H_4LA$	38.7(1)	36.8(1)	$L + 5H^+ + A^{5-} = H_5LA$	45.12(4)	
$H_2L^{2+} + A^{4-} = H_2LA^{2-}$	2.8	—	$H_2L^{2+} + HA^{4-} = H_3LA^{2-}$	2.18(3)	
$H_2L^{2+} + HA^{3-} = H_3LA^-$	2.4	3.0	$H_3L^{3+} + HA^{4-} = H_4LA^-$	3.02(3)	
$H_3L^{3+} + HA^{3-} = H_4LA$	2.9	3.4	$H_4L^{4+} + HA^{4-} = H_5LA$	3.61(4)	

[a] Values in parentheses are standard deviations on the last significant figure.

macrocycles in their triprotonated forms. A similar increase of stability with the increasing protonation degree of the receptors is generally observed for the other systems. An increasing number of protonated polyammonium functions increases the receptor's ability to give charge–charge and hydrogen bonding interactions with the anionic substrates. High protonation degrees imply protonation of nucleotides and, therefore, a low negative charge of the substrates. As a consequence, hexaprotonated adducts with ATP and ADP are not detected by potentiometry or display low formation constants. Finally, no interaction between protonated macrocycles and the H_3ADP and H_4ATP uncharged substrates was found in our experimental conditions. As a consequence, the formation of the substrate–polyammonium receptor adducts takes place mainly from weakly alkaline to slightly acidic pHs, as shown in Fig. 3, which displays the distribution diagrams for the ADP–**L1** and ATP–**L1** systems. For the system ATP–**L1** the percentage of overall complexed species is more than 70% in the pH range 4–7, while in the case of the system ADP–**L1** the percentage is more than 50% in the pH range 4.5–7.5. In both cases, the overall percentages of substrate–receptor complexes decrease at more strongly alkaline or acidic pH. For instance the percentage of ATP–**L1** adduct is less than 25% at pH 2 and no interaction between ATP and **L1** is detected above pH 10.

The comparison of Fig. 3a and 3b also shows a lower interaction of ADP with **L1** with respect to ATP. As can be seen from Table 2, for the same protonation degree, the ADP complexes with **L1** and **L2** show a lower stability in comparison with the ATP adducts. This behaviour can be reasonably ascribed to weaker charge–charge interactions in the ADP complexes, due to a lower negative charge on this anion.

Finally, for a determined protonation degree of both reagents, anion and macrocycle, a decrease in stability occurs from **L2** to **L1**. This may be ascribed to a decrease in charge density as the macrocyclic size increases.

Anion complexation has also been followed by recording ^{31}P NMR spectra on solutions containing receptors and substrates in 1:1 molar ratio at different pH values. Fig. 4 shows the ^{31}P chemical shifts of the phosphate groups of ATP in the presence of **L1** (1:1 molar ratio) at different pH values, together with those of free ATP, and Table 3 reports the complexation

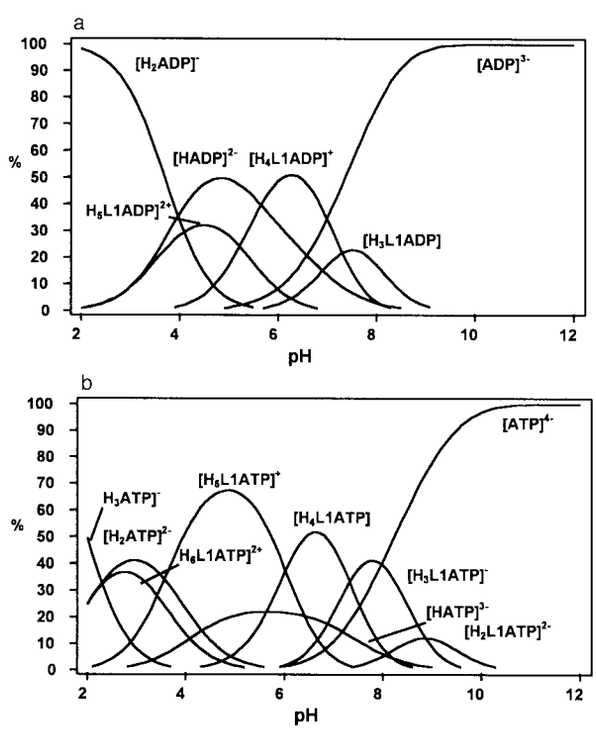


Fig. 3 Distribution diagrams for the systems **L1**–ADP (a) (0.1 mol dm^{-3} NMe_4Cl, 298.1 K, [**L1**] = [ADP] = 1×10^{-3} mol dm^{-3}) and **L1**–ATP (b) (0.1 mol dm^{-3} NMe_4Cl, 298.1 K, [**L1**] = [ATP] = 1×10^{-3} mol dm^{-3}).

induced ^{31}P chemical shifts (CIS) for the systems nucleotides–**L1** or –**L2**.

Considering the data in Fig. 4 and Table 3, some general features can be outlined: (a) complexation of substrates produces significant variations in the ^{31}P chemical shifts, as already observed for analogous complexes with other polyammonium macrocyclic receptors.[2,3] Considering ADP complexation, the resonances of both the phosphate groups shift downfield in the presence of the macrocycles. In the case of the ATP complexes with **L1** and **L2**, only the signals of two phosphate

Table 3 ^{31}P NMR shifts (δ, ppm) for the **L1** and **L2** adducts with ATP and ADP and complexation-induced ^{1}H NMR chemical shifts (CIS, ppm) for selected protons, measured in D_2O solution at pH 4.5 (systems ATP–**L1** and ADP–**L1**) and 4.8 (systems ATP–**L2** and ADP–**L2**), 298 K

	L1		**L2**	
ADP	P_α	P_β	P_α	P_β
δ(ppm)	−7.69[a]	−4.21	−7.97	−6.09
CIS	0.49[b]	3.33	0.21	1.45

	L1			**L2**		
ATP	P_α	P_β	P_γ	P_α	P_β	P_γ
δ(ppm)	−8.21	−19.59	−6.84	−8.22	−19.35	−6.95
CIS	0.05	0.52	0.82	0.0	0.67	0.73

[a] From measurements in D_2O solution at pH 4.5 (systems ATP–**L1** and ADP–**L1**) and 4.8 (systems ATP–**L2** and ADP–**L2**), 298 K, with a receptor–substrate 1:1 molar ratio. In these conditions the complexation degrees are 40% (system ADP–**L1**), 72% (ATP–**L1**), 49% (ADP–**L2**) and 53% (ATP–**L2**). [b] CIS (for 100% complexation) calculated based on equilibrium constants from Table 2.

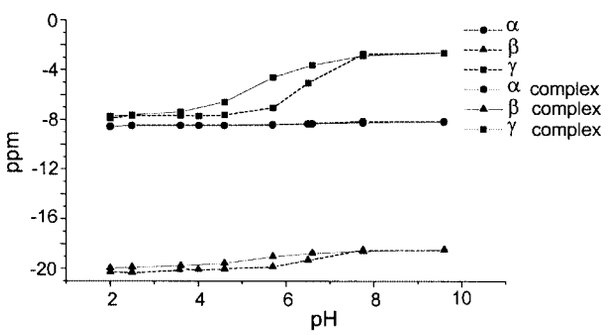


Fig. 4 Experimental ^{31}P chemical shifts of free ATP (-----) and of ATP in the presence of **L1** (——) as a function of pH ([**L1**] = [ATP] = 1×10^{-2} mol dm^{-3}, 298 K).

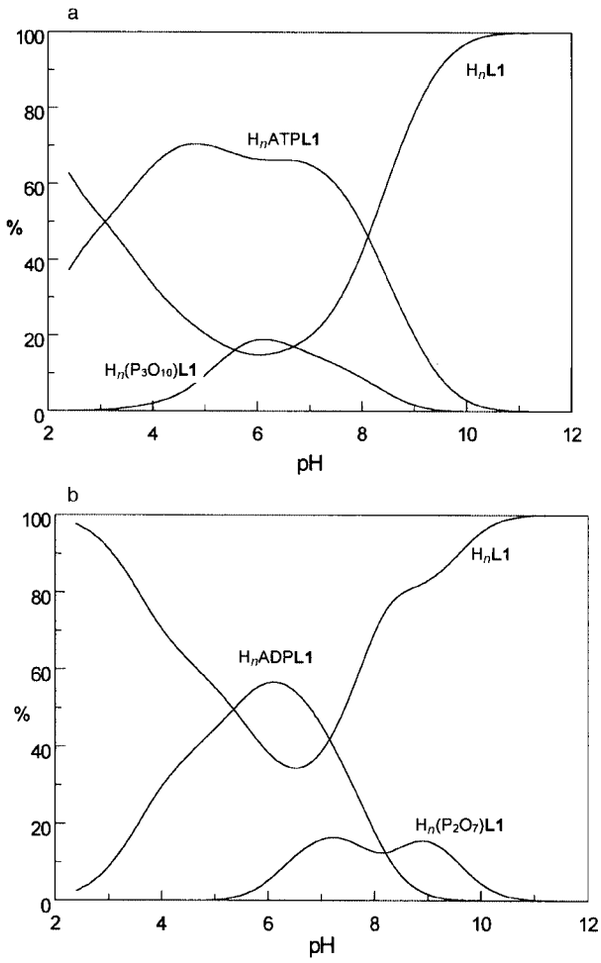


Fig. 5 Overall percentages of **L1** complexed species as a function of pH in competing systems containing ATP and triphosphate ([**L1**] = [ATP] = [triphosphate] = 1×10^{-3} mol dm^{-3}) (a) and ADP and diphosphate ([**L1**] = [ADP] = [diphosphate] = 1×10^{-3} mol dm^{-3}) (b). Percentages are calculated with respect to **L1**.

groups, namely P_β and P_γ, show a clear downfield shift upon complexation, while the chemical shift of P_α is not influenced by the interaction with the receptors. These observations seem to indicate that in both ATP and ADP binding the polyammonium functions of the macrocycles interact with two contiguous phosphate groups of nucleotides (P_β and P_γ in the case of ATP). (b) As shown in Fig. 4 for the system ATP–**L1**, the variations of chemical shifts are strongly pH dependent, being greater in the pH range 5–7 and almost negligible above pH 8 and below pH 3.5. A similar behavior is also observed for the other systems under investigation. This result is in good accord with the potentiometric study of these systems, which has shown that large amounts of the 1:1 receptor–substrate adducts are formed from slight alkaline to acidic pHs, *i.e.*, in the pH region where highly protonated species of the receptors and anionic species of ADP or ATP are simultaneously present in solution.

Both potentiometric and ^{31}P NMR data confirm the important roles played by electrostatic forces and hydrogen bonding in this kind of interaction. The formation of the adducts takes place mainly *via* the charged polyphosphate chains of ADP or ATP, which allows the formation of multiple electrostatic and hydrogen bond interactions with the polyammonium macrocycles. In the case of ATP, however, one of the phosphate groups (P_α) does not interact with the polyammonium sites.

Comparing nucleotide and inorganic phosphate anion ($P_2O_7^{4-}$ and $P_3O_{10}^{5-}$) binding, the data in Table 2 seem to show that pyrophosphate gives weaker interactions with **L1** and **L2** than ADP, which contains a diphosphate chain similar to that of $P_2O_7^{4-}$. Similarly, the stability of the triphosphate adducts with **L1** is lower than the ATP one. On the other hand, such comparisons may sometimes be misleading in the analysis of selectivity in anion coordination, due to the different protonation degrees of different substrates at the same pH values. An appropriate way to visualize selectivity in anion coordination is to consider a ternary system containing the ligands and the two anions in equimolar concentrations and calculate the overall percentages of the two complexed anions over a wide pH range.[11b] Plots of the percentages *vs.* pH produce species distribution diagrams from which the binding ability of the receptor can be interpreted in terms of selectivity. In Fig. 5 are reported similar diagrams calculated for the ATP–$P_3O_{10}^{5-}$–**L1** and ADP–$P_2O_7^{4-}$–**L1** systems. As can be seen, the formation of nucleotide adducts with **L1** prevails over a wide pH range, *i.e.*, nucleotide anions are selectively bound with respect to their inorganic counterparts (all over the pH range investigated in the case of the ATP–$P_3O_{10}^{5-}$–**L1** system, from pH 8 to acidic pH in the case of the ADP–$P_2O_7^{4-}$–**L1** system).

These experimental observations suggest that, besides electrostatic interactions between the phosphate chain and the polyammonium macrocycles, other effects (hydrogen bond interactions between adenine nitrogens and/or hydroxy groups of nucleotides and polyammonium functions, hydrophobic and/or π-stacking interactions between the heteroaromatic moieties and cation–π system interactions between the charged ammonium group and adenine) may contribute to the complex stability.

Indeed, ^{1}H NMR spectra of these systems provide unambiguous evidence for the participation of π-stacking

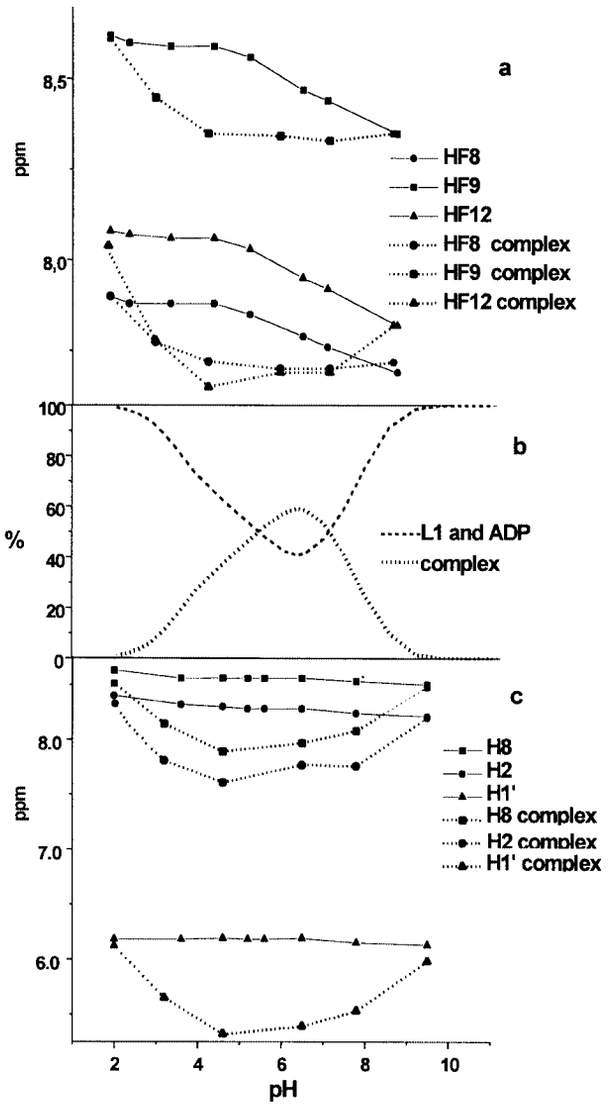


Fig. 6 (a) Experimental ^{1}H chemical shifts for the aromatic protons of free **L1** (——) and of **L1** in the presence of ADP (·····); (b) overall percentages of free ADP, **L1** and ADP–**L1** complexes; (c) experimental ^{1}H chemical shifts for the H2, H8 and H1′ protons of free ADP (——) and of ADP in the presence of **L1** (·····) ([**L1**] = [ADP] = 1×10^{-2} mol dm^{-3}, 298 K).

interactions in the stabilization of the adduct species with **L1** and **L2** (for the labeling, see Scheme 1). For both ligands, throughout the pH ranges in which interaction occurs, significant upfield displacements are observed for the resonances of the adenine protons H2, H8 and for the anomeric proton H1′ of the nucleotides as well as for the signals of phenanthroline (HF8, HF9 and HF12). Minor shifts are observed for the benzylic protons F6 (less than 0.3 ppm) and for the other protons of the aliphatic chains (less than 0.2 ppm). Fig. 6 shows the ^{1}H chemical shifts for the phenanthroline hydrogens of **L1** in the absence and in the presence of ADP (Fig. 6a) and for the hydrogens H2, H8 and H1′ of ADP (Fig. 6c) in the absence and in the presence of **L1** (1 : 1 molar ratio). A distribution diagram displaying the overall percentages of complexed ADP is also reported (Fig. 6b). As previously observed for the ^{31}P resonances of the phosphate chains, the ^{1}H NMR displacements are strongly pH dependent, being larger at neutral or slight acidic pHs, where the largest extent of complexation occurs (Fig. 6). It is interesting to remark that, once more, these differences are remarkably reduced at strongly acidic or alkaline pHs, where the interaction vanishes. Similar considerations can be made also for ATP complexation by **L1** (Fig. S5, supplementary material).† Table 4 reports the complexation induced chemical shift (CIS) for the interaction of **L1** and **L2** with ADP and ATP. It is to be noted that the CIS values for ATP and ADP complexation are remarkably high (more than 1 ppm for adenosine protons of ADP complexed by **L1** or **L2**), if compared with the CIS value found for ATP or ADP complexation by polyammonium macrocycles containing phenylene spacers, such as **2** in Scheme 1.[3] This can be attributed to the insertion into the macrocyclic framework of a large heteroaromatic system, phenanthroline, which can give strong π-stacking interactions with the adenine moiety of nucleotides. Furthermore, high CIS values may be indicative of a partial inclusion of the substrate inside the macrocyclic cavity, as already observed in the case of the receptors **2**[3] and **3**.[5c] A partial inclusion may allow for a simultaneous involvement of electrostatic and π-stacking interactions in the stabilization of the adducts and, additionally, adenine nitrogens and/or hydroxy groups of the ribose subunits could be properly disposed to give hydrogen bonds with the polyammonium groups of the receptor. A proposed mode of interaction between ATP and H_4**L1**$^{4+}$ is reported in Fig. 7.

Table 4 also shows that the CIS values of the adenine protons of both ATP and ADP are larger for the interaction with macrocycle **L1**, which presents a larger cavity than **L2**. Furthermore, for the same macrocycle, the CIS values of adenine protons of

Table 4 ^{1}H NMR shifts (δ, ppm) for the **L1** and **L2** adducts with ATP and ADP and complexation-induced ^{1}H NMR chemical shifts (CIS, ppm) for selected protons, measured in D_2O solution at pH 4.5 (systems ATP–**L1** and ADP–**L1**) and 4.8 (systems ATP–**L2** and ADP–**L2**), 298 K

		HF8	HF9	HF12	HF6	H8	H2	H1
L1	δ	7.69[a]	8.21	7.47	4.64			
ATP	δ					7.61	7.31	5.02
	CIS	−0.19[b]	−0.36	−0.52	−0.12	−0.91	−0.95	−1.12
L2	δ	7.71	8.27	7.55	4.579			
ATP	δ					7.98	7.77	5.46
	CIS	−0.13	−0.31	−0.46	−0.03	−0.52	−0.46	−0.66
L1	δ	7.48	7.91	7.05	4.44			
ADP	δ					7.2	6.58	4.04
	CIS	−0.44	−0.74	−1.01	−0.25	−1.36	−1.73	−2.15
L2	δ[a]	7.6	8.23	7.49	4.61		7.34	4.7
ADP	δ					7.50		
	CIS	−0.14	−0.35	−0.52	−0.10	−1.06	−0.97	−1.49

[a] From measurements in D_2O solution at pH 4.5 (systems ATP-**L1** and ADP–**L1**) and 4.8 (systems ATP–**L2** and ADP–**L2**), 298 K, with a receptor–substrate 1 : 1 molar ratio. In these conditions the complexation degrees are 40% (system ADP–**L1**), 72% (ATP–**L1**), 49% (ADP–**L2**) and 53% (ATP–**L2**). [b] CIS (for 100% complexation) calculated based on equilibrium constants from Table 2.

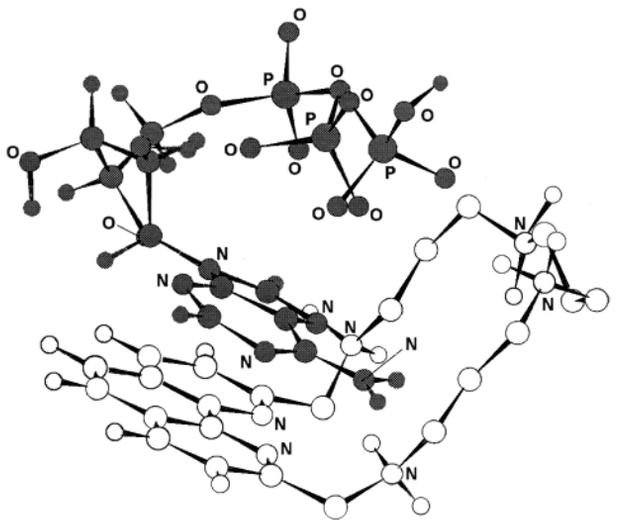


Fig. 7 Schematic representation for the interaction of tetraprotonated receptor **L1** with HATP in the $[H_5\mathbf{L1}ATP]^+$ adduct.

ADP are remarkably higher than those of ATP. These data suggest that the extent of the inclusion of nucleotides inside the macrocyclic cavity depends on the dimensions of both the receptor cavity and the nucleotide, *i.e.*, the smaller nucleotide, ADP, is better accommodated into the larger cavity of **L1**.

Concluding remarks

Protonation of **L1** and **L2** takes place on the amine groups of the aliphatic chain, while the heteroaromatic moieties are not involved in protonation. In the case of **L1**, the aromatic nitrogens protonate only at strongly acidic pHs. Therefore, **L1** and **L2** present in their protonated species a molecular organization which enables their multipoint binding with anionic forms of nucleotides. The protonated macrocycles contain two main binding sites: i) the polyammonium groups, which can form salt bridges with the phosphate chains and, at the same time, charge–dipole interactions with the π-system of nucleotides, ii) the phenanthroline unit, which can give π-stacking and hydrophobic interactions with the adenine moiety of substrates.

Electrostatic and hydrogen bonding interactions between the polyammonium binding sites and the anionic phosphate chain give the most important contribution to the stability of the complexes. On the other hand, both ADP and ATP give a stronger interaction with the protonated receptors than their inorganic counterparts, namely di- and triphosphate, indicating that the adenosine moiety also contributes to complex stabilization. In particular, the presence of a large heteroaromatic unit as an integral part of the macrocyclic frameworks leads to a marked π-stacking interaction with the adenine moiety of nucleotides. The extent of this mode of interaction seems to be modulated by the dimensions of the macrocyclic cavity and the length of the phosphate chain of nucleotides. Larger staking effects are observed in the case of the interaction of macrocycle **L1** with ADP, suggesting a better "fitting" of the smaller ADP anion inside the larger macrocyclic cavity of this polyammonium receptor.

Experimental

Synthesis

Macrocycle **L2**,[21a] 1,5,9,13-tetratosyl-1,5,9,13-tetraazatridecane (**4**) and 2,9-bis(bromomethyl)-1,10-phenanthroline (**5**) were prepared as previously described.[30]

2,6,10,14-Tetratosyl-2,6,10,14-tetraaza[15](2,9)cyclo(1,10)-phenanthrolinophane (6). A solution of **5** (2.3 g, 6.2 mmol) in dry CH_3CN (200 cm^3) was added over a period of 6 h to a refluxing and vigorously stirred suspension of **4** (5 g, 6.2 mmol) and K_2CO_3 (8.6 g, 0.062 mol) in CH_3CN (400 cm^3). After the addition was completed, the solution was refluxed for an additional two hours. The resulting suspension was filtered out and the solution was vacuum evaporated to give a crude oil. The product was purified by chromatography on neutral alumina using CH_2Cl_2–ethyl acetate (100 : 4) as eluting solvent, affording product **6** as a white solid. Yield: 1.6 g (27%). Elemental anal., found: C, 62.3; H, 5.6; N, 5.6. Calc. for $C_{51}H_{56}N_6S_4O_8$: C, 62.43; H, 5.75; N, 5.71%.

2,6,10,14-Tetraaza[15](2,9)cyclo(1,10)phenanthrolinophane tetrahydrobromide (L1·4HBr). **6** (2.2 g, 2.19 mmol) and phenol (29 g, 0.308 mol) were dissolved in 33% HBr–CH_3COOH (240 cm^3). The reaction mixture was kept under stirring at 90 °C for 22 hours until a precipitate was formed. The solid was filtered out and washed several times with CH_2Cl_2. The tetrahydrobromide salt was recrystallized from an EtOH–water 2 : 1 mixture, yield 1.5 g (96%). Elemental anal., found: C, 38.5; H, 5.0; N, 11.6. Calc. for $C_{23}H_{32}N_6$·4HBr: C, 38.57; H, 5.07; N, 11.73%. ^{1}H NMR (D_2O, pH = 4): δ(ppm): 2.24 (m, 4H), 2.37 (m, 4H), 3.36 (t, 4H), 3.42 (t, 4H), 3.61 (t, 4H), 4.76 (s, 4H), 7.88 (d, 2H), 8.06 (s, 2H), 8.59 (d, 2H); ^{13}C NMR (D_2O, pH = 4): δ(ppm): 22.1, 23.6, 44.4, 44.6, 46.1, 53.3, 124.5, 128.3, 130.1, 140.2, 145.3, 152.3.

EMF Measurements

The protonation constants of **L1** and the formation constants of the anion complexes with **L1** and **L2** were determined by means of potentiometric measurements (pH = −log [H^+]), carried out in 0.1 mol dm^{-3} NMe_4Cl at 298.1 ± 0.1 K, in the pH range 2.5–11, using the equipment that has already been described.[31] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by Gran's method[32] which allows us to determine the standard potential E^o, and the ionic product of water (pK_w = 13.83 ± 0.01). Ligand concentration was about 1×10^{-3} M, while substrate concentration was in the range 5×10^{-3}–5×10^{-4} M. At least three measurements (about 100 experimental points each one) were performed for each system. The computer program HYPERQUAD[29] was used to calculate the protonation constants and the stability constants of the ATP and ADP complexes from EMF data. The titration curves for each system were treated either as a single set or as separate entities without significant variations in the values of the basicity constants.

NMR Spectroscopy

200.0 MHz ^{1}H and 50.32 MHz ^{13}C NMR spectra in D_2O solutions at different pH values were recorded at 298 K in a Bruker AC-200 spectrometer. In ^{1}H NMR spectra peak positions are reported relative to HOD at 4.75 ppm. Dioxane was used as reference standard in ^{13}C NMR spectra (δ = 67.4 ppm). ^{1}H–^{1}H and ^{1}H–^{13}C 2D correlation experiments were performed to assign the signals. Small amounts of 0.01 mol dm^{-3} NaOD or DCl solutions were added to a solution of **L1**·4HBr or **L2**·4HBr to adjust the pD. The pH was calculated from the measured pD values using the following relationship:[33] pH = pD − 0.40. The ^{31}P NMR spectra were recorded at 81.01 MHz in a Bruker AC-200 spectrometer. Chemical shifts are relative to an external reference of 85% H_3PO_4.

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