Inorg. Chem. **2003**, *42*, 6929–6939

Coordination Properties of New Bis(1,4,7-triazacyclononane) Ligands: A Highly Active Dizinc Complex in Phosphate Diester Hydrolysis

Massimiliano Arca,[†] Andrea Bencini,*[,‡] Emanuela Berni,[‡] Claudia Caltagirone,[†] Francesco A. Devillanova,[†] Francesco Isaia,[†] Alessandra Garau,[†] Claudia Giorgi,[‡] Vito Lippolis,*[,†] Alessandro Perra,[†] Lorenzo Tei,[†] and Barbara Valtancoli[‡]

Dipartimento di Chimica Inorganica ed Analitica, Università degli Studi di Cagliari, SS 554 Bivio per Sestu, 09042 Monserrato (CA), Italy, and Dipartimento di Chimica, Polo Scientifico, Università di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy

Received January 17, 2003

The synthesis and characterization of three new bis([9]aneN$_3$) ligands, containing respectively 2,2′-bipyridine (L^1), 1,10-phenanthroline (L^2), and quinoxaline (L^3) moieties linking the two macrocyclic units, are reported. Proton binding and Cu(II), Zn(II), Cd(II), and Pb(II) coordination with L^1–L^3 have been studied by potentiometric titrations and, for L^1 and L^2, by spectrophotometric UV–vis measurements in aqueous solutions. All ligands can give stable mono- and dinuclear complexes. In the case of L^1, trinuclear Cu(II) complexes are also formed. The stability constants and structural features of the formed complexes are strongly affected by the different architecture and binding properties of the spacers bridging the two [9]aneN$_3$ units. In the case of the L^1 and L^2 mononuclear complexes, the metal is coordinated by the three donors of one [9]aneN$_3$ moiety; in the [ML^2]$^{2+}$ complexes, however, the phenanthroline nitrogens are also involved in metal binding. Finally, in the [ML^3]$^{2+}$ complexes both macrocyclic units, at a short distance from each other, can be involved in metal coordination, giving rise to sandwich complexes. In the binuclear complexes each metal ion is generally coordinated by one [9]aneN$_3$ unit. In L^1, however, the dipyridine nitrogens can also act as a potential binding site for metals. The dinuclear complexes show a marked tendency to form mono-, di-, and, in some cases, trihydroxo species in aqueous solutions. The resulting M–OH functions may behave as nucleophiles in hydrolytic reactions. The hydrolysis rate of bis(*p*-nitrophenyl)phosphate (BNPP) was measured in aqueous solution at 308.1 K in the presence of the L^2 and L^3 dinuclear Zn(II) complexes. Both the L^2 complexes [Zn$_2$L^2(OH)$_2$]$^{2+}$ and [Zn$_2$L^2(OH)$_3$]$^+$ and the L^3 complex [Zn$_2$L^3(OH)$_3$]$^+$ promote BNPP hydrolysis. The [Zn$_2$L^3(OH)$_3$]$^+$ complex is ca. 2 orders of magnitude more active than the L^2 complexes, due both to the short distance between the metal centers in [Zn$_2$L^3(OH)$_3$]$^+$, which could allow a bridging interaction of the phosphate ester, and to the simultaneous presence of single-metal bound nucleophilic Zn–OH functions. These structural features are substantially corroborated by semiempirical PM3 calculations carried out on the mono-, di-, and trihydroxo species of the L^3 dizinc complex.

Introduction

The rationale design and synthesis of ligand systems capable of accommodating two or more metal centers in close proximity and in predetermined spatial arrangements is an active area of research. Indeed, polynuclear complexes, in which cation−cation interactions can be tuned via functionality variations of the ligand structure, can find applications as models for important metallobiosites,[1−4] for catalysts,[5] and in the study of the mutual influence of proximal metal centers on the redox, electronic, and magnetic properties of such

* To whom correspondence should be addressed. E-mail: lippolis@unica.it (V.L.). Phone: +39 0706754467 (V.L.). Fax: +39 0706754456 (V.L.).
[†] Università di Cagliari.
[‡] Università di Firenze.

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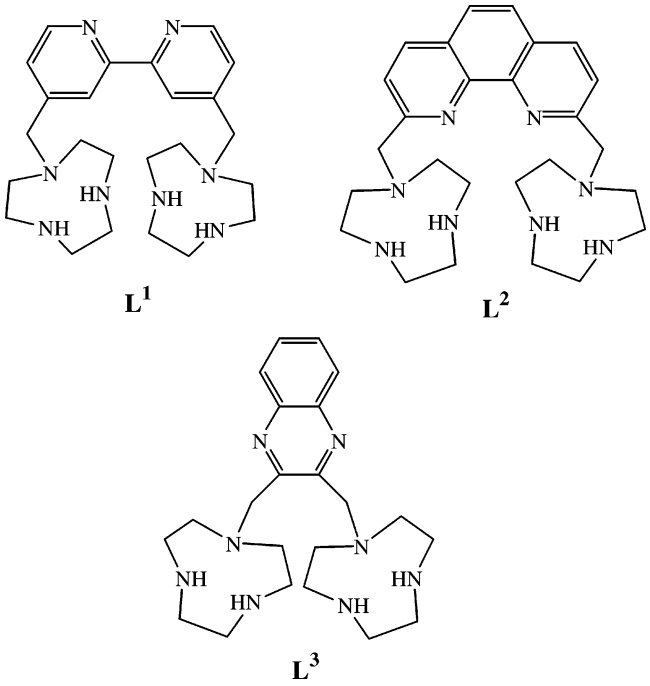
10.1021/ic034050r CCC: $25.00 © 2003 American Chemical Society
Published on Web 09/23/2003

systems.[6−9] In this regard, a whole range of bis(1,4,7-triazacyclonon-1-yl) ligands featuring for example alkane,[10] ether,[11] xylene,[12] durene,[13] phenol,[14] pyrazol,[15] naphthalene,[16] and calixarene[17] as groups bridging two or more [9]aneN_3 (1,4,7-triazacyclononane) nine-membered ring crowns has been prepared and the coordinating properties studied. These types of ligands can afford kinetically and thermodinamically stable polynuclear complexes with two or three coordination sites on each metal center, available for additional ligands or bridge formation.

Here we report the synthesis of three new bis([9]aneN_3) dinucleating systems (L^1−L^3), featuring respectively 2,2′-bipyridine (L^1), 1,10-phenanthroline (L^2), and quinoxaline (L^3) as noninnocent spacers of differing length, rigidity, and geometry connecting the two [9]aneN_3 macrocyclic units (Chart 1). The coordination properties of these ligands toward Cu(II), Zn(II), Cd(II), and Pb(II) have been studied in solution. The hydrolytic properties of the dizinc complexes with L^2 and L^3 toward the phosphate ester bond have been examined using bis(*p*-nitrophenyl)phosphate (BNPP) as a substrate.

Chart 1. Ligands



Experimental Section

General Methods. Microanalytical data were obtained using a Fison EA CHNS-O instruments operating at 1000 °C. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Varian VXR300 or a Varian VXR400 spectrometer. UV−vis absorption spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer. ESI-TOF mass spectra were recorded on a Applied Biosystems Mariner instrument. HNO_3 and NaOH were used to adjust the pH values which were measured on a Metrohm 713 pH meter.

Synthesis of the Compounds. 2,9-Bis(chloromethyl)-1,10-phenanthroline,[18] 4,4′-bis(chloromethyl)-2,2′-bipyridine,[19] and 1,4,7-triazatricyclo[5.2.1.0^{4,10}]decane[20] were prepared according to published methods. 2,3-Bis(bromomethyl)quinoxaline and other reagent or analytical grade materials were obtained from commercial suppliers and used without further purification. All solvents were dried by conventional methods, freshly distilled, and degassed.

L^1·4HBr·2H_2O. A solution of 1,4,7-triazatricyclo[5.2.1.0^{4,10}]-decane (1.0 g, 7.2 mmol) in dry MeCN (30 cm^3) was added dropwise to a stirred solution of 4,4′-bis(chloromethyl)-2,2′-bipyridine (0.91 g, 3.6 mmol) in MeCN (50 cm^3) over a period of 1 h, under a nitrogen atmosphere. After 48 h of stirring at room temperature, the formed pale pink precipitate was filtered off, washed with MeCN and Et_2O, and dried under reduced pressure. A further amount of this solid was recovered from the MeCN solution by partial removal of the solvent under reduced pressure. All the solid collected was then dissolved in water (40 cm^3), refluxed for 4 h, and stirred overnight at room temperature. NaOH pellets (1 g, 25 mmol) were carefully added to the solution, and the mixture was refluxed for a further 4 h. It was then extracted with CH_2Cl_2, and the resulting organic layers were combined and dried over $MgSO_4$. Following removal of solvent, L^1 was obtained as a white solid (0.82 g, 52% yield). The HBr salt of the ligand can be prepared by dissolving the crude product in ethanol and adding concentrated HBr resulting in the formation of a white precipitate of L^1·4HBr·2H_2O. Anal. Found (calcd for $C_{24}H_{46}Br_4N_8O_2$): C, 36.46 (36.11); H, 5.85 (5.81); N, 14.07 (14.04). ^{1}H NMR (D_2O, 298 K, 300 MHz): δ_H 3.23 (8H, t, J = 5.6 Hz, ArCH_2-NCH_2), 3.48 (8H, t, J = 5.6 Hz, ArCH_2NCH_2CH_2N), 3.80 (8H, s, NCH_2CH_2N), 4.27 (4H, s, ArCH_2N), 7.97 (2H, d, J = 5.4 Hz, Ar*H*), 8.61 (2H, s, Ar*H*), 8.88 (2H, d, J = 5.4 Hz, Ar*H*). ^{13}C NMR (D_2O, 298 K): δ_C 44.89, 46.45 (*C*H_2N(H)*C*H_2), 50.35 (*C*H_2N(*C*H_2Ar)), 59.81 (Ar*C*H_2N), 127.50, 130.44, 148.78, 150.35, 154.92 (*C* aromatic carbons). MS (ESI): *m*/*z* 220 ([M + 2H]$^{2+}$).

L^2·4HBr·4H_2O. L^2 can be synthesized by starting from 2,9-bis-(chloromethyl)-1,10-phenanthroline (0.7 g, 2.53 mmol) and following the methods described for L^1. The HBr salt of L^2 can be prepared by dissolving the crude product in ethanol and adding concentrated HBr resulting in the formation of a white precipitate,

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which after several recrystallizations from EtOH/H_2O proved to be L^2·4HBr·4H_2O (1.00 g, 46% yield). Anal. Found (calcd for $C_{26}H_{50}Br_4N_8O_4$): C, 36.70 (36.38); H, 5.75 (5.87); N, 12.84 (13.05). ^{1}H NMR (D_2O, 298 K, 300 MHz): δ_H 2.05−3.40 (24H, m), 3.72 (4H, s, ArC*H*$_2$N), 6.80−8.00 (6H, m, Ar*H*). ^{13}C NMR (D_2O, 298 K): δ_C 44.41, 45.88 (*C*H_2N(H)*C*H_2), 51.18 (*C*H_2N(CH_2Ar)), 62.35 (ArCH_2N), 122.94, 125.58, 127.65, 137.05, 143.52, 165.88 (C aromatic carbons). MS (ESI): *m*/*z* 231 ([M + 2H]$^{2+}$).

L^3·4HBr·4H_2O. The reaction of 2,3-bis(bromomethyl)quinoxaline (1.51 g, 4.77 mmol) with the orthoamide derivative of [9]aneN_3 (in a 1:2 molar ratio) followed by base hydrolytic workup according to the methods described for L^1 and L^2 gives L^3 as a brown solid. Analogously, the HBr salt of the ligand L^3 can be prepared by dissolving the crude product in ethanol and adding concentrated HBr, which results in the formation of a white hygroscopic precipitate of L^3·4HBr·4H_2O (3.01 g, 78% yield). Anal. Found (calcd for $C_{22}H_{48}Br_4N_8O_4$): C, 32.85 (32.69); H, 6.23 (5.98); N, 13.69 (13.86). ^{1}H NMR (D_2O, 298 K, 300 MHz): δ_H 3.48 (8H, s, NC*H*$_2$C*H*$_2$N), 3.50−4.00 (16H, m, ArCH_2NC*H*$_2$C*H*$_2$N), 7.80−8.3 (4H, m, Ar*H*). ^{13}C NMR (D_2O, 298 K): δ_C 44.28, 45.79, (*C*H_2N(H)*C*H_2), 49.98 (*C*H_2N(CH_2Ar)), 55.66 (Ar*C*H_2N), 127.52, 131.31, 139.89, 154.15 (*C* aromatic carbons). MS (ESI): *m*/*z* 207 ([M + 2H]$^{2+}$).

Complexes. Many attempts were made to try to isolate and crystallize metal complexes of L^1−L^3. Generally, solid compounds that did not give a satisfactory elemental analysis were isolated from the reaction of the ligands with metal salts in either organic solvents or water at controlled pH, and their recrystallization was unsuccessful. Presumably the precipitation of more than one species occurred in the experimental condition used. However, solid microcrystalline compounds corresponding to the stoichiometry $[Cu_3L^1]Cl_6$, $[Zn_2L^2](NO_3)_4$·2H_2O, $[Zn_2L^3](NO_3)_4$·3H_2O, and $[Cu_2L^3]Br_4$ were isolated from the reaction of the appropriate metal salts with L^1, L^2, or L^3 in MeCN. All attempts to grow single crystals for X-ray diffraction analysis failed. The stoichiometry of the complexes was evaluated by elemental analysis and by analysis of the metal content via atomic absorption spectrometry using a Varian Liberty 200 ICP emission spectrometer.

Potentiometric Measurements. Ligand protonation constants and equilibrium constants for the complexation reactions were determined by pH-metric measurements (pH = −log [H^+]) in 0.1 mol dm^{-3} NMe_4NO_3 at 298.1 or 308.1 ± 0.1 K, using the potentiometric equipment already described.[21] The combined glass 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,[22] which allows to determine the standard potential $E°$, and the ionic product of water (pK_w = 13.83(1) at 298.1 and 13.40 at 308.1 K, in 0.1 mol dm^{-3} NMe_4NO_3, K_w = [H^+][OH^-]). Ligand concentration was 1×10^{-3} mol dm^{-3}, while metal concentration varied from 0.5×10^{-3} to 3×10^{-3} mol dm^{-3}. At least three titration experiments (of about 100 data points each) were performed in the pH range 2.5−11. The computer program HYPERQUAD[23] was used to calculate equilibrium constants from emf data. All titrations were treated either as single sets or as separate entities for each system without significant variation in the values of the determined constants. In the HYPERQUAD program the sum of the weighted square residuals on the observed emf values is minimized. The weights were derived from the estimated errors in emf (0.2 mV) and titrant volume (0.002 cm^3). The most probable chemical model was selected by following a strategy based on the statistical inferences applied to the variance of the residuals, σ^2. The sample standard deviation should be 1, in the absence of systematic errors and when a corrected weighting scheme is used. However, the agreement is considered good for standard deviation values smaller than 3 (σ^2 < 9). Values of σ^2 lower than 6 were obtained for all the refined equilibrium models in the present works. If more than one model gave acceptable σ^2, the reliability of the proposed speciation models was checked by performing *F* tests at the 0.05 confidence level, using the method reported in ref 24 for two different proposed models, A and B. Assuming that the minimum value of the sample variance, σ_A^2, has been reached for the proposed model A, an alternative model B, which supplies a value of the variance σ_B^2 was rejected if $\sigma_B^2/\sigma_A^2 > F$, where σ_A and σ_B are given directly by data treatment with the HYPERQUAD[23] program. The *F* values were taken from ref 24a. For all the systems investigated, this method leads one to define univocally one acceptable system.

Kinetics of Bis(*p*-nitrophenyl) Phosphate (BNPP) Hydrolysis. The hydrolysis rate of BNPP to give mono(*p*-nitrophenyl) phosphate (MNPP) and *p*-nitrophenate (the hydrolysis products were identified by means of ^{1}H and ^{31}P NMR spectra) in the presence of the zinc complexes with L^2 and L^3 was measured by an initial slope method monitoring the increase in 403 nm absorption of the *p*-nitrophenate at 308.1 ± 0.1 K using the procedure reported in ref 25. 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 6.5−8.5), TAPS (pH 7.8−9.1), CHES (pH 8.6−10.1), and CAPS (pH 9.7−11.1) buffers were used (50 mM). Freshly prepared stock solutions of $Zn(NO_3)_2$ (1−10 mM) and ligands in 1:1 (L^2) or 2:1 (L^2 or L^3) molar ratio and of BNPP (1−10 mM) were used in the measurements. In a typical experiment, immediately after BNPP and the zinc complexes with L^2 or L^3 were mixed in aqueous solutions at the appropriate pH (the reference experiment does not contain the Zn(II) complex), the UV absorption spectrum was recorded and followed generally until 5−10% decay of BNPP. For L^2, two species, $[Zn_2L^2(OH)_2]^{2+}$ and $[Zn_2L^2(OH)_3]^+$ promote BNPP hydrolysis. Measurements in the pH range 6.7−7.8, where the $[Zn_2L^2(OH)_3]^+$ complex is absent from the solution, allow to determine second-order k_{BNPP2} constants for promoted hydrolysis by the dihydroxo species $[Zn_2L^2(OH)_2]^{2+}$ and to extrapolate the k_{BNPP2} values for this species in the pH range 7.8−10.5, where both species are present in solution. Measurements in the pH range 7.8−10.5 lead to determine k_{OBS} values. The second-order rate constants k_{BNPP3} for the trihydroxo complex $[Zn_2L^2(OH)_3]^+$ are calculated by subtracting, from the k_{OBS} value at a given pH, the k_{BNPP2} value at the same pH. Plots of the k_{BNPP2} and k_{BNPP3} as a function respectively of the $[Zn_2L^2(OH)_2]^{2+}$ and $[Zn_2L^2(OH)_3]^+$ molar concentrations give straight lines and allow one to determine the k'_{BNPP} values for 100% formation of $[Zn_2L^2(OH)_2]^{2+}$ and $[Zn_2L^2(OH)_3]^+$. A similar method was used for the L^3 complexes. Errors in k'_{BNPP} values were about 5%. The rate hydrolysis promoted by the dinuclear L^3 complexes was also determined by ^{31}P NMR measurements; solutions were prepared using the method described above for the spectrophotometric measurements. The increase of

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Table 1. Protonation Constant (log K) of Ligands L^1-L^3 (0.1 mol dm^{-3} NMe_4NO_3, 298.1 ± 0.1 K)

reacn	L^1	L^2	L^3
$H^+ + L \rightleftharpoons [HL]^+$	10.44(2)	10.01(2)	11.2(1)
$[HL]^+ + H^+ \rightleftharpoons [H_2L]^{2+}$	9.00(2)	9.23(2)	9.91(8)
$[H_2L]^{2+} + H^+ \rightleftharpoons [H_3L]^{3+}$	5.99(3)	6.39(6)	6.77(9)
$[H_3L]^{3+} + H^+ \rightleftharpoons [H_4L]^{4+}$	4.86(3)	4.24(7)	4.58(9)
$[H_4L]^{4+} + H^+ \rightleftharpoons [H_5L]^{5+}$	3.31(4)	2.88(7)	
$[H_5L]^{5+} + H^+ \rightleftharpoons [H_6L]^{6+}$	2.07(5)	2.0(1)	

Table 2. Protonation Constants and Metal Stability Constants (log K) for [9]aneN$_3$, 2,2′-Bipyridine, and 1,10-Phenanthroline

	[9]aneN$_3$	2,2-bipyridine	1,10-phenanthroline
$L + H^+ \rightleftharpoons [LH]^+$	10.42[a]	4.42[b]	4.96[b]
$[LH]^+ + H^+ \rightleftharpoons [H_2L]^{2+}$	6.80[a]	1.50[b]	1.90[b]
$Cu^{2+} + L \rightleftharpoons [CuL]^{2+}$	15.5[a]	9.00[c]	9.25[d]
$Zn^{2+} + L \rightleftharpoons [ZnL]^{2+}$	11.6[a]	4.95[e]	6.55[d]
$Cd^{2+} + L \rightleftharpoons [CdL]^{2+}$	9.5[f]	4.25[g]	5.78[g]
$Pb^{2+} + L \rightleftharpoons [PbL]^{2+}$	10.8[f]	2.90[g]	4.65[g]

[a] From ref 32. [b] From ref 31. [c] From ref 33. [d] From ref 34. [e] From ref 35. [f] From ref 36. [g] From ref 37. All data were obtained by means of potentiometric measurements at 298 K, with $I = 0.1$ mol dm^{-3} [a−d,g] or 0.2 mol dm^{-3}.[e,f]

the ^{31}P resonance for MNPP and the decrease of the ^{31}P resonance for BNPP were monitored in time. BNPP cleavage was generally followed until 40−50% BNPP decay.

Calculations. Molecular mechanics MMFF94[26] calculations were performed on the ligand L^3, and semiempirical PM3(tm)[27] geometry optimization was performed on the dizinc complexes $[Zn_2L^3(OH)]^{3+}$, $[Zn_2L^3(OH)_2]^{2+}$, and $[Zn_2L^3(OH)_3]^+$ on a Digital 500au Personal Workstation running Digital Unix using the Spartan 5.0 program.[28] The structures were analyzed by the Molekel program.[29]

Results and Discussion

Ligand Protonation. The protonation properties of ligands L^1-L^3 have been studied by means of potentiometric and, in the case of L^1 and L^2, by UV−vis spectrophotometric measurements in aqueous solutions. The protonation constants, determined by potentiometry, are reported in Table 1. L^1 and L^2 can bind up to six protons in aqueous solutions, while in the case of L^3 the fifth and sixth protonation constants are too low to be determined under our experimental conditions ($\log K < 2$). On the other hand, it is known that pyrazine nitrogens do not display a significant proton affinity in aqueous solution and can protonate only below pH 1.5.[30] Therefore, the four protonation steps found for L^3 should occur on the aliphatic amine groups. Furthermore, 1,10-phenanthroline and 2,2′-dipyridine are characterized by a lower basicity than aliphatic amines ($\log K = 4.96$ and 4.42[31] for the equilibrium $L + H^+ \rightleftharpoons LH^+$, with L = 1,10-phenanthroline and 2,2′-dipyridine, respectively, Table 2). Therefore, it is expected that in L^1 and L^2 at least the first protonation steps take place on the polyamine macrocycles. Actually, protonation of the aromatic nitrogens of 1,10-phenanthroline[38] or 2,2′-dipyridine[39] gives rise to a new red-shifted band in their UV absorption spectra. In the case of L^1 and L^2, a marked red shift of their UV bands (from 279 to 299 nm for L^1 and from 267 to 279 nm for L^2) is observed below pH 4.5 and 4, respectively, with the formation of the pentaprotonated species $[H_5L^1]^{5+}$ or $[H_5L^2]^{5+}$ (Figures S1 and S2, Supporting Information). This indicates that for both ligands protonation of the heteroaromatic nitrogens occurs at the fifth protonation step.

L^1 and L^2 display a first protonation constant similar to that found for [9]aneN$_3$ ($\log K = 10.42$).[32] Surprisingly, L^3 shows a higher basicity than L^1 and L^2, at least in the first two protonation steps. This result can be explained by considering that in L^3 the two macrocyclic rings are kept at close distance by the short quinoxaline linkage. A simple MM conformational search shows that in L^3 the two [9]aneN$_3$ units can assume a face to face conformation, with a distance between the two macrocycles of ca. 3.5 Å. Such a distance, in principle, would allow the formation of stabilizing hydrogen bond interactions between the two macrocyclic units, involving the protonated amine groups in the $[HL^3]^+$ and $[H_2L^3]^{2+}$ species.

Metal Complexation in Aqueous Solution. At a first glance, ligands L^1-L^3 display a similar molecular architecture, with two [9]aneN$_3$ binding units separated by a heteroaromatic spacer. Several bis(macrocycles) containing two polyamine moieties separated by aromatic spacers have been reported recently.[12−17,40−49] These ligands may behave as ditopic receptors for metal ions, giving rise to stable binuclear complexes, where each metal is generally coordi-

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Table 3. Stability Constants (log *K*) of the Metal Complexes with L^1 (0.1 mol dm^{-3} NMe_4NO_3, 298.1 ± 0.1 K)

reacn	Cu	Zn	Cd	Pb
$M^{2+} + L \rightleftharpoons [ML]^{2+}$	14.9(1)	10.65(5)	9.30(4)	10.0(1)
$[ML]^{2+} + H^+ \rightleftharpoons [MHL]^{3+}$	7.85(6)	8.13(5)	8.15(4)	8.20(4)
$[MHL]^{3+} + H^+ \rightleftharpoons [MH_2L]^{4+}$		5.52(6)	5.44(4)	5.61(4)
$[MH_2L]^{4+} + H^+ \rightleftharpoons [MH_3L]^{5+}$		4.44(6)	4.31(5)	4.31(5)
$[ML]^{2+} + OH^- \rightleftharpoons [ML(OH)]^+$	3.7(1)	3.75(9)		4.1(1)
$[ML(OH)]^+ + OH^- \rightleftharpoons ML(OH)_2$		3.27(9)		3.5(1)
$[ML]^{2+} + M^{2+} \rightleftharpoons [M_2L]^{4+}$	8.9(2)	5.3(1)	4.01(7)	4.58(8)
$[M_2L]^{4+} + H^+ \rightleftharpoons [M_2HL]^{5+}$		4.6(1)	5.04(5)	
$[M_2L]^{4+} + OH^- \rightleftharpoons [M_2L(OH)]^{3+}$	7.25(5)	4.9(3)	3.86(9)	5.27(8)
$[M_2L(OH)]^{3+} + OH^- \rightleftharpoons [M_2L(OH)_2]^{2+}$	4.01(6)	4.6(3)	3.14(8)	3.75(9)
$[M_2L]^{4+} + M^{2+} \rightleftharpoons [M_3L]^{6+}$	3.70(3)			

Table 4. Stability Constants (log *K*) of the Metal Complexes with L^2 (0.1 mol dm^{-3} NMe_4NO_3, 298.1 ± 0.1 K)

reacn	Cu	Zn	Cd	Pb
$M^{2+} + L \rightleftharpoons [ML]^{2+}$	16.9(1)	13.5(1)	13.2(2)	13.9(1)
$[ML]^{2+} + H^+ \rightleftharpoons [MHL]^{3+}$	9.1(1)	8.4(1)	8.5(2)	8.34(7)
$[MHL]^{3+} + H^+ \rightleftharpoons [MH_2L]^{4+}$	3.9(1)	4.95(7)	3.5(2)	3.7(1)
$[MH_2L]^{4+} + H^+ \rightleftharpoons [MH_3L]^{5+}$	2.9(1)			3.6(1)
$[ML]^{2+} + OH^- \rightleftharpoons [ML(OH)]^+$	4.5(1)	3.8(1)		3.6(1)
$[ML(OH)]^+ + OH^- \rightleftharpoons ML(OH)_2$		3.1(1)		
$[ML]^{2+} + M^{2+} \rightleftharpoons [M_2L]^{4+}$			6.9(1)	
$2M^{2+} + L + OH^- \rightleftharpoons [M_2L(OH)]^{3+}$	27.9(1)			
$2M^{2+} + L + 2OH^- \rightleftharpoons [M_2L(OH)_2]^{2+}$		29.7(1)		
$[M_2L]^{4+} + OH^- \rightleftharpoons [M_2L(OH)]^{3+}$			4.4(1)	
$[M_2L(OH)]^{3+} + OH^- \rightleftharpoons [M_2L(OH)_2]^{2+}$	3.9(1)		4.3(1)	
$[M_2L(OH)_2]^{2+} + OH^- \rightleftharpoons [M_2L(OH)_3]^+$	3.7(2)	4.4(1)	4.2(1)	

nated by one macrocyclic unit. In our case, however, the presence of a couple of heteroaromatic nitrogens could in principle affect the ligational properties of the ligands. At the same time, the different arrangement and binding ability of the heteroaromatic N-donors could differentiate the coordination properties of L^1−L^3. 1,10-Phenanthroline and 2,2′-dipyridine generally show a good binding ability toward transition and post-transition metal ions (Table 2). In L^2 the heteroaromatic nitrogens are placed close to the two macrocyclic units, and therefore, the triamine units and the phenanthroline moiety could act cooperatively in binding cations. This is not the case with L^1, where the two heteroaromatic nitrogens are located far from the [9]aneN_3 units. Therefore, in L^1 the dipyridine nitrogens constitute a potential independent binding unit for metal cations. Finally, pyrazine nitrogens are much poorer electron donors and do not display a significant binding ability toward metals.[30] On the other hand, in L^3 the two [9]aneN_3 macrocycles are placed close to each other and may act cooperatively in the coordination of a single metal cation, which would result sandwiched between the two [9]aneN_3 units.

With this in mind, we decided to perform a potentiometric and UV−vis spectrophotometric study on the binding features of L^1−L^3 in aqueous solutions. Tables 3−5 include the formed species and the corresponding stability constants, potentiometrically determined, for the Cu(II), Zn(II), Cd(II), and Pb(II) complexations with L^1−L^3.

Table 5. Stability Constant (log *K*) of the Metal Complexes with L^3 (0.1 mol dm^{-3} NMe_4NO_3, 298.1 ± 0.1 K)

reacn	Cu	Zn	Cd	Pb
$M^{2+} + L \rightleftharpoons [ML]^{2+}$	18.2(1)	15.8(1)	14.5(1)	15.1(9)
$[ML]^{2+} + H^+ \rightleftharpoons [MHL]^{3+}$	9.8(1)	8.9(1)	9.1(1)	9.48(6)
$[MHL]^{3+} + H^+ \rightleftharpoons [MH_2L]^{4+}$	5.7(1)	4.8(1)	5.2(6)	4.96(7)
$[ML]^{2+} + 2OH^- \rightleftharpoons ML(OH)_2$	8.9(1)	7.05(9)	6.2(1)	6.57(9)
$[ML]^{2+} + M^{2+} \rightleftharpoons [M_2L]^{4+}$	6.3(1)		3.8(1)	
$[M_2L]^{4+} + OH^- \rightleftharpoons [M_2L(OH)]^{3+}$	6.5(1)		4.1(1)	
$2M^{2+} + L + OH^- \rightleftharpoons [M_2L(OH)]^{3+}$		25.9(1)		
$[M_2L(OH)]^{3+} + OH^- \rightleftharpoons [M_2L(OH)_2]^{2+}$	6.3(1)	5.6(1)	4.0(1)	
$[M_2L(OH)_2]^{2+} + OH^- \rightleftharpoons [M_2L(OH)_3]^+$	5.2(1)	3.8(1)		

As expected considering the presence of two [9]aneN_3 binding units, ligands L^1−L^3 can generally form both mono- and dinuclear metal complexes in aqueous solution. Ligand L^1, however, can also form a trinuclear Cu(II) complex. In the case of L^2 and L^3, the low solubility of the dinuclear Pb(II) complexes precludes the study of these systems with a Pb(II)/L molar ratio greater than 1:1, and only the stability of the mononuclear complexes was determined.

Mononuclear Complexes. Considering the mononuclear $[ML]^{2+}$ complexes, for all three ligands, Cu(II) forms the most stable complexes, while the stability of the Zn(II), Cd(II), and Pb(II) is rather similar, as is often found in complexes with polyamine ligands.[50] All the $[ML]^{2+}$ complexes present a high tendency to bear protonation, forming mono-, di-, and, in some cases, also triprotonated species in aqueous solutions. The first protonation constant is only 1−2 logarithmic units lower than the corresponding basicity constants of the free ligands. As a consequence, protonated species are largely prevalent in acidic to slightly alkaline solutions containing M(II) and the ligands in 1:1 molar ratio (see Figure S3, Supporting Information, for the Cu(II) and Zn(II) complexes with L^2). These characteristics are generally related to the presence of nitrogen atoms not bound to the metal cations, which can easily protonate. Furthermore, the $[ML]^{2+}$ ($L = L^1$−L^3) complexes show a marked tendency to form stable mono- and, in some cases, dihydroxo species. Such behavior is usually related to a metal coordination sphere not saturated by the ligand donors. The coordinated water molecules, in fact, can easily be deprotonated to produce hydroxylated species.

At a first glance, these common characteristics for the complexes with L^1−L^3 would suggest that in the mononuclear complexes the metal is simply coordinated by a single [9]aneN_3 unit. A more accurate analysis of the data in Tables 3−5, however, reveals some important differences in the coordination properties of ligands L^1−L^3. Actually, the formation constant of the mononuclear complexes with the dipyridine-containing ligand L^1 are similar to those reported for the corresponding complexes with [9]aneN_3 (log K = 15.5 vs 14.9, 11.6 vs 10.65, 9.5 vs 9.30, and 10.8 vs 10.0 for the Cu(II), Zn(II), Cd(II), and Pb(II) complexes with [9]aneN_3 and L^1, respectively; see Tables 2 and 3), accounting for metal coordination by a single macrocyclic unit, without any involvement in the metal binding of the other. The

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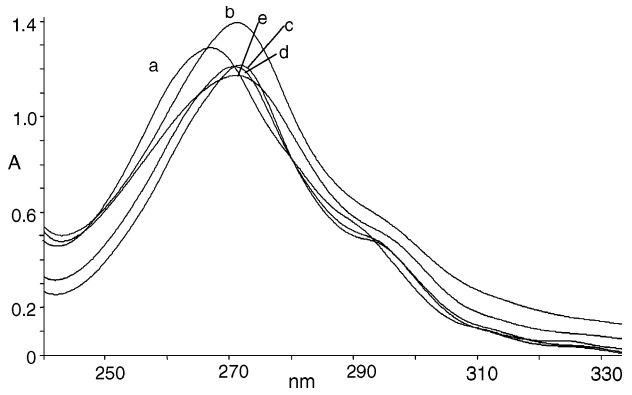

Figure 1. Absorption spectra of L^2 in the absence (a spectrum, pH 12) and in the presence of equimolecular amounts of Cu(II) (b spectrum), Zn(II) (c), Cd(II) (d), and Pb(II) (e) at pH 9.

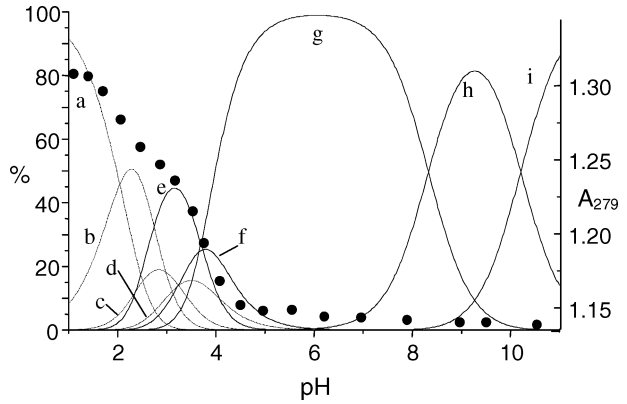

Figure 2. Distribution diagram of the protonated and complexed species for the system Pb/L^2 in 1:1 molar ratio (left *y* axis; a, $[H_6L^2]^{6+}$; b, $[H_5L^2]^{5+}$; c, $[H_4L^2]^{4+}$; d, $[H_3L^2]^{3+}$; e, $[PbH_3L^2]^{5+}$; f, $[PbH_2L^2]^{4+}$; g, $[PbHL^2]^{3+}$; h, $[PbL^2]^{2+}$; i, $[PbL^2(OH)]^{+}$) and absorbance at 279 nm (●, right *y* axis) as function of pH ($[L^2] = 4.3 \times 10^{-5}$ mol dm^{-3}).

somewhat higher stability of the [9]aneN_3 complexes compared to those with L^1 can be ascribed to the replacement in the coordination sphere of the metal of a secondary nitrogen of [9]aneN_3 with a tertiary one, a poorer σ-donor in aqueous solution.[51]

Unlike L^1, the mononuclear complexes with L^2 and L^3 display a marked higher stability than the corresponding complexes with [9]aneN_3.

Considering the phenanthroline-containing ligand L^2 (Table 4), the stability constants of the $[CdL^2]^{2+}$ and $[PbL^2]^{2+}$ complexes are increased by 3.7 and 3.1 log units with respect to the corresponding [9]aneN_3 complexes. A minor gain in stability is found for the Cu(II) and Zn(II) complexes (1.4 and 1.9 log units). These data suggest that the phenanthroline nitrogens are involved in metal coordination, thus leading to complex stabilization. To obtain further information on the role played by the phenanthroline unit in metal coordination, the reaction of complex formation was followed by UV spectra recorded on aqueous solutions containing ligands and M(II) in a 1:1 molar ratio. It has been reported that metal binding by phenanthroline-based polyamine ligands usually leads to a slight red shift of the typical UV band of this heterocycle, accompanied by a marked change in molar absorbance.[52] UV spectra of L^2 and its metal complexes are shown in Figure 1. Metal coordination induces a 6−7 nm red shift of the band at 267 nm for the uncomplexed ligand, with a decrease in molar absorbance in the case of Zn(II), Cd(II), and Pb(II) and an increase in the case of Cu(II). These spectrophotometric data indicate that at least one of the phenanthroline nitrogens is involved in metal coordination.

In both L^1 and L^2 mononuclear complexes, one [9]aneN_3 unit is not involved in metal coordination and can easily protonate. Besides stable mono- and diprotonated species, in the case of the mononuclear Zn(II), Cd(II), and Pb(II) complexes with L^1 and the Cu(II) and Pb(II) complexes with L^2, triprotonated species are also detected in aqueous solution. On the other hand, [9]aneN_3 can bind three acidic protons in aqueous solution only at strongly acidic pH values (pH < 1).[53] This suggests that, in the $[MH_3L]^{5+}$ species (L = L^1 or L^2), one acidic proton is localized on the heteroaromatic nitrogens. Actually, as in the case of protonation of the uncomplexed ligands, protonation of the heteroaromatic nitrogens in the mononuclear complexes with L^1 and L^2 leads to an increase in absorbance at 299 nm or at 278 nm, respectively. As shown in Figure 2 for the L^2 complex with Pb(II), this occurs below pH 5, with formation of the triprotonated $[PbH_3L^2]^{5+}$ species. Interestingly, a further increase in absorbance is observed below pH 2.5, where highly protonated species of the ligand, $[H_5L^2]^{5+}$ and $[H_6L^2]^{6+}$, which contain a protonated heteroaromatic nitrogen, are prevalent in solution (see above). A similar behavior is also found in the other metal complexes.

As far as L^3 is concerned (Table 5), the increased complex stability with respect to [9]aneN_3 cannot be ascribed to the involvement of the heteroaromatic nitrogens in metal binding, due to the very poor electron donor properties of pyrazine nitrogens.[30] On the other hand, the macrocyclic rings are in close proximity and may act cooperatively in metal binding. The gain in stability with respect to [9]aneN_3 is greater than that found for the corresponding complexes with L^2, since it is 2.7 log unit in the case of Cu(II) and 4.2−5 log units in the case of Zn(II), Cd(II), and Pb(II). The stability constant values, however, are by far lower than those generally found for the corresponding penta- or hexacoordinated metal complexes with polyamine ligands.[50] Actually, ligand 1,2-bis(1,4,7-triaza-1-cyclononyl)ethane (L^4), which contains two [9]aneN_3 units bridged by an ethylene chain, only forms Cu(II) and Zn(II) mononuclear complexes, where the metal is hexacoordinated by six nitrogen donors. The stability constants of these complexes are remarkably higher than those of the L^3 complexes (log K = 27.82 and 22.78 for the Cu(II)[10b,c] and Zn(II)[10d] complexes with L,[4] while log K = 18.2 and 15.8 for the corresponding L^3 complexes). Furthermore,

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the values of the first two protonation constants of the mononuclear L^3 complexes (log $K > 9$ for the equilibria $[ML^3]^{2+} + H^+ \rightleftharpoons [MH\,L^3]^{3+}$ and log $K > 5$ for the equilibria $[MHL^3]^{3+} + H^+ \rightleftharpoons [MH_2L^3]^{4+}$) are generally attributed to the protonation of amine groups not involved, or weakly involved, in metal coordination. These observations would suggest a tetracoordination of the metal ions in the mononuclear L^3 complexes.

Dinuclear Complexes. As previously anticipated, ligands L^1-L^3 can form stable dinuclear complexes in aqueous solution. For all the investigated systems, the formation of dinuclear complexes is accompained by deprotonation of the metal-bound water molecules to give mono- and dihydroxo complexes, which are the prevalent species in solution from neutral to alkaline pHs. In the case of the Cu(II), Zn(II), and Cd(II) complexes with L^2 and the Cu(II) and Zn(II) with L^3, trihydroxo complexes are also formed in aqueous solution. Finally, in the case of the Cu(II), Zn(II), and Pb(II) complexes with L^2 and of the Zn(II) complexes with L^3, the formation of $[M_2L]^{4+}$ complexes is not detected in our experimental conditions and dinuclear complexes are only present in their hydroxylated forms. As in the case of the mononuclear complexes, a marked tendency to form hydroxylated species may be related to metal coordination environments not saturated by the ligand donors, which lead to a facile deprotonation of the coordinated water molecules. In the case of ligand L^2 and L^3, it can simply be suggested that in the dinuclear complexes each metal is coordinated by one [9]aneN_3 unit. Unfortunately, the formation constants of $[M_2L^2]^{4+}$ (M = Cu, Zn, or Pb) were not determined (see above), precluding a clear comparison between the dinucleating ability of the two ligands. In the case of Cd(II), however, the data in Tables 4 and 5 allow one to determine the constants for the equilibrium $2M^{2+} + L \rightleftharpoons [M_2L]^{4+}$ (log $K = 20.1$ and 18.3 for $L = L^2$ and L^3, respectively). The higher stability of the dinuclear Cd(II) complex with L^2 may be due both to the stabilizing interaction with the phenanthroline nitrogens and to the larger distance between the two charged metal centers.

Unlike L^2 and L^3, L^1 contains two potential binding sites for the second metal ion, i.e., a [9]aneN_3 unit and the heteroaromatic nitrogens of dipyridine. For all the metals under investigation, the macrocyclic ligand [9]aneN_3 shows a binding ability by far higher than that of 2,2′-dipyridine (Table 2). On the other hand, coordination of the metal ions by the two [9]aneN_3 units, at a rather short distance, would lead to a marked electrostatic repulsion between two positively charged metal centers. Instead, coordination of the two metal ions by one [9]aneN_3 unit and by the dipyridine nitrogens would localize the two metals at a larger distance, giving a more energetically favored metal arrangement from an electrostatic point of view. As in the case of proton binding, metal coordination by dipyridine gives rise to a new red shift band in its UV spectrum. The formation of mono- and dinuclear Zn(II), Cd(II), and Pb(II) complexes does not significantly affect the dipyridine band at 283 nm of ligand L^1, indicating that the metals are coordinated by the [9]aneN_3 units. Interestingly, in the case of the dinuclear Zn(II) and Cd(II) complexes with L^1, the formation of a protonated complex, $[M_2HL^1]^{5+}$ is also detected in solution. Once again, this reflects the particular molecular topology of ligand L^1. In the $[M_2HL^1]^{5+}$ complexes, the heteroaromatic nitrogens, not involved in metal coordination, may act as binding sites for protons.

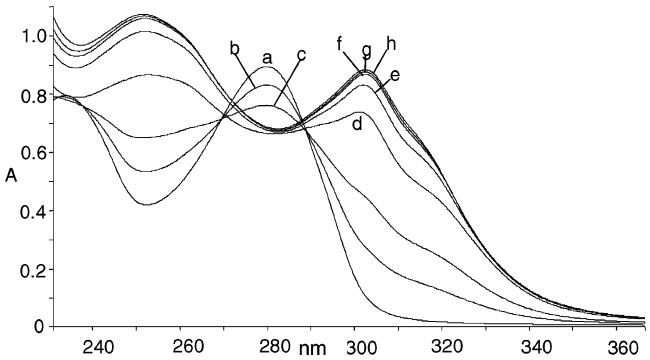

Figure 3. Absorption spectra of L^1 in the presence of Cu(II) at pH 6 and different metal to ligand molar ratios (a, free ligand; b, $R = 0.5$; c, $R = 1$; d, $R = 1.5$; e, $R = 1.75$; f, $R = 2$; g, $R = 2.5$; h, $R = 3$. $R = [Cu(II)]/[L^1]$ ($[L^1] = 7.5 \times 10^{-5}$ mol dm^{-3}).

Surprisingly, L^1 displays a different behavior toward Cu(II). Figure 3 reports the UV spectra recorded in aqueous solution containing Cu(II) and L^1 in different molar ratios at pH 6. The UV spectrum obtained for $R = 2$ ($R = [Cu^{2+}]/[L^1]$), where dinuclear species predominate in solution (see Figure S4, Supporting Information, for the distribution diagrams for the system Cu(II)/L^1 in 1:1 and 2:1 molar ratio (R)), clearly shows the presence of a band 25 nm red-shifted with respect to that of the free ligand at the same pH. This band does not show any significant variations for $R > 2$. These data point out that in the dinuclear Cu(II) complexes one of the metal cations is coordinated by the dipyridine nitrogens. For $R \leq 1$, minor significant amounts of dinuclear complexes are still present in aqueous solution at pH 6 (Figure S4). The UV spectra recorded for $R = 0.5$ and 1 therefore display a decrease in absorbance at 273 nm and, for $R = 1$, a shoulder at ca. 305. Obviously, the dinuclear Cu(II) complex contains an uncoordinated [9]aneN_3 unit, which can act as a binding site for a third metal to give the trinuclear $[Cu_3L^1]^{6+}$ complex.

Our data do not allow us to rationalize the different behavior of Cu(II) with respect to the other metals in their dinuclear complexes with L^1, but they point out that metal localization in the $[M_2L^1]^{4+}$ complexes is modulated by a subtle balance between binding features of the metal and structural characteristics of the ligand.

Bis(*p*-nitrophenyl) Phosphate (BNPP) Hydrolysis. Ligands L^1-L^3 are capable of holding two metal centers in proximity, mimicking the dinuclear metal arrays at the active sites of several metalloenzymes. Actually, many metalloenzymes with hydrolytic properties use Zn(II) ions in a bifunctional catalytic mechanism. In particular, dizinc enzymes are responsible for the hydrolysis of the phosphate ester bond in a variety of low molecular weight metabolites and nucleic acids.[54−58] The catalytic role of zinc is ascribed to two main functions: (i) binding and activation of the substrates; (ii) deprotonation of Zn(II)-coordinated water

molecules to give Zn−OH functions, which can act as nucleophiles in the hydrolytic mechanism. To this purpose, several dinuclear metal complexes with macrocyclic ligands have been used as simple structural or functional models for hydrolytic metalloenzymes.[1,25,59−81]

All three ligands (L^1−L^3) meet the necessary requisites to give dinuclear complexes that can be used as functional model systems for hydrolytic enzymes. They can in fact form stable dizinc complexes at neutral pH, and at the same time, a facile deprotonation of the coordinated water molecules can occur at neutral or slightly alkaline pH values to give Zn−OH functions, which are potential nucleophiles in hydrolytic processes. These observations prompted us to carry out a kinetic study on the hydrolytic properties of the dizinc complexes with ligands L^1−L^3 toward the phosphate ester bond, using bis(*p*-nitrophenyl) phosphate (BNPP) as a substrate. BNPP hydrolysis to give mono(*p*-nitrophenyl) phosphate (MNPP) and *p*-nitrophenate was followed by the appearance of the *p*-nitrophenate band at 403 nm at 308.1 K (I = 0.1 mol dm^{-3} NMe_4NO_3). For this reason, the formation constants of the Zn(II) complexes were potentiometrically redetermined at 308.1 K and have been deposited as Supporting Information. The formation constant values are not much affected by temperature, the most relevant difference being a slightly lower stability of the $[ZnL]^{2+}$ and $[Zn_2L]^{4+}$ complexes at the higher temperature generally, probably due to the exothermicity of the process of metal coordination. The dinuclear $[Zn_2L^3]^{4+}$ complex, however, is not detected at 298.1 K, preventing any comparison with the stability of the corresponding dinuclear complexes at 308.1 K. The species $[Zn_2L^2]^{4+}$ is not detected at either temperature.

Since the hydrolytic properties of Zn(II) complexes are generally related to the acidity of the coordinated water molecules, it is important to compare the tendency of the dinuclear zinc complexes with L^1−L^3 to give the Zn−OH function. In the case of L^1, water deprotonation occurs only in the alkaline pH region (pH > 8), while in the case of L^3 the $[Zn_2L^3(OH)]^{3+}$ complex is already present in solution at neutral pH. For L^2 a simultaneous deprotonation of two coordinated water molecules occurs at pH > 7 to give the species $[Zn_2L^2(OH)_2]^{2+}$; this prevents the calculation of the corresponding pK_a values. From the formation constant values at 308.1 K (Table S1, Supporting Information), the pK_a values for the coordinated water molecules can be calculated, at least for the $[Zn_2L^1]^{4+}$ and $[Zn_2L^3]^{4+}$ complexes. The dinuclear L^3 complex displays rather low pK_a values compared to the corresponding L^1 complex, at least in the first two deprotonation steps (pK_{a1} = 7.7 and 8.7, pK_{a2} = 7.8 and 8.9 for $[Zn_2L^3]^{4+}$ and $[Zn_2L^1]^{4+}$, respectively). Similar low values are generally attributed to a bridging coordination of the hydroxide anions between two metal centers.[25,78] Actually, in $[Zn_2L^3(OH)_2]^{2+}$, the two Zn(II)−[9]aneN_3 units are kept at a short distance by the small quinoxaline bridge, thus favoring the assembly of a $Zn_2(\mu$-$OH)_2$ unit, which is enclosed in the cavity generated by the two macrocyclic moieties and the aromatic linkage. The

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Table 6. Second-Order Rate Constants k'_{BNPP} (M^{-1} s^{-1}) for Hydrolysis of Bis(*p*-nitrophenyl) Phosphate

nucleophile	$10^5 k_{BNPP}$ (M^{-1} s^{-1})	pK_a
$[ZnL^2(OH)]^+$	7.5 ± 0.3	9.3[a]
$[ZnL^2(OH)_2]$	6.9 ± 0.3	10.7[b]
$[Zn_2L^2(OH)_2]^{2+}$	6.9 ± 0.3	
$[Zn_2L^2(OH)_3]^+$	6.3 ± 0.3	9.0[c]
$[Zn_2L^3(OH)_3]^+$	420 ± 20	9.3[c]
$[ZnL^5(OH)]^+$	8.5[e]	7.2
$[Zn_2L^6(OH)_2]^{2+}$	11.5[f]	9.2[d]

[a] $K_a = [ZnL(OH)^+][H^+]/[ZnL^{2+}]$. [b] $K_{a2} = [ZnL(OH)_2][H^+]/[ZnL(OH)^+]$. [c] $K_{a3} = [Zn_2L(OH)_3^+][H^+]/[Zn_2L(OH)_2^{2+}]$. [d] $K_{a2} = [Zn_2L(OH)_2^{2+}][H^+]/[Zn_2L(OH)^{3+}]$. [e] From ref 64a, $I = 0.2$ mol dm^{-3} $NaClO_4$, pH = 8.6. [f] From ref 25, $I = 0.15$ mol dm^{-3} NaCl, pH = 10.5.

dinuclear L^3 complex also forms the trihydroxo $[Zn_2L^3(OH)_3]^+$ species at alkaline pH's with pK_{a3} value of 9.3. This pK_a value is much higher than those usually found for bridging hydroxide groups and is generally related to the formation of a single-metal bound hydroxide function. Obviously, the molecular architecture of L^1 keeps the two metal centers at too long a distance to give bridging hydroxide groups, in agreement with the higher pK_{a1} and pK_{a2} values observed for the L^1 complexes with respect to the corresponding pK_a values found for the L^3 complexes. Therefore, the $[Zn_2L^1(OH)_2]^{2+}$ complex contains two independent [9]aneN$_3$–Zn−OH units. Similar structural features can also be proposed for the $[Zn_2L^2(OH)_2]^{2+}$ complex, where the two [9]aneN$_3$ binding unit are separated by a large phenanthroline moiety.

The different characteristics of the dinuclear hydroxo complexes strongly affect their ability in BNPP hydrolysis. Unfortunately, in the case of L^1, precipitation of the adduct between the dizinc complex and BNPP at neutral or slightly alkaline pH does not allow a reliable study of the hydrolytic capability of the L^1 complexes.[82] Both the mono- and dinuclear Zn(II) complexes with L^2 and the dinuclear Zn(II) complexes with L^3 promote BNPP hydrolysis following second-order kinetics. The mononuclear complexes with L^3 are not active, due to the absence of mononuclear hydroxo species at 308.1 K. The active species in the hydrolytic process and the corresponding second-order rate constants k'_{BNPP} are reported in Table 6. The k'_{BNPP} values for the mononuclear Zn(II) complexes with the triazamacrocycles [12]aneN$_3$ (L^5)[64a] and for the dinuclear Zn(II) complex with ligand [30]aneN$_6$O$_4$ (L^6),[25] which contains two separated triamine binding units, are also reported for comparison.

Considering the dinuclear Zn(II) complexes L^2, both the $[Zn_2L^2(OH)_2]^{2+}$ and $[Zn_2L^2(OH)_3]^+$ species promote BNPP hydrolysis in aqueous solution, to give MNPP and *p*-nitrophenate. The plot of the second-order rate constants k_{OBS} for the Zn/L^2 (2:1 molar ratio) system as a function of the pH points out that the $[Zn_2L^2(OH)_2]^{2+}$ and $[Zn_2L^2(OH)_3]^+$ complexes are the kinetically active species (Figure 4a). No effect is observed below pH 7.5, where these species are

(82) A solid compound of stoichiometry $[Zn_2L^1](BNPP)(NO_3)_3 \cdot 2H_2O$ was isolated and characterised by elemental analysis. Anal. Found (calcd for $C_{36}H_{50}N_{13}O_{19}PZn_2$): C, 38.51 (38.24); H, 4.54 (4.46); N, 16.25 (16.11); P, 2.53 (2.74). The very low solubility in water as well as in organic solvents prevented a more detailed characterization of this complex, and all attempts of crystallization failed.

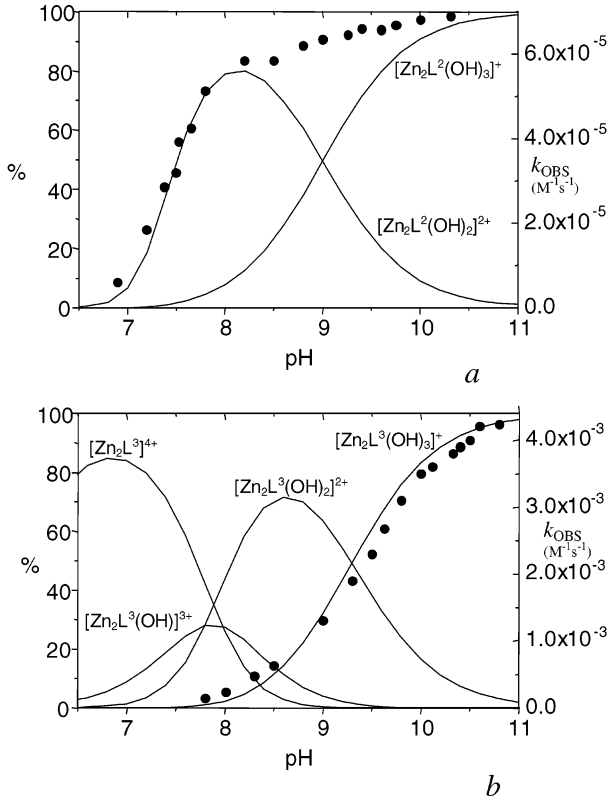


Figure 4. Plots of the distribution curves of selected species for the Zn/L^2 (a) and Zn/L^3 (b) systems ([Zn(II)] = 2×10^{-3} M, [L] = 1×10^{-3} M (solid line, left *y* axes) and k_{obs} values (●, right *y* axes) as a function of pH (0.1 mol dm^{-3} NMe_4NO_3, 308.1 K).

absent from the solution. These data clearly indicate that Zn−OH functions act as nucleophiles in the hydrolytic process. BNPP hydrolysis promoted by mono- and dinuclear Zn(II) complexes is generally explained in terms of an "associative" mechanism,[25,64,78] in which the substrate would approach the Zn(II) complex and the oxygens of BNPP start associating with the electrophilic Zn(II) ion. Simultaneously a zinc-bound hydroxide operates a nucleophilic attack on the phosphorus. However, the hypothesis of a two-steps mechanism, involving a rapid preequilibrium with substrate binding followed by a nucleophilic attack of a Zn(II)-coordinated hydroxide, is also reasonable. In both cases, substrate interaction with two electrophilic metal centers favors the nucleophilic attack of a Zn−OH function and thus enhances the rate of the hydrolytic process. This is the case of the dinuclear $[Zn_2L^6(OH)_2]^{2+}$ complex, where the two metals, 5.35 Å apart, play a cooperative role in BNPP binding and activation, through a bridging coordination of the phosphate ester.[25] The two complexes $[Zn_2L^2(OH)_2]^{2+}$ and $[Zn_2L^2(OH)_3]^+$ show an equal ability, within the experimental error, in BNPP cleavage; their activity is also similar to that of the mononuclear $[ZnL^2(OH)]^+$ and $[ZnL^2(OH)_2]$ complexes but lower than that reported for the dinuclear complex $[Zn_2L^6(OH)_2]^{2+}$ and even lower than that found for the mononuclear $[ZnL^5(OH)]^+$ complex (Table 6). These data would indicate that in the dinuclear Zn(II) complexes with L^2 the two metal ions act independently in the hydrolytic process, in agreement with the large distance which separates the two [9]aneN$_3$ binding units.

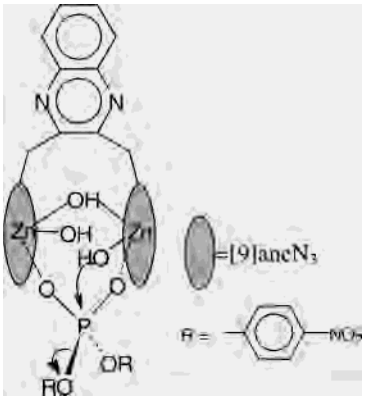
The dinuclear Zn(II) complex with L^3 displays a different behavior in BNPP cleavage with respect to the L^2 complexes. According to Figure 4b, only the trihydroxo complex $[Zn_2L^3(OH)_3]^+$ promotes BNPP hydrolysis, while $[Zn_2L^3(OH)]^{3+}$ and $[Zn_2L^3(OH)_2]^{2+}$ are totally inactive. The pK_{a3} value for the equilibrium $[Zn_2L^3(OH)_2]^{2+} + H_2O \rightleftharpoons [Zn_2L^3(OH)_3]^+ + H^+$ can be calculated by means of a least-squares fitting of the rate constants (k_{OBS}) collected at different pHs. Its value, 9.4 ± 0.1 log units, is in good agreement with that obtained by potentiometry (9.3 log units).

As already found with other binucleating ligands,[25,78] the lack of hydrolytic ability of the $[Zn_2L^3(OH)]^{3+}$ and $[Zn_2L^3(OH)_2]^{2+}$ species can be attributed to the bridging coordination of the hydroxide anion. In fact, simultaneous binding to two electrophilic metal centers reduces the nucleophilicity of the generated hydroxide.

Addition of a third hydroxide anion to $[Zn_2L^3(OH)_2]^{2+}$ gives rise to a dramatic increase of the hydrolysis rate. The trihydroxo complex $[Zn_2L^3(OH)_3]^+$ is almost 2 orders of magnitude more active than the corresponding L^2 complex and gives rise to the highest rate increase in BNPP hydrolysis in aqueous solutions at 308 K among Zn(II) complexes. At a first glance, this result is rather surprising since the presence of three Zn(II)-bound hydroxide function in $[Zn_2L^3(OH)_3]^+$ and the consequent decreased overall positive charge of the complex would reduce the substrate interaction with the metals and its activation to the nucleophilic attack. The unexpected high activity of this complex prompted us to confirm the spectrophotometrically determined k'_{BNPP} value by means of ^{31}P NMR measurements, monitoring the increase of the MNPP signal at 0.94 ppm and the simultaneous decrease of the BNPP signal at −10.07 ppm in time (Figure S5, Supporting Information) The measured k'_{BNPP} value of $(390 \pm 20) \times 10^{-5}$ M^{-1} s^{-1} is equal, within the experimental error, to that found by means of spectrophotometric measurements.

As previously discussed, the pK_{a3} value (9.0) for the formation of $[Zn_2L^3(OH)_3]^+$ is too high to be ascribed to a bridging hydroxide anion. Indeed, the bridging hydroxide anions in $[Zn_2L^3(OH)_2]^{2+}$ reduce the positive charge on the metal ions. This would determine the rather high pK_{a3} value found for deprotonation of a Zn(II)-bound water molecule to give the $[Zn_2L^3(OH)_3]^+$ complex and increase the nucleophilic character of the resulting Zn−OH function. The high activity in BNPP cleavage of $[Zn_2L^3(OH)_3]^+$, however, cannot simply be due to the enhanced nucleophilicity of a Zn−OH function, since the pK_{a3} value for $[Zn_2L^3(OH)_3]^+$ is equal or similar to those reported for the dinuclear complexes $[Zn_2L^6(OH)_2]^{2+}$ and $[Zn_2L^2(OH)_3]^+$. A tentative explanation could reside in the particular molecular architecture of L^3, which contains two [9]aneN$_3$ binding units at a slightly variable distance from each other. Most likely, in the dinuclear L^3 complexes the two Zn(II) ions are placed at an optimal distance to achieve a strong interaction and activation of the substrate, through a bridging interaction of BNPP with both the two electrophilic metal centers. The high activity of this complex would exclude the possible interaction of BNPP with a single metal center. Furthermore, binding of the third hydroxide could also lead to detachment of the bridging OH from one of the metals or to a weakening of the Zn(μ-OH) bonds, giving a more "open" conformation of the complex, which can leave catalytic sites accessible on the two Zn(II) ions. The simultaneous presence of single-metal bound, highly nucleophilic Zn−OH functions and of two Zn(II) ions at the appropriate distance to interact with the phosphate ester may explain the high activity of our $[Zn_2L^3(OH)_3]^+$ complex in BNPP cleavage (see Scheme 1).

Scheme 1. Proposed Mechanism for BNPP Hydrolysis Promoted by the $[Zn_2L^3(OH)_3]^+$ Complex



This hypothesis is sustained by semiempirical PM3 calculations carried out on $[Zn_2L^3(OH)]^{3+}$, $[Zn_2L^3(OH)_2]^{2+}$, and $[Zn_2L^3(OH)_3]^+$. The optimized structure of the complex $[Zn_2L^3(OH)]^{3+}$ features each Zn(II) coordinated to the nitrogens of a [9]aneN$_3$ moiety (Zn−N distances ranging between 2.055 and 2.105 Å), with the hydroxo group bridging the two metal ions (Zn−O distances 2.023 and 2.037 Å) and imposing a Zn−Zn distance of 3.77Å (Figure 5a). In the case of $[Zn_2L^3(OH)_2]^{2+}$, the optimized structure (Zn−O distances ranging between 2.071 and 2.108 Å, Zn−N distances ranging between 2.118 and 2.193 Å) shows both hydroxo groups bridging the metal ions which are therefore kept at a remarkably shorter distance (3.082 Å) compared to the complex $[Zn_2L^3(OH)]^{3+}$ (Figure 5b). As expected, addition of a third OH^- anion to $[Zn_2L^3(OH)_2]^{2+}$ causes a weakening of the two Zn(μ-OH) bonds with one of the metal centers. The optimized structure for $[Zn_2L^3(OH)_3]^+$ shows that the Zn(II) ion bonded to the free OH^- features longer distances to the two bridging hydroxo anions (Zn−O = 2.373 and 2.442 Å) as compared to the second metal center in the complex, for which the two Zn(μ-OH) bonds (Zn−O = 2.007 and 1.998 Å) are very similar to those calculated for the dihydroxo complex $[Zn_2L^3(OH)_2]^{2+}$. The different coordination at the two Zn(II) ions results in a lengthening of the Zn−Zn distance which reaches a value of 3.270 Å.

Concluding Remarks

Compared to previously reported bis([9]aneN$_3$) ligands, in L^1−L^3 the heteroaromatic bridges between the two macrocyclic units behave as noninnocent spacers; their different length and coordination characteristics, in fact, strongly affect both the basicity and metal binding properties of the ligands.

Considering proton binding, in the case of L^1 and L^2 the heteroaromatic nitrogens are effective binding sites for

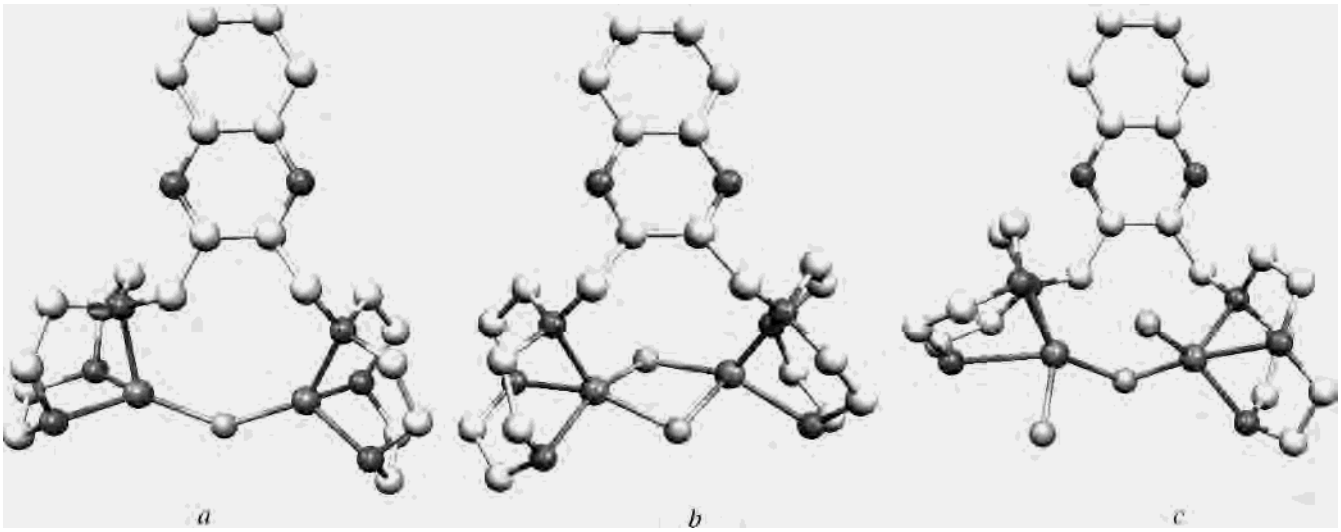


Figure 5. Optimized structures of the complexes $[Zn_2L^3(OH)]^{3+}$ (*a*), $[Zn_2L^3(OH)_2]^{2+}$ (*b*), and $[Zn_2L^3(OH)_3]^{+}$ (*c*) at the PM3 level. Hydrogen atoms have been omitted for clarity.

protons. On the contrary, protonation of L^3 does not involve the quinoxaline nitrogens, due to their extremely poor electron donor properties. At the same time, however, L^3 is the most basic ligand, at least in the early protonation steps. Most likely, in the $[HL^3]^+$ and $[H_2L^3]^{2+}$ species the acidic protons are involved in a hydrogen bond network involving both [9]aneN_3 units, at a short distance apart. Similarly, in the mononuclear $[ML^3]^{2+}$ complexes both [9]aneN_3 macrocyclic units act cooperatively in metal coordination. This is not the case with $[ML^1]^{2+}$ and $[ML^2]^{2+}$ complexes, where the metal ion is hosted by a single [9]aneN_3 macrocycle. Unlike L^3, however, L^1 and L^2 heteroaromatic nitrogens can be involved in metal coordination. In L^2 phenanthroline nitrogens are close to the macrocyclic units and participate in metal coordination; in L^1 the two dipyridine donors behave as a potential separate binding site for the metals, and in the $[Cu_2L^1]^{4+}$ and $[Cu_3L^1]^{6+}$ complexes the Cu(II) cation is coordinated by this binding unit.

The different molecular architecture of L^2 and L^3 strongly affects also the reactivity of their dizinc complexes in the hydrolysis of the BNPP diester. Both dizinc complexes in their hydroxylated forms promote BNPP cleavage. In the case of L^2 the hydrolysis rate is similar or lower than those generally reported for tricoordinated Zn(II) complexes; in fact, the two metal ions act independently in the cleavage process, due to the large distance between the two catalytic sites. The dinuclear L^3 complex, instead, $[Zn_2L^3(OH)_3]^+$, is ca. 2 orders of magnitude more active in BNPP hydrolysis. This surprising rate increase can be related to the optimal intermetallic distance, which allows a bridging interaction of the phosphate ester, and to the simultaneous presence of highly nucleophilic Zn−OH units.

Acknowledgment. Financial support by the "Regione Autonoma della Sardegna", the Università degli Studi di Cagliari, and the Italian Ministero della Ricerca Scientifica e Tecnologica (Grant COFIN 2002) is gratefully acknowledged. We thank the reviewers for helpful comments.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra in D_2O for the species L^1·4HBr, L^2·4HBr, and L^3·4HBr, stability constant of the Zn(II) complexes with L^1, L^2 , and L^3 at 308.1 ± 0.1 K in 0.1 mol dm^{-3} NMe_4NO_3, absorption spectra of L^2 and L^1 at different pH values, distribution diagrams of the protonated L^2 and L^1 species and pH dependence of the absorbance at 279 nm (L^2) and at 299 nm (L^1), distribution diagrams for the systems Cu(II)/L^2 and Zn(II)/L^2 with a metal to ligand 1:1 molar ratio, distribution diagrams for the system Cu(II)/L^1 (1:1 and 2:1 molar ratio), and ^{31}P NMR spectra recorded during the hydrolysis of BNPP in the presence of $[Zn_2L^3(OH)_3]^+$.

IC034050R