

# Synthesis and properties of a dizinc complex of a novel macrocyclic ligand with two alcohol-pendants: a model for alkaline phosphatase †

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A novel 24-membered macrocyclic compound having two hydroxyethyl pendants L, 3,6,9,17,20,23-hexazatricyclo[23.3.1.1.1.<sup>11,15</sup>]tricycanta-1(29),11(30),12,14,25,27-hexaene-6,20-bis(2-hydroxyethyl), has been synthesized as a dinucleating ligand. A new complex  $[\text{Zn}_2\text{H}_{-2}\text{L}]\text{Br}_2(\text{H}_2\text{O})_2$  has been synthesized as a model to mimic the active center of alkaline phosphatase. The bridging coordination of the two alkoxides to two  $\text{Zn}^{\text{II}}$  ions was confirmed by the crystal structure of  $[\text{Zn}_2\text{H}_{-2}\text{L}]\text{Br}_2(\text{H}_2\text{O})_2$ . The protonation constants of L as well as complexation constants of L with  $\text{Zn}^{\text{II}}$  have been determined at 298.1 K by means of potentiometric titration.  $[\text{Zn}_2\text{H}_{-2}\text{L}]^{2+}$  and  $[\text{Zn}_2\text{H}_{-2}\text{L}(\text{OH})]^+$  are the dominant species in aqueous solution at  $7 < \text{pH} < 9$ . The kinetics of promoted hydrolysis of *p*-nitrophenyl acetate has also been studied; the second-order rate constant is  $0.018 \text{ M}^{-1} \text{ s}^{-1}$  at  $\text{pH} = 9.0$ .

## Introduction

Some multinuclear metalloenzymes such as alkaline phosphatase contain two  $\text{Zn}^{\text{II}}$  in each active site region and are responsible for the cleavage of phosphate monoester.<sup>1–3</sup> The two  $\text{Zn}^{\text{II}}$  (*ca.* 3.9 Å separation) show different coordination environments and seem to work cooperatively. The activation of the proximal alcohol of Ser-102 under the effect of  $\text{Zn}^{\text{II}}$  at the active center is involved in the substrate hydrolysis process and a proposed mechanism is shown in Scheme 1.<sup>4</sup> It is now considered that the phosphate substrate is initially attacked by the deprotonated Ser-102 in **1** to yield a transient phosphoserine intermediate **2** that is attacked intramolecularly by the adjacent  $\text{Zn}^{\text{II}}$ -bound hydroxide in **3** to complete the hydrolysis and reproduce **1** and thus restart the catalytic cycle.

Synthetic metal complexes acting as model compounds for the hydrolytic metalloenzymes have attracted much attention.<sup>5–17</sup> Much effort to design models for alkaline phosphatase or nucleases to elucidate the mechanisms by which metal ions promote hydrolysis has focused primarily on mononuclear zinc or copper complexes.<sup>5–11</sup> For instance, by studying several macrocyclic polyamine  $\text{Zn}^{\text{II}}$  complexes bearing an alcohol pendant, Kimura's group discovered that the pendant alcohol can be deprotonated to form a  $\text{Zn}^{\text{II}}$ -bound alkoxide anion, which acts as a strong nucleophile at physiological pH.<sup>5,6</sup> The synthesis of dinuclear complexes to probe the cooperative mechanism between two metal ions in native enzymes has also ignited considerable interest.<sup>12–17</sup> However, only a few model complexes combining two  $\text{Zn}^{\text{II}}$  ions with a pendant alcohol inside a macrocycle have been synthesized successfully. The first reported alkoxide O<sup>−</sup>-bridged dinuclear  $\text{Zn}^{\text{II}}$  cryptate is shown as **4** in Scheme 2.<sup>12</sup> In this case, instead of acting as a nucleophile, the alcohol deprotonates to act as a bridging group to connect two  $\text{Zn}^{\text{II}}$ . Another dinuclear  $\text{Zn}^{\text{II}}$  complex shown as **5** in Scheme 2 given by a macrocyclic ligand with an ethanolic sidearm

was recently reported.<sup>13</sup> Zn-assisted deprotonation of the alcoholic group takes place at neutral pH and at slightly alkaline pH the species containing both a Zn-bound alkoxide and a terminally bound hydroxide group as nucleophilic functions can promote the hydrolysis of phosphate or carboxy ester cooperatively.<sup>13</sup>

To further explore the influence of the cavity size and rigidity of the macrocycle ligand on the behavior of corresponding  $\text{Zn}^{\text{II}}$  complexes, in addition to investigating the nucleophilicity of alkoxide under the effect of two adjacent  $\text{Zn}^{\text{II}}$ , we have synthesized a novel 24-membered hexaza ligand containing two *m*-xylyl spacers with 2-hydroxyethyl pendants and its binuclear  $\text{Zn}^{\text{II}}$  complex (**6** in Scheme 2). Its crystal structure revealed a novel  $\text{Zn}_2(\mu\text{-alkoxide})_2$  center located inside a macrocycle. The protonation constants of L as well as the complexation constants of L with  $\text{Zn}^{\text{II}}$  at 25 °C have also been studied. The behavior toward the hydrolysis of *p*-nitrophenyl acetate has been studied too.

## Experimental

### Materials

2-[Bis(2-aminoethyl)amino]ethanol was prepared as a colorless sticky liquid according to the literature method.<sup>18</sup> Isophthalaldehyde was synthesized and purified by a Schmelet reaction with slight modifications.<sup>19</sup> All of the other chemicals used in the experiments were of analytical grade from commercial sources and were used without further purification.

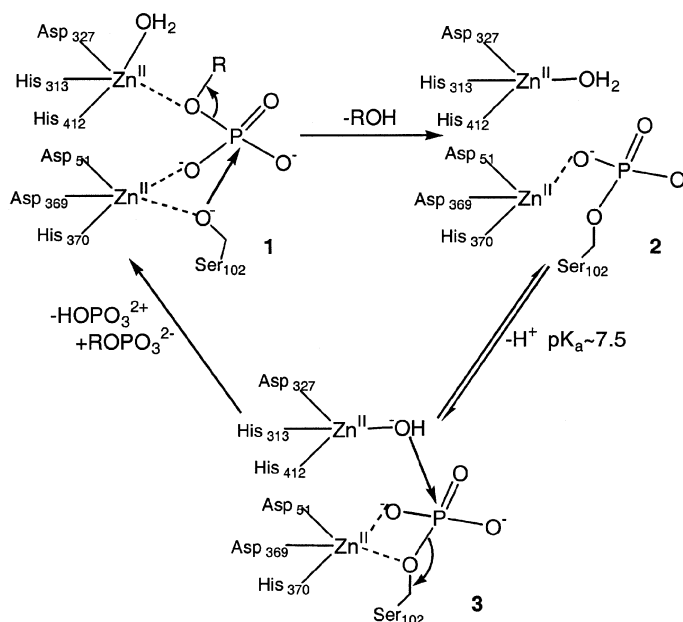
### Physicochemical measurements

<sup>13</sup>C NMR spectra were measured with a Bruker AM 500 spectrometer. Element analysis was performed on a Perkin-Elmer 240C. ES-MS spectra were obtained from a Finnigan MAT LCQ ES mass spectrometer with a mass to charge (*m/z*) range of 2000 and MeOH/H<sub>2</sub>O was used as the mobile phase.

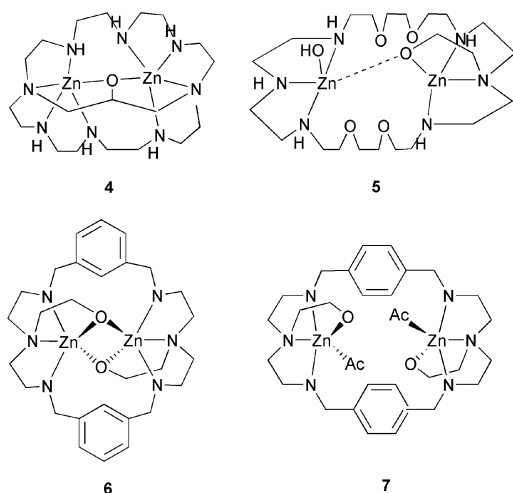
### Synthesis of the ligand

A solution of isophthalaldehyde (0.804 g, 0.006 mol) in 100 mL CH<sub>3</sub>CN was added dropwise to a solution of 2-[bis(2-amino-

† Electronic supplementary information (ESI) available: typical titration curves for L with and without ZnCl<sub>2</sub>; possible species present in solution; species distribution curve as a function of pH; ES-MS spectrum of  $\text{Zn}^{\text{II}}$  with L in a 2 : 1 ratio. See <http://www.rsc.org/suppdata/dt/b2/b206033g/>



Scheme 1



Scheme 2

ethyl)amino]ethanol (0.882 g, 0.006 mol) in 150 mL  $\text{CH}_3\text{CN}$  under magnetic stirring over 8 hours at  $0^\circ\text{C}$  and a white suspension appeared. After stirring for another 12 hours, a white microcrystalline precipitate appeared and was filtered off. The microcrystals were dissolved in 100 mL  $\text{CH}_3\text{CH}_2\text{OH}$  at  $45^\circ\text{C}$  and 2.5 g  $\text{NaBH}_4$  was added little by little over 3 hours under stirring. After removing the solvent under reduced pressure, 5 mL  $\text{H}_2\text{O}$  and 100 mL  $\text{CH}_2\text{Cl}_2$  were added sequentially to extract the product. A colorless viscous oil was obtained after removing the  $\text{CH}_2\text{Cl}_2$  from the organic phase. Several hours after the addition of 5 mL 48%  $\text{HBr}$  to the product at  $0^\circ\text{C}$ , white microcrystals appeared with a yield of 1.61 g (60%). Colorless crystals of  $\text{C}_{28}\text{H}_{46}\text{N}_6\text{O}_2 \cdot 4\text{HBr} \cdot 4\text{H}_2\text{O}$  were obtained by evaporating an aqueous solution of the white microcrystals described above.<sup>20</sup> The whole procedure is shown in Scheme 3. The 500 MHz  $^{13}\text{C}$  NMR spectrum in  $\text{D}_2\text{O}$  gave peaks at  $\delta = 42.6$  ( $\text{CH}_2$  attached to phenyl), 51.0 and 57.1 ( $\text{NCH}_2\text{CH}_2\text{OH}$ ), 55.4 and 49.8 ( $\text{NCH}_2\text{CH}_2\text{N}$ ), 131.0, 131.4, 131.5 and 132.0 (phenyl). Elemental analysis, Calcd for  $\text{C}_{28}\text{H}_{46}\text{N}_6\text{O}_2 \cdot 4\text{HBr} \cdot 4\text{H}_2\text{O}$ : C, 37.6%; H, 6.48%; N, 9.39%, Found: C, 37.5%; H, 6.36%; N, 9.28%.

#### $\text{Zn}_2\text{C}_{28}\text{H}_{48}\text{Br}_2\text{N}_6\text{O}_2$

An aqueous solution (1 mL) of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (58 mg, 0.2 mmol) was added to an aqueous solution (2 mL) of  $\text{L} \cdot 4\text{HBr} \cdot 4\text{H}_2\text{O}$

(89 mg, 0.1 mmol). After adjustment to  $\text{pH} = 9.0$  by adding 1 M  $\text{NaOH}$ , the colorless solution was stirred for 2 hours at  $50^\circ\text{C}$ . The resultant solution was diffused with acetone, and colorless prisms suitable for X-ray analysis were obtained a week later. Elemental analysis, Calc. for  $\text{Zn}_2\text{C}_{28}\text{H}_{48}\text{Br}_2\text{N}_6\text{O}_2$ : C, 40.8%; H, 5.83%; N, 10.2%; Zn, 15.9%, Found: C, 40.6%; H, 5.81%; N, 10.1%; Zn, 16.1%.

#### X-Ray crystallographic study of $\text{Zn}_2\text{C}_{28}\text{H}_{48}\text{Br}_2\text{N}_6\text{O}_2$

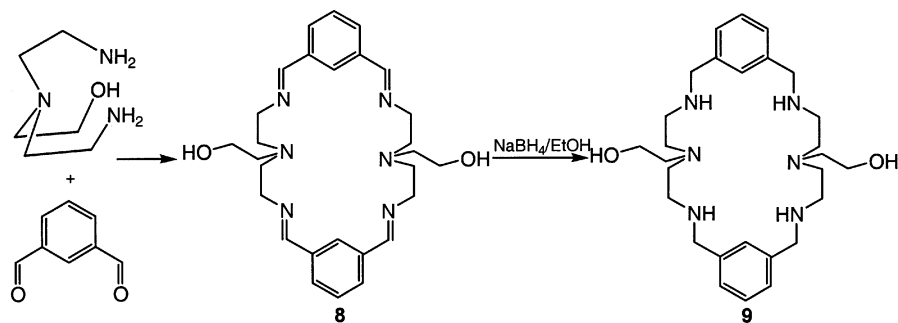
Intensity data for the crystal of  $\text{Zn}_2\text{C}_{28}\text{H}_{48}\text{Br}_2\text{N}_6\text{O}_2$  were collected on a Siemens P4 four-circle diffractometer with monochromated  $\text{Mo-K}\alpha$  ( $\lambda = 0.71073 \text{ \AA}$ ) radiation using the  $\omega/2\theta$  scan mode at  $25^\circ\text{C}$  in the range  $2.24 \leq \theta \leq 25.00^\circ$ . An empirical absorption correction based on  $\psi$  scans was also applied. Data were corrected for Lorenz-polarization effects during data reduction using XSCANS.<sup>21</sup> The structure was solved by direct methods and refined using SHELXL97 software.<sup>22</sup> All non-hydrogen atoms were refined anisotropically by full-matrix least squares. Crystallographic data and details about the data collection are presented in Table 1.

CCDC reference number 176430.

See <http://www.rsc.org/suppdata/dt/b2/b206033g/> for crystallographic data in CIF or other electronic format.

#### Potentiometric measurements

Equilibrium data for the potentiometric experiments were determined by using the procedure described in our previous works with an Orion 91-04 glass-combined pH electrode on an microprocessor ionalyser/901.<sup>11,17</sup> The pH meter was calibrated using commercial pH standards prior to each titration. A humidified nitrogen gas stream was introduced to protect the solution from the effect of air and the solution temperature was maintained at  $25.0 \pm 0.1^\circ\text{C}$ . Double-distilled water with  $\text{pH} = 6.4$  was used in all solutions.  $\text{NaNO}_3$  (1.0 M) was used to maintain the ionic strength at  $I = 0.1 \text{ M}$ , the  $\text{Zn}(\text{NO}_3)_2$  stock solution was calibrated with EDTA. 2.00 mM pure ligand with 6 equiv of  $\text{HCl}$  (12.0 mM) in the presence or absence of 2 equiv of  $\text{Zn}(\text{NO}_3)_2$  was employed in the titration measurement in the pH range 2.5–11 by addition of  $\text{NaOH}$  (0.1 M,  $\text{CO}_2$  free) with a spiral micro-injector. Program BEST<sup>23</sup> was used to determine each equilibrium constant and about 90 data points were calculated every time, using  $K_w (= a_{\text{H}^+} a_{\text{OH}^-}) = 10^{-13.69}$  for  $25^\circ\text{C}$ .<sup>11</sup> All data represent the average of at least two independent titration experiments.



Scheme 3

Table 1 Crystallographic data for Zn<sub>2</sub>C<sub>28</sub>H<sub>48</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub>

Formula weight	823.28
Crystal system	Orthorhombic
Space group	<i>Pbca</i>
<i>T</i> / <i>K</i>	298(2)
$\lambda/\text{\AA}$	0.71073
Color of crystal, habit	Colorless, prism
<i>a</i> / $\text{\AA}$	18.149(2)
<i>b</i> / $\text{\AA}$	8.3650(10)
<i>c</i> / $\text{\AA}$	21.169(3)
$\alpha = \beta = \gamma/^\circ$	90
<i>V</i> / $\text{\AA}^3$	3213.8(7)
<i>Z</i>	4
$\mu/\text{mm}^{-1}$	4.032
Reflections collected	3520
Independent reflections	2814 ( $R_{\text{int}} = 0.0357$ )
Data/restraints/parameters	2814/0/191
$R1^a [I > 2\sigma(I)]$	0.0349
$wR2^b [I > 2\sigma(I)]$	0.0685

<sup>a</sup>  $R1 = \sum ||F_o| - |F_c|| / \sum |F_o|$ ,  $wR2 = \sum w(|F_o|^2 - |F_c|^2) / \sum w(F_o)^2^{1/2}$ .  
<sup>b</sup>  $w = 1 / [(F_o)^2 + (0.0354P)^2 + 0.0000P]$ , where  $P = (F_o^2 + 2F_c^2) / 3$ .

Table 2 Selected bond lengths [ $\text{\AA}$ ] and angles [ $^\circ$ ] for Zn<sub>2</sub>C<sub>28</sub>H<sub>48</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub>

Zn–O1A	1.976(3)	Zn–N2	2.119(3)
Zn–O1	2.031(3)	Zn–N1	2.229(3)
Zn–N3	2.109(3)	Zn ⋯ ZnA	3.0669(10)
O1A–Zn–O1	80.12(12)	O1–Zn–N1	79.51(11)
O1A–Zn–N3	112.60(13)	N3–Zn–N2	104.39(13)
O1–Zn–N3	120.18(13)	O1A–Zn–N1	159.16(12)
O1A–Zn–N2	106.46(12)	N3–Zn–N1	81.74(13)
O1–Zn–N2	128.58(12)	N2–Zn–N1	83.14(13)
O1A–Zn–ZnA	40.73(8)	O1–Zn–ZnA	39.39(7)
N3–Zn–ZnA	125.49(10)	N2–Zn–ZnA	126.55(9)
N1–Zn–ZnA	118.78(9)		

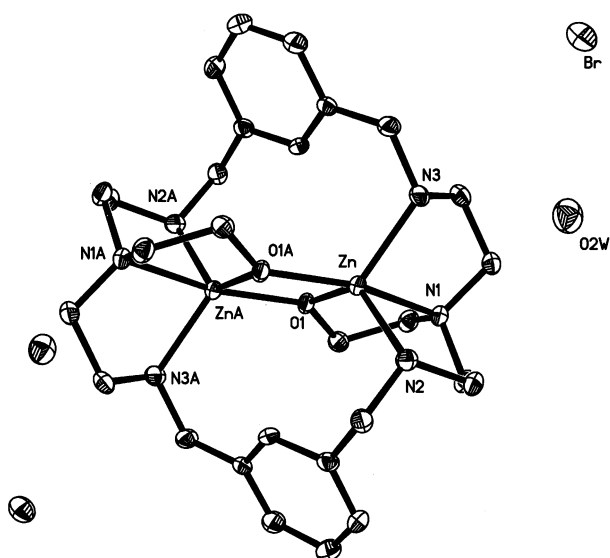


Fig. 1 An ORTEP<sup>24</sup> drawing of Zn<sub>2</sub>C<sub>28</sub>H<sub>48</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub>. All hydrogen atoms are omitted for clarity.

### Hydrolysis of *p*-nitrophenyl acetate

The hydrolysis rate of *p*-nitrophenyl acetate (NA) was measured by the initial slope method following the increase in the 400 nm absorption of the released *p*-nitrophenolate in 10% (v/v) aqueous CH<sub>3</sub>CN at 25.0 ± 0.5 °C on a UV-3100 spectrophotometer by using the procedure previously reported.<sup>11,17</sup> Buffered solutions containing 20 mM Tris buffer (pH 7.5, 8.5) or 20mM CHES buffer (pH 9.0) were used, and the ionic strength was adjusted to  $I = 0.10$  M with 1 M NaNO<sub>3</sub>. For the initial rate determination, the following procedure was employed. Each time, after the NA (0.4–1.0 mM) and the Zn complex (0.5–2.0 mM) were mixed in the buffered solution, the UV absorption at 400 nm was recorded immediately and followed generally until 2% formation of *p*-nitrophenolate which gave the total velocity  $v_{\text{total}}$ . After subtracting the velocity of spontaneous hydrolysis of *p*-nitrophenyl acetate (promoted by buffered solutions which were identical in composition to the above except lacking the Zn complex) denoted as  $v_{\text{buffer}}$ ,  $v_{\text{Zn}} = v_{\text{total}} - v_{\text{buffer}}$  was obtained. The slope of  $v_{\text{Zn}}$  vs. [NA] was denoted as the first-order rate constant  $k_{\text{obs}}$  (s<sup>-1</sup>) and the slope of  $k_{\text{obs}}$  vs. [Zn(II) complex]<sub>total</sub> was denoted as the second-order rate constant  $k_{\text{NA}}$  (M<sup>-1</sup> s<sup>-1</sup>). Experiments were conducted at least in duplicate.

## Results and discussion

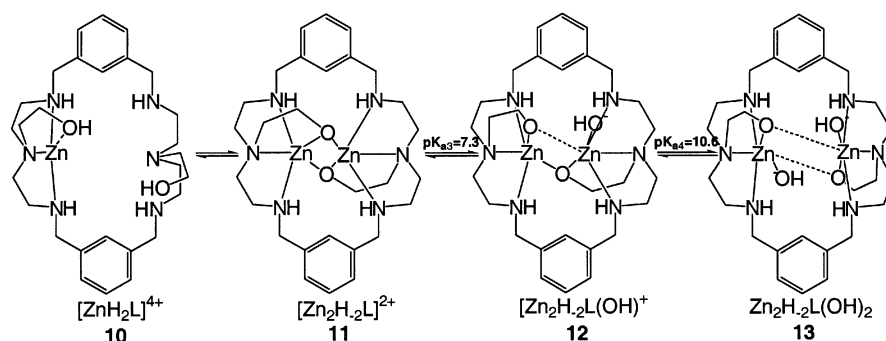
### Crystal structure of Zn<sub>2</sub>C<sub>28</sub>H<sub>48</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub>

A perspective view of Zn<sub>2</sub>C<sub>28</sub>H<sub>48</sub>Br<sub>2</sub>N<sub>6</sub>O<sub>2</sub> is shown in Fig. 1. Selected bond lengths and bond angles relevant to the Zn coordination sphere are given in Table 2.

A chair conformation is adopted by the macrocyclic complex. The whole structure is centrosymmetric with the center

located in the middle of the Zn and ZnA ions. The two zinc ions show analogous coordination environments and each Zn is penta-coordinated. Analysis of the Zn coordination polyhedron according to the method of Muettterties<sup>25</sup> and Galy<sup>26</sup> provide the degree of distortion  $\Delta$  from an ideal trigonal bipyramid ( $\Delta = 0$ ) to a square pyramid ( $\Delta = 1$ ), and each Zn ion has a coordination geometry close to trigonal bipyramid in the N3O2 coordination environment, indicated by  $\Delta = 0.202$ . The Zn ion deviates 0.313 Å from the equatorial plane defined by N2, N3 and O1 with O1–Zn–N3 = 120.18(13)°, N2–Zn–N3 = 104.39(13)°, O1–Zn–N2 = 128.58(12)°, and the axial sites are coordinated by O1A from the pendant alcohol and N1 from the diethylenetriamine moiety with the angle O1A–Zn–N1 = 159.16(12)°.

A Zn<sub>2</sub>(μ-alkoxide)<sub>2</sub> structure is located inside the macrocycle with the angle Zn–O–ZnA = 99.88°. The two zinc ions are bridged by the two deprotonated alcohol pendants located on



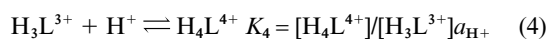
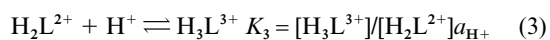
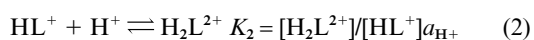
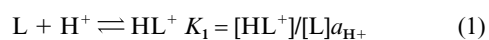
Scheme 4

two different sides of the plane defined by the nitrogen atoms of the macrocycle; the Zn, ZnA, O1, O1A atoms are coplanar. The distance between Zn and ZnA is 3.0669(10) Å and this short distance is primarily governed by the nature and mode of coordination of the bridging groups. Each alkoxide almost symmetrically bridges the two Zn<sup>II</sup> ions with Zn–O1 = 2.031(3) Å and Zn–O1A = 1.976(3) Å. Compared with the structure of the ligand,<sup>20</sup> the whole macrocycle is distorted. The distance between the two tertiary amino N atoms N1 and N1A is only 6.541 Å and the two alcohol O atoms are 2.579 Å away from each other. The two tertiary amino N atoms deviate from the plane defined by the four secondary amino N atoms with a value of 2.277 Å. Two factors contribute to the distortion of the conformation: i) the bridging coordination has brought the two alcohol O atoms together and accordingly the size of the cavity shrinks; ii) by adopting a bipyramidal coordination geometry, Zn has folded the N1, N2, N3 plane, and distance between N1 and N1A is thereby shortened because of the coordination of the Zn<sup>II</sup> ions to O1, O1A, N1 and N1A.

The two aromatic rings are parallel to each other and tilt with an angle of 48.5° to the plane defined by the two Zn and two O atoms. The distance between the two aromatic rings is 8.103 Å, therefore  $\pi$ – $\pi$  stacking interactions do not exist.

### Protonation constants of L

A typical titration curve of 2.0 mM pure ligand with 6 equiv HCl with the addition of 0.1 M NaOH solution at 298.1 K is shown in the ESI (Supplement 1(a)†). Only one inflection point at  $a = 2$  (where  $a$  = moles of added base per mole of ligand) is shown in the potentiometric equilibrium curve, which reveals that the two tertiary nitrogen atoms are of very low basicity and release their protons easily into aqueous solution at low pH.<sup>23</sup> The buffer region occurring at higher pH corresponds to the neutralization of the remaining four ammonium groups of the ligand. The titration data were analyzed for equilibria (1)–(4). The protonation constants  $K_1$ – $K_4$  ( $a_{H^+}$  is the activity of H<sup>+</sup>) are defined as follows:



Logarithms of the protonation constants  $\log K_1$ – $\log K_4$  are  $9.39 \pm 0.02$ ,  $9.18 \pm 0.02$ ,  $7.86 \pm 0.03$ ,  $7.59 \pm 0.02$ , respectively.

### Complexation constants of L with Zn<sup>II</sup>

The complexation constants of the species formed by L and Zn(II) in a 1 : 2 (L : Zn(II)) system with  $I = 0.10$  M (NaNO<sub>3</sub>)

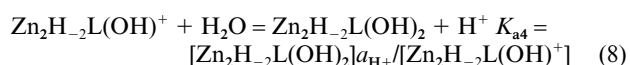
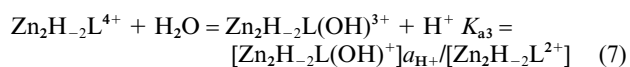
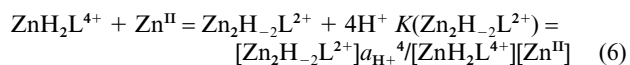
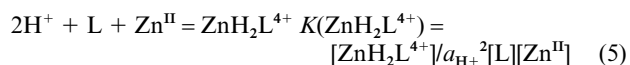
in aqueous solution at 298.1 K have been determined by potentiometric titration; 6 equiv HCl was initially added to acidify the pure ligand. A typical pH titration curve is shown in the ESI (Supplement 1(b)†).

The titration curve reveals the formation of stable complexes corresponding to the buffer region at  $6 < \text{pH} < 10$ . In this region, two continuous inflection points at  $a = 8$  and  $a = 9$  are observed. The inflection at  $a = 8$  corresponds to the completion of the neutralization of the six protonated ammonium groups and deprotonation of the two pendant hydroxyethyl groups during the formation of dinuclear Zn complexes. Further deprotonation was evidenced by the buffer region between  $a = 8$  and  $a = 9$ , and the inflection point at  $a = 9$  is ascribed to the completion of deprotonation of a Zn<sup>II</sup>-bound water molecule. The buffer region above  $a = 9$  suggests further deprotonation of water molecules and the appearance of a white precipitate, Zn(OH)<sub>2</sub>, at pH > 12 suggests a partial decomposition of the dinuclear Zn<sup>II</sup> complex at higher pH.

The program BEST was used to analyze the titration data. All possible species used for model construction shown in the ESI (Supplement 2†) have been used for calculation. Reasonable results were given by BEST, with a  $\sigma$  fit value optimized to be 0.003. Four species: [ZnH<sub>2</sub>L]<sup>4+</sup>, [Zn<sub>2</sub>H<sub>2</sub>L]<sup>2+</sup>, [Zn<sub>2</sub>H<sub>2</sub>L(OH)]<sup>+</sup>, [Zn<sub>2</sub>H<sub>2</sub>L(OH)<sub>2</sub>] were confirmed to be dominant species under the employed experimental conditions, and other species were less than 1 ppm in concentration.

The species distribution as a function of pH based on the calculation results is shown in the ESI (Supplement 3†). The mononuclear complex [ZnH<sub>2</sub>L]<sup>4+</sup> appears in the pH range 5.5–7.2 (maximum at pH 6.4, 38.6%). The binuclear Zn complex with two alkoxide bridges [Zn<sub>2</sub>H<sub>2</sub>L]<sup>2+</sup> exists in the pH range 6–8 (maximum at pH 7.0, 51.3%). As the pH is raised, a binucleating complex with a Zn-bound hydroxide [Zn<sub>2</sub>H<sub>2</sub>L(OH)]<sup>+</sup> is formed with a preferred pH of 9.0, above which it is gradually converted to [Zn<sub>2</sub>H<sub>2</sub>L(OH)<sub>2</sub>]. To illustrate the process clearly, the stepwise complexation of L with Zn<sup>II</sup> via these four species is shown in Scheme 4.

The complexation constants  $K$  and deprotonation constants  $K_a$  for the four species are defined as follows:



[ZnH<sub>2</sub>L]<sup>4+</sup> is a mono-Zn complex with  $\log K(ZnH_2L) = 23.9$ . As another Zn<sup>II</sup> ion binds to [ZnH<sub>2</sub>L]<sup>4+</sup>, the dinuclear Zn<sup>II</sup>

complex  $[\text{Zn}_2\text{H}_{-2}\text{L}]^{2+}$  is formed directly and several processes, involving deprotonation of two substituted ammonium groups, deprotonation of two alcohols and dimerization of two tri-podal moieties in the macrocycle, take place simultaneously. ES-MS spectra (shown as ESI Supplement 4†) for the complex at pH = 7.0 in aqueous solution evidences the stable existence of  $[\text{Zn}_2\text{H}_{-2}\text{L}]^{2+}$  ( $m/z = 313.3$ ) with a relative abundance of 100%. The stable existence of  $[\text{Zn}_2\text{H}_{-2}\text{L}]^{2+}$  at pH = 7.0 indicated that deprotonation of hydroxyethyl bound to  $\text{Zn}^{\text{II}}$  happened below pH = 7.0, that is, the  $pK_a$  value for  $\text{Zn}^{\text{II}}$  bridged hydroxyethyl denoted as  $pK_{a1,2}$  is less than 7.0.

It is worthy of mention that the simultaneous formation of binuclear Zn complexes together with the formation of bridging alkoxide or hydroxide has also been reported previously in other 24-membered macrocycles.<sup>12,27</sup> The simultaneous deprotonation of a water molecule with a  $pK_a$  value less than 7 to bridge two  $\text{Zn}^{\text{II}}$  was reported in the work of Bencini *et al.* for the ligand [24]aneN<sub>8</sub>.<sup>27</sup> Kimura *et al.*, also observed the simultaneous coordination of two Zn ions to the propanol-bridged [24]aneN<sub>8</sub>.<sup>12</sup>

$pK_{a3} = 7.3$  for equilibrium (7) is almost identical with the  $pK_a$  value for  $\text{H}_2\text{O}$  bound to  $\text{Zn}^{\text{II}}[12]\text{andN}_3$  ( $pK_a = 7.3$ )<sup>9</sup> and may correspond to the formation of a terminal  $\text{Zn}^{\text{II}}$ -bound hydroxide ion to give  $[\text{Zn}_2\text{H}_{-2}\text{L}(\text{OH})]^+$ , a situation encountered by many dinuclear  $\text{Zn}^{\text{II}}$  complexes with or without pendant alcohol groups.<sup>13,14</sup> During this process, the binding of hydroxide to a  $\text{Zn}^{\text{II}}$  ion will lead to detachment of a chelating alkoxide from that  $\text{Zn}^{\text{II}}$  ion to a terminal position by binding to the other  $\text{Zn}^{\text{II}}$ , or cause a weakening of the binding of  $\text{Zn}-\text{OR}$  as sketched in Scheme 4.  $pK_{a4} = 10.6$  for equilibrium (8) is assigned to the deprotonation of another water molecule coordinated to the second  $\text{Zn}^{\text{II}}$  ion.

### Hydrolysis of *p*-nitrophenyl acetate

The reactivity of the dinuclear complex toward the hydrolysis of phosphate ester or carboxy ester has been checked. Since phosphomonoester underwent low hydrolysis with the complex, here we only describe the hydrolytic cleavage of *p*-nitrophenyl acetate (NA). The activity of the binuclear species has been investigated by following the hydrolysis of *p*-nitrophenyl acetate (0.4 mM–1.0 mM) promoted by the Zn complex (0.5 mM–2.0 mM) at different pH (7.5, 8.5, 9.0) as described in the Experimental section, and the spontaneous hydrolysis of *p*-nitrophenyl acetate by the buffered solution alone has been subtracted from the Zn-complex promoted hydrolysis. An initial slope method has been applied to the data processing procedure and the initial velocity  $v_{\text{total}}$ , spontaneous hydrolysis velocity  $v_{\text{buffer}}$ , Zn complexes induced hydrolysis velocity  $v_{\text{Zn}}$ , first-order rate constant  $k_{\text{obs}}$  ( $\text{s}^{-1}$ ), and second-order rate constant  $k_{\text{NA}}$  ( $\text{M}^{-1} \text{s}^{-1}$ ) are defined as follows:

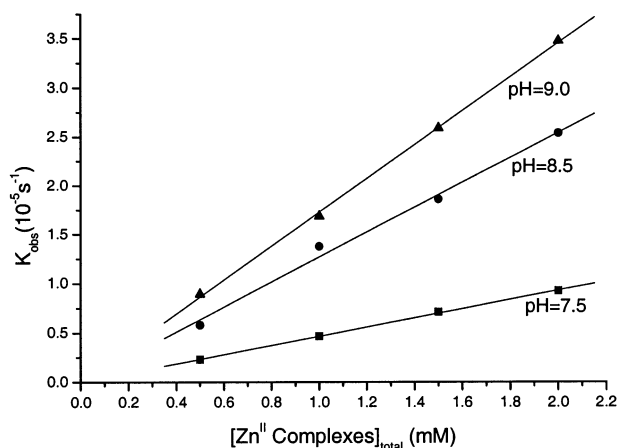
$$v_{\text{total}} = v_{\text{Zn}} + v_{\text{buffer}}$$

$$v_{\text{buffer}} = (k_{\text{OH}^-}[\text{OH}^-] + \dots)[\text{NA}]$$

$$v_{\text{Zn}} = k_{\text{obs}}[\text{NA}] = k_{\text{NA}}[\text{Zn(II) complex}]_{\text{total}}[\text{NA}]$$

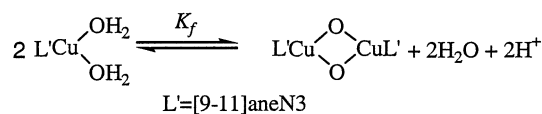
Initial velocities  $v_{\text{total}}$  were collected by following the absorbance at 400 nm increasing up to 2% formation of *p*-nitrophenolate with the slope being  $v_{\text{total}}$ . Zn complex induced hydrolysis velocity  $v_{\text{Zn}}$  was obtained by subtracting the spontaneous hydrolysis velocity promoted by the buffered solution ( $v_{\text{buffer}}$ ) from  $v_{\text{total}}$ . The first-order rate constant  $k_{\text{obs}}$  ( $\text{s}^{-1}$ ) was calculated from the slope of the straight line  $v_{\text{Zn}}$  vs. [NA]. The plot of  $k_{\text{obs}}$  vs.  $[\text{Zn(II) complexes}]_{\text{total}}$  gave a straight line with the slope denoted as  $k_{\text{NA}}$  ( $\text{M}^{-1} \text{s}^{-1}$ ).<sup>17</sup> The pH-dependant curve of  $k_{\text{obs}}$  vs.  $[\text{Zn(II) complexes}]_{\text{total}}$  is shown in Fig. 2.

The second-order rate constant  $k_{\text{NA}}$  is  $0.0046 \text{ M}^{-1} \text{ s}^{-1}$  at pH = 7.5, where  $[\text{Zn}_2\text{H}_{-2}\text{L}]^{2+}$  is the main species in aqueous solution.



**Fig. 2** Change in  $k_{\text{obs}}$  ( $\text{s}^{-1}$ ) as a function of total concentration of Zn complex  $[\text{Zn(II) complexes}]_{\text{total}}$  at pH = 7.5, 8.5 and 9.0 with  $I = 0.1 \text{ M}$  ( $\text{NaNO}_3$ ) at  $25^\circ \text{C}$  in the presence of 10% (v/v) aqueous  $\text{CH}_3\text{CN}$ . Spontaneous hydrolysis by the buffered solution alone has been subtracted at each pH and the slope of the straight line illustrated is denoted as  $k_{\text{NA}}$ .

The low activity of  $[\text{Zn}_2\text{H}_{-2}\text{L}]^{2+}$  containing  $\text{Zn}_2(\mu\text{-alkoxide})_2$  is to some degree similar to the inactivity of the dimers of some mono-copper model complexes  $\text{Cu}[9-11]\text{aneN}_3\text{X}_2$ .<sup>7,8</sup> In these complexes, the activity is decided by the concentration of monomers controlled by the monomer–dimer equilibrium, with the monomer being the active species and the dimer being an inactive form of corresponding complexes.



A higher activity is achieved when the pH is raised above 8.0 with  $k_{\text{NA}} = 0.013 \text{ M}^{-1} \text{ s}^{-1}$  at pH = 8.5, and  $k_{\text{NA}} = 0.018 \text{ M}^{-1} \text{ s}^{-1}$  at pH = 9.0, which parallels the increase in the concentration of  $[\text{Zn}_2\text{H}_{-2}\text{L}(\text{OH})]^+$ . The obvious correlation between concentration of  $[\text{Zn}_2\text{H}_{-2}\text{L}(\text{OH})]^+$  and hydrolysis rate at  $7.0 < \text{pH} < 9.0$  suggests that the latter complex which is characterized as having a Zn-bound alkoxide and a terminally bound hydroxide group has higher hydrolytic activity than  $[\text{Zn}_2\text{H}_{-2}\text{L}]^{2+}$ . Since no intermediate has been isolated the hydrolysis mechanism is still uncertain. A possible catalytic process is suggested as being similar to that described in the work of Bencini *et al.*, where both  $\text{Zn}-\text{OH}^-$  and  $\text{Zn}-\text{OR}$  are involved in the *p*-nitrophenyl acetate hydrolysis.<sup>13</sup>

For comparison, we have recently synthesized a di-Zn complex of a novel 26-membered binucleating macrocyclic ligand  $[\text{Zn}_2\text{L}1(\text{CH}_3\text{CO}_2)_2](\text{ClO}_4)_2(\text{H}_2\text{O})_2$  (7 in Scheme 2), and it is considerably more efficient in promoting hydrolysis of NA with  $k_{\text{NA}} = 0.32 \text{ M}^{-1} \text{ s}^{-1}$  at pH = 9.0,<sup>28</sup> a value that is remarkably higher than that of the title complex. This can be explained by considering the structural differences between the two compounds. The rigidity of the 26-membered macrocycle has kept the two Zn(II) ions 8.74 Å apart and therefore cannot form the species containing  $\text{Zn}_2(\mu\text{-alkoxide})_2$  while the cavity size and rigidity of the 24-membered macrocycle facilitates the formation of the inactive species containing  $\text{Zn}_2(\mu\text{-alkoxide})_2$ . The difference in their hydrolytic activity is to some degree similar to some copper complexes described previously whose activities are determined by the concentration of the active monomer species in the monomer–dimer equilibrium.<sup>7,8</sup>

### Conclusion

A new macrocyclic ligand containing two pendant alcohol groups was synthesized and characterized by potentiometric

analysis. The corresponding dizinc complex was crystallized from aqueous solution. X-Ray crystal analysis reveals that the two pendant alcohol groups are deprotonated to bridge two  $Zn^{II}$  ions from two different sides of the plane determined by the nitrogen atoms of the macrocycle. Potentiometric investigation shows that the two alcohol groups deprotonate simultaneously with  $pK_{a1,2} < 7$  at 298K. Further deprotonation of the terminally coordinated water molecule was observed with  $pK_{a3} = 7.3$  and  $pK_{a4} = 10.6$  respectively. The catalyzed hydrolysis rate of *p*-nitrophenyl acetate by the complex has been investigated at  $7.0 < pH < 9.0$  and results show that the catalytic activity of  $[Zn_2H_{-2}L(OH)]^+$  is higher than  $[Zn_2H_{-2}L]^{2+}$ .

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## References

- 1 B. L. Vallee and D. S. Auld, *Acc. Chem. Res.*, 1993, **26**, 543.
- 2 E. E. Kim and H. W. Wyckoff, *J. Mol. Biol.*, 1991, **218**, 449.
- 3 W. N. Lipscomb and N. Strater, *Chem. Rev.*, 1996, **96**, 2396.
- 4 M. J. Jędrzejak and P. Setlow, *Chem. Rev.*, 2001, **101**, 607.
- 5 E. Kimura, I. Nakamura, T. Koike, M. Shionoya, Y. Kodama, T. Ikeda and M. Shiro, *J. Am. Chem. Soc.*, 1994, **116**, 4764.
- 6 T. Koike, S. Kajitani, I. Nakamura, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1995, **117**, 1210.
- 7 E. L. Hegg, S. H. Mortimore, C. L. Cheung, J. E. Huyett, D. R. Powell and J. N. Burstyn, *Inorg. Chem.*, 1999, **38**, 2961.
- 8 K. M. Deck, T. A. Tseng and J. N. Burstyn, *Inorg. Chem.*, 2002, **41**, 669.
- 9 E. Kimura, T. Shiota, T. Koike, M. Shiro and M. Kodama, *J. Am. Chem. Soc.*, 1990, **112**, 5805.
- 10 J. A. Connolly, M. Banaszczuk, R. C. Hynes and J. Chin, *Inorg. Chem.*, 1994, **33**, 665.
- 11 J. Xia, Y. Xu, S-a. Li, K-b. Yu and W-x. Tang, *Inorg. Chem.*, 2001, **40**, 2394.
- 12 T. Koike, M. Inoue, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1996, **118**, 3091.
- 13 C. Bazzicalupi, A. Bencini, E. Berni, A. Bianchi, V. Fedi, V. Fusi, C. Giorgi, P. Paoletti and B. Valtancoli, *Inorg. Chem.*, 1999, **38**, 4115.
- 14 A. Bencini, E. Berni, A. Bianchi, V. Fedi, C. Giorgi, P. Paoletti and B. Valtancoli, *Inorg. Chem.*, 1999, **38**, 6323.
- 15 P. Jurek and A. E. Martell, *Inorg. Chim. Acta*, 1999, **287**, 47.
- 16 W. H. Chapman and Jr. R. Breslow, *J. Am. Chem. Soc.*, 1995, **117**, 5462.
- 17 S-a. Li, J. Xia, D-x. Yang, Y. Xu, D-f. Li, M-f. Wu and W-x. Tang, *Inorg. Chem.*, 2002, **41**, 1807.
- 18 V. A. Bobylev and V. D. Chechik, *Zh. Obshch. Khim.*, 1990, **60**, 2721.
- 19 A. Roger, *Organic Reaction*, Wiley, New York, 1954, vol. 8.
- 20 D-x. Yang, S-a. Li, D-f. Li and W-x. Tang, *Acta Crystallogr., Sect. C*, 2002, **58**, o11–o13.
- 21 XSCANS (Version 2.1), Siemens Analytical X-ray Instruments Inc., Madison, MI, 1994.
- 22 G. M. Sheldrick, SHELXTL (version 5.1), Program for Crystal Structure Determinations, University of Göttingen, 1997.
- 23 A. E. Martell and R. J. Motekaitis, *Determination and Use of Stability Constants*, VCH Publishers, Inc., New York, 2nd edn., 1992.
- 24 C. K. Johnson, ORTEP, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, TN, 1976.
- 25 E. L. Muetterties and L. J. Guggenberger, *J. Am. Chem. Soc.*, 1974, **96**, 1748.
- 26 J. Galy, J. I. Bownet and S. Anderson, *Acta Chem. Scand.*, 1979, **33**, 383.
- 27 A. Bencini, A. Bianchi, P. Dapporto, E. Garcia-España, M. Micheloni and P. Paoletti, *Inorg. Chem.*, 1989, **28**, 1188.
- 28 S-a. Li, D-x. Yang, D-f. Li, J. Huang and W-x. Tang, *New. J. Chem.*, 2002, to be published.