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Protonated macrocyclic Zn(II) complexes as polyfunctional receptors for ATP †

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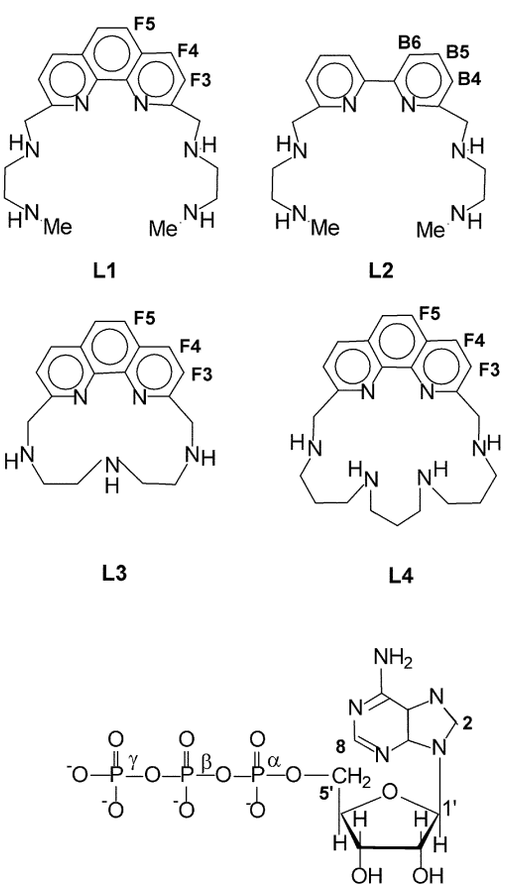
Zn(II) coordination by the phenanthroline-containing macrocycle 2,6,10,14-tetraaza[15](2,9)cyclo(1,10)-phenanthrolinophane (**L4**) has been studied by means of potentiometric measurements in aqueous solution. Its coordination properties have been compared with those of other phenanthroline- or dipyridine-containing open-chain (**L1**, **L2**) or cyclic (**L3**) ligands. ATP binding to the Zn(II) complexes with **L1**–**L4** has been examined by means of potentiometric and ^{1}H and ^{31}P NMR measurements in aqueous solution. In the ATP adducts with the $[ZnL]^{2+}$ complexes, the nucleotide interacts with the metal *via* the terminal P_{γ} phosphate group; the equilibrium constants for the addition of ATP to the complexes depend on the number and arrangement of the nitrogen donors coordinated to the metal ion. Protonation of the $[ZnL]^{2+}$ complexes gives $[ZnH_xL]^{(x+2)+}$ species, which contain two binding sites for the phosphate chain of ATP; while the P_{γ} phosphate group gives a coordination bond with the metal, the P_{β} one interacts *via* $P–O^{-} \cdots H–N^{+}$ salt bridges with the ammonium functions of the complex. In consequence, protonated complexes are better ATP receptors than the simple $[ZnL]^{2+}$ species and even than the protonated forms $[H_xL]^{x+}$ of the ligands, due to the synergetic action of the metal ion and of ammonium functions in ATP binding.

Among biologically important anions, a great deal of interest has been focused on the design of receptors for nucleotide polyphosphates.[1] In particular adenosine triphosphate is one of the basic components in bioenergetic processes of living organisms, its polyphosphate chain being the centers for chemical energy storage and transfer.

Two different classes of receptors, polyammonium cations and metal complexes with simple ligands, have been developed as potential selective binders for nucleotide anions. In the first approach, protonated polyamines or quaternary ammonium cations have been used to bind to nucleotides *via* electrostatic interactions between the cationic binding sites (ammonium groups) of the receptor and the negatively charged polyphosphate chain.[1–14] Among synthetic receptors, polyamine macrocycles are the most versatile receptors for nucleotides, since their protonation may occur readily in aqueous solutions, even at neutral pH, yielding protonated species which are well suited to the study of anion coordination. Selective coordination, however, requires the incorporation of sites for multiple interactions with the substrates. To achieve a better recognition of nucleotides, receptors may contain other binding sites capable of interactions with the sugar moiety[15–17] or the nucleic base,[2,4,7,9,12,18–21] 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. To this purpose, the binding properties of macrocyclic ligands containing both a polyammonium chain, as anion binding site, and an aromatic or heteroaromatic unit, able to give stacking interactions with the nucleobase, have been recently studied by several groups.[2,4,7,12]

In the second approach, metal complexes bind to nucleotides through the formation of covalent bonds between the metal ion and the phosphate chain of the nucleotides.[3c,d,22–25] Stacking interactions between the nucleobase and aromatic units of the ligand may reinforce the stability of the resulting ternary complexes. The simplest class of these receptors are transition metal complexes with 2,2′-dipyridine and 1,10-phenanthroline, earlier investigated by Sigel[22] and Cini.[25]

We have recently reported on the synthesis of a series of phenanthroline- and dipyridine-containing open chain or macrocyclic polyamine,[12a,26] such as **L1**–**L4** (Scheme 1).



Scheme 1 Ligands and ATP drawing with atom labelling used in NMR experiments.

† Electronic supplementary information (ESI) available: Protonation constants of ligands **L1**–**L4**, formation constants of the ATP complexes with ligands **L1**–**L4** and the crystal packing of [ZnH**L4**Br]Br(ClO_4). See http://www.rsc.org/suppdata/dt/b3/b303264g/

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These ligands can form polyprotonated species in aqueous solution able to bind phosphate nucleotides through the formation of salt bridges between the anionic phosphate chain of the nucleotides and the polyammonium groups of the ligand and stacking interactions between the nucleobase and dipyridine or phenanthroline.[12] Phenanthroline- and dipyridine-containing polyamines form also stable Zn(II) complexes in aqueous solutions.[27] The rather high stability of the complexes is mainly due to the heteroaromatic nitrogens, which can offer an optimal binding site for the metal ion. At the same time, the rigidity of the heteroaromatic units generally does not allow the involvement of all the aliphatic amine groups in metal binding. Some amine groups are not bound, or weakly bound, to the metal, and can easily bind acidic protons in aqueous solution, giving protonated metal complexes.[27] This would allow one, in principle, to combine within the same receptor the special binding features toward nucleotides of both polyammonium cations and metal complexes. In other words, protonated metal complexes with these ligands could behave as multifunctional receptors for nucleotides, through the formation of coordination bonds with the metal ion, salt bridges between the protonated amines and the anionic phosphate groups and, finally, stacking interaction with the dipyridine or phenanthroline moieties. To verify this hypothesis, we have investigated the binding properties of the Zn(II) complexes with **L1**–**L4** toward ATP by means of potentiometric and ^{1}H and ^{31}P NMR measurements. The Zn(II) binding properties of **L4**, not previously reported, are also analyzed and compared with those of **L1**–**L3**.

Results and discussion

Crystal structure of [ZnHL4Br]Br(ClO_4)

The molecular structure consists of protonated mononuclear complex cations $[ZnH\mathbf{L4}Br]^{2+}$, bromide and perchlorate anions. In $[ZnH\mathbf{L4}Br]^{2+}$ the metal is coordinated to the two phenanthroline nitrogens N(1) and N(2), two adjacent amine groups of the aliphatic chain, N(3) and N(4), and an exogenous bromide anion (Br(1)) (Fig. 1). The coordination geometry can be best described as a slightly distorted trigonal bipyramid, N(2), N(4) and Br(1) defining the equatorial plane. N(1) and N(3) occupy the apical position, coordinated at a longer distance than N(2) and N(4) (2.370(5) and 2.276(5) Å for N(1) and N(3) *vs.* 2.047(5) and 2.072(5) Å for N(2) and N(4), respectively, Table 1).

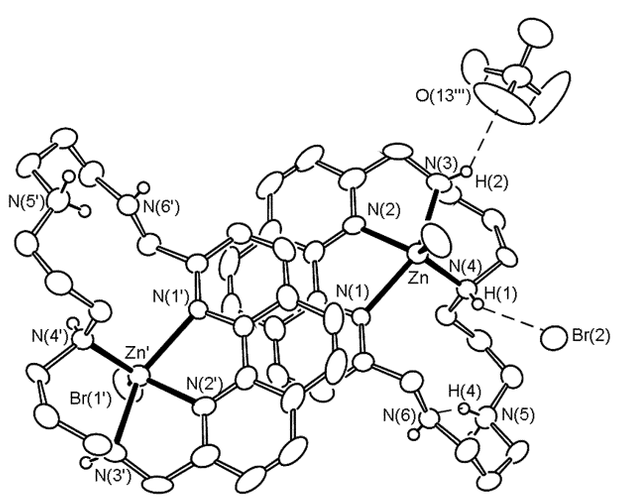

Fig. 1 ORTEP drawing of two $[ZnH\mathbf{L4}Br]^{2+}$ symmetry related complexes ($1 - x$, $-y$, $-z$), associated *via* π-stacking interactions between the two phenanthroline units. Hydrogen bonding interactions between one macrocyclic complex and bromide and perchlorate anions are also shown.

Table 1 Bond lengths [Å] and angles [°] for the metal coordination environment in the of $[Zn\mathbf{L4}H(Br)]^{2+}$ cation

Zn–N(2)	2.047(5)
Zn–N(4)	2.072(5)
Zn–N(3)	2.276(5)
Zn–Br(1)	2.3541(14)
Zn–N(1)	2.370(5)
N(2)–Zn–N(4)	126.6(2)
N(2)–Zn–N(3)	76.4(2)
N(4)–Zn–N(3)	90.5(2)
N(2)–Zn–Br(1)	115.20(16)
N(4)–Zn–Br(1)	118.17(16)
N(3)–Zn–Br(1)	102.54(19)
N(2)–Zn–N(1)	74.98(17)
N(4)–Zn–N(1)	97.96(19)
N(3)–Zn–N(1)	149.4(2)

The N(5) and N(6) aliphatic amine groups are not coordinated, with the acidic proton localized on N(5). These two nitrogens interact each other *via* a strong H-bond (N(5) ··· N(6) 2.770(8) Å, H(4) ··· N(6) 1.97(7) Å).

The overall ligand conformation can be best visualized by considering the two mean planes defined respectively by the phenanthroline unit and the aliphatic polyamine chain. The macrocycle is bent along the ideal line joining N(3) and the benzylic carbon atom adjacent to N(6), giving rise to a folded conformation with a dihedral angle of 114.7(1)° between the planes defined, respectively, by the aromatic and aliphatic ligand moieties.

As shown in Fig. 1, each $[ZnH\mathbf{L4}Br]^{2+}$ cation is coupled with a second complex, symmetry related by an inversion centre, *via* π-stacking between the two phenanthroline moieties. This interaction is characterized by a parallel disposition of the phenanthroline units with an interplanar distance of 3.47(1) Å. Only two adjacent aromatic rings of each phenanthroline unit, however, are involved in the π-stacking interaction. Hydrogen bond interactions are also found between the amine groups N(4) and N(3) and the bromide (N(4)–H(1) ··· Br(2) 2.85(5) Å) and perchlorate (N(3)–H(2) ··· O(13''') 2.23(6) Å, symmetry operation $2 - x$, $1 - y$, $-z$ for O(13''')) anions. Interestingly, inspection of the crystal packing (Fig. S1, Supplementary Information†) reveals that the Br(2) anion also interacts *via* hydrogen bonding with the N(5) nitrogen of a second symmetry related Zn(II) complex (symmetry operation $x + 1$, y, z) (N(5)–H(3) ··· Br(2) 2.32(8) Å). In consequence, the crystal lattice is composed of infinite pillars of $[ZnH\mathbf{L4}Br]^{2+}$ units, associated *via* hydrogen bonding with the Br(2) anion and π-stacking interactions between the phenanthroline moieties.

Zn(II) complexation by ligands L1–L4

Zn(II) coordination by **L4** was studied by means of potentiometric measurements in 0.1 M NMe_4NO_3 aqueous solution at 298.1 K. The coordination features of **L1**, **L2** and **L3** have been previously studied in NMe_4Cl. Unfortunately, the Zn(II) complexes with **L4** are insoluble in this ionic medium, and, therefore, the stability constants for the **L1**, **L2** and **L3** complexes have been redetermined in NMe_4NO_3. Their values do not differ significantly in the two ionic media. The results of this potentiometric study are presented in Table 2.

Considering the stability of the Zn(II) complexes, the most interesting finding in Table 2 is the much lower stability of the $[Zn\mathbf{L4}]^{2+}$ complex with respect to the **L1** and **L2** ones, which contain the same number of nitrogen donors available for metal coordination. This seems to be in contrast with the generally higher stability of macrocyclic complexes with respect to open chain ligands. The $[Zn\mathbf{L4}]^{2+}$ complex also displays a higher tendency to protonate in aqueous solution to give up to a triprotonated species, $[Zn\mathbf{L4}H_3]^{5+}$ (Fig. 2a). In the case of the $[Zn\mathbf{L1}]^{2+}$ and the $[Zn\mathbf{L2}]^{2+}$ complexes it was found that the

Table 2 Stability constants (log K) of the Zn(II) complexes with ligands **L1**–**L4** (NMe_4NO_3 0.1 M, 298.1 K)

Reaction	L1	L2	L3	L4
$ZnLH_2^{4+} + H^+ \rightleftharpoons ZnLH_3^{5+}$				6.01(4)
$ZnLH^{3+} + H^+ \rightleftharpoons ZnLH_2^{4+}$	4.40(4)	4.39(4)		7.15(8)
$ZnL^{2+} + H^+ \rightleftharpoons ZnLH^{3+}$	4.51(3)	4.49(4)		8.41(3)
$Zn^{2+} + L \rightleftharpoons ZnL^{2+}$	16.37(2)	16.64(3)	16.30(2)	9.83(2)
$ZnL^{2+} + OH^- \rightleftharpoons ZnL(OH)^+$	3.31(4)		4.48(6)	3.90(5)
$ZnL(OH)^+ + OH^- \rightleftharpoons ZnL(OH)_2$			2.89(8)	2.47(7)

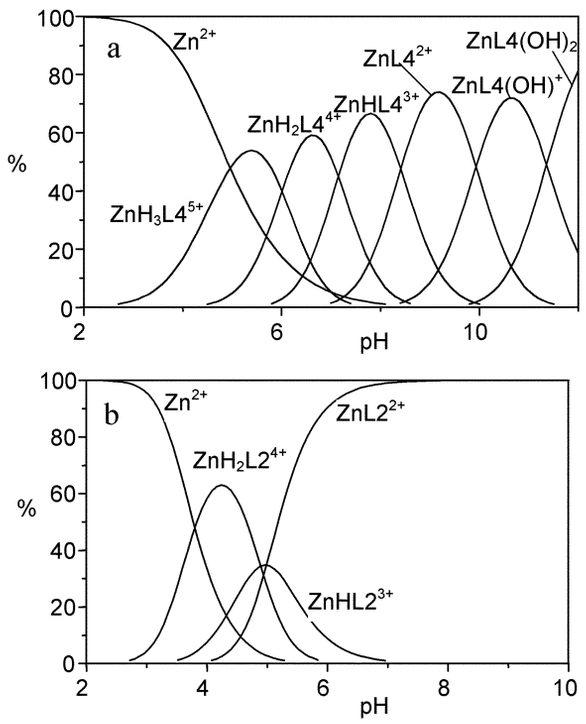

Fig. 2 Species distribution diagrams for the systems Zn^{2+}/**L4** (a) and Zn^{2+}/**L2** (b) ([**L2**] = [**L4**] = [Zn^{2+}] = 1 × 10^{-3}, NMe_4NO_3 0.1 M, 298.1 K).

metal ion is encapsulated inside the cleft delimited by the heteroaromatic unit and the two ethylendiamine chains, hexacoordinated by all the nitrogen donors.[12b] Both complexes, however, can form a mono- and a diprotonated species in slightly acidic aqueous solutions (Fig. 2b). The crystal structure of the $[ZnL2]^{2+}$ cation shows that two nitrogens, the terminal methylated amine groups, are only weakly involved in metal coordination, with Zn–N distances of *ca.* 2.3 Å.[12b] Most likely, protonation of $[ZnL1]^{2+}$ and $[ZnL2]^{2+}$ to give the $[ZnLH]^{3+}$ and $[ZnLH_2]^{4+}$ species (**L** = **L1** or **L2**) of the complex implies protonation and simultaneous detachment from the metal of the terminal amine groups.

The much lower stability of the $[ZnL4]^{2+}$ complex can be simply ascribed to the replacement of the ethylenic chains linking the amine groups in **L1** and **L2** with propilenic ones. It is well known, in fact, that open-chain or macrocyclic tetraamines containing propilenic chains linking the nitrogen donors form less stable Zn(II) complexes than the corresponding ligands with ethylenic chains (for instance log K = 12.14 for the formation of the Zn(II) complex with 1,4,7,10-tetraazadecane,[28] while log K = 9.30 in the case of the Zn(II) complex with 1,5,9,13-tetraazatridecane).[29] As a matter of fact, the crystal structure of the $[ZnHL4Br]^{2+}$ cation shows the metal coordinated by only four nitrogens of the macrocycle [N(1), N(2), N(3) and N(4)] and by one bromide ion in a strongly distorted trigonal bipyramidal coordination environment, two of the nitrogens [N(1) and N(3)] being just weakly bound to the metal. N(6) and the protonated nitrogen N(5) are not coordinated. In consequence, the ligand donors do not saturate the metal coordination sphere, leaving accessible binding sites at the metal.

Both the solution and solid state data account for a relatively low number of Zn(II)-bound amine groups, which can easily protonate in aqueous solutions; actually Fig. 2a shows that protonated metal complexes are present in solution from slightly alkaline to acidic pH values. Therefore, the protonated Zn(II) complexes possess two potential sites for cooperative anion binding, *i.e.*, the metal ion and the charged ammonium groups. The $[ZnL1]^{2+}$ and the $[ZnL2]^{2+}$ complexes seem to be less promising anion receptors, due to the saturated coordination sphere of the metal, which would reduce their binding ability toward ATP. Both complexes, however, can form mono- and diprotonated species at slightly acidic pH values. These protonated complexes would contain both an unsaturated Zn(II) ion and protonated amine groups and, therefore, they could also act as polyfunctional receptor for anionic species. Finally, the $[ZnL3]^{2+}$ complex does not form any protonated species in aqueous solution. On the other hand, the crystal structure of the $[ZnL3(H_2O)]^{2+}$ complex[27a] shows that the metal coordination sphere is not saturated by the ligand donors, both the benzylic nitrogens being very weakly interacting with the metal (Zn ··· N distances 2.4–2.5 Å).[27a] The absence of protonated forms is probably due to the small macrocyclic cavity, which does not allow the simultaneous binding in close proximity of the metal cation and acidic protons. Obviously, the binding properties of this complex cannot be affected by protonation. In $[ZnL3]^{2+}$ only the metal ion can act as binding site for anionic species and this complex can be used as a "reference" compound in the study of the effects of complex protonation in anion binding.

ATP binding by the Zn(II) complexes with ligands L1–L4

ATP complexation by the Zn(II) complexes with ligands **L1**–**L4** was studied by means of potentiometric, ^1H and ^{31}P NMR measurements in aqueous solutions; the species formed and the corresponding overall and partial stability constants, potentiometrically determined, are reported in Table 3. The data in Table 3 outline two main features. First, the equilibrium constants for the addition of ATP^{4-} to the $[ZnL]^{2+}$ complexes increase in the order **L1** < **L2** < **L3** < **L4**. The higher ability of the $[ZnL3]^{2+}$ and $[ZnL4]^{2+}$ complexes in ATP binding can be simply related to the metal coordination environment less saturated by ligand donors in the **L3** and **L4** complexes; as previously discussed, while in $[ZnL1]^{2+}$ and $[ZnL2]^{2+}$ the metal is encapsulated in the ligand cleft and shielded by the ligand donors, in $[ZnL3]^{2+}$ and $[ZnL4]^{2+}$ the Zn(II) ion displays a more "open" coordination sphere, being tri- or, at most, tetracoordinated by the ligand nitrogens. Other factors, however, such as steric hindrance of the ligands or the presence of π-stacking interactions between the receptor heteroaromatic moieties and the ATP adenine unit can contribute to the stability of the ternary complexes. Second, the nucleotide forms 1 : 1 complexes with protonated forms of the Zn(II) complexes of **L1**, **L2** and **L4**. The addition constants of ATP to the protonated Zn(II) complexes, $[ZnLH_n]^{(n+2)+}$, are larger than those found for ATP binding by the corresponding unprotonated $[ZnL]^{2+}$ complexes and increase with the complex protonation degree. For instance, the binding constants of ATP^{4-} to the Zn(II) complexes with **L4** progressively increase, passing from $[ZnL4]^{2+}$ (log K = 5.18) to $[ZnHL4]^{3+}$ (Log K = 6.16) and

Table 3 Stability constants (log *K*) of the ternary complexes $[ZnLH_nA]^{(n-2)+}$ (A = ATP, **L** = **L1**–**L4**) (NMe_4NO_3 0.1 M, 298.1 K)

Reaction	L1	L2	L3	L4
$L + Zn^{2+} + A^{4-} \rightleftharpoons ZnLA^{2-}$	19.62 (8)	20.38 (8)	20.65 (2)	15.01 (6)
$L + Zn^{2+} + H^{+} + A^{4-} \rightleftharpoons ZnHLA^{-}$	26.32 (7)	27.16 (8)	26.61 (2)	24.40 (5)
$L + Zn^{2+} + 2H^{+} + A^{4-} \rightleftharpoons ZnH_2LA$	32.63 (5)	33.00 (8)		33.13 (6)
$L + Zn^{2+} + 3H^{+} + A^{4-} \rightleftharpoons ZnH_3LA^{+}$	38.40 (4)	38.47 (4)		39.90 (3)
$L + Zn^{2+} + A^{4-} + 4H^{+} \rightleftharpoons ZnH_4LA^{2+}$				45.90 (3)
$L + Zn^{2+} + A^{4-} + H_2O \rightleftharpoons ZnLA(OH)^{3-} + H^{+}$	8.61 (5)		10.82 (3)	3.89 (5)
$ZnL^{2+} + A^{4-} \rightleftharpoons ZnLA^{2-}$	3.25	3.74	4.35	5.18
$ZnL^{2+} + HA^{3-} \rightleftharpoons ZnHLA^{-}$	2.92	3.69	3.48	
$ZnLH^{3+} + A^{4-} \rightleftharpoons ZnHLA^{-}$				6.16
$ZnHL^{3+} + HA^{3-} \rightleftharpoons ZnH_2LA$	4.72	5.04		
$ZnH_2L^{4+} + A^{4-} \rightleftharpoons ZnH_2LA$				7.74
$ZnH_2L^{4+} + HA^{3-} \rightleftharpoons ZnH_3LA^{+}$	6.15	6.12		7.68
$ZnH_3L^{5+} + HA^{3-} \rightleftharpoons ZnLAH_4^{2+}$				8.67
$ZnL(OH)^{+} + A^{4-} \rightleftharpoons ZnLA(OH)^{3-}$	2.76		3.86	3.41

$[ZnH_2L4]^{4+}$ (log $K = 7.74$). Similar enhancements are also found in the case of **L1** or **L2** for addition of $HATP^{3-}$ to $[ZnHL]^{3+}$ and $[ZnH_2L]^{4+}$. These observations suggest that, beside coordination bonds between Zn(II) and the phosphate groups, the formation of salt bridges between the ammonium functions of the receptor and the anionic phosphate chain of ATP also contributes to complex stability.

Finally, hydroxylated forms of the ATP complexes are also observed at alkaline pHs. The addition constants of the nucleotide to the $[ZnLOH]^{+}$ complexes, however, are lower than those found for ATP binding by the $[ZnL]^{2+}$ complexes, as expected considering the lower positive charge on the metal ion.

To shed further light on the structural characteristics of these complexes, we decided to perform ^{31}P measurements on solutions containing the $[ZnL]^{2+}$ complexes and the nucleotide at different pH values. Figs. 3 and 4 display the pH dependence of

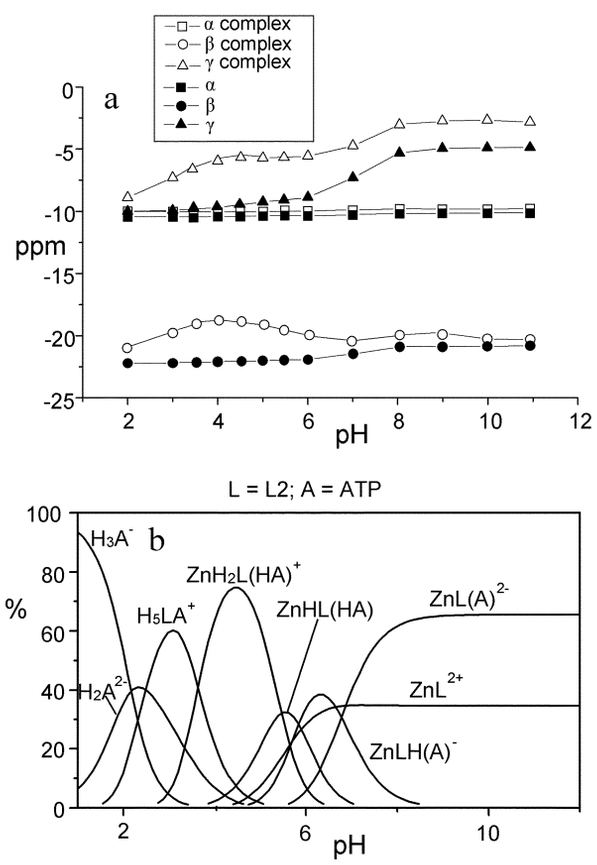

Fig. 3 (a) pH dependence of the ^{31}P NMR chemical shifts of ATP in the absence and in the presence of the Zn(II) complex with **L2**. (b) Species distribution diagram for the systems Zn^{2+}/**L2**/ATP ([**L2**] = [Zn(II)] = [ATP] = 1×10^{-3} M, NMe_4NO_3 0.1 M, 298.1 K).

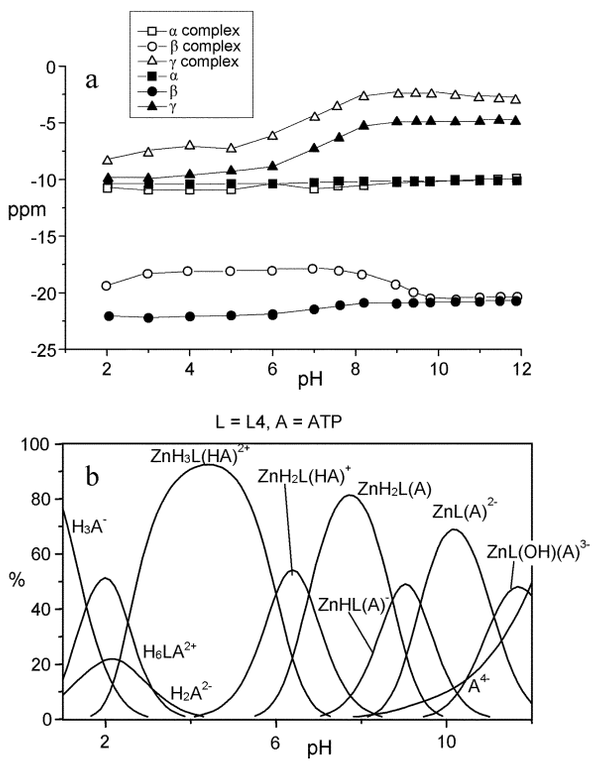

Fig. 4 (a) pH dependence of the ^{31}P NMR chemical shifts of ATP in the absence and in the presence of the Zn(II) complex with **L4**. (b) Species distribution diagram for the systems Zn^{2+}/**L4**/ATP ([**L4**] = [Zn(II)] = [ATP] = 1×10^{-3} M, NMe_4NO_3 0.1 M, 298.1 K).

the ^{31}P ATP signals in presence and in absence of the $[ZnL2]^{2+}$ and $[ZnL4]^{2+}$complexes, compared with the corresponding distribution diagrams of the ternary complexes. Similarly to ATP coordination by polyammonium macrocycles, ATP binding by $[ZnL2]^{2+}$ and $[ZnL4]^{2+}$ gives significant variation in the ^{31}P chemical shifts of the P_β and P_γ resonances of ATP, while the chemical shift of P_α is not influenced by the interaction with the metallo-receptors. In the case of the **L2** complex, ATP binding at alkaline pHs to yield the $[ZnL2(ATP)]^{2-}$ and $[ZnL2(HATP)]^{-}$ complexes produces a remarkable downfield shift of signal of the P_γ terminal phosphate group. In this pH range the P_β resonance is almost unaffected by the interaction with the Zn(II) complex. Below pH 7, the formation of the $[ZnHL2(HATP)]$ and $[ZnH_2L2(HATP)]^{+}$ complexes, which contain positively charged ammonium functions on the ligand, is accompanied by a progressive downfield shift of the P_β resonance. The ^{31}P signals of ATP in the presence of the **L1** Zn(II) complex display shifts almost equal to those found in the case of **L2**. A similar behavior is also observed in the case of ATP

Table 4 ^{1}H NMR shifts [δ (ppm)] for the **L1**–**L4** adducts with ATP and complexation-induced ^{1}H NMR chemical shifts [CIS (ppm)] for selected protons, measured in D_2O solution at pH 5 (systems ATP–**L1**, ATP–**L2** and ATP–**L4**) and 7 (system ATP–**L3**) at 298 K, with a receptor : substrate 1 : 1 molar ratio. In these conditions the complexation degrees are 61% (system ATP–**L1**), 70% (ATP–**L2**), 82% (ATP–**L3**) and 97% (ATP–**L4**). CIS (for 100% complexation) calculated based on equilibrium constants from Table 3

	HF3	HF4	HF6	HB4	HB5	HB6	H8	H2	H1′
L1	7.84	8.6	7.76						
ATP							8.21	7.95	5.79
CIS	−0.05	−0.175	−0.730				−0.51	−0.42	−0.56
L2				7.46	7.95	8.11			
ATP							8.19	7.99	5.70
CIS				−0.11	−0.16	−0.21	−0.51	−0.30	−0.57
L3	7.51	8.23	7.59						
ATP							8.08	7.95	5.58
CIS	−0.37	−0.49	−0.57				−0.54	−0.34	−0.66
L4	7.57	8.3	7.7						
ATP							8.24	8.01	5.82
CIS	−0.16	−0.14	−0.13				−0.31	−0.28	−0.31

binding by the Zn(II) complex with the macrocyclic ligand **L4** (Fig. 4). While the chemical shift of the P_γ signals is downfield shifted over all the pH range investigated, the P_β signal displays a downfield shift only with the formation, at slightly alkaline pHs, of protonated forms of the ternary complex, which contains protonated amine group in the macrocyclic framework. Finally, in the case of the Zn(II) complex with **L3**, which does not gives any $[ZnH_nL]^{(n+2)+}$ species, only the P_γ resonance is affected by ATP coordination, while the P_β signal remains unchanged.

These data point out that ATP coordination by the unprotonated $[ZnL]^{2+}$ complexes (**L** = **L1**–**L4**) takes place through the interaction of the terminal phosphate group (P_γ) with the metal, while nucleotide binding by the protonated forms of the Zn(II) complexes also involves the central phosphate group (P_β), most likely through the formation of salt bridges with the ammonium function of the macrocycle. This conclusion is in accord with the increase in the stability of the adducts observed with protonation of the $[ZnL]^{2+}$ complexes.

It is known that polyammonium macrocycles containing aromatic[2c–e,4,7,12] units as well as the Zn(II) complexes[22,25] with 1,10-phenanthroline or 2,2′-dipyridine can also interact with ATP through π pairing of the adenine and the heteroaromatic rings of the receptor. Indeed, ^{1}H NMR spectra carried out on solutions containing ATP and our Zn(II) complexes provide unambiguous evidence for the participation of π-stacking interactions in the stabilization of the ternary adducts (for labelling, see Scheme 1). For all 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 (F3, F4, and F6) or dipyridine (B4, B5 and B6). Fig. 5 shows the ^{1}H chemical shifts for the hydrogens H2, H8 and H1′ of ATP in absence and in presence of ATP (Fig. 5a) and for the phenanthroline hydrogens of **L4** (Fig. 5b) in absence and in presence of **L4** (1 : 1 molar ratio), while Table 4 reports the complexation induced chemical shifts (CIS) for the phenanthroline or dipyridine protons of **L1**–**L4**, as well for the ATP hydrogens. For all complexes the ^{1}H NMR displacements are pH dependent, being generally larger at neutral or slight acidic pHs, where the largest extent of complexation occurs (see, for instance, Figs. 3b and 4b for the **L2** and **L4** complexes). The CIS values, however, are by far lower than those reported in the case of ATP coordination by the protonated forms of ligand **L1**–**L4** (generally 0.8–1.2 ppm),[12] indicating a weaker π-stacking interaction in the ATP adducts with the Zn(II) complexes with respect to the corresponding complexes with protonated ligands.

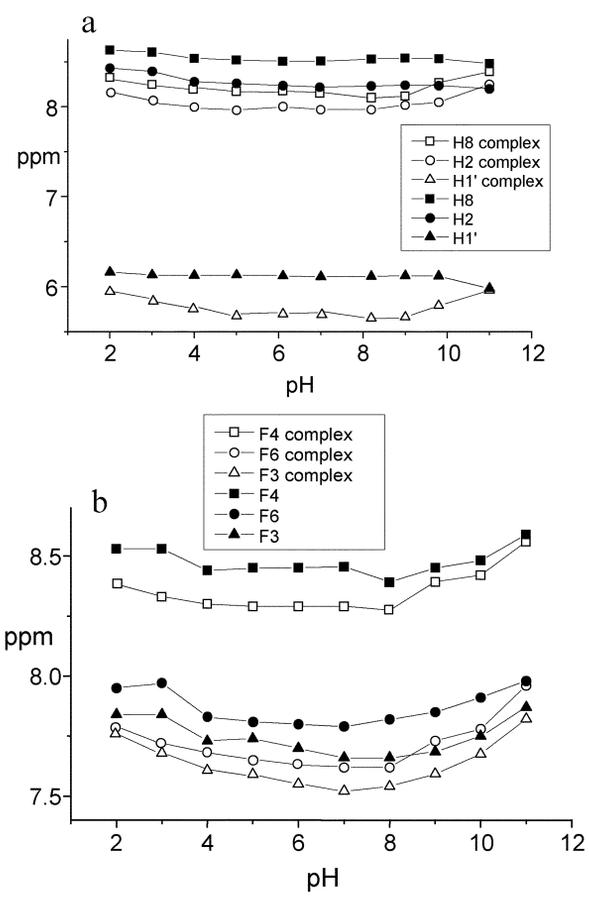

Fig. 5 (a) Experimental ^{1}H chemical shifts for the H2, H8 and H1′ protons of ATP in the absence and in the presence of the Zn complex with **L4**. (b) Experimental ^{1}H chemical shifts for the aromatic protons of the Zn complex with **L4** in presence and in the absence of ATP. In all experiments the Zn(II)–**L4** complex and ATP were in a 1 : 1 molar ratio (both 1×10^{-3} mol dm^{-3}), T = 298 K.

A comparison between the ATP binding ability of the L1–L4 protonated forms and their Zn(II) complexes

A previous study on ATP binding features of the present ligands in their protonated forms, carried out in NMe_4Cl 0.1 M, showed that they form stable complexes with ATP, through the formation of both salt bridges between the protonated nitrogens and the phosphate chain of the nucleotide and π-stacking interactions between the heteroaromatic units of substrate and receptors. The complex stability depends on the protonation degree of both receptors and ATP and large amounts of the

1 : 1 receptor–substrate adducts are formed in aqueous solutions that vary from slightly acidic to weakly alkaline, *i.e.*, in the pH region where highly protonated species of the receptors and anionic species of ATP are simultaneously present in solution. The interaction vanishes at strongly acidic or basic pHs, where the fully protonated form of ATP or the unprotonated polyamine receptors prevail in solution. The stability constants of the ATP complexes, now redetermined in NMe_4NO_3 0.1 M, are almost equal to those previously found in NMe_4Cl 0.1 M [12] and are supplied within the Supplementary Information. †

It can be of interest, instead, to compare the binding abilities of the protonated receptors **L1**–**L4** with those of their Zn(II) complexes. In this respect, it is to be noted that a direct comparison between the equilibrium constants for ATP interaction with the protonated receptors and the equilibrium constants for ATP interaction with the corresponding Zn(II) complexes could be misleading, due to the different species, *i.e.*, metal complexes or protonated ligands, involved in ATP binding. An appropriate way to visualize selectivity in ATP coordination can be to consider a ternary system containing the ligand, Zn(II) and ATP in 2 : 1 : 1 molar ratio. Since Zn(II) is completely complexed by ligands **L1**–**L4** to form 1 : 1 species in a wide pH range, this provides the simultaneous presence in solution of the ligand, its Zn(II) complex and ATP in equimolecular concentrations, *i.e.*, a system containing the ligand and the corresponding Zn(II) complex as competing ATP binding agents. Plots of the calculated overall percentages of the two different ATP complexes, with protonated ligand and with metal complex, as a function of pH,[11b,30] produce species distribution diagrams from which the binding ability of the receptors can be interpreted in terms of selectivity at a given pH. Fig. 6 reports similar diagrams calculated for the ATP/[Zn(II)**L3**]/**L3**, ATP/[Zn(II)**L4**]/**L4** and ATP/[Zn(II)**L2**]/**L2** systems. The corresponding ternary diagram for **L1** is similar to that of **L2**. The plots in Fig. 6 show different binding patterns among the three ligands. Among the Zn(II) complexes investigated, the **L3** one does not form any protonated species. According to Fig. 6a, **L3**, in its protonated forms or as Zn(II) complex shows a comparable binding ability toward ATP in a wide pH range (4–10.5). Only at strongly acidic pH values (pH < 3.5) where decomposition of the $[Zn\mathbf{L3}]^{2+}$ complex occurs upon ligand protonation, adducts between ATP and the protonated ligand are largely predominant in solution. In absence of complex protonated forms, therefore, $[Zn\mathbf{L3}]^{2+}$ and the protonated **L3** ligand display a similar affinity for the nucleotide. The Zn(II) complex with **L4** displays a different behavior. As shown in Fig. 6b, ATP is selectively bound by the Zn(II) complexes with **L4** almost over all the pH range investigated. While the preferred ATP binding to the Zn(II) complex with respect to the uncomplexed ligand at strongly alkaline pHs is due to the simultaneous presence in solution of the unprotonated form of **L4** (unable to bind ATP) and of the $[Zn\mathbf{L4}]^{2+}$ complex (an efficient ATP receptor), nucleotide recognition by the Zn(II) complexes from slightly alkaline to acidic pHs is instead due to the presence in solution of protonated complexed species of the type $[ZnH_x\mathbf{L4}]^{(x+2)+}$, which are better receptors for ATP than the simply protonated **L4** forms. The most interesting selectivity profile, however, is displayed by ligands **L1** and **L2**. As shown in Fig. 6c for the **L2** complexes, ATP is selectively bound by the protonated species forms of the ligand from alkaline up to pH 6. In this pH range, in fact, the Zn(II) complex is present in solution as its unprotonated complex $[Zn\mathbf{L2}]^{2+}$, which shows only a weak tendency to bind ATP. On the contrary, below pH 6 the nucleotide is preferentially bound to the Zn(II) complex, present in solution in its protonated forms $[ZnH\mathbf{L2}]^{3+}$ and $[ZnH_2\mathbf{L2}]^{4+}$. Obviously, below pH 3.8 Zn(II) decomplexation occurs upon ligand protonation, and the adducts between ATP and the protonated ligand forms become again predominant in solution.

These results point out that protonated Zn(II) complexes are better receptors for ATP than protonated ligand forms, due to the simultaneous presence of the metal ion and ammonium functions which can act cooperatively in ATP binding. On the basis of the potentiometric and ^1H and ^{31}P results, we propose an interaction mode between ATP and the protonated $[ZnH\mathbf{L4}]^{3+}$ complex involving Zn^{2+}–$^-$OP, $NH^+ \cdots {}^-$OP interactions and π-stacking pairing between the nucleobase and phenanthroline, as shown in Fig. 7.[31]

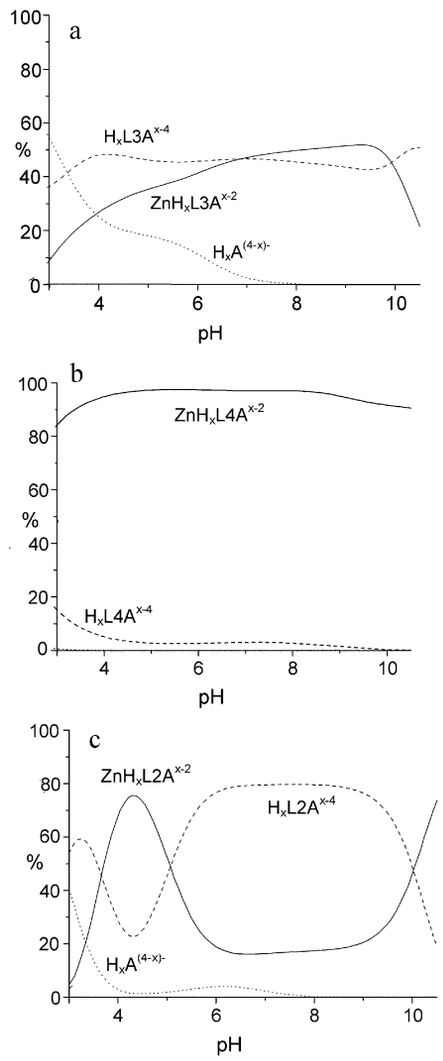

Fig. 6 Overall percentages of ATP complexed species as a function of pH in competing systems containing **L3** and $Zn\mathbf{L3}^{2+}$ ([**L3**] = 2 × 10^{-3} mol dm^{-3}, $[Zn^{2+}]$ = [ATP] = 1 × 10^{-3} mol dm^{-3}) (a), **L4** and $Zn\mathbf{L4}^{2+}$ ([**L4**] = 2 × 10^{-3} mol dm^{-3}, $[Zn^{2+}]$ = [ATP] = 1 × 10^{-3} mol dm^{-3}) (b), and **L2** and $Zn\mathbf{L2}^{2+}$ ([**L2**] = 2 × 10^{-3} mol dm^{-3}, $[Zn^{2+}]$ = [ATP] = 1 × 10^{-3} mol dm^{-3}) (c). Percentages are calculated with respect to ATP.

Among the **L1**–**L4** complexes, the Zn–**L4** one is most efficient ATP receptor from acidic to alkaline pHs. Solutions containing two different ligands, Zn(II) and ATP in 1 : 1 : 2 : 1 molar ratio provide simple competing systems where two different metal complexes, in equimolecular ratio, may act as competing ATP receptors. Plots of these selectivity diagrams for the systems ATP/[Zn(II)**L4**]/[Zn(II)**L**] (**L** = **L1**, **L2** or **L3**) point out that ATP is almost completely bound to the Zn–**L4** complex in presence of equimolecular amounts of the Zn–**L1**, Zn–**L2** or Zn–**L3** complexes (Fig. 8). The protonated Zn(II) complexes with **L4** are also better receptors than those with 2,2′-dipyridine (bipy) and 1,10-phenanthroline (phen), where the nucleotide is bound to Zn(II) through the coordination bonds with the phosphate chain and π-stacking interactions. Actually, the equilibrium constant for the addition of ATP to the $[Zn\mathbf{L4}]^{2+}$ complex is almost equal to those found for the Zn(II) complexes with bipy and phen (log K = 5.18, 5.32 [22d] and 5.26 [22f] for the equilibrium

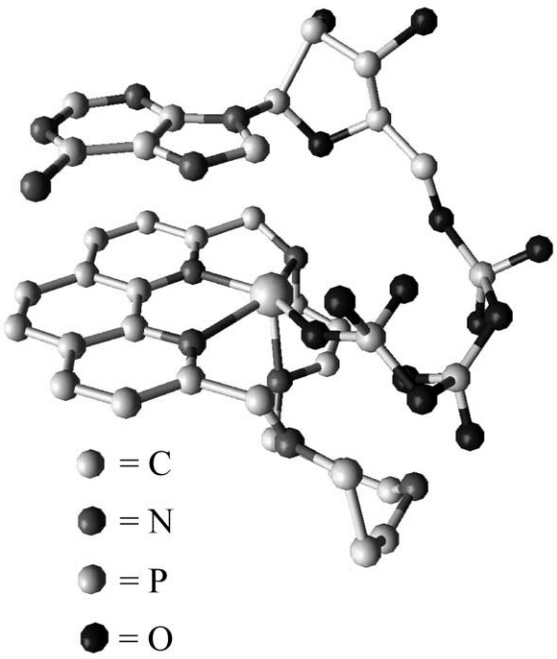

Fig. 7 Proposed interaction mode between the ZnH**L4**$^{3+}$ complex and ATP^{4-}, deduced on the basic of both potentiometric and ^{1}H and ^{31}P NMR measurements.

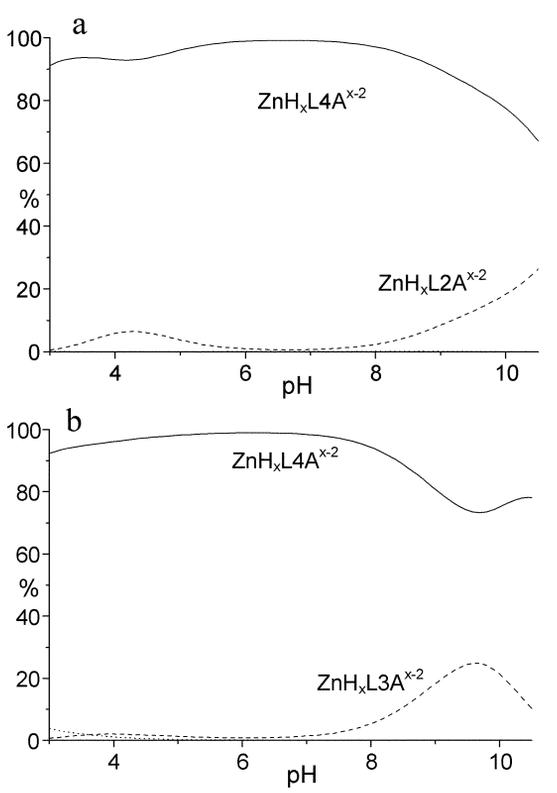

Fig. 8 Overall percentages of ATP complexed species as a function of pH in competing systems containing Zn**L2**$^{2+}$ and Zn**L4**$^{2+}$ ([**L2**] = [**L4**] = [ATP] = 1 × 10^{-3} mol dm^{-3}, [Zn^{2+}] = 2 × 10^{-3} mol dm^{-3}) (a) and Zn**L3**$^{2+}$ and Zn**L4**$^{2+}$ ([**L3**] = [**L4**] = [ATP] = 1 × 10^{-3} mol dm^{-3}, [Zn^{2+}] = 2 × 10^{-3} mol dm^{-3}) (b). Percentages are calculated with respect to ATP.

$ZnL^{2+} + ATP^{4-} \rightleftharpoons [ZnL(ATP)]^{2-}$, with L = **L4**, phen and bipy, respectively). The protonated species of the [Zn**L4**]$^{2+}$ complexes, which are largely prevalent in solution in the neutral pH region, display by far higher constants for ATP addition; for instance, the addition constant of ATP^{4-} to the diprotonated [ZnH_2**L4**]$^{4+}$ receptor is more than two order of magnitude higher then that of [Zn(bipy)]$^{2+}$ or Zn[(phen)]$^{2+}$. Once again, this result evidences the relevant contribution of the salt bridges between the phosphate chain of ATP and the ammonium groups of the receptor in the stabilization of the adducts.

The interaction between the Zn(II) complexes with **L4** and ATP is enhanced by the increasing charge of the metallo-receptor and nucleotide, mainly due to the increased number of salt bridge contacts. A tentative calculation of the number of salt bridge contacts can be made by considering that the contribution of a single P–O$^-$ ··· HN$^+$ salt bridge to the complex stabilization is generally considered to be 5 ± 1 kJ mol^{-1}.[6,12] The free energy change for ATP^{4-} addition to the Zn(II) complexes increases of *ca.* 5 kJ mol^{-1} from [Zn**L4**]$^{2+}$ to [ZnH**L4**]$^{3+}$ ($\Delta G°$ = −29.5 and −35.4 kJ mol^{-1} for the reactions [Zn**L4**]$^{2+}$ + ATP^{4-} = [Zn**L4**(ATP)]$^{2-}$ and [ZnH**L4**]$^{3+}$ + ATP^{4-} = [ZnH**L4**(ATP)]$^-$). This would account for the presence of a single salt bridge in the monoprotonated [ZnH**L4**(ATP)]$^-$ complex. Similarly, the $\Delta G°$ value for ATP^{4-} addition increases of *ca.* 8.8 kJ mol^{-1} from [ZnH**L4**]$^{3+}$ to [ZnH_2**L4**]$^{4+}$ ($\Delta G°$ = −44.2 kJ mol^{-1} for the reaction [ZnH_2**L4**]$^{4+}$ + ATP^{4-} = [ZnH_2**L4**(ATP)]), suggesting that the formation of the diprotonated complex [ZnH_2**L4**-(ATP)] is accompanied by the further formation of two P–O$^-$ ··· HN$^+$ contacts.

Experimental

General procedures

Ligand **L1**,[26c] **L2**,[26c] **L3**,[26a] and **L4**[12a] were prepared as already described. Crystals of [ZnH**L4**Br]Br(ClO_4) were obtained by slow evaporation of an aqueous solution containing **L4**·4HBr and $Zn(ClO_4)_2$·6H_2O in equimolecular ratio at neutral pH.

Single crystal X-ray diffraction analysis

Analysis on prismatic colourless single crystal of [ZnH**L4**Br]-Br·(ClO_4) ($C_{23}H_{33}Br_2ClN_6O_4Zn$, M = 718.19) was carried out on a Siemens P4 X-ray diffractometer (λ = 1.54180 Å, T = 298 K). Crystals belong to the monoclinic family, space group $P2_1/c$ (a = 7.351(2) Å, b = 17.809(6) Å, c = 21.469(5) Å, β = 91.73(3)°, V = 2809.3(14) Å^3, Z = 4, μ(Cu-Kα = 1.698 mm^{-1}). 4778 Reflections were collected (3380 unique, R(int) = 0.0258). No loss of intensity was observed during data collection. Empirical absorption correction (PSI-scan method) was applied. The structure was solved by direct methods (SIR97).[32] Refinements were performed by means of full-matrix least-squares using the SHELXL-97 program.[33]

All non-hydrogen atoms were anisotropically refined while the aromatic and aliphatic hydrogen atoms were introduced in calculated positions and their coordinates and thermal factors were refined according to the linked atoms. The hydrogens linked to the nitrogen atoms were localized in the ΔF map, introduced in the calculation and isotropically refined.

Final agreement factors for 354 parameters were $R(F)$ = 0.0599 (for 3145 reflections with $I > 2\sigma(I)$) and $wR(F_2)$ = 0.1688 (all data) ($R = \Sigma \, ||F_o| - |F_c|| \, / \, \Sigma \, |F_o|$; $wR_2 = [\Sigma \, w(F_o^2 - F_c^2)^2 \, / \, \Sigma \, wF_o^4]^{1/2}$).

CCDC reference number 206806.

See http://www.rsc.org/suppdata/dt/b3/b303264g/ for crystallographic data in CIF or other electronic format.

Potentiometric measurements

All the pH metric measurements (pH = −log [H$^+$]) were carried out in degassed 0.1 mol dm^{-3} NMe_4NO_3 solutions, at 298.1 K by using equipment and procedure that have been already described.[12] The combined Ingold 405 S7/120 electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NMe_4OH solutions and determining the equivalent point by Gran's method[34] (which allows one to determine the standard potential $E°$), and the

ionic product of water (pK_w = 13.83(1) at 298.1 K in 0.1 mol dm^{-3} NMe_4NO_3). All equilibria involved in the studied systems were determined under the present experimental conditions in order to obtain a consistent set of data. Ligand protonation constants and formation constant of ATP complexes with the protonated ligands are supplied within the supplementary material. In the experiments to determine the stability of the ternary complexes, the Zn(II) and ligand concentrations were both 1×10^{-3} mol dm^{-3}, while the concentration of ATP was varied in the range 1×10^{-3}–4×10^{-3} mol dm^{-3}. At least three measurements (about 150 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[35] which furnished the relevant equilibrium constants reported in Table 2 and 3.

NMR spectroscopy

200.0 MHz ^{1}H and 81.01 MHz ^{31}P 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. In the ^{31}P NMR spectra, the chemical shifts are relative to an external reference of 85% H_3PO_4. 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:[36]

$$pH = pD - 0.40$$

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