Dinuclear Zn^{II} Complexes of Polydentate Polyamines as Minimalist Models of Hydrolytic Reactions

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The synthesis of the novel macrocycle 2,6,9,12,16-pentaaza-[17](2,9)(1,10)phenanthrolinophane (L3) is reported. Speciation studies on the systems Zn^{II}–L3 and Zn^{II}–L2 (L2 = 2,6,10,13,17,21-hexaaza[22]metacyclophane) performed in aqueous solution show the formation of mono- and dinuclear Zn^{II} complexes. In the two systems, the dinuclear complexes readily hydroxylate, with the hydroxo species being the main ones in solution at relatively low pH values. This feature makes these complexes promising hydrolytic agents for carboxy and phosphate esters. The hydrolytic ability of the L1–L3 dinuclear complexes toward the carboxy and phosphate ester bond was tested by addition of *p*-nitrophenyl acetate (NA) and bis(*p*-nitrophenyl)phosphate (BNP). While in the case of NA the cleavage takes place through a simple bimolecular mechanism and the hydrolysis rate depends on the nucleophilicity of the Zn^{II}–OH functions, in the case of BNP the hydrolytic mechanism involves substrate interaction with the metal ions and nucleophilic attack of a Zn^{II}-bound hydroxide ion at the phosphorus atom. The L3 complex gives rise to the highest hydrolysis rate constant {$k = 62 \times 10^{-5}$ $M^{-1}\cdot s^{-1}$ vs. $k = 8 \times 10^{-5}$ and 4×10^{-5} $M^{-1}\cdot s^{-1}$ for $[Zn_2L2(OH)_2]^{2+}$ and $[Zn_2L1(OH)_2]^{2+}$, respectively}. This may be related to a stronger interaction with the substrate due to the synergetic role in BNP binding played by the phenanthroline unit, which can give π-stacking and hydrophobic interactions with the aromatic units of the substrate, and by the two Zn^{II} ions, which can act cooperatively in BNP coordination.

Introduction

A model compound of a metalloprotein can be defined as a structurally simpler metal complex, without the protein ligand, that is able to reproduce key features of its active center and/or its spectroscopic properties and/or its biochemical function. In this respect, throughout the last few years a great deal of experimental work has been addressed to the development of Zn^{II} complexes with unsaturated coordination spheres able to mimic features of the active site of Zn^{II} hydrolases.[1–23] Many of these enzymes use two Zn^{II} centers for the activation of the substrate and very often the nucleophile is a hydroxy group generated from deprotonation of a coordinated water molecule.[22,23] The right compromise between the thermodynamic stability of the complex and its rapid dynamic reorganization following the mechanism of the reaction, the hydrophilic/hydrophobic balance of the environment, the possibility of formation of hydrogen bonds with the substrate, and the presence of noncoordinated basic groups that can assist the process are other factors that influence the catalytic activity.

Macrocyclic structures are very appealing for the construction of these mimics because they intrinsically provide a certain organization of their reactive functions. In this context many aza or oxaaza macrocycles have been shown to yield interesting performances as models for the cleavage of carboxy or phosphate esters. One representative example was provided by the binucleating oxaaza ligand 1,4,7,16,19,22-hexaaza-10,13,25,28-tetraoxacyclotriacontane ([30]aneN_6O_4), which contains two triamine moieties separated by two dioxa chains.[24]

Here we report on the catalytic ability towards carboxy- and phosphate-ester cleavage of three compounds of different structure. L1 is an open-chain hexaamine consisting of a symmetrical array of four propylenic chains and one central ethylenic chain connecting the set of nitrogen atoms,[25] L2 presents a classical 1:1 cyclophane structure in which the

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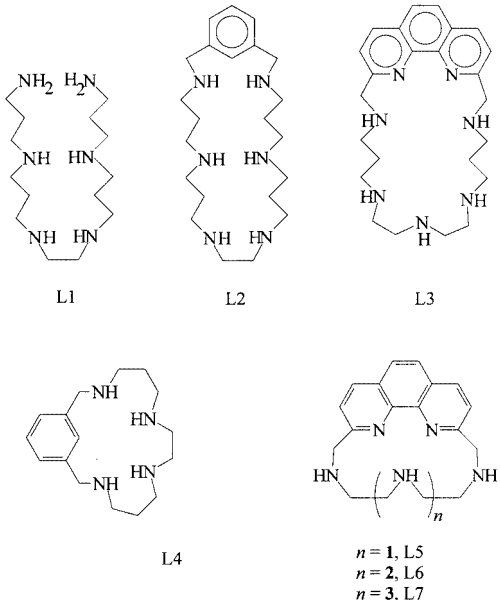
hexamine L1 has been linked through methylene groups to a *meta*-benzene spacer,[26] and L3 is a novel phenanthrolinophane ligand consisting of the pentaamine 4,7,10-triazatridecane-1,13-diamine linked through methylene groups to the 2- and 9-positions of a 1,10-phenanthroline moiety (Scheme 1). We present the synthesis of L3 and we discuss the Zn^{II} coordination capabilities of the three ligands L1, L2 and L3 as well as their potentialities as hydrolytic mimics.



Scheme 1

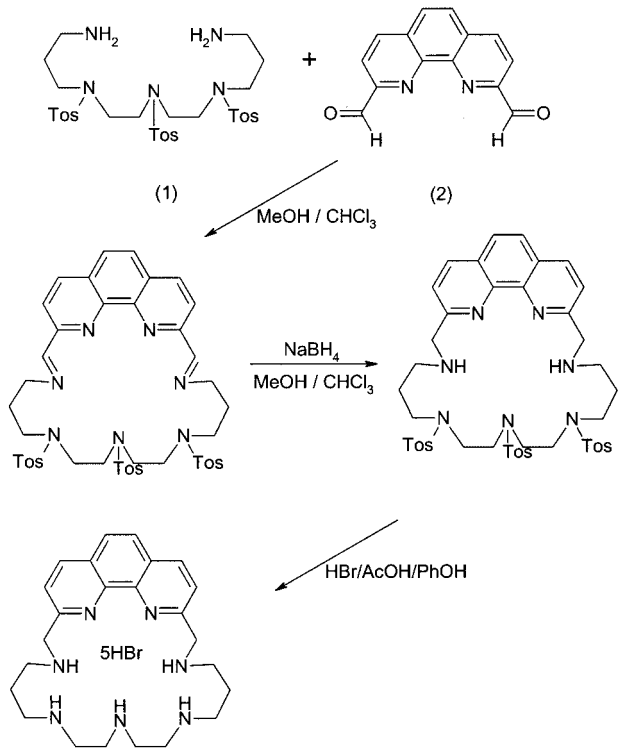
Results and Discussion

Synthesis of L3

The synthesis of L3 was achieved according to a route different to that reported for the preparation of related cyclophanes[27] and similar phenanthrolinophanes.[28] The procedure consists of a condensation reaction of the partly tosylated amine 4,7,10-tris(*p*-tolylsulfonyl)-4,7,10-triaazatridecane-1,13-diamine (**1**) with 1,10-phenanthroline-2,9-dicarboxaldehyde (**2**), which yields the tosylated imine **3**. Compound **3** is then reduced with $NaBH_4$ to give the partly tosylated macrocycle (**4**; Scheme 2). The reductive cleavage of the amine to give L3 was performed with HBr/AcOH in PhOH. L3 was handled as its hexahydrobromide salt. The overall yield of the reaction was about 40%.

Speciation Studies

Table 1 gathers the stability constants for the formation of Zn^{II} complexes of L1, L2 and L3. Formation of both mono- and dinuclear complexes was detected in all three systems. L1 and L2 form protonated mononuclear complexes with protonation degrees varying between 2 and 0 and between 2 and −1, respectively. The speciation studies



Scheme 2

for the Zn^{II}−L3 system show the formation of mononuclear complexes with stoichiometries $[ZnH_xL]^{(2+x)}$ with x ranging from 4 to 0. With respect to the dinuclear complexes, while in the systems Zn^{II}−L1 and Zn^{II}−L2 only hydroxylated species are detected, in the system Zn^{II}−L3 formation of a neutral and of a monoprotonated dinuclear complex is also observed.

In a previous report,[25] some of us analysed the coordination capabilities towards Zn^{II} of the open-chain ligand L1. Taking into account the values of the stability constants, the NMR spectra of the complex and the comparison with related polyamines and the other metal ions of the second part of the first transition series, a coordination number of four was confidently established for its mononuclear Zn^{II} complexes. A similar analysis can be performed for the system Zn^{II}−L2. First of all, a direct comparison with L1 shows that the formation constant of $[ZnL2]^{2+}$ is even a little bit lower than that of $[ZnL1]^{2+}$ (Table 1). The formation constant of $[ZnL2]^{2+}$ is, on the other hand, similar to those of open-chain tetraamines like 3,6-diazaoctane-1,8-diamine (trien) ($\log K_{ZnL/Zn\cdot L} = 12.0$) with all ethylenic chains, 4,7-diazadecane-1,10-diamine (2,2,2-tet, $\log K_{ZnL/Zn\cdot L} = 11.25$) with a symmetrical disposition of two propylenic and one ethylenic chain or 4,8-diazadecane-1,11-diamine (3,2,3-tet, $\log K_{ZnL/Zn\cdot L} = 10.46$) with all ethylenic chains and larger than those obtained for open-chain triamines like 3-azapentane-1,5-diamine (2,2-tri, $\log K_{ZnL/Zn\cdot L} = 8.8$).[29] If we compare tetraazacycloalkanes like 1,4,8,11-tetraazacyclotetredecane (cyclam, $\log K_{ZnL/Zn\cdot L} = 15.5$) or 1,4,7,10-tetraazacyclododecane (cyclen, $\log K_{ZnL/Zn\cdot L} = 16.2$), then the formation constant of $[ZnL]^{2+}$ is clearly

Table 1. Logarithms of the stability constants for the formation of Zn^{2+} complexes of ligands L1, L2 and L3 determined at 298.1 K in 0.15 M ionic strength

Reaction	L1[a][b]	L2[b]	L3[c]
$Zn^{2+} + L + 4H^+ \rightarrow ZnH_4L^{6+}$			36.44(3)
$Zn^{2+} + L + 3H^+ \rightarrow ZnH_3L^{5+}$			32.48(2)
$Zn^{2+} + L + 2H^+ \rightarrow ZnH_2L^{4+}$	28.25(1)	26.31(2)	26.37(2)
$Zn^{2+} + L + H^+ \rightarrow ZnHL^{3+}$	20.09(1)	18.65(2)	19.41(2)
$Zn^{2+} + L \rightarrow ZnL^{2+}$	10.53(2)	10.19(3)	10.96(2)
$Zn^{2+} + L + H_2O \rightarrow ZnL(OH)^+ + H^+$		0.39(3)	
$Zn^{2+} + L + H_2O \rightarrow ZnL(OH)_2 + 2H^+$		−10.18(5)	
$2Zn^{2+} + L + H^+ \rightarrow Zn_2HL^{5+}$			22.54(7)
$2Zn^{2+} + L \rightarrow Zn_2L^{4+}$			16.26(3)
$2Zn^{2+} + L + H_2O \rightarrow Zn_2L(OH)^{3+} + H^+$	7.55(3)	5.80(4)	8.48(4)
$2Zn^{2+} + L + 2H_2O \rightarrow Zn_2L(OH)_2^{2+} + 2H^+$	−1.98(8)	−2.63(3)	−1.35(5)
$ZnH_3L^{5+} + H^+ \rightarrow ZnH_4L^{6+}$			4.0[d]
$ZnH_2L^{4+} + H^+ \rightarrow ZnH_3L^{5+}$			6.1
$ZnHL^{3+} + H^+ \rightarrow ZnH_2L^{4+}$	8.2	7.7	7.0
$ZnL^{2+} + H^+ \rightarrow ZnHL^{3+}$	9.6	8.6	8.5
$Zn_2L^{4+} + H^+ \rightarrow Zn_2HL^{5+}$			6.3
$Zn_2L^{4+} + OH^- \rightarrow Zn_2L(OH)^{3+}$			6.0
$Zn_2L^{4+} + H_2O \rightarrow Zn_2L(OH)^{3+} + H^+$			−7.8
$Zn_2L(OH)^{3+} + OH^- \rightarrow Zn_2L(OH)_2^{2+}$	4.2	5.3	3.9
$Zn_2L(OH)^{3+} + H_2O \rightarrow Zn_2L(OH)_2^{2+} + H^+$	−9.5	−8.4	−9.8

[a] Taken from ref.[25] [b] 0.15 M $NaClO_4$. [c] 0.15 M NaCl. [d] Just one decimal figure is included in the calculated stepwise constants.

lower as a result of the important macrocyclic effect that is operative in these small-sized saturated azamacrocycles. A comparison with the triazamacrocycles 1,4,7-triazacyclononane ([9]aneN_3, log $K_{ZnL/Zn\cdot L}$ = 11.6) and 1,5,10-triazacyclododecane ([12]aneN_3, log $K_{ZnL/Zn\cdot L}$ = 8.6) shows an intermediate constant for $[ZnL2]^{2+}$ between the macrocycle with all ethylenic chains and that with only propylenic chains. A last comparison of relevance refers to tetraazametacyclophanes like 2,6,9,13-tetraaza[14]metacyclophane (L4; Scheme 1) for which the number of coordinated nitrogen atoms was deduced to be three (log $K_{ZnL/Zn\cdot L}$ = 8.73).[30] Therefore, all these data suggest that in the Zn^{II}−L1 mononuclear complexes the metal ion is coordinated by four nitrogen donors of the macrocycle. The two high, stepwise protonation constants obtained for $[ZnL2]^{2+}$ also give support to the tetracoordination of the metal ion. The nitrogen atoms undergoing protonation would be noncoordinated ones and this is the reason for such high values of their protonation constants.

The analysis of the stability constants for the interaction of Zn^{II} with the phenanthrolinophane L3 leads to similar conclusions. Firstly, the value for the formation constant of $[ZnL3]^{2+}$ (Table 1) is of the same order of magnitude as found for the other polyamines studied in the present work (L1 and L2); this could indicate that, as has been just discussed, four is the most likely coordination number. However, in this case the presence of the strong π-acceptor phenanthroline spacer can produce some modification in the analysis. Therefore, it is interesting to compare L3 with similar phenanthrolinophanes reported previously.[31] Indeed the $[ZnL]^{2+}$ complex of the phenanthrolinophane receptor 2,5,8-triaza[9](2,9)(1,10)phenanthrolinophane (L5; Scheme 1) displays a stability constant of 16.15, which is much higher than those reported here for the analogous complexes of L1−L3. In spite of this, the crystal structure of its complex cation $[ZnL(H_2O)]^{2+}$ revealed that Zn^{II} is coordinated only by the phenanthroline donors, by the central nitrogen atom of the polyamine bridge and by a water molecule, with a tetrahedral disposition of the donor atoms. This again points out the difficulties of deriving coordination numbers just from free-energy terms. Nevertheless, one has to consider that such an enhancement of the stability of Zn^{II} complexes by ligands favoring tetrahedral arrangements is quite usual as can be seen from the relevant data for the tripodal tetraamine tris(2-aminoethyl)amine, which has a higher stability constant than any other open-chain tetraamine.[29]

However, the larger 2,5,8,11-tetraaza[12](2,9)(1,10)phenanthrolinophane (L6 in Scheme 1) and 2,5,8,11,13-pentaaza[14](2,9)(1,10)phenanthrolinophane (L7) have lower constants — log $K_{ZnL/Zn\cdot L}$ = 14.29 and 12.38, respectively.[31] In the case of L3, the stability constant obtained follows this tendency, being even lower (Table 1). Additionally, the number of protonated species found is very large. All this can be interpreted on the grounds of an increasing strained coordination geometry imposed by the macrocyclic conformation and to a low coordination number that should involve either three or at most four nitrogen donors, including those of the spacer.

The most relevant aspect with respect to the dinuclear complexes is the ready hydroxylation observed for the three systems at relatively low pH values (see Figure 1). In the case of L1 and L2, $[Zn_2L]^{4+}$ species are not even detected in solution due to the marked tendency of the dinuclear Zn^{II} complexes to form hydroxylated complexes. In the case of L3, a pK_a value of 7.8 for deprotonation of a Zn^{II}-coordinated water molecule of $[Zn_2L3]^{4+}$ was found. Similar low pK_a values are generally observed in the case of a bridging

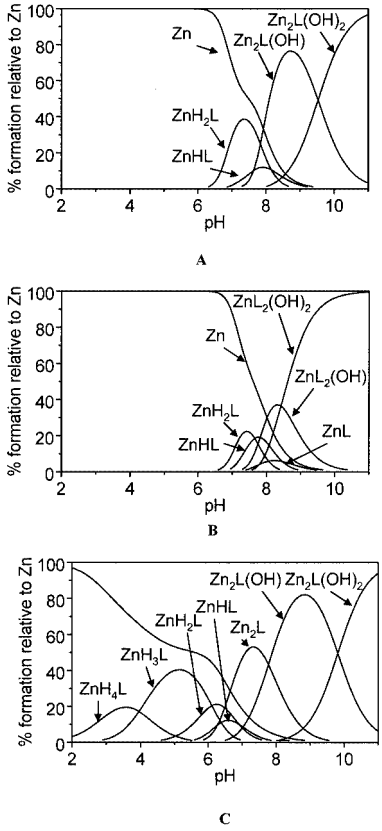


Figure 1. Distribution diagrams for the systems $Zn^{II}-L1$, $Zn^{II}-L2$ and $Zn^{II}-L3$ for molar ratios $Zn^{II}/L = 2:1$ ($[Zn^{II}] = 2 \times 10^{-3}$ M)

disposition of hydroxide between two metal ions[24,32,33] or in the case of mononuclear complexes containing a water molecule placed as a fourth ligand of a Zn^{II} moiety coordinated by three nitrogen donors.[4,30] Therefore, the coordinatively unsaturated first coordination spheres of these dinuclear complexes provide nucleophilic OH^- functions, which could play a key role in the hydrolytic process (vide infra).

Kinetics of p-Nitrophenyl Acetate (NA) Hydrolysis

NA hydrolysis was monitored by UV/Vis spectroscopy by monitoring the appearance of the *p*-nitrophenolate anion at 403 nm (298.1 K, $I = 0.15$ M $NaClO_4$). All ligands form dinuclear complexes in aqueous solution. In the case of L3 the formation of the dinuclear $[Zn_2L3]^{4+}$ complex takes place at a pH > 6, with almost simultaneous deprotonation of a coordinated water molecule to give the monohydroxo species $[Zn_2L3(OH)]^{3+}$. The rather low pK_a (7.8 log units) suggests a bridging coordination of the hydroxide ion between the two metal centers, as is often found in dizinc complexes with macrocyclic ligands, where the two metal atoms are kept at a short distance by the cyclic framework, thus favoring the assembly of $Zn_2(\mu\text{-OH})$ units.[24,32,33] The formation of a dihydroxo $[Zn_2L3(OH)_2]^{2+}$ complex is observed at alkaline pH values, with $pK_{a2} = 9.8$ (298 K). This pK_a value is higher than those usually found for bridging hydroxide ions and is generally related to the formation of a single-metal-bound hydroxide function.[24,32,33] L1 and L2 display a somewhat different behavior, since the formation of the $[Zn_2L]^{4+}$ complex is not observed and only the dinuclear hydroxylated species $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ are present in neutral and alkaline aqueous solutions, respectively. For all the three ligands, both the dinuclear complexes $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ promote NA hydrolysis; second-order kinetics is monitored at different pH values. As shown in Figure 2 for the dinuclear L1 complexes, plots of the k_{NA} values as a function of the percentages of $[Zn_2L1(OH)]^{3+}$ (Figure 2a) and $[Zn_2L1(OH)_2]^{2+}$ (Figure 2b) give rise to straight lines. Similar plots were also obtained for the dinuclear complexes with L2 and L3. For all the dinuclear complexes under investigation, no effect is observed at pH < 7.5, where such species are absent in solutions. Finally, experiments carried out on solutions containing ligands L1, L2 or L3 and Zn^{II} in a 1:1 molar ratio in the pH range 7−9 (under these conditions mononuclear complexes are largely prevalent in solution) do not show significant increases of the hydrolysis rate, indicating that the mononuclear Zn^{II} complexes are not active in this hydrolytic process.

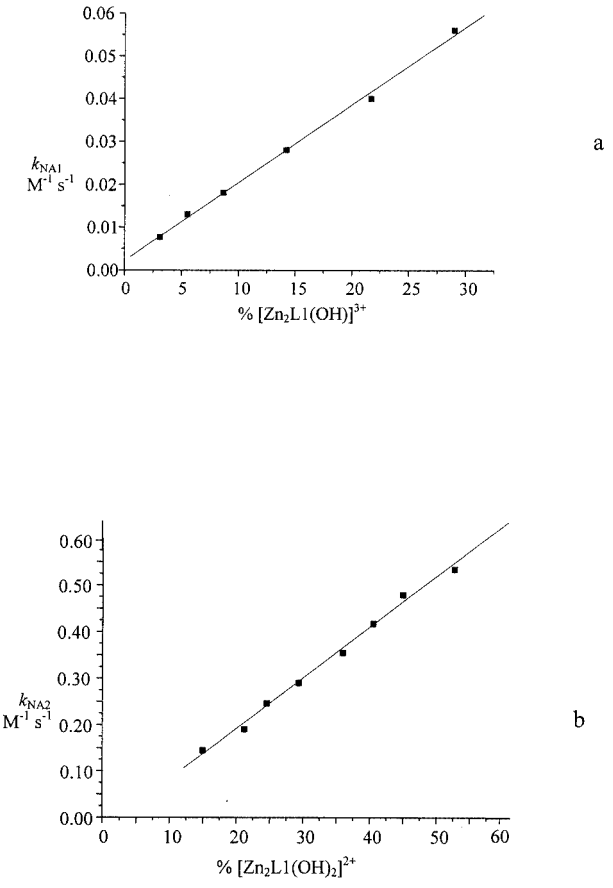


Figure 2. Second-order rate constants for NA hydrolysis (k_{NA}) as a function of the percentage of $[Zn_2L1(OH)]^{3+}$ (a) and as a function of the percentage of $[Zn_2L1(OH)_2]^{2+}$ (b)

Only the mono- and dihydroxo complexes $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ are the kinetically active species, meaning that the $Zn^{II}-OH$ function is indeed nucleophilic. Furthermore, Figure 2 clearly shows that the dihydroxo complex $[Zn_2L1(OH)_2]^{2+}$ is much more active in NA hydrolysis than the monohydroxo complex $[Zn_2L1(OH)]^{3+}$. Similarly, the dinuclear dihydroxo complexes with L2 and L3 show a higher activity in promoting NA cleavage than the monohydroxo ones.

The $[Zn_2L(OH)]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ complexes (L = L1, L2 or L3) are formed in at most 70% yield in the pH ranges used in the kinetic measurements. Therefore, in order to quantify the different activity in NA hydrolysis of the present complexes, second-order rate constants k'_{NA} have been determined from the maximum k_{NA} values by using the equation:

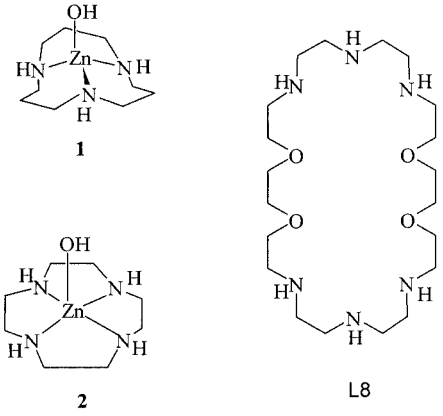
$$v = k_{NA}[\text{total } Zn^{II}\text{complex}][NA] = k'_{NA}[Zn_2L(OH)_x^{4-x}][NA];\ x = 1 \text{ or } 2$$

The k'_{NA} values for the present complexes are listed in Table 2, together with the rate constants found for Kimura's mononuclear Zn^{II} complexes **1** and **2** (Scheme 3).[34]

Table 2. Second-order rate constants k'_{NA} [M^{-1} s^{-1}] for hydrolysis of *p*-nitrophenyl acetate

Nucleophile	k'_{NA} [M^{-1} s^{-1}]	pK_a
$[Zn_2L1(OH)]^{3+}$	0.18 ± 0.01	–
$[Zn_2L1(OH)_2]^{2+}$	1.1 ± 0.05	9.5
$[Zn_2L2(OH)]^{3+}$	0.06 ± 0.003	–
$[Zn_2L2(OH)_2]^{2+}$	0.80 ± 0.4	8.4
$[Zn_2L3(OH)]^{3+}$	0.069 ± 0.04	7.8
$[Zn_2L3(OH)_2]^{2+}$	2.2 ± 0.05	9.8
1	0.041[a]	7.2
2	0.11[a]	7.9

[a] From ref.[34]; I = 0.15 M $NaClO_4$.



Scheme 3

Table 2 clearly shows that the monohydroxo $[Zn_2L(OH)]^{3+}$ complexes exhibit a remarkably lower rate constant than the corresponding dihydroxo $[Zn_2L(OH)_2]^{2+}$ complexes. The much lower hydrolytic properties of the $[Zn_2L(OH)]^{3+}$ species can be ascribed to the bridging coordination of the hydroxide ion, as is often found in dizinc complexes with hexa- and heptaamine ligands.[22,24,32] In fact, the simultaneous binding to two electrophilic metal centers reduces the nucleophilicity of the generated hydroxide ion. The k'_{NA} of $[Zn_2L(OH)]^{3+}$ is, however, similar to those found for the monohydroxo complexes **1** and **2**.

Addition of a second hydroxide anion to a Zn^{II} ion may lead to detachment of the bridging OH ion from one of the metal atoms or to a weakening of the Zn(μ-OH) bond, giving a more "open" conformation of the complex, which can leave catalytic sites accessible on the two Zn^{II} ions. As a matter of fact, the dihydroxo complexes $[Zn_2L(OH)_2]^{2+}$ give much higher rate constants in NA hydrolysis than the monohydroxo complexes **1** and **2**, indicating an enhanced nucleophilicity of the $Zn-OH$ functions in $[Zn_2L(OH)_2]^{2+}$. Actually, a bridging hydroxide ion in $[Zn_2L(OH)]^{3+}$ reduces the positive charge on the metal ions. This determines the rather high pK_{a2} values found for deprotonation of a Zn^{II}-bound water molecule to give the $[Zn_2L(OH)_2]^{2+}$ complexes and increases the nucleophilic character of the resulting $Zn-OH$ functions.

Comparing the different abilities of these species in NA cleavage, Table 2 shows that the k'_{NA} values increase in the sequence L2 < L1 < L3. The mechanism generally accepted for NA hydrolysis involves a simple nucleophilic attack of the metal-bound hydroxide ion to the carbonyl group of the ester and release of *p*-nitrophenolate.[8,35] The two Zn^{II} ions do not play any cooperative role in substrate activation and a simple bimolecular mechanism is predominant. For this reason, for a series of analogous complexes, such as **1** and **2**, the ability of $Zn-OH$ functions in NA hydrolysis increases with their pK_a values. From this point of view, the increasing pK_{a2} value observed on passing from L2 to L1 would explain the higher k'_{NA} value found for $[Zn_2L1(OH)_2]^{2+}$ with respect to $[Zn_2L2(OH)_2]^{2+}$. On the other hand the $[Zn_2L3(OH)_2]^{2+}$ complex displays a pK_{a2} value similar to that of the corresponding L1 complex {pK_{a2} = 9.8 and 9.5 for $[Zn_2L3(OH)_2]^{2+}$ and $[Zn_2L1(OH)_2]^{2+}$, respectively}, but, at the same time, is twice as active in NA hydrolysis {k'_{NA} = 2.2 and 1.1 $M^{-1}{\cdot}s^{-1}$ for $[Zn_2L3(OH)_2]^{2+}$ and $[Zn_2L1(OH)_2]^{2+}$, respectively}. A tentative explanation for this behavior could reside in the presence of a large heteroaromatic unit — phenanthroline — in the L3 cyclic framework. It is known, in fact, that phenanthroline-containing receptors can give host–guest adducts with substrates containing aromatic moieties, stabilized by strong π-stacking and hydrophobic interactions between the two aromatic units.[2,36–38] In the present case, these interactions between phenanthroline and the aromatic moiety of NA would stabilise the transient state of the nucleophilic attack, leading to the observed higher hydrolytic ability of the L3 complex. At the same time phenanthroline is poorly solvated in water. Therefore, the presence of this unit within the cyclic framework can provide a rather hydrophobic environment for the $Zn-OH$ function, enhancing its nucleophilic activity.

The high activity of the present complexes in NA hydrolysis prompted us to study their hydrolytic ability toward the phosphate ester bond, by using bis(*p*-nitrophenyl)phosphate (BNP) as substrate. It is known, in fact, that BNP hydrolysis in the presence of Zn^{II} complexes generally takes place through an "associative" mechanism,[33−35] in which substrate binding and activation by the metal center is followed by a nucleophilic attack at the phosphorus atom. Hydrophobic and π-stacking interactions would lead, in principle, to a stronger association between BNP and the Zn^{II} complex with L3, and, therefore, to an increased substrate activation of the hydrolytic process

BNP Binding by the Zn^{II} Complexes with L1−L3

Binding of BNP to the Zn^{II} complexes with ligands L1−L3 was monitored by means of ^{31}P NMR spectra recorded on MeOD/CD_3CN (50:50, v/v) solutions containing BNP (1×10^{-2} M) and the complexes in different molar ratios. Solutions containing BNP and increasing amounts of the dinuclear complexes display a progressive upfield shift of the BNP signals (Figure 3). In the case of the dizinc complexes with L3 (Figure 3c), the plot of $\Delta\delta$ (where $\Delta\delta$ is the difference between the chemical shift of BNP in the presence and in absence of the complex) as a function of the $[Zn_2L3](ClO_4)_4{\cdot}2H_2O$/[BNP] molar ratio ($R$) gives a straight line for $R < 0.8$. Then, the slope changes almost suddenly to give a straight line parallel to the x axis for $R > 1.2$ (at this point $\Delta\delta = 2.8$ ppm). This behavior indicates the formation of a stable 1:1 complex between the dizinc complex and BNP. A similar plot is obtained with the dizinc complex with L2; the slope change, however, is less sudden than in the case of the L3 complex and a lower $\Delta\delta$ value ($\delta = 1.9$ ppm) is reached for $R > 1.5$ (Figure 3b). Finally, in the presence of the dizinc complex with L1 a very smooth curve is observed up to $R = 5$ ($\Delta\delta = 0.55$ ppm), where precipitation of a 1:1 complex between the dizinc complex and BNP takes place. {A solid compound of stoichiometry $[Zn_2L1](BNP)(ClO_4)_3{\cdot}2H_2O$ was isolated and characterized by elemental analysis. $C_{26}H_{48}Cl_3N_8O_{20}PZn_2$ (1060.82): calcd. C 29.54, H 4.58, N 10.61, P 2.93; found C 28.99, H 4.50, N 10.25, P 2.73.} These data are in agreement with the formation of 1:1 complexes with BNP, whose stability increases in the order $[Zn_2L1]^{4+} < [Zn_2L2]^{4+} < [Zn_2L3]^{4+}$. Furthermore, the rather large shift for the signal of the phosphate ester bound to the L2 and L3 dinuclear complexes would suggest interaction of BNP with both Zn^{II} ions through a bridging coordination of the phosphate anion. From this point of view, it is of interest to note that no shift of the BNP signal is observed in the presence of the mononuclear Zn^{II} complexes with ligands L1−L3. Most likely, in the mononuclear complexes the Zn^{II} ion is almost coordinately saturated by the ligand donors and no free binding site is available at the metal for BNP coordination.

The ^{31}P NMR spectra recorded in D_2O solution display minor shifts of the BNP signal, even in the presence of a large excess of the dinuclear complexes ($\delta = 0.5$ and 0.3 ppm in the case of L3 and L2, respectively, and $\delta \approx$ 0.1 ppm for L1), probably due to a much lower percentage of coordinated BNP in this more solvating medium. In the case of L2 and L3, ^{31}P NMR titrations at pD = 10 (see Supporting Information), carried out by adding increasing amounts of $[Zn_2L3](ClO_4)_4{\cdot}2H_2O$ or $[Zn_2L2](ClO_4)_4{\cdot}4H_2O$ to a BNP solution lead to calculate a log K value of 2.5 ± 0.2 and 1.7 ± 0.1 for the addition of BNP to the dinuclear

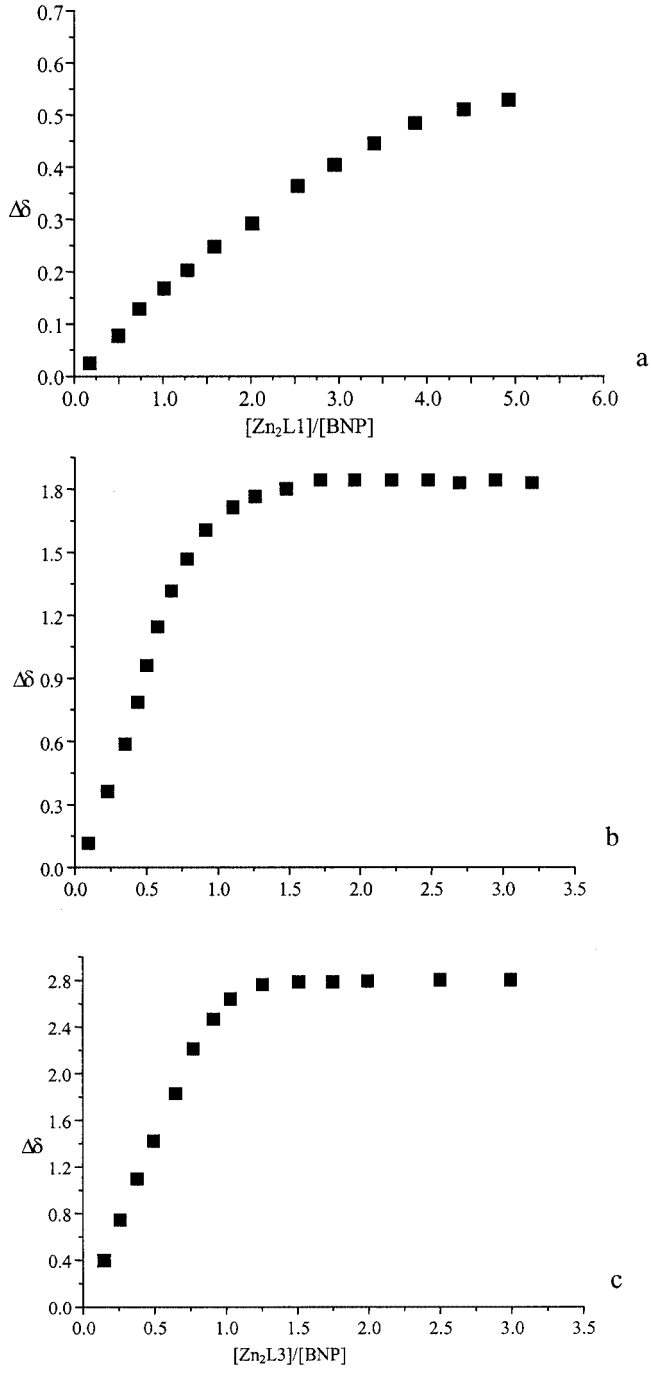
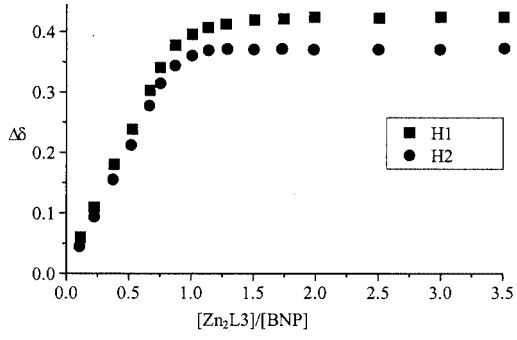


Figure 3. Plot of the ^{31}P NMR chemical shifts of BNP ($\Delta\delta = \delta_{BNP} - \delta_{OBS}$, where δ_{BNP} = chemical shift of the unbound BNP ester and δ_{OBS} = observed chemical shift in the presence of the dinuclear complexes with L1−L3) as a function of the [$[Zn_2L1](ClO_4)_4{\cdot}2H_2O$]/[BNP] (a), [$[Zn_2L2](ClO_4)_4{\cdot}4H_2O$]/[BNP] (b) and [$[Zn_2L3](ClO_4)_4{\cdot}2H_2O$]/[BNP] molar ratios (MeOD/CD_3CN solution, 298 K, [dizinc complex] = 1×10^{-2} M)

Zn^{II} complex with L3 and L2, respectively. In the case of the dizinc complex with L1, the observed shift of the ^{31}P NMR signal is too low to confidently determine the addition constant of BNP to the complex.

To obtain further information on the interaction mode of the BNP anion with our dizinc complexes, we also recorded ^{1}H NMR spectra of solutions containing BNP and increasing amounts of dinuclear Zn^{II} complexes with L1−L3. Although the ^{1}H NMR spectra of the ligands in their Zn^{II} complexes are rather fluxional and do not allow a correct determination of the ^{1}H chemical shifts, the resonances of the aromatic protons of BNP [two doublets at δ = 7.98 (H1; for atom labelling see Figure 4) and δ = 7.13 ppm (H2) in the absence of the Zn^{II} complexes] are generally easily recognizable. The ^{1}H NMR signals of BNP are almost unaffected by the presence, even in large excess, of the dizinc complexes with ligand L1 and L2. On the contrary, addition of the dizinc complex with L3 to a BNP solution gives rise to a significant upfield shift of both the BNP signals (Figure 4). As in the case of the ^{31}P NMR spectra, the plot of the $\Delta\delta$ values as a function of the [dinuclear L3 complex]/[BNP] molar ratio (R) gives a straight line for $R < 0.8$; subsequently, the slope changes to give a straight line parallel to the x axis for $R > 1.2$ (at this point $\Delta\delta$ = 0.42 and 0.37 ppm for the protons H1 and H2, respectively). The upfield shifts observed for the BNP signals are indicative of a π-stacking interaction between the aromatic rings of the ester and the large phenanthroline unit. This interaction seems to be absent in the case of the zinc complexes with L2, which contains a smaller aromatic unit.



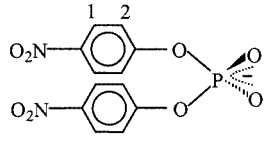


Figure 4. Plot of the ^{1}H NMR chemical shifts of BNP ($\Delta\delta = \delta_{BNP} - \delta_{OBS}$, where δ_{BNP} = chemical shift of the unbound BNP ester and δ_{OBS} = observed chemical shift in the presence of the dinuclear complexes with L1−L3) as a function of the $[[Zn_2L3](ClO_4)_4{\cdot}2H_2O]$/[BNP] molar ratios (MeOD/CD_3CN solution, 298 K, $[[Zn_2L3](ClO_4)_4{\cdot}2H_2O] = 1 \times 10^{-2}$ M)

Both the ^{31}P and ^{1}H NMR experiments account for a higher overall interaction between the phosphate ester and the dizinc complex with L3, probably due to the synergetic effect in BNP binding exerted by the two metal centers, which can act cooperatively in substrate coordination, and by the phenanthroline moiety, which can interact with BNP by π-stacking.

To shed further light on the role played by substrate interaction and activation by the present metal complexes, we decided to carry out a comparative kinetic study on the ability of the L1−L3 complexes to hydrolyze BNP.

BNP Hydrolysis Promoted by the Zn^{II} Complexes with L1−L3

A kinetic analysis of BNP cleavage shows that only the dinuclear complexes with L1−L3 promote BNP hydrolysis, while the mononuclear complexes are totally unable to hydrolyze this ester. Figure 5 shows the second-order rate constants for BNP cleavage (k_{BNP}) in the presence of the ligand L3 and increasing amounts of Zn^{II} at pH = 10. No hydrolytic effect is observed with molar ratios $R < 1$ for Zn^{II}/L3, when only mononuclear complexes are formed in aqueous solutions; for $1 < R < 2$, the k_{BNP} values increase almost linearly with R, due to the formation of increasing amounts of the dinuclear complex. The maximum k_{BNP} value is obtained when $R = 2$, where only the dinuclear complex is present in solution at pH = 10. Addition of further Zn^{II} ($R > 2$) does not change the hydrolysis rate.

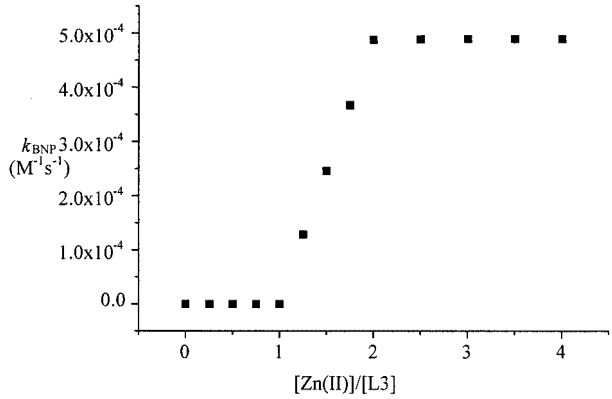


Figure 5. Second-order rate constants for BNP cleavage (k_{BNP}) as a function of the $[Zn^{II}]$/[L3] molar ratio at pH = 10 (CHES buffer)

For all the three ligands, only the dihydroxo complexes $[Zn_2L(OH)_2]^{2+}$ promote BNP hydrolysis in aqueous solution, to give mono(*p*-nitrophenyl phosphate) (MNP) and *p*-nitrophenolate, while the monohydroxo species $[Zn_2L(OH)]^{3+}$ do not promote this process (Figure 5), in agreement with the very much lower activity in NA hydrolysis found for this species. Plots of the second-order rate constants k_{BNP} for the L1, L2 and L3 dizinc complexes as a function of pH fit the distribution curves of the $[Zn_2L1(OH)_2]^{2+}$, $[Zn_2L2(OH)_2]^{2+}$, and $[Zn_2L3(OH)_2]^{2+}$ species (Figure 6), pointing out that these dihydroxo complexes are the kinetically active species. As in the case of NA hydrolysis, second-order rate constants k'_{BNP} can be

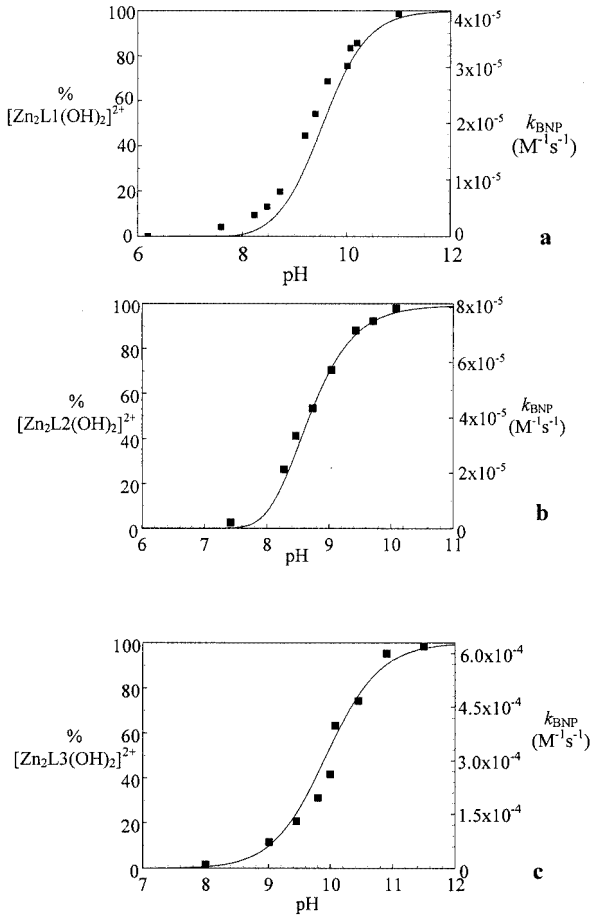


Figure 6. Plots of the distribution curves of $[Zn_2L1(OH)_2]^{2+}$ (a), $[Zn_2L2(OH)_2]^{2+}$ (b) and $[Zn_2L3(OH)_2]^{2+}$ (c) (solid line, right *y* axes) and k_{BNP} values (squares, left *y* axes) as a function of pH

calculated from the maximum k_{BNP} value by using the expression:

$$v = k_{BNP}[\text{total } Zn^{II}\text{complex}][BNP] = k'_{BNP}[Zn_2L(OH)_2{}^{2+}][BNP]$$

Table 3. Second-order rate constants *k'BNP* [M^{-1} s^{-1}] for hydrolysis of bis(4-nitrophenyl)phosphate

Nucleophile	$k'_{BNP}\cdot10^5$ [M^{-1} s^{-1}]	pK_a
$[Zn_2L1(OH)_2]^{2+}$	4.0 ± 0.2	9.5
$[Zn_2L2(OH)_2]^{2+}$	8.0 ± 0.4	8.4
$[Zn_2L3(OH)_2]^{2+}$	62 ± 3	9.8
1	8.5[a]	7.2
2	2.1[a]	7.9
$[Zn_2L8(OH)_2]^{2+}$	11.0[b]	9.1

[a] From ref.[40]; I = 0.15 M $NaClO_4$. [b] From ref.[24]; I = 0.1 M NaCl.

Table 3 reports the k'_{BNP} values for the $[Zn_2L(OH)_2]^{2+}$ complexes, in comparison with the corresponding pK_{a2} values. The k'_{BNP} values reported for the mononuclear complexes **1** and **2**[34] and for the dinuclear complex with L8[24] (Scheme 3) are also reported for comparison. Considering L1 and L2, the activity of their complexes in BNP hydrolysis decreases from L2 to L1, i.e., the hydrolytic properties decrease as the pK_a of the complexes increases, which is the opposite behavior to that found in NA cleavage. Thus, in BNP cleavage the hydrolytic properties of these complexes are not only determined by the nucleophilicity of the Zn−OH functions. This behavior has been already observed in both mono- and dinuclear Zn^{II} complexes and can be explained in terms of an "associative" mechanism,[24,32−34] in which the substrate approaches the $[Zn_2L(OH)_2]^{2+}$ complexes and the oxygen atoms of BNP associate with the electrophilic Zn^{II} ions, probably through a bridging coordination. A zinc-bound hydroxide ion then nucleophilically attacks the substrate (Figure 7). Lower pK_a values generally imply a better ability in substrate binding. The L2 complex, which contains the less nucleophilic Zn^{II}−OH functions, would give a stronger interaction with BNP during the hydrolytic process, leading to enhanced substrate activation and to higher rate acceleration. This is in agreement with the ^{31}P NMR results, which point out a higher interaction of BNP with the dinuclear L2 complex.

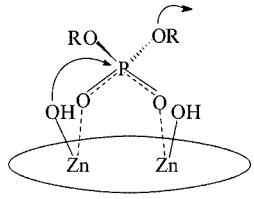


Figure 7. Proposed mechanism for BNP hydrolysis promoted by the dinuclear Zn^{II} complex with L2 and L3

Actually, the hydrolytic activity of the dinuclear complex with L2 is similar to that found for the mononuclear complex **1** and somewhat lower than that of the dinuclear Zn^{II} complex with [30]aneN_6O_4 (L8 in Scheme 3). The L2 complex, however, shows a better ability in BNP hydrolysis than the mononuclear complex **2**. However, experiments performed on solutions containing ligand L2 and Zn^{II} in 1:1 molar ratio in the alkaline pH region (pH = 7−10) showed that the mononuclear complex with L2 is completely unable to promote BNP hydrolysis. This suggests a cooperative effect of the two metal ions in BNP hydrolysis through a bridging coordination of the substrate to the two Zn^{II} ions, as actually confirmed by the marked upfield shift observed for the ^{31}P NMR signal of BNP upon coordination to the L2 complex.

The most interesting finding in Table 2, however, is the remarkably high k_{BNP} value found for $[Zn_2L3(OH)_2]^{2+}$, which is almost eight times more active than the corresponding L2 complex. A tentative explanation for this effect could reside in the stronger interaction of the L3 complex with BNP suggested by the ^1H and ^{31}P NMR results. Although precipitation of a solid complex {a compound of stoichiometry $[Zn_2L3](BNP)_2(ClO_4)_2$ precipitates at pH = 10 with a molar ratio of BNP/dizinc complex > 5; $C_{48}H_{56}Cl_2N_{11}O_{24}P_2Zn_2$ (1434.67): calcd. C 40.22, H 3.93, N 10.74, P 4.32; found C 40.76, H 4.08, N 10.55, P 4.5} at high molar ratios between BNP and the L3 dizinc complex prevents a Michaelis−Menten analysis of this hydrolytic

process, the simultaneous bridging coordination of the phosphate ester to the two metal centers and π-stacking and/or hydrophobic interactions between phenanthroline and the *p*-nitrophenylene groups of BNP could reinforce the interaction between the dizinc complex and the substrate.

Conclusions

Ligands L1−L3 present similar binding features toward Zn^{II}, giving rise to both mono- and dinuclear complexes in aqueous solutions. The dizinc complexes are characterized by a marked tendency to form hydroxo species; the relatively low number of nitrogen donors, in fact, cannot fulfil the coordination sphere of two metal ions and, therefore, facile deprotonation of Zn^{II}-bound water molecules occurs on going from from neutral to alkaline pH values. This feature parallels the behavior of Zn^{II}-based hydrolytic enzymes, where Zn−OH functions behave as nucleophilic agents in the overall catalytic process.

Despite the similar coordination properties of L1−L3, their dizinc complexes display remarkably different hydrolytic ability. While the dinuclear Zn^{II} complexes with L1 and L2 show a hydrolytic activity toward NA and BNP similar to that reported for other Zn^{II} complexes developed as functional models for hydrolytic metalloenzymes, the dinuclear Zn^{II} complexes with L3, which contain a large and hydrophobic heteroaromatic moiety, give remarkably higher rate enhancements; among synthetic dizinc complexes, the L3 complex is one of the most active in both NA and BNP hydrolysis. The synergetic role in BNP binding of phenanthroline, which can give π-stacking and hydrophobic interactions with the aromatic rings of the substrate, and of the two metal ions, which act cooperatively in substrate coordination, probably gives a higher BNP activation.

Experimental Section

General Procedures: Ligands L1 and L2 were prepared as described elsewhere and handled as their hydrochloride salts.[25,26] UV/Vis spectra were recorded with a Shimadzu UV-2101PC spectrophotometer.

NMR Measurements: The ^1H and ^{13}C NMR spectra were recorded with Varian UNITY 300 and UNITY 400 spectrometers, operating at 299.95 and 399.95 MHz for ^1H and at 75.43 and 100.58 MHz for ^{13}C. The spectra were obtained at room temperature in D_2O or $CDCl_3$ solutions. For the ^{13}C NMR spectra dioxane was used as a reference standard (δ = 67.4 ppm) and for the ^1H NMR spectra the solvent signal. Adjustments to the desired pH were made by adding drops of DCl or NaOD solutions. The pH was calculated from the measured pD values using the correlation pH = pD − 0.4.[39] In the ^{31}P and ^1H NMR titrations, increasing amounts of 1 × 10^{-2} M solutions in MeOD/CD_3CN (50:50, v/v) or in D_2O at pH = 10 of the preformed Zn^{II} complexes with L1−L3 (see below) were added to a 5 × 10^{-3} M solution of BNP. The addition constants of BNP to dizinc complexes with L2 and L3 were calculated by means of the HYP NMR program.[40] TMS or DSS were used as internal standard.

2,6,9,12,16-Pentaaza[17](2,9)(1,10)phenanthrolinophane Pentahydrobromide (L3·5HBr): 4,7,10-Tris(*p*-tolylsulfonyl)-4,7,10-triaazatridecane-1,13-diamine (2.5 g, 3.68 mmol)[41] and 1,10-phenanthroline-2,9-dicarboxaldehyde (0.88 g, 3.73 mmol) were stirred overnight in a mixture of $CHCl_3$ and MeOH (1:5; 300 mL) to give the imine. Sodium borohydride (0.57 g, 15.2 mmol) was added and the mixture was stirred at room temperature for 24 h. The solvent was then removed at reduced pressure. The resulting residue was treated with water (50 mL) and extracted with dichloromethane (2 × 45 mL). Then, it was dried with Na_2SO_4 and the solvent removed by rotary evaporation. The tosyl groups of tritosylated L3 (3 g, 3.39 mmol) were removed by reductive cleavage with a 33% HBr/AcOH mixture (120 mL) and phenol (15 g, 160 mmol) heating at 90 °C for 18 h and then cooling to obtain a white solid. The resulting solid was filtered off and washed several times with CH_2Cl_2 and EtOH. The macrocycle was obtained as its hydrobromide salt (1.2 g, yield 44%). ^1H NMR (D_2O): δ = 1.95−2.03 (m, 4 H), 2.99 (t, J = 8 Hz, 4 H), 3.12 (t, J = 8 Hz, 4 H), 3.31−3.39 (m, 8 H), 4.6 (s, 4 H), 7.63 (d, J = 8 Hz, 2 H), 7.75 (s, 2 H), 8.34 (d, 2 H) ppm. ^{13}C NMR (D_2O): δ = 22.9, 43.3, 43.7, 45.1, 45.5, 51.9, 123.7, 127.5, 129.4, 139.9, 150.9 ppm. $C_{24}H_{40}N_7$·5HBr (831.19): calcd. C 34.89, H 4.88, N 11.88; found C 35.0, H 4.9, N 12.0.

Synthesis of the Dinuclear Zn^{II} Complexes with L1−L3: The Zn^{II} complexes $[Zn_2L1](ClO_4)_4$·$2H_2O$, $[Zn_2L2](ClO_4)_4$·$4H_2O$ and $[Zn_2L3](ClO_4)_4$·$2H_2O$ were obtained by slow evaporation of the solvent from butanol solutions containing ligands L1−L3 and $Zn(ClO)_4$·$6H_2O$ in a 1:2 molar ratio and characterized by means of elemental analysis. $[Zn_2L1](ClO_4)_4$·$2H_2O$: $C_{14}H_{40}Cl_4N_6O_{18}Zn_2$ (853.19): calcd. C 19.71, H 4.73, Cl 16.62, N 9.85; found C 19.99, H 4.78, Cl 16.3, N 10.01. $[Zn_2L2](ClO_4)_4$·$4H_2O$: $C_{22}H_{50}Cl_4N_6O_{20}Zn_2$ (991.26): calcd. C 26.66, H 5.08, Cl 14.31, N 8.48; found C 26.75, H 5.12, Cl 14.2, N 8.57. $[Zn_2L3](ClO_4)_4$·$2H_2O$: $C_{24}H_{39}Cl_4N_7O_{18}Zn_2$ (986.20): calcd. C 29.23, H 3.39, Cl 14.38, N 9.94; found C 29.79, H 3.51, Cl 14.2, N 10.05.

emf Measurements: The potentiometric titrations were carried out at 298.1 ± 0.1 K using 0.15 M $NaClO_4$ as supporting electrolyte. The experimental procedure (burette, potentiometer, cell, stirrer, microcomputer, etc.) has been fully described elsewhere.[42] The acquisition of the emf data was performed with the computer program PASAT.[43] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen-ion-concentration probe by titration of previously standardized amounts of HCl with CO_2-free NaOH solutions and determining the equivalent point by Gran's method,[44] which gives the standard potential, $E^{0\prime}$, and the ionic product of water [pK_w = 13.73(1)]. The computer program HYPERQUAD was used to calculate the protonation and stability constants.[45] The protonation constants of L1 and L2 were taken from refs.[25,26], respectively. Protonation constants of L3 determined in 0.15 M NaCl at 298.1 K are as follows: log $K_{HL/H\cdot L}$ = 10.0(1), log $K_{H2L/HL\cdot H}$ = 9.15(1), log $K_{H3L/H2L\cdot H}$ = 7.81(1), log $K_{H4L/H3L\cdot H}$= 6.42(1), log $K_{H5L/H4L\cdot H}$ = 2.97(2). The pH range investigated was 2.5−10.5 and the concentration of the Zn^{II} ions and that of the ligands ranged from 1 × 10^{-3} to 5 × 10^{-3} M with Zn^{II}/L molar ratios varying between 2:1 and 1:2. The different titration curves for each system (at least two) were treated either as a single set or as separate curves without significant variations in the values of the stability constants. Finally, the sets of data were merged and treated simultaneously to give the final stability constants.

Kinetics of *p*-Nitrophenyl Acetate (NA) and Bis(*p*-nitrophenyl)phosphate (BNP) Hydrolysis: The hydrolysis rate of 4-nitrophenyl acetate in the presence of the dizinc complexes of L1, L2 and L3 was

measured by an initial-slope method monitoring the increase in the 403-nm absorption of the released 4-nitrophenolate ion at 298.1 ± 0.1 K by using the procedure reported previously.[24] The ionic strength was adjusted to 0.15 M with $NaClO_4$. The reaction solution was maintained at 298.1 ± 0.1 K. MOPS (pH = 7.0−7.8), TAPS (pH = 7.8−8.9), CHES (pH = 8.9−9.7) and CAPSO (pH = 9.1−10.2) buffers were used (50 mM). In a typical experiment, after 4-nitrophenyl acetate and the dizinc complexes $[Zn_2L1](ClO_4)_4{\cdot}2H_2O$, $[Zn_2L2](ClO_4)_4{\cdot}4H_2O$ and $[Zn_2L3](ClO_4)_4{\cdot}2H_2O$ (0.1−1.0 mM) in 10% CH_3CN solutions at appropriate pH (the reference experiment does not contain the Zn^{II} complex) were mixed, the UV absorption decay was recorded immediately and was monitored generally until 2% decay of 4-nitrophenyl acetate. For all three ligands, two species — $[Zn_2LOH]^{3+}$ and $[Zn_2L(OH)_2]^{2+}$ — promote NA hydrolysis; in the case of L1, measurements in the pH range 7.5−8.2, where the $[Zn_2L1(OH)_2]^{2+}$ complex is absent from the solution, allowed us to determine second-order k_{NA1} rate constants for promoted hydrolysis by the monohydroxo species $[Zn_2L1OH]^{3+}$ and to extrapolate the k_{NA2} values for this species in the pH range 8.7−10.0, where both species are present in solution. Measurements in the pH range 8.7−10.0 allowed us to determine k_{OBS} values. The second-order rate constants k_{NA2} for the dihydroxo complex $[Zn_2L1(OH)_2]^{2+}$ are calculated by subtracting from the k_{OBS} value at a given pH the k_{NA1} value at the same pH. An analogous procedure was used for the L2 and L3 complexes. Errors in k_{NA} values were generally 5%. The hydrolysis rate of BNP to give mono(*p*-nitrophenyl)phosphate (MNP) and *p*-nitrophenolate (the hydrolysis products were identified by means of ^1H and ^{31}P NMR spectroscopy) in the presence of the dizinc complexes with L1−L3 was measured by an initial-slope method monitoring the increase in the 403 nm absorption of the *p*-nitrophenolate at 308.1 ± 0.1 K using a similar procedure to that reported for NA. The ionic strength was adjusted to 0.1 with NMe_4NO_3. The reaction solution was maintained at 308.1 ± 0.1 K. MOPS (pH = 6.5−7.8), TAPS (pH = 7.8−8.9), CHES (pH = 8.9−10.1) and CAPS (pH = 10.1−11.4) buffers were used (50 mM). Freshly prepared stock solutions of the zinc complexes and of BNP (1−10 mM) where used in the measurements. In a typical experiment, immediately after BNP and the zinc complexes were mixed in aqueous solutions at the appropriate pH value (the reference experiment does not contain the Zn^{II} complex), the UV absorption spectrum was recorded and monitored generally until 2% decay of BNP (for each second-order rate constant determination at least five experiments were monitored until 20−30%). For all systems, only the dihydroxo complexes $[Zn_2L(OH)_2]^{2+}$ (L = L1−L3) promote BNP hydrolysis. A plot of the hydrolysis rate vs. BNP concentration (0.1−1.0 mM) at a given pH value gave a straight line, and then we determined the slope/[zinc complex] ratio as the second-order rate constants k_{BNP} [M^{-1} s^{-1}]. Errors in k_{BNPP} values were about 5%.

Acknowledgments

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