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Ester Hydrolysis by a Cyclodextrin Dimer Catalyst with a Metallophenanthroline Linking Group

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Abstract: A novel β-cyclodextrin dimer, 1,10-phenanthroline-2,9-dimethyl-bridged-bis(6-monoammonio-β-cyclodextrin) (phenBisCD, L), was synthesized. Its zinc complex (ZnL) has been prepared, characterized, and applied as a new catalyst for diester hydrolysis. The formation constant ($\log K_{ML} = 9.56 \pm 0.01$) of the complex and deprotonation constant ($pK_a = 8.18 \pm 0.04$) of the coordinated water molecule were determined by a potentiometric pH titration at (298 ± 0.1) K. Hydrolytic kinetics of carboxylic acid esters were performed with bis(4-nitrophenyl) carbonate (BNPC) and 4-nitrophenyl acetate (NA) as substrates. The obtained hydrolysis rate constants showed that ZnL has a very high rate of catalysis for BNPC hydrolysis, giving a 3.89×10^4-fold rate enhancement over uncatalyzed hydrolysis at pH 7.01, relative to only a 42-fold rate enhancement for NA hydrolysis. Moreover, the hydrolysis second-order rate constants of both BNPC and NA greatly increases with pH. Hydrolytic kinetics of a phosphate diester catalyzed by ZnL was also investigated by using bis(4-nitrophenyl) phosphate (BNPP) as the substrate. The pH dependence of the BNPP cleavage in aqueous buffer shows a sigmoidal curve with an inflection point around pH 8.11, which was nearly identical to the pK_a value from the potentiometric titration. The k_{cat} of BNPP hydrolysis promoted by ZnL was found to be $9.9 \times 10^{-4}\,M^{-1}\,s^{-1}$, which is comparatively higher than most other reported Zn^{II}-based systems. The possible intermediate for the hydrolysis of BNPP, BNPC, and NA catalyzed by ZnL is proposed on the basis of kinetic and thermodynamic analysis.

Keywords: cyclodextrins · hydrolysis · kinetics · phosphate diesters · zinc

Introduction

Over these years, a great deal of interest has been focused on cyclodextrin dimers (BisCDs) that are covalently linked by some flexible or rigid groups because of the favorable cooperative binding interactions between the two hydrophobic cyclodextrin (CDs) cavities and guest molecules.[1] Relative to α- or γ-CDs, β-CDs have an appropriately sized cavity and appropriate water solubility; the result of this is that the synthesis and properties of CD dimers through their primary sides are well documented.[2] Breslow, Nolte, Fujita, and Liu have synthesized a series of BisCDs with a variety of functional linkers that can be further coordinated to a metal ion as the active site of a metalloenzyme,[3–6] such as porphyrin, bipyridine, and so on. These BisCDs have various comprehensive applications in chiral discrimination,[7] drug carriers,[8] molecular recognition,[9] and enzyme mimics.[10] Marsura et al. synthesized a metallohydrolase model with a BisCD linked by a long-chained group, and the catalytic hydrolysis activity of the ester was low relative to a BisCD linked by an appropriately lengthened metallobipyridine.[11,12] It is well known that in biological systems a substrate is rigidly immobilized around the catalytic group by an enzyme exquisitely tailoring both the first and the second coordination spheres of its active site to afford efficient and selective catalytic systems for the reactive geometry.[12,13] Recently, Liu et al. reported a β-cyclodextrin dimer linked by telluroxy group, in which the catalytic rate of hydrolysis of bis(4-nitrophenyl) carbonate (BNPC) was remarkably accelerated.[14] However, tellurium compounds are toxic to living organisms and have not been found in natural biomacromolecules, including DNA, proteins, and lipids. To our best knowledge, artificial hydrolase systems are usually based on Zn^{2+}, Cu^{2+}, Co^{3+}, Fe^{3+}, and lanthanide ions (Eu^{3+},

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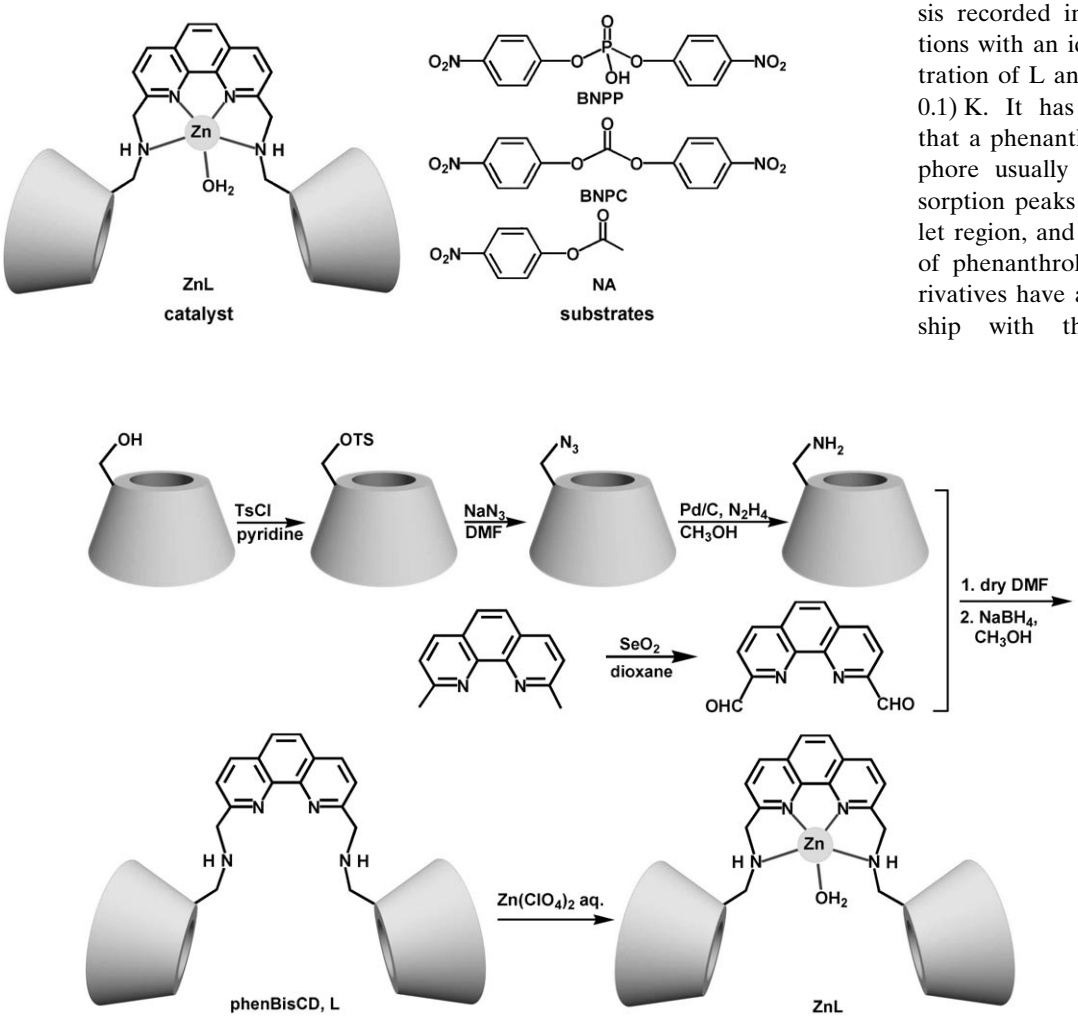
Ce^{4+}), but nature's choices fall mainly on Mg^{2+}, Zn^{2+}, Ca^{2+}, and Fe^{2+}. Therefore, the Zn^{II} ion is the only metal frequently encountered in both natural and artificial agents, due to a variety of factors: The Zn^{II} ion is a good Lewis acid, exchanges ligands rapidly, is not toxic, and is not redox active.[15] Moreover, it has no ligand-field stabilization energy and, as a consequence, it can easily adapt its coordination geometry to best fulfill the structural requirement of a reaction.[16] For all of these reasons, the development of a Zn^{II}-based artificial hydrolase would be highly valuable.

Recently, we reported some supramolecular models of metallohydrolase and superoxide dismutase with the cyclodextrin inclusion complexes constructed by metal-complex binding into CD or its derivatives. We also reported that their catalytic activities can be greatly improved due to second coordination sphere interactions.[17–19] We found that the inclusion complex with CD or its derivatives has potential in the development of an effective model for metalloenzymes. On the basis of the above work, we synthesized a cyclodextrin dimer linked by 1,10-phenanthroline-2,9-ammoniomethyl (phenBisCD, L) and its zinc complex (ZnL). In this complex, the zinc ion has a stable ZnN_4–OH_2 configuration, which was deemed to be an appropriate catalytic site for hydration or hydrolysis.[20] The investigation of esterase activity was performed by using ZnL to promote the hydrolysis of BNPC, 4-nitrophenyl acetate (NA), and bis(4-nitrophenyl) phosphate (BNPP). Herein, we report the synthesis, characterization, and thermodynamic properties of ZnL as well as detailed hydrolytic kinetics of esters catalyzed by ZnL.

Results and Discussion

Synthesis and characterization of ZnL: As illustrated in Scheme 1, the dimer (L) is synthesized in a yield of 32% by the reaction of a 6-monodeoxy-6-monoamino-β-cyclodextrin with 1,10-phenanthroline-2,9-dicarboxaldehyde and is characterized by spectroscopy (see Figures S1–S3 in the Supporting Information). The further reaction of L and zinc(II) perchlorate gave the zinc(II) complex in a moderate yield (68%).

To obtain information on the role played by the phenanthroline unit in metal coordination, complex formation was followed by UV spectral analysis recorded in aqueous solutions with an identical concentration of L and Zn^{II} at (298 ± 0.1) K. It has been reported that a phenanthroline chromophore usually shows two absorption peaks in the ultraviolet region, and the UV spectra of phenanthroline and its derivatives have a great relationship with their complexa-



Scheme 1. Synthetic scheme of the catalyst ZnL.

tion.[21] As illustrated in Figure S4 (see the Supporting Information), the ligand shows two absorption peaks at 232 ($\varepsilon = 50\,967\ mol^{-1}\,L\,cm^{-1}$) and 273 nm ($\varepsilon = 33\,177\ mol^{-1}\,L\,cm^{-1}$), respectively, which are assigned to absorptions of the phenanthroline chromophore. In the presence of $Zn(ClO_4)_2$, a new broad peak of 300 nm appears, and the absorption intensity at 232 nm obviously decreases (ε from 50 967 to $45\,509\ mol^{-1}\,L\,cm^{-1}$) accompanying a slight violetshift. Meanwhile, zinc(II)-ion coordination induces a 4 nm redshift of the band at 277 nm over the uncomplexed ligand, which slightly increases in absorbance (ε from 32 050 to $33\,766\ mol^{-1}\,L\,cm^{-1}$). Since the solution of the Zn^{II} ion does not cause UV spectral changes, these spectrophotometric data indicate that the phenanthroline group is involved in metal coordination.

ESIMS spectrometry of ZnL is a more important method for the investigation into the formation of complexation in aqueous/MeOH solution. Figure 1 shows both the experimental and calculated isotopic distribution for the peak at m/z: 1268.31, which was assigned to $[ZnL]^{2+}$. This result indicates that the complexation binding between the zinc ion and L exists. To access the complexation, ^1H NMR spectra was used to investigate and to distinguish the difference after L complexation. Some changes in the chemical shift of the phenanthroline protons in ZnL were observed relative to those in L (see Figure S5 in the Supporting Information): the Ha, Hb, and Hc protons shifted downfield by approximately 0.32, 0.40, and 0.37 ppm, respectively, which indicated that a complex had formed between the L and zinc(II) ion.

Formation and deprotonation constants of ZnL: The protonation constants (K_n) of the ligand, their inclusion complex formation constants (K_{ML}), and the deprotonation constant (pK_a) of the coordinated water molecule as well as species distribution in solution were determined by pH potentiometric titration at $I = 0.10$ M $NaClO_4$ and (298 ± 0.1) K. The pH profiles of the titration curves, which include the distribution curves of the Zn^{II} species as a function of pH (Figure 2), were analyzed by the Hyperquad program. The calculated results are summarized in Table 1, which includes data from the [Zn(pdma)] complex (pdma = *N*,*N*′-dimethyl-1,10-phenanthroline-2,9-dimethanamines),[22] a simple analogue of ZnL.

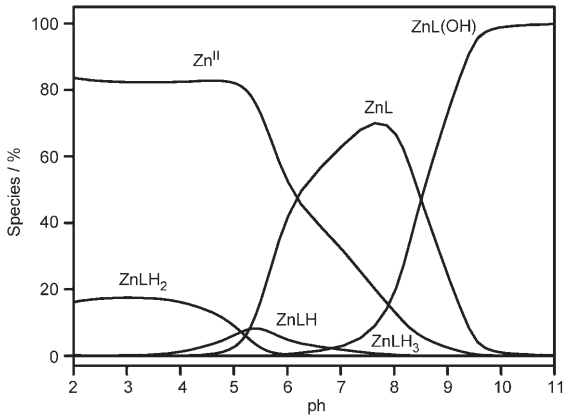


Figure 2. Distribution plots of species with ZnL (1.00 mM) as a function of pH at 0.10 M $NaClO_4$ and (298 ± 0.1) K.

Table 1. Equilibrium constants of the ligand and its metal complex.

Chemical equilibrium		Equilibrium constant	
	pdma[a]	phenBisCD	
$H_3L^{3+} = H_2L^{2+} + H^+$	pK_1	2.04	1.34 ± 0.03
$H_2L^{2+} = HL^+ + H^+$	pK_2	8.35	5.06 ± 0.06
$HL^+ = L + H^+$	pK_3	9.71	12.6 ± 0.04
$[Zn(H_2O)_6]^{2+} + L = [ZnL(H_2O)]^{2+}$	$\log K_{ML}$	8.98	9.56 ± 0.01
$[ZnL(H_2O)]^{2+} = [ZnL(OH)]^+ + H^+$	pK_a	8.64	8.18 ± 0.04

[a] See reference [22], pdma = *N*,*N*′-dimethyl-1,10-phenanthroline-2,9-dimethanamine.

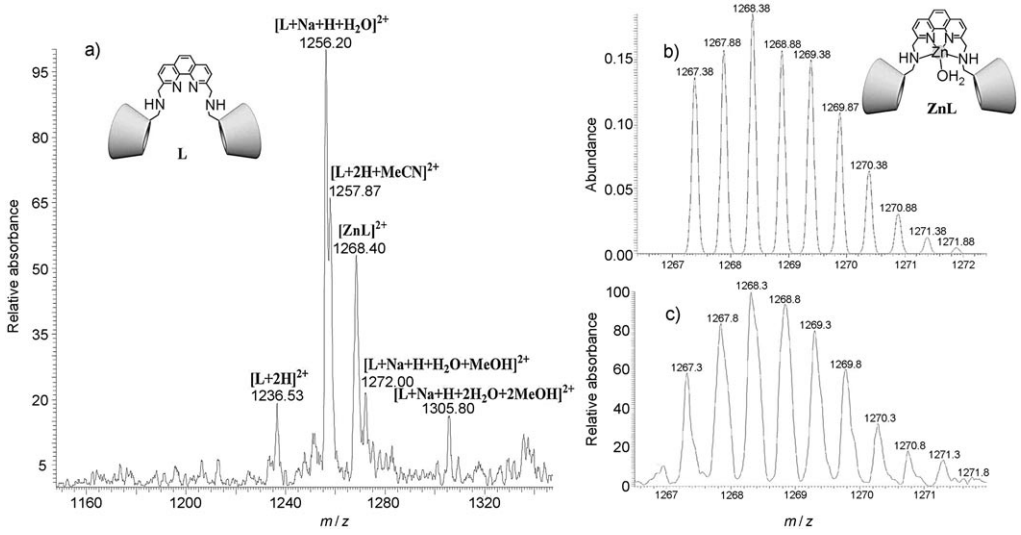


Figure 1. ESIMS spectra of ZnL. a) Full-range spectra. b) Bivalence ion isotope spectra determined by computer simulation. c) Detected bivalence ion isotope spectra.

It is indicated in Figure 2 that five Zn^{II} species, $[ZnL]^{2+}$, $[ZnLH]^{3+}$, $[ZnLH_2]^{4+}$, $[ZnLH_3]^{5+}$, and $[ZnLH_{-1}]^{+}$, corresponding to Equations (1–5), respectively, are involved in the complex formation at pH 2–11. Since the addition of $NaClO_4$ does not cause spectral changes, it was also confirmed that the remaining coordination sites of Zn^{II} are occupied by one water molecule.[19]

$$Zn^{2+} + L = [ZnL]^{2+} \quad K_{ZnL} \tag{1}$$

$$Zn^{2+} + L + H^{+} = [ZnLH]^{3+} \quad K_{ZnLH} \tag{2}$$

$$Zn^{2+} + L + 2H^{+} = [ZnLH_2]^{4+} \quad K_{ZnLH2} \tag{3}$$

$$Zn^{2+} + L + 3H^{+} = [ZnLH_3]^{5+} \quad K_{ZnLH3} \tag{4}$$

$$[ZnL]^{2+} = [ZnLH_{-1}]^{+} + H^{+} \quad K_{a1} \tag{5}$$

It might be of interest to compare the complex formation constants of ZnL with that of [Zn(pdma)] complex, which is an analogue of ZnL. From the catalytic mechanism analysis of [Zn(pdma)] complex, it was found that three nitrogen donors are involved in metal coordination. The formation constant (log$K$) of ZnL is slightly higher than that of [Zn(pdma)] complex (9.56 ± 0.01 for ZnL and 8.98 for [Zn(pdma)], respectively), which implies that all benzylic nitrogen donors in the ZnL are coordinated to the zinc ion. Furthermore, a marked change is that the pK_a of $[ZnL(H_2O)]^{2+}$ (8.18 ± 0.04) is approximately 0.5 pH units lower than that of $[Zn(pdma)(H_2O)]^{2+}$ (8.64). This change is probably due to the effect of both the hydrophobic environment around the metal ion center and weak interactions after complexation, similar to those in carbonic anhydrase or alkaline phosphatase.[23]

Hydrolysis of carboxylic acid esters: To demonstrate the effect of hydrophobic interactions on the catalytic activities of ZnL, BNPC and NA were also selected as testing substrates on the basis of their structural features. Studies into the hydrolysis kinetics of BNPC and NA were performed in a 10% MeCN solution of Tris-HCl (50 mM, pH 7.01) at (298 ± 0.1) K (Figure 3). By means of spectrophotometrical detection of product 4-nitrophenol at 400 nm ($\varepsilon_{obs} = 8700\ M^{-1}\ cm^{-1}$),[24] the initial hydrolysis rates of BNPC (50 μM) and NA (200 μM) in the presence of catalysts were calculated (Table 2).

The measured initial rate of spontaneous cleavage of BNPC (50 μM) is very slow ($\vartheta_{control} = 2.45 \times 10^{-10}\ M\,s^{-1}$), which is consistent with the value reported.[14] Almost no enhancement in the hydrolysis rate was observed when only the zinc ion was added to the substrate of BNPC or NA. However, an enhancement was observed when L was added, which was 312-fold higher than BNPC self-hydrolysis. A similar observation has been recently reported in which a cyclodextrin dimer linked by tris(2-aminoethyl)amine showed a 150-fold rate enhancement for BNPC hydrolysis.[25] Most interestingly, under identical conditions, ZnL exhibits an abrupt enhancement in the rate of BNPC hydrolysis, which is 2.61×10^3-fold higher than BNPC self-hydrolysis. In the case of NA, however, hydrolysis rates catalyzed by L and ZnL are only 1.47- and 1.95-fold higher than that of the self-hydrolysis, respectively.

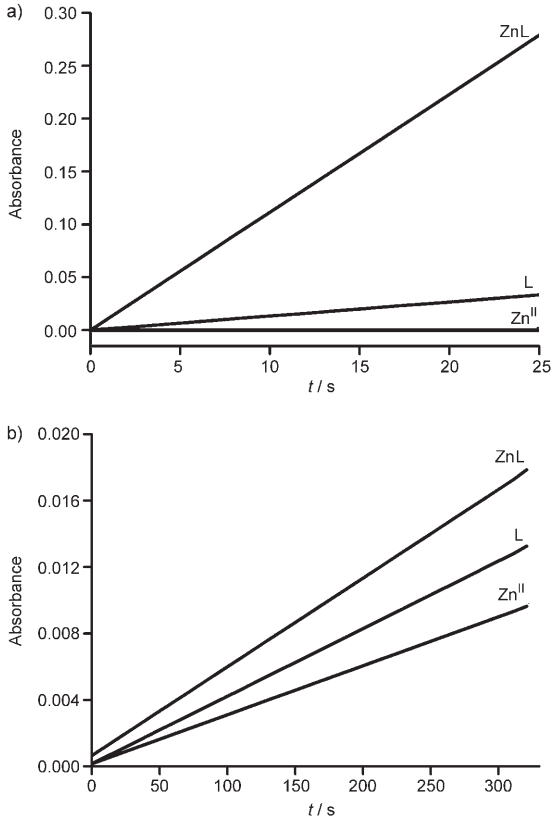


Figure 3. Plots of absorbance versus time during BNPC (a) and NA hydrolysis (b) catalyzed by $Zn(ClO_4)_2$, L, and ZnL in a 10% MeCN solution of pH 7.01 Tris-HCl buffer with 0.10 M $NaClO_4$ at (298 ± 0.1) K ([catalyst] = 50 μM, [BNPC] = 50 μM, [NA] = 200 μM).

Table 2. Initial rate (v) for ester hydrolysis promoted by different catalysts.

Catalyst[a]	BNPC		NA	
	$v(10^{-9}\ M\,s^{-1})$	v/v_{contr}	$v(10^{-9}\ M\,s^{-1})$	v/v_{contr}
buffer	$(2.45 \pm 0.03) \times 10^{-1}$	1.00	3.17 ± 0.10	1.00
Zn^{II}	$(2.62 \pm 0.03) \times 10^{-1}$	1.07	3.33 ± 0.17	1.05
L	$(7.65 \pm 0.20) \times 10$	3.12×10^2	4.67 ± 0.3	1.47
ZnL	$(6.40 \pm 0.23) \times 10^2$	2.61×10^3	6.17 ± 0.20	1.95

[a] Reaction conditions: 50 μM BNPC or 200 μM NA, 50 μM catalyst, 0.10 M $NaClO_4$, 50 mM pH 7.01 Tris-HCl buffer, (298 ± 0.1) K.

To fully assess the hydrolysis ability of ZnL for BNPC, a detailed kinetic study was undertaken. Saturation kinetics were observed (Figure 4) and thus kinetic parameters deduced from the Michaelis–Menten equation for the hydrolysis are listed in Table 3. Turnover numbers of k_{cat} ($(1.88 \pm 0.20) \times 10^{-1}\ s^{-1}$) were obtained for BNPC hydrolysis catalyzed by ZnL. The value of k_{cat}/k_{uncat} was used to describe the catalytic ability of hydrolase mimics, and it showed in our case values up to 3.89×10^4. However, for NA hydrolysis, the value of k_{cat}/k_{uncat} was found to be 42.3, which is almost

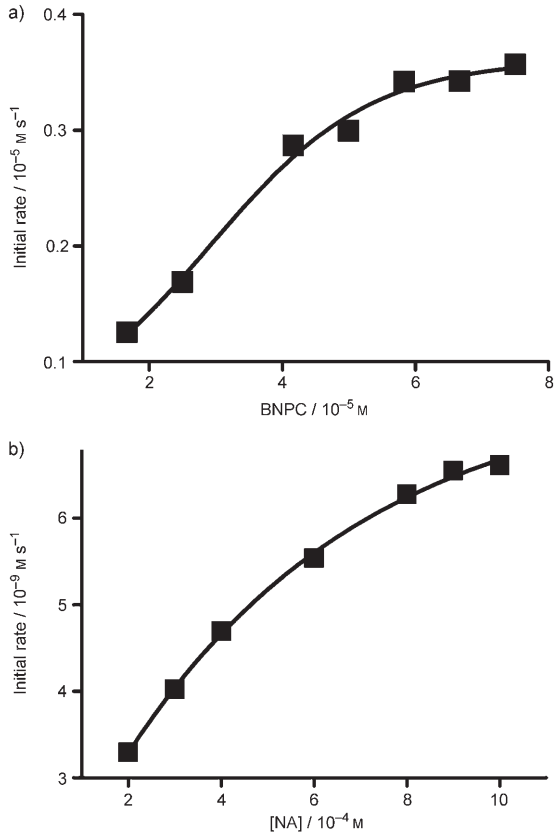


Figure 4. Saturation kinetics of BNPC (a) and NA hydrolysis (b) catalyzed by ZnL. Each reaction mixture contained ZnL (50 μM), Tris-HCl buffer (50 mM pH 7.01) with 0.10 M $NaClO_4$ at (298 ± 0.1) K.

Table 3. Kinetic parameters for ester hydrolysis in the presence of ZnL (50 μM) in a 10% MeCN solution of Tris-HCl buffer (50 mM pH 7.01) at (298 ± 0.1) K.

Substrate	BNPC	NA
$k_{uncat.}$ [s^{-1}]	$(4.83\pm0.16)\times10^{-6}$	$(3.95\pm0.03)\times10^{-6}$
$k_{cat.}$ [s^{-1}]	$(1.88\pm0.20)\times10^{-1}$	$(1.67\pm0.33)\times10^{-4}$
K_m [mM]	0.11 ± 0.02	0.35 ± 0.03
$k_{cat.}/K_m$ [M^{-1} s^{-1}]	1.71×10^{3}	4.77×10^{-1}
$k_{cat.}/k_{uncat.}$	3.89×10^{4}	42.3

three orders of magnitude lower than that of BNPC. Furthermore, a much better catalytic efficiency k_{cat}/K_m was obtained for BNPC hydrolysis (1.71×10^{3} M^{-1} s^{-1}) in contrast to NA hydrolysis (4.77×10^{-1} M^{-1} s^{-1}), which had about a 3.58×10^{3}-fold higher activity than that of NA.

To further demonstrate the effect of pH value on catalytic hydrolysis, the kinetic experiment with BNPC and NA was performed at different concentrations of catalyst at pH 8.85 relative to neutral conditions.

$$v = k_{in}[\text{substrate}] = (k_{obs}[Zn^{II}\ \text{complex}]_{total} + k_{OH^-}[OH]^{+})[\text{substrate}] \quad (6)$$

On the basis of Equation 6, the observed second-order rate constants (k_{obs}) of ester hydrolysis can be calculated from the slope of the straight line of the initial rate constants (k_{in}) versus $[ZnL]_{total}$ (Figure 5). Calculated k_{obs} values of BNPC and NA hydrolysis are 55.5 and 4.87×10^{-2} M^{-1} s^{-1} at pH 7.01, and 1.33×10^{3} and 0.39 M^{-1} s^{-1} at pH 8.85, respectively. Calculated k_{obs} values of BNPC hydrolysis at pH 8.85 is about 20-fold higher than that of at pH 7.01, whereas NA increases about 8-fold. More interestingly, the k_{obs} value of BNPC hydrolysis promoted by ZnL at pH 8.85 is 3.41×10^{3}-fold higher than that of NA hydrolysis.

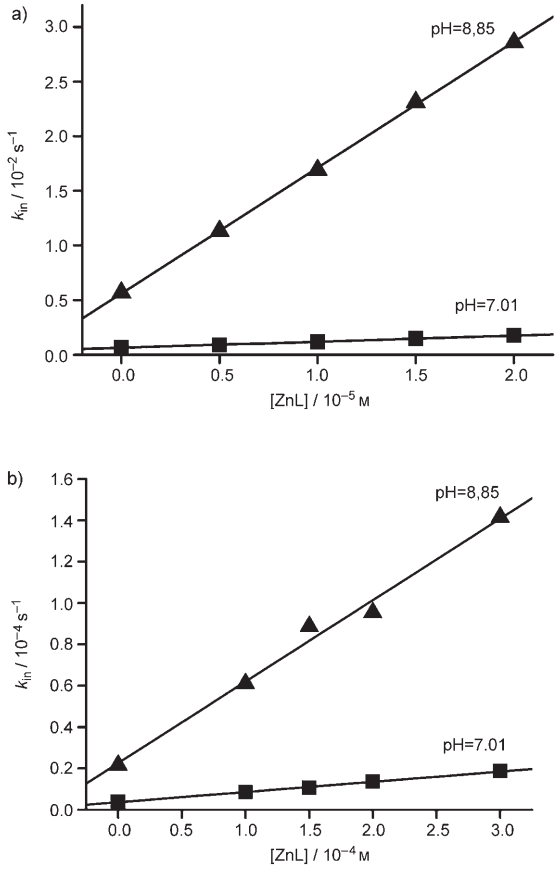


Figure 5. Dependence of the first-order rate constants for BNPC (a) and NA hydrolysis (b) on different concentrations of ZnL in a 10% MeCN solution of 50 mM Tris-HCl buffer with 0.10 M $NaClO_4$ at (298 ± 0.1) K ([BNPC] = 50 μM, [NA] = 200 μM).

Hydrolysis of phosphate diester: BNPP is often used as a DNA model compound in the investigation of phosphodiesterase activity. The test was carried out in buffers to mimic biological conditions. The initial phosphorylation rate in aqueous solution at (308 ± 0.1) K and pH 6.50–9.31 (50 mM Good's buffer) was followed by the appearance of *p*-nitrophenolate at 400 nm.[26] Since the substrate concentration was essentially constant during the measurement, the initial first-order rate constant (k_{in}, in = initial) of the total catalyst was calculated as in Equation (7):[19]

$$v = k_{in}[BNPP] = (k_{BNPP}[ZnL]_{total} + k_{OH-}[OH^-]^{+})[BNPP] \quad (7)$$

in which v is the p-nitrophenolate releasing rate. At a given pH value, the k_{in} values were measured at different concentrations of catalyst. Figure 6 shows the effect of ZnL concentrations on the k_{in} for the cleavage of BNPP at pH 8.85 and (308 ± 0.1) K. The rate of BNPP cleavage initially increases linearly with the increase of ZnL concentration but gradually deviates from linearity. The calculated second-order rate constant of BNPP hydrolysis catalyzed by ZnL, k_{BNPP}, was determined from the slope of the linear plot. Thus, the slope of k_{in} versus $[ZnL]_{total}$ from Equation (7) resulted in the second-order rate constant (k_{BNPP}). The dependence of the second-order rate (k_{BNPP}) on the pH for the BNPP (1.00 mM) cleavage promoted by ZnL (0.10 mM) is illustrated in Figure 7. k_{BNPP} increases sharply as the pH increases from 7.50 to 8.85 and then slows at higher pH, displaying a sigmoidal curve for the cleavage reaction. The result indicates a kinetic process controlled by an acid/base equilibrium. The data were fitted by means of a Boltzman model, which resulted in an inflection point at 8.11; this is almost the same as the pK_a value of the coordinated water molecule deprotonation in $[ZnL(H_2O)]^{2+}$ obtained from the potentiometric pH titration (Table 1). Therefore, one can conclude that the deprotonated $[ZnL(OH)]^+$ ion is the only reactive species of the catalytic system. Consequently, the catalytic second-order rate constant k_{MOH} of $[ZnL(OH)]^+$ must be expressed as in Equation (8):

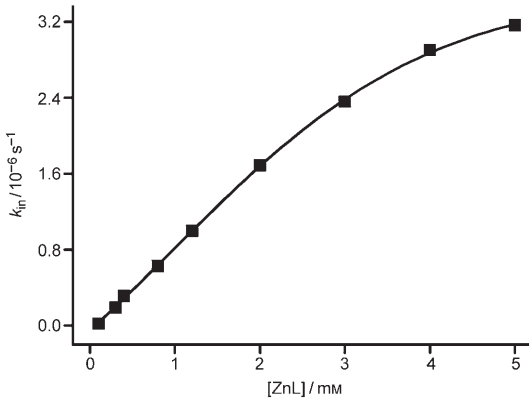


Figure 6. Dependence of the initial first-order rate constants on the concentration of ZnL at pH 8.85 and (308 ± 0.1) K (I = 0.10 M $NaClO_4$, [BNPP] = 0.20 mM, [buffer] = 50 mM).

$$k_{BNPP} = \frac{k_{MOH}[ZnL(OH)]}{[ZnL]_{total}} = \frac{k_{MOH}K_a}{[H^+] + K_a} \quad (8)$$

The k_{MOH} and pK_a values were found by curve-fitting from Equation (8) to be 9.9×10^{-4} M^{-1} s^{-1} and 8.11 for $[ZnL(OH)]^+$, respectively (R = 0.995).

Catalytic mechanism: The enhancement of the rate of hydrolysis of the carboxylic acid ester with pH increase is in good agreement with the percentage of nucleophilic active species $[ZnL(OH)]^+$. This result is in agreement with the analysis of the k_{BNPP}/pH profile in phosphate diester hydrolysis.

Under identical conditions, the BNPC hydrolysis dramatically increases, whereas NA hydrolysis increases only a little. To the best of our knowledge, some catalytic groups introduced into the primary side of monocyclodextrin can enhance catalytic hydrolysis of the substrate owing to the hydrophobic interactions between the mimics and substrate.[3,27,28] In addition, a cyclodextrin dimer bridged with metallobipyridyl can effectively catalyze ester hydrolysis, in which the substrate can be immobilized rightly near the metal ion by the hydrophobic binding interaction between the cavity of the cyclodextrin and substrate.[12] BNPC is a kind of ditopic hydrophobic substrate and can aesthetically bind into the two cavities of cyclodextrin,[14] However, such binding hardly appears for NA because it has only one hydrophobic group. A cyclodextrin dimer can bind strongly to such a ditopic substrate, which can occupy both cyclodextrin cavities as a result of its size/shape,[29] and once the substrate is split in half by the catalytic group, the monotopic hydrophobic product would easily leave because of weakened binding.[10,12,26]

To observe the role of cyclodextrin cavities on hydrolysis, we prepared a BNPC analogue, di(*p-tert*-butylbenzyl) amine (DBBA). The rate of catalytic hydrolysis of BNPC can be dramatically decreased by 59% in the presence of DBBA (see Figure S9 in the Supporting Information), which indicates that it is significantly inhibited by DBBA. Because the *p-tert*-butyl group can strongly bind into the CD cavity by hydrophobic interactions,[12,17–19] DBBA occupied a position in the BisCD cavity. As a result, BNPC cannot again bind into the cavity to form the favorable conformation in which BNPC is nicely near the zinc ion, which results in the inhibition of catalytic hydrolysis. To further confirm the above observation, the inclusion complexation of inhibitor DBBA and ZnL was investigated by means of 2D NMR spectroscopy. In an observed 2D NMR spectrum (Figure 8), interactions between the aryl protons of DBBA and the protons inside of cyclodextrin were found, which indicated that DBBA markedly inhibits the catalytic reaction of [ZnL].

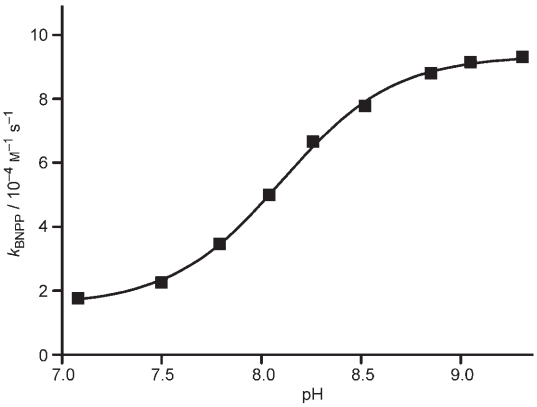


Figure 7. The pH dependence of the second-order rate constants of BNPP hydrolysis ([ZnL] = 0.10 mM, [BNPP] = 1.00 mM, [buffers] = 50 mM, I = 0.10 M $NaClO_4$, (308 ± 0.1) K.

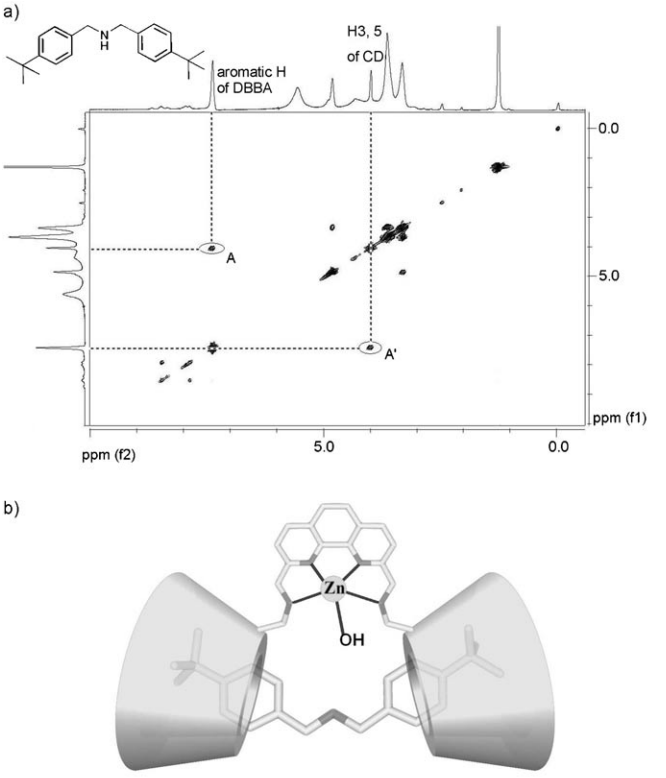


Figure 8. COSY spectrum of the inclusion complexation of ZnL and inhibitor DBBA.

These observations unambiguously demonstrated that hydrophobic interactions play an essential role in catalytic hydrolysis. In BNPP hydrolysis, the k_{MOH} value is comparatively higher than most other reported Zn^{II}-based systems with a similar pK_a in the catalytic hydrolysis of BNPP.[30–33] Surprisingly, the k_{MOH} of $[ZnL(OH)]^+$ is about 50-fold higher than that of its a macrocyclic polyamine analogue, [Zn(OH)([12]aneN4)]$^+$ ([12]aneN$_4$=1,4,7,10-tetraazacyclododecane, $k_{MOH}=2.1\times10^{-5}\,M^{-1}\,s^{-1}$),[34] and is even higher than those of other reported binuclear complexes (Table 4).[35] It is well-known that the *p*-nitrophenate group can bind into the hydrophobic cavity of β-cyclodextrin,[36,37] and thus BNPP with two ditopic groups of *p*-nitrophenole can strongly bind into both cavities of some appropriate BisCDs.[38]

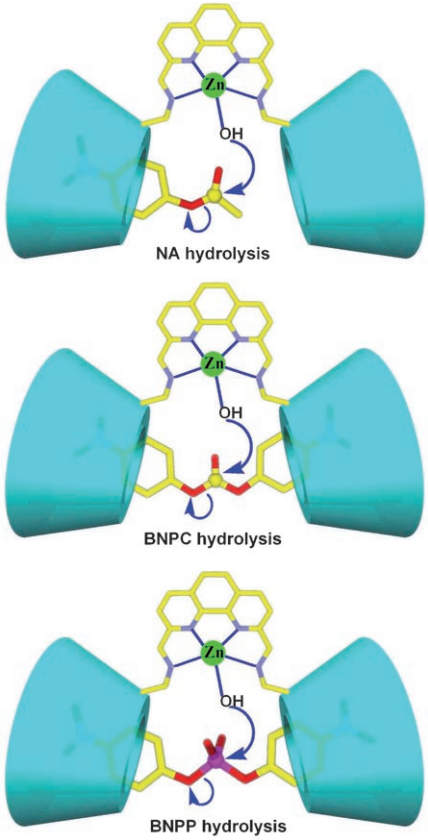
Table 4. The second-order rate constants k_{MOH} of BNPP hydrolysis promoted by Zn^{II} complex at (308±0.1) K.[a]

Species	k_{MOH} [$M^{-1}\,s^{-1}$]	pK_a	Ref.
[Zn(OH)([12]aneN4)]$^+$	2.1×10^{-5}	7.9	[34]
[Zn(OH)(mecyclen)]$^+$	5.2×10^{-6}	7.68	[35]
[Zn(OH)([15]aneN$_3$O$_2$)]$^+$	1.31×10^{-5}	8.8	[31]
[Zn(L)(OH)]$^+$	9.9×10^{-4}	8.18	this work
[Zn$_2$(bmxd)(OH)]$^+$	5.6×10^{-6}	6.94	[32]
[Zn$_2$(OH)$_2${([9]aneN$_3$)$_2$-phen}]$^{2+}$	6.9×10^{-5}	10.7	[33]

[a] [12]aneN$_4$=1,4,7,10-tetraazacyclododecane, mecyclen=*N*-methyl-1,4,7,10-tetraazacyclododecane, [15]aneN$_3$O$_2$=1,4-dioxa-7,10,13-triazacyclopentadecane, bmxd=3,6,9,17,20,23-hexaazatricyclotriaconta-1,11,13,15,25,27-hexaene, and ([9]aneN$_3$)$_2$-phen=1,10-phenanthroline-2,9-(1,4,7-triazacyclododecane).

Therefore, the enhanced second-order rate constant should be contributed to both the binding sites (two hydrophobic cavities) and the catalytic site (metal ion and its coordinated groups). The two hydrophobic cavities cooperatively bind the substrate and juxtapose the ester functional group and the catalytic metal ion.

To all appearances, the rate acceleration of diester hydrolysis catalyzed by ZnL should be ascribed to the cooperative binding action of two hydrophobic cavities and the zinc hydroxyl active species of metallophenanthroline complex. A possible intermediate is suggested for ester hydrolysis in Scheme 2.



Scheme 2. Suggested intermediates for ester hydrolysis catalyzed by ZnL.

Conclusion

A novel cyclodextrin dimer has been prepared as a catalytic precursor. Its zinc complex has been successfully synthesized and fully characterized, and thus demonstrated as a potent catalyst of diester hydrolysis. The hydrophobic interactions between catalyst and substrate play an important role in the catalytic hydrolysis. Therefore, such a stable supramolecular complex is a promising model compound for mononuclear metallohydrolase and has potential in the development of an effective model for metalloenzymes.

Experimental Section

Materials: NA, BNPC, 4-*tert*-butylbenzyl amine, and *p*-tertbutyl benzaldehyde were purchased from Aldrich. β-CD of reagent grade was recrystallized twice from H_2O and dried in vacuo for 12 h at 373 K, DMF was dried over CaH_2 for 2 days and then distilled under reduced pressure prior to use. Common organic reagents were reagent grade and redistilled before use. Water used in all physical measurement experiments was Milli-Q grade. The materials of 6-monodeoxy-6-monoamino-β-cyclodextrin were prepared from 6-monodeoxy-6-monoazido-β-cyclodextrin according to a previous procedure reported by Jicsinszky et al., with a minor modification,[39] whereas 6-mono(*p*-toluenesulfonyl)-β-cyclodextrin was prepared in dry pyridine solution rather than aqueous solution.[40] 1,10-phenanthroline-2,9-dicarbaldehyde was obtained according to the literature procedure.[41] All compounds were confirmed by their elemental analyses, ESIMS, and ^{1}H NMR spectra.

General methods: ^{1}H NMR spectra were recorded on a Varian INOVA-300NB or Mercury plus 300 spectrometers. IR spectra were recorded on a Brucker FTIR EQUINOX 55 spectrometer. Elemental contents were analyzed by a Perkin–Elemer 240 elemental analyzer. ESIMS spectra were performed on a Thremo LCQ-DECA-XP spectrometer. UV/Vis spectra were monitored with a Varian Cary 300 UV/Vis spectrophotometer equipped with a temperature controller (±0.1 K).

1,10-Phenanthroline-2,9-bis(6-ammoniomethyl-β-cyclodextrin) (L): A solution of 1,10-phenanthroline-2,9-dicarboxaldehyde (0.249 g, 1.056 mmol) in dry DMF (5 mL) was added dropwise to a solution of 6-monodeoxy-6-monoamino-β-cyclodextrin (2.395 g, 2.112 mmol) in dry DMF and anhydrous MeOH (v/v 2:1) with vigorous stirring. The mixture was then heated to 353 K for 6 h under nitrogen gas. After this time, the reaction was cooled to room temperature, then a slight excess of $NaBH_4$ (0.096 g, 2.543 mmol) was added to reduce the imine over a period of 2 h. The mixture was then washed with water and filtered. The filtrate was evaporated to dryness under a reduced pressure, and the resulting residue was dissolved in a small amount of hot water, and the aqueous solution was poured into acetone (200 mL) to give a light-yellow precipitate. The crude product obtained was dried and purified by column chromatography over Sephadex G-25 with distilled deionized water as the eluent to give the pure compound in 32% yield. UV/Vis (H_2O): λ_{max} (ε) = 273 (33177), 232 nm (50967 $mol^{-1}Lcm^{-1}$); ^{1}H NMR (300 MHz, DMSO): δ = 8.41 (d, ^3J(H,H) = 8.2 Hz, 2H; phen-H-4,7), 7.89 (s, 2H; phen-H-5,6), 7.84 (d, ^3J(H,H) = 8.2 Hz, 2H; phen-H-3,8), 5.68–5.72 (m, 28H; OH-2,3), 4.88–4.82 (m, 14H; H-1), 4.45 (m, 12H; OH-6), 4.22–3.57 (m, 60H; H-3,5,6, phen-CH_2), 3.36–2.72 (m, 28H; H-2,4, overlaps with H_2O), 2.10 ppm (brs, 2H; NH); IR (KBr): $\tilde{\nu}$ = 3382, 2927, 1624, 1595, 1503, 1421, 1369, 1157, 1081, 1029, 943, 858, 756, 707, 578 cm^{-1}; MS (ESI, H_2O): *m*/*z*: calcd: 1236.4 $[M+2H]^{2+}$, 1247.4 $[M+Na+H]^{2+}$; found: 1236.6, 1247.7; elemental analysis calcd (%) for $C_{98}H_{150}N_4O_{68}\cdot 14H_2O$: C 43.20, H 6.59, N 2.06; found: C 43.04, H 6.58, N 2.06.

Zinc complex (ZnL): A solution of phenBisCD (0.100 g, 0.040 mmol) in water (5 mL) was added dropwise to a dilute aqueous solution of a slight excess of $Zn(ClO_4)_2\cdot 6H_2O$ (0.016 g, 0.044 mmol) with magnetic stirring at room temperature. The resultant solution was kept stirring for 2 h, and then the solution was evaporated under reduced pressure. The precipitate formed was collected by filtration, washed successively with a small amount of ethanol and diethyl ether, and then dried in vacuo to give the pure complex as a pale-yellow solid in 68% yield. UV/Vis (H_2O): λ_{max} (ε): $\tilde{\nu}$ = 277 (33766), 230 nm (45743 $mol^{-1}Lcm^{-1}$); MS (ESI, H_2O): *m*/*z*: calcd: 1268.38 $[M]^{2+}$; found: 1268.31; elemental analysis calcd (%) for $C_{98}H_{150}N_4O_{68}\cdot Zn(ClO_4)_2\cdot 14H_2O$: C 39.38, H 6.00, N 1.87; found: C 39.45, H 6.35, N 1.52.

Di(*p*-*tert*-butylbenzyl) amine (DBBA): *p*-*tert*-Butyl benzaldehyde (1.76 mL, 10 mmol) in absolute ethanol (5 mL) was added dropwise to a stirred solution of 4-*tert*-butylbenzyl amine (1 mL, 6 mmol) in absolute ethanol (5 mL) in an ice bath. After 4 h, the solution was cooled to 273 K and sodium borohydride (0.45 g, 12 mmol) was added in small portions. After the reaction had been stirred for 12 h at room temperature, aqueous HCl (5 mL, 2 M) was added slowly and the mixture was stirred for 1 h. An aqueous solution of sodium hydroxide (2 M) was then added until a pH of 11 was reached. The mixture was extracted with methylene chloride, dried over sodium sulfate, filtered, and acidified with concentrated HCl. After evaporation of the solvents, the oil product was dissolved in ethanol (95%), acidified with concentrated HCl to pH 3, and the volatiles removed. The crude product obtained was recrystallized from an ethanol/acetone mixture and afforded pure DBBA (HCl salt) in 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.01 (brs, 1H; NH), 7.44 (d, ^3J(H,H) = 7.7 Hz, 4H; phenyl-H-1,1′), 7.33 (d, ^3J(H,H) = 7.7 Hz, 4H; phenyl-H-2, 2′), 3.79 (s, 4H; benzyl-3,3′), 1.21 ppm (s, 18H; *tert*-butyl-H); MS (ESI, MeOH): *m*/*z*: calcd: 310.3 $[M+H]^+$; found: 310.0; elemental analysis calcd (%) for $C_{22}H_{31}N\cdot HCl\cdot 0.5H_2O$: C 74.44, H 9.37, N 3.95; found: C 74.29, H 9.27, N 3.74.

***Caution**: Perchlorate salts of organic compounds are potentially explosive; these compounds must be prepared and handled with great care!*

Potentiometric pH titration: An automatic titrator (Metrohm 702GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to the Gran method.[42] The electrode system was calibrated with buffers and checked by titration of $HClO_4$ with NaOH solution (0.10 M). The thermostated cell contained 25 mL of 1.00 mM species in aqueous solutions with the ionic strength maintained at 0.10 M by sodium perchlorate. All titrations were carried out in the aqueous solutions under nitrogen at (298 ± 0.1) K, and initiated by adding fixed volumes of 0.10 M standard NaOH in small increments to the titrated solution. Duplicate measurements were performed, for which the experimental error was below 1%. The titration data were fitted from the raw data with the Hyperquad 2000 program to calculate the ligand protonation constants K_n, the complex formation constant K_{ML}, and the deprotonation constants of the coordinated water pK_a.

Kinetics of BNPC and NA hydrolysis: The hydrolysis rate of BNPC and NA in the presence of ZnL complex was measured by an initial slope method following the increase in the 400 nm absorption of the released 4-nitrophenolate.[14,25] The reaction solution was maintained at (298 ± 0.1) K. Tris-HCl (pH 7.01, 8.85) buffers were used (50 mM), and the ionic strength was adjusted to 0.10 with $NaClO_4$. In a typical experiment, after substrate (NA or BNPC) and ZnL complex in 10% (v/v) CH_3CN solution at an appropriate pH were mixed, the UV absorption decay was recorded immediately and was followed generally until 2% decay of 4-nitrophenyl acetate. Errors on k_{obs} values were about 5%.

Kinetics of BNPP hydrolysis: The rate of hydrolysis of BNPP to give mono(4-nitrophenyl) phosphate and *p*-nitrophenolate was measured by an initial slope method following the increase in the 400 nm absorption of the released *p*-nitrophenolate in aqueous solution at (308 ± 0.1) K.[26] At this wavelength, the absorbance of the ester substrate was negligible. MES (pH 6.00–6.60), MOPS (pH 6.60.-7.40), HEPES (pH 7.40–8.20), TAPS (pH 8.20–8.90), and CHES (pH 8.90–9.50) buffers were used (50 mM), and the ionic strength was adjusted to 0.10 with $NaClO_4$. The pH of the solution was measured after each run, and all kinetic runs with pH variation larger than 0.1 were excluded. The substrate BNPP, buffers, and ZnL in aqueous solution were prepared freshly. The reactions were initiated by injecting a small amount of BNPP into the buffer solutions of ZnL and followed by fully mixing at (308 ± 0.1) K. The visible absorption increase was recorded immediately and was followed generally until 2% formation of *p*-nitrophenolate, in which ε values for 4-nitrophenolate were 4069 (pH = 6.5), 9610 (pH = 7.08), 13745 (pH = 7.50), 15788 (pH = 7.79), 16163 (pH = 7.86), 16943 (pH = 8.04), 17600 (pH = 8.26), 18079 (pH = 8.52), 18512 (pH = 9.05), and 18569 (pH = 9.31) at 400 nm. The initial first-order rate constants, k_{in} (s^{-1}), for the cleavage of BNPP were obtained directly from a plot of the 4-nitrophenolate concentration versus time by the method of initial rates, which was linear with $R > 0.996$. The second-order rate constants (k_{BNPP}) for the catalyzed reactions were determined as the slope of the linear plots of k_{in} versus ZnL concentration. To correct for the spontaneous cleavage of BNPP, each reaction was measured against a reference cell that was identical to the sample cell in composition except for the absence of ZnL. Errors on k_{BNPP} values were about 5%.

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