Hydrolysis of Lipophilic Esters Catalyzed by a Zinc(II) Complex of a Long Alkyl-Pendant Macrocyclic Tetraamine in Micellar Solution

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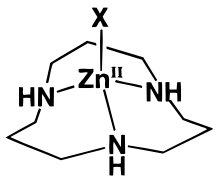
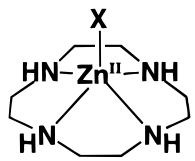
Abstract: A novel lipophilic zinc(II) complex with 1-hexadecyl-1,4,7,10-tetrazacyclododecane (hexadecylcyclen, L) has been synthesized, which is almost insoluble in water, but becomes soluble in the presence of Triton X-100 surfactant. Analysis of the potentiometric pH titration of Zn^{II}−hexadecylcyclen (1 mM) in the presence of Triton X-100 (10 mM) disclosed monodeprotonation of the Zn^{II}-bound H_2O, yielding an OH^--bridged complex $(ZnL)_2OH^-$ ($pK_d = -\log([(ZnL)_2OH^-]a_{H^+}/[ZnL]^2) = 3.92 \pm 0.05$) and a monomeric $Zn^{II}-OH^-$ complex $ZnL-OH^-$ ($pK_a = -\log([ZnL-OH^-]a_{H^+}/[ZnL]) = 7.56 \pm 0.05$) at 25 °C with $I = 0.10$ ($NaNO_3$). The zinc(II) complex (ZnL) possesses higher catalytic activity than the parent zinc(II) complex of 1,4,7,10-tetrazacyclododecane (cyclen, L′) in hydrolysis of 4-nitrophenyl acetate (NA), bis(4-nitrophenyl) phosphate (BNP^-), and tris(4-nitrophenyl) phosphate (TNP) in aqueous comicellar solution with 10 mM Triton X-100. The NA hydrolysis activity of Zn^{II}−hexadecylcyclen increased with the pH and leveled off at pH > 10 and 25 °C, from which the active species was estimated to be a $ZnL-OH^-$ complex. The second-order rate (first-order in [NA] and $[ZnL-OH^-]$) of NA hydrolysis ($k_{NA} = 5.0 \pm 0.2\ M^{-1}\ s^{-1}$) in the presence of 10 mM Triton X-100 is 50 times greater than that with a reference $ZnL'-OH^-$ in 10% (v/v) CH_3CN aqueous solution. Hydrolysis of TNP in 10% (v/v) MeOH aqueous solution with $ZnL-OH^-$ and 10 mM Triton X-100 ($k_{TNP} = (1.1 \pm 0.1) \times 10^3\ M^{-1}\ s^{-1}$) is 290 times more efficient than with $ZnL'-OH^-$ ($3.8 \pm 0.2\ M^{-1}\ s^{-1}$) at 25 °C. The higher effective molarity of the lipophilic substrate coexisting with $ZnL-OH^-$ in the micelles accounts for the extraordinary catalytic activity. From comparison with previously reported metal catalysts, the present lipophilic zinc(II) complex with hexadecylcyclen is probably one of the best candidate catalyst for detoxification of poisonous phosphotriesters.

Introduction

The catalytic hydrolysis of acetate, phosphates, and β-lactam by zinc(II) complexes with macrocyclic triamine ([12]aneN$_3$, 1,5,9-triazacyclododecane) **1** and tetraamine (cyclen, 1,4,7,10-tetraazacyclododecane) **2** in aqueous solution have been intensively studied[1,2] to elucidate the role of zinc(II) in hydrolytic zinc enzymes such as carbonic anhydrase, alkaline phosphatase, and β-lactamase II. Hydrolysis is thought to involve the $Zn^{II}-OH^-$ species as the active nucleophile for the enzyme models (**1b** and **2b**) and is now widely accepted for hydrolytic zinc enzymes.[3] We have demonstrated that the zinc(II) ions in the macrocyclic polyamine complexes (**1** and **2**) act as an acid to deprotonate the Zn^{II}-bound water and generate the latent nucleophiles at neutral pH: e.g., the pK_a values are 7.3 for **1a** ⇄ **1b** + H^+ and 7.9 for **2a** ⇄ **2b** + H^+ at 25 °C.[2] Recently, organophosphorus hydrolase that catalyzes hydrolysis of organophosphate triesters was characterized by X-ray crystal analysis.[4] A site-directed mutagenesis study disclosed two zinc(II) ions at the active center: one surrounded by three histidine (His) residues and the other by two histidine residues.[5] Here again, the reactive nucleophile was proposed to be the $Zn^{II}-OH^-$ species generated from the $Zn^{II}-(His)_3$ environment.



1a; X = OH_2
b; X = OH^-



2a; X = OH_2
b; X = OH^-

As an extension from these simple model systems in aqueous solution, we now have synthesized a cyclen derivative attached to a long alkyl chain ($C_{16}H_{33}$), which was designed to provide

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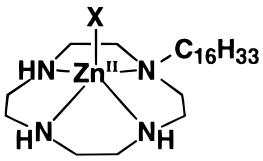
a Zn^{II}−cyclen complex **3** with micelle-forming properties. Anticipated supramolecular assembly between the zinc(II) complex and lipophilic substrates [such as 4-nitrophenyl acetate **4** (NA), bis(4-nitrophenyl) phosphate **5** (BNP^-), and tris(4-nitrophenyl) phosphate **6** (TNP)] coexisting in micelles would make hydrolysis of lipophilic esters more effective.



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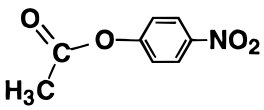
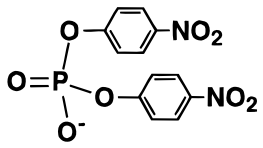
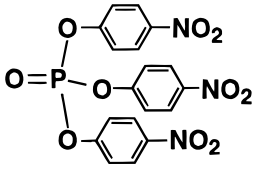
b; X = OH^-



4
NA

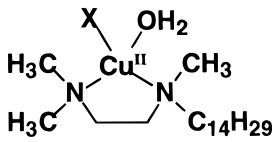


5
BNP^-

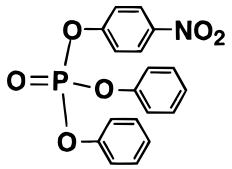


6
TNP

Earlier, Menger et al.[6] reported that a $C_{14}H_{29}$-attached copper(II) complex (metallomicelles) **7** possesses a remarkable catalytic activity in hydrolysis of phosphotriester **8**. The **7**-promoted hydrolysis is more than 200 times faster than the hydrolysis of **8** catalyzed by an equivalent concentration of a nonmicellar homologue, Cu^{II}−tetramethylethylendiamine complex. They also showed that nerve gases, such as soman **9**, were very efficiently detoxified with **7** at pH 7.0 (detected by the release of F^-). The possible reasons for the rate accelerations were the enhanced electrophilicity of the micellized copper(II) ion, the acidity of Cu^{II}-bound water, or the intramolecular type of reaction due to the micellar formation. The Cu^{II}−OH^- species **7b** was postulated to be an active catalytic species. On the basis of the rate−pH profile for pH 6.0−8.3, the pK_a value for Cu^{II}−OH_2 (**7a**) ⇄ Cu^{II}−OH^- (**7b**) + H^+ was estimated to be less than 6. Gutsche et al.[7] observed that a lipophilic dioxime metal complex such as **10** (M = Cu, Ni, and Zn) at pH 11.5 accelerates the hydrolysis of acetyl phosphate under comicelle-forming conditions with cetyl trimethylammonium bromide. Tagaki et al.[8] synthesized surfactant imidazole ligands which, in the presence of Cu^{II} and Zn^{II} (a possible structure **11**), catalyzed the hydrolysis of 4-nitrophenyl picolinate in a comicellar system with hexadecyl trimethylammonium bromide. Gellman et al.[9] synthesized a lipophilic macrocyclic zinc(II) complex **12**, which was an effective catalyst for the hydrolysis of phosphotriester **8** in a Brij micelle [neutral surfactant, $C_{12}H_{25}(OCH_2CH_2)_{23}OH$]. The active species was presumed to be Zn^{II}−OH^- species **12b** from the sigmoidal profile of the rate−pH plot with a kinetic pK_a value of 9.1. However, its catalytic mechanism was not simple, requiring consideration of other undefined species such as aggregates, as indicated by the kinetic term higher than first-order for **12**.



7a; X = OH_2
b; X = OH^-



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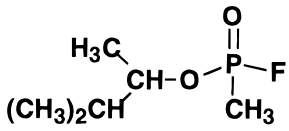
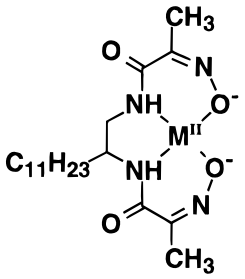


9
Soman



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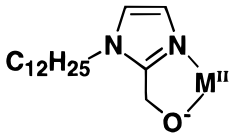

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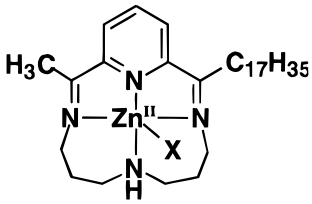

12a; X = OH_2
b; X = OH^-

Despite intensive studies, fundamental hydrolysis mechanisms by lipophilic metal catalysts have not been thoroughly studied yet. A common drawback in those past micellar systems (such as **7** and **10**−**12**) was that the metal complexes with long alkyl chains were not fully characterized in solution (e.g., complex stabilities or deprotonation constants of metal-bound water remained totally unstudied thermodynamically), and thus the reactive species were not discretely resolved. Hence, speculative pictures were given for the accelerated catalytic rates. On the foundation of our accumulated knowledge of the hydrolysis with a *discrete and very stable* Zn^{II}−cyclen complex, we hoped to find a well-defined reactive species using hexadecylcyclen zinc(II) complex **3** to add clearer mechanistic knowledge for the metal ion-catalyzed hydrolysis in micellar solution. Our ultimate objective is to find a better and a more practical catalyst than those reported earlier.

Results and Discussion

Synthesis (Scheme 1) and Properties of the Ligand 15. The 12-membered macrocyclic dioxotetraamine **13**[10] was treated with a 0.8 equiv of 1-bromohexadecane ($C_{16}H_{33}Br$) in DMF at 50 °C for 1 day and yielded the monoalkylated product **14** as colorless needles in 47% yield after crystallization from CH_2Cl_2/hexane. The alkylated position (N_{10}) of **14** was discerned by its 1D (^1H and ^{13}C) and 2D (NOESY and HMBC) spectra in $CDCl_3$. The 12-membered dioxotetraamine **14** is a good candidate for a lipophilic copper(II) ion carrier,[11] which will be described elsewhere. The reduction of **14** with BH_3·THF complex in THF at 60 °C for 1 week yielded the desired product 1-hexadecyl-1,4,7,10-tetraazacyclododecane **15** (hexadecylcyclen), which was isolated as its 3HCl salt (colorless needles) in 45% yield.

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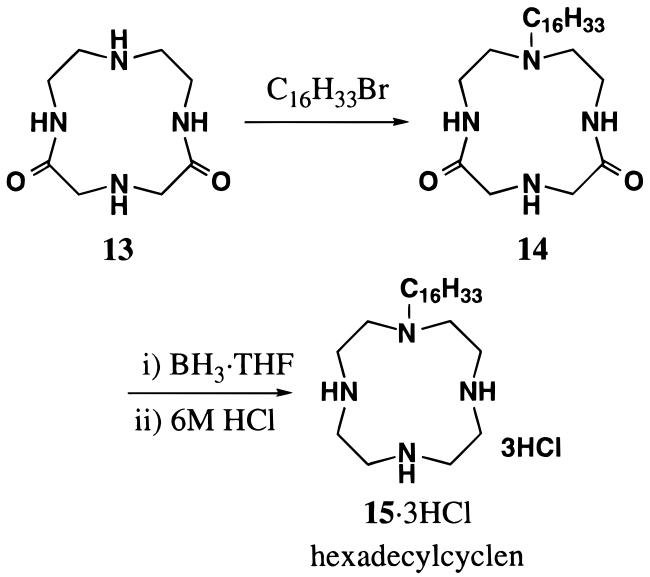
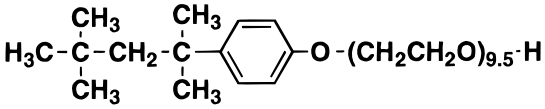
Scheme 1



13 **14**

15·3HCl
hexadecylcyclen

Since the new ligand **15** (e.g., 1 mM) was not soluble enough in H_2O for potentiometric pH titration, a neutral detergent Triton X-100 **16** (10 mM) was added to obtain a homogeneous solution (50 mL) at 25 °C with $I = 0.10$ ($NaNO_3$). The concentration of Triton X-100 was much higher than its critical micelle concentration of 0.08 ± 0.01 mM determined by vertical plate method under the same conditions. A typical titration curve is shown in Figure 1a. The titration data were analyzed for equilibria 1−3. The mixed protonation constants log K_{1-3} (a_{H^+} is the activity of H^+) are 9.95 ± 0.05, 8.27 ± 0.05, and 2.3 ± 0.2. The corresponding log K_n values for cyclen in the absence of Triton X-100 are 11.04, 9.86, and <2.[2h] The lower basicity of **15** with respect to that of cyclen may be due to the diminished solvation of the protonated species under comicelle conditions (and hence lesser stability).



16
Triton X-100

$$L + H^+ \rightleftarrows HL \qquad K_1 = [HL]/[L]a_{H^+} \qquad (1)$$

$$HL + H^+ \rightleftarrows H_2L \qquad K_2 = [H_2L]/[HL]a_{H^+} \qquad (2)$$

$$H_2L + H^+ \rightleftarrows H_3L \qquad K_3 = [H_3L]/[H_2L]a_{H^+} \qquad (3)$$

Lipophilic Zinc(II) Complex 3. The acid-free ligand **15** (L) was extracted with CH_2Cl_2 from an aqueous alkaline (pH ca. 12) suspension of **15**·3HCl. A crystalline zinc(II) complex **3a** ($ZnL-OH_2$) was obtained by mixing **15** and 1.3 equiv of $Zn(ClO_4)_2 \cdot 6H_2O$ in MeOH followed by slow evaporation. The elemental analysis (C, H, N) suggested a $ZnL-OH_2 \cdot 2ClO_4^-$ formula, which represents the first distinct 1:1 zinc(II) macrocyclic polyamine complex appended with a long alkyl chain. Complex **3a** is soluble in MeOH and CH_3CN, slightly soluble in $CHCl_3$, but hardly soluble (<0.1 mM) in H_2O. The critical micelle concentration of **3a**·$2ClO_4^-$ at 25 °C with $I = 0.10$ ($NaNO_3$) was determined to be 0.3 ± 0.1 μM. Triton X-100 (10 mM) was selected as a surfactant that allowed sufficient solubility (e.g., 1 mM) of **3** for all the following studies.

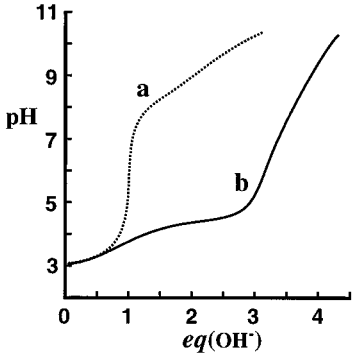
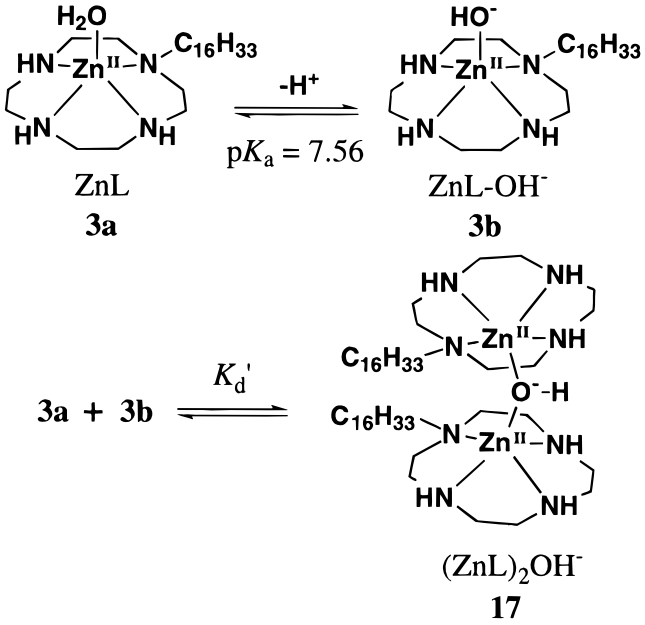


Figure 1. Typical titration curves for hexadecylcyclen **15** in the presence of 10 mM Triton X-100 at 25 °C with $I = 0.10$ ($NaNO_3$): (a) 1.0 mM **15**·3HCl; (b) a + 1.0 mM $ZnSO_4$. eq(OH^-) is the number of equivalents of base added.

Scheme 2



The potentiometric pH titration curve (Figure 1b) of **15**·3HCl (1 mM) in the presence of an equimolar amount of $ZnSO_4$ and 10 mM Triton X-100 revealed zinc(II) complexation at 3.5 < pH < 5.5, followed by dissociation of 1 equiv proton at 5 < pH < 9. The same titration curve was obtained with **3a** (1 mM) and 3 equiv of $HClO_4$ under the same conditions. Up to 3 equiv of base the equilibration was very slow (requiring more than 1 h between each titration point). The titration data were best treated for equilibria of a 1:1 ZnL complex (eq 4), an OH^--bridged dinuclear complex $(ZnL)_2OH^-$ **17** (eq 5), and an OH^--bound mononuclear complex $ZnL-OH^-$ (eq 6, see Scheme 2). A complexation constant K_d' for the OH^--bridged complex from **3a** and **3b** was derived from eqs 5 and 6 (eq 7, see Scheme 2). No further deprotonation or precipitation of $Zn(OH)_2$ was observed over pH 10, indicating the stability of those zinc(II) complexes under comicellar conditions.

$$L + Zn^{2+} \rightleftarrows ZnL\ (\mathbf{3a}) \qquad K(ZnL) = [ZnL]/[Zn^{2+}][L] \qquad (4)$$

$$2ZnL \rightleftarrows (ZnL)_2OH^-\ (\mathbf{17}) + H^+ \qquad pK_d = -\log([(ZnL)_2OH^-]a_{H^+}/[ZnL]^2) \qquad (5)$$

$$ZnL \rightleftarrows ZnL-OH^-\ (\mathbf{3b}) + H^+ \qquad pK_a = -\log([ZnL-OH^-]a_{H^+}/[ZnL]) \qquad (6)$$

$$ZnL + ZnL-OH^- \rightleftarrows (ZnL)_2OH^- \qquad K_d' = [(ZnL)_2OH^-]/[ZnL-OH^-][ZnL] \qquad (7)$$

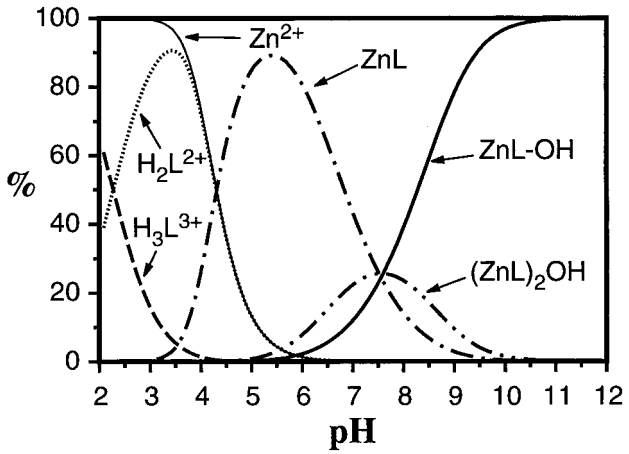
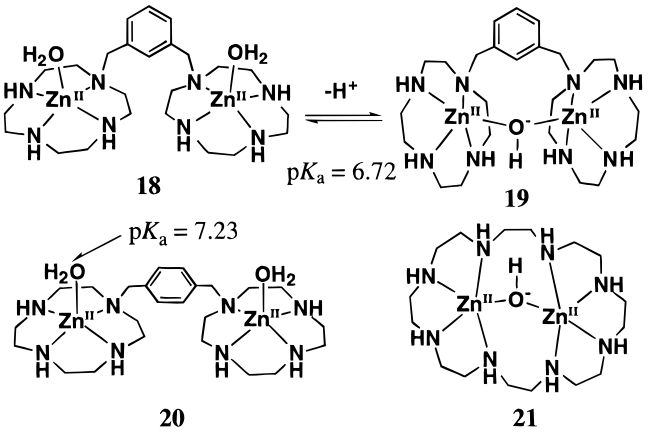


Figure 2. Species distribution resulting from a 1:1 solution [1 mM of both hexadecylcyclen **15** (L) and Zn^{2+}] in the presence of 10 mM Triton X-100 at 25 °C with $I = 0.10$ ($NaNO_3$).

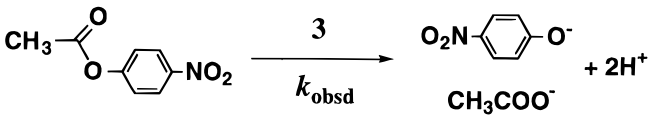
The obtained log K(ZnL), pK_d, pK_a, and log K_d' values are 12.90 ± 0.05, 3.92 ± 0.05, 7.56 ± 0.05, and 3.64 ± 0.05, respectively. A distribution diagram for the hexadecylcyclen−Zn^{2+} system as a function of pH at [total zinc] = [total L] = 1 mM, [Triton X-100] = 10 mM, and 25 °C with $I = 0.10$ ($NaNO_3$) is displayed in Figure 2. It was found that although the stability of the ZnL complex **3a** is lower than that of Zn^{II}−cyclen **2a** (log K(ZnL′) = 15.3, **3a** remains sufficiently stable at physiological pH (see Figure 1b). After formation of the 1:1 zinc(II) complex completed at eq(OH^-) = 3, the deprotonation occurs to initially form an OH^--bridged dinuclear complex **17** and then an OH^--bound complex **3b**. From the pK_d value of 3.92 (see eq 5), one can predict pK_a values for the bridging water between the two Zn^{II}−hexadecylcyclen complexes at various concentration of **3a** (e.g., the pK_a is the same as pK_d of 3.92 at [**3a**] = 1 M). The pK_a value of 7.56 for the Zn^{II}-bound water of **3a** is almost the same as that for **2a**: i.e., in the presence and absence of Triton X-100 (10 mM) 7.83 ± 0.03 and 7.86,[2h] respectively, under the same conditions. In the deprotonation of **2a** (at [total **2**] = 1.0 mM), no dimeric complex homologous to **17** was found. The similar pK_a values with or without Triton X-100 implies little interaction between the neutral surfactant and hydrophilic Zn^{II}−cyclen.

Recently,[12] we found that a bis(Zn^{II}−cyclen) closely linked with a *m*-xylyl unit **18** yielded an OH^--bridged complex **19** [or a water and HO^--bridging dizinc(II) complex] with a pK_a value of 6.72 at 25 °C. This value is significantly lower than that of 7.86 for the monomeric complex **2a**. On the other hand, a homologous bis(Zn^{II}−cyclen) separated with a *p*-xylyl unit **20**[13] showed a corresponding pK_a value of 7.23. Moreover, a 24-membered macrocyclic octaamine preferentially forms a stable OH^--bridged dinuclear zinc(II) complex **21** below pH 7,[14] while its protonated complex (i.e., H_2O-bound species) is not observed under the same conditions. These facts are somewhat relevant to our findings for the OH^--bridged complex **17**. Hence, the occurrence of the OH^--bridged dinuclear complex **17** at lower pH is accounted for by postulating the proximity of the two Zn^{II}−cyclen units embedded in Triton X-100 micelles (i.e., the local concentration of **3** in the micelles being higher than total concentration of 1 mM).[15] As found in the next kinetic study, **17** unlike **3b** is kinetically inactive as a nucleophile toward esters such as 4-nitrophenyl acetate.



Hydrolysis of 4-Nitrophenyl Acetate (NA) by 3. Hydrolysis of 4-nitrophenyl acetate (NA) (0.5−2.0 mM) was promoted by **3** (0.1 and 0.2 mM) in 10% (v/v) CH_3CN aqueous solution under the comicellar conditions with 10 mM Triton X-100 at pH 9.2 (20 mM CHES buffer) and 25 °C with $I = 0.10$ ($NaNO_3$) (see Scheme 3). The second-order dependence of the rate constant, k_{obsd} on the concentration of NA (10, 25, and 50.0 μM) and **3** (0.2, 0.5, and 1.0 mM) at pH 10.2 (2 mM CAPS buffer) and 25 °C with $I = 0.10$ ($NaNO_3$) fits to the kinetic eq 8. No other reaction such as acetate transfer to Triton X-100 was observed, which was confirmed by a ^1H NMR experiment with a 10% D_2O solution of 2.0 mM NA, 0.2 mM **3**, and 10 mM Triton X-100. Since the second-order kinetics held after several catalytic cycles, the NA hydrolysis was concluded to be catalytic (see Experimental Section). In eq 8, ν_{obsd} is the observed NA hydrolysis rate catalyzed by **3**, which was derived by subtraction of the buffer-promoted NA hydrolysis rate from total NA hydrolysis rate.

Scheme 3



$$\nu_{obsd} = k_{obsd}[\text{total zinc(II) complex}][\text{NA}] \quad (8)$$

$$= k_{NA}[\mathbf{3b}][\text{NA}] \quad (9)$$

The critical micelle concentration of Triton X-100 was determined to be 0.12 ± 0.05 mM under the reaction conditions. The effect of the surfactant concentration (0.75−30 mM) on the pseudo-first-order rate constant (k'_{NA}) for NA hydrolysis promoted by 0.2 mM **3** is shown in Figure 3, where 10% (v/v) CH_3CN aqueous solution was used. In the range of 0 ≤ [Triton X-100] ≤ 10 mM, with an increase in the concentration of Triton X-100, the hydrolysis rate increased, probably because the lipophilic NA goes preferentially into the comicelle to react with **3**. Increases of Triton X-100 concentrations above 10 mM results in a gradually decreased rate, probably because **3** and NA are thinly spread and separated in different comicellar phases. For a control experiment, the hydrolysis of NA (0.10 mM) promoted by Zn^{II}−cyclen **2** (1.0 mM) and [Triton X-100] (0−30 mM) under the same conditions was determined. A similar plot of k'_{NA} is shown in Figure 4. In this case, an increase in the concentration of Triton X-100 induces a slower NA hydrolysis, further explained by increased transfer of the lipophilic NA into micellar phase, while the nonlipophilic **2**

(12) Fujioka, H.; Koike, T.; Yamada, N.; Kimura, E. *Heterocycles* **1996**, *42*, 775−787.

(13) Koike, T.; Takashige, M.; Kimura, E.; Fujioka, H.; Shiro, M. *Chem. Eur. J.* **1996**, *2*, 617−623.

(14) Bencini, A.; Bianchi, A.; Dapporto, P.; Garcia-España, E.; Micheloni, M. *Inorg. Chem.* **1989**, *28*, 1188−1191.

(15) A Zn^{II}−cyclen complex having $(ZnL')_2OH{\cdot}H_2O{\cdot}(ClO_4)_3$ formula was isolated as crystals from an alkaline MeOH/H_2O solution. Its structure was proposed to be a water and a OH^--bridged dizinc(II) complex. Norman, P. R. *Inorg. Chim. Acta* **1987**, *130*, 1−4.

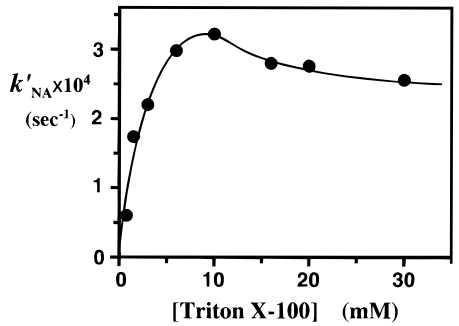


Figure 3. Effect of Triton X-100 concentration on the pseudo-first-order rate constant (k'_{NA}) for the hydrolysis of NA (0.1 mM) catalyzed by **3** (0.2 mM) in 10% (v/v) CH_3CN aqueous solution [pH 9.2 CHES buffer (20 mM)] at 25 °C with $I = 0.10$ ($NaNO_3$).

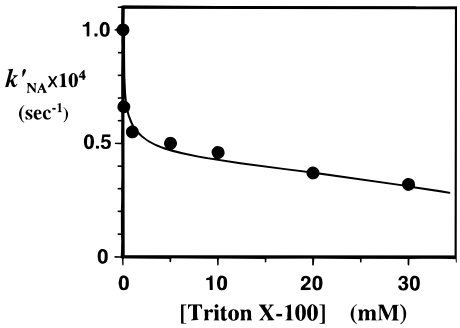


Figure 4. Effect of Triton X-100 concentration on the pseudo-first-order rate constant (k'_{NA}) for the hydrolysis of NA (0.1 mM) catalyzed by **2** (1.0 mM) in 10% (v/v) CH_3CN aqueous solution [pH 9.2 CHES buffer (2.0 mM)] at 25 °C with $I = 0.10$ ($NaNO_3$).

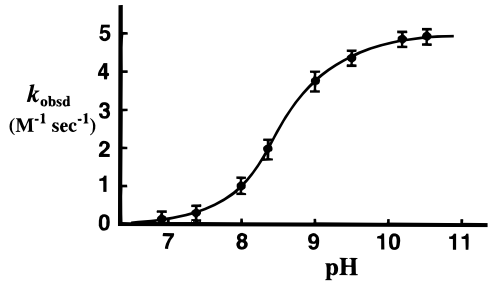


Figure 5. Rate−pH profile for the second-order rate constant (k_{obsd}) in the hydrolysis of NA (50 μM) catalyzed by **3** (1.0 mM) and Triton X-100 (10 mM) in aqueous solution at 25 °C with $I = 0.10$ ($NaNO_3$).

remains in aqueous phase. Since the NA hydrolysis catalyzed by **3** became maximal with 10 mM Triton X-100 (see Figure 3), we chose this concentration of Triton X-100 for the kinetic studies, where NA (≤1 mM) is sufficiently soluble, and we conducted the following kinetics for NA hydrolysis without organic cosolvent such as 10% (v/v) CH_3CN, as previously done with **1b** and **2b**.[2a,2h]

The observed rate constant k_{obsd} under the conditions employed for the potentiometric pH titrations (i.e., 25 °C, $I = 0.10$ $NaNO_3$, 10 mM Triton X-100, 1 mM **3**) is plotted as a function of pH (= 6.9−10.5) (see Figure 5). The resulting sigmoidal curve with an inflection point at pH 8.5 almost overlaps with the sigmoidal distribution curve for ZnL−OH^- species **3b** obtained in the equilibrium studies (see Figure 2). Therefore, the kinetically involved species among **3a**, **3b**, and **17** is concluded to be only the monomeric OH^--bound zinc(II) complex **3b**, as defined by the kinetic eq 9.[16] The second-order rate constant k_{NA} was determined to be 5.0 ± 0.2 M^{-1} s^{-1} from the maximum k_{obsd} values at 25 °C and pH 10.5. Unless the OH^--bridged zinc(II) complex **17** is considered as one of the deprotonated species, the kinetic inflection should appear around

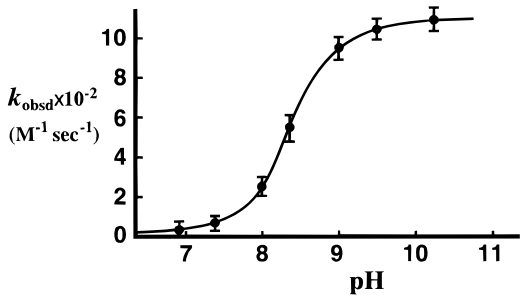


Figure 6. Rate−pH profile for the second-order rate constant (k_{obsd}) in the hydrolysis of TNP (50 μM) catalyzed by **3** (0.1 mM) and Triton X-100 (10 mM) in 10% MeOH aqueous solution at 25 °C with $I = 0.10$ ($NaNO_3$).

pH 7.6 (see Figure 2). This fact is a kinetic support to the thermodynamic consideration of **17** (see Scheme 2). It should be recalled that, in the hydrolysis of NA with **2** (ZnL′), the kinetic sigmoidal curve in the rate−pH profile (corresponding to Figure 5) has an inflection at pH 7.9 and almost overlaps with the thermodynamic distribution curve for the monomeric OH^--bound species **2b**, where no other species like the present dimeric $(ZnL')_2OH^-$ was involved.[2b]

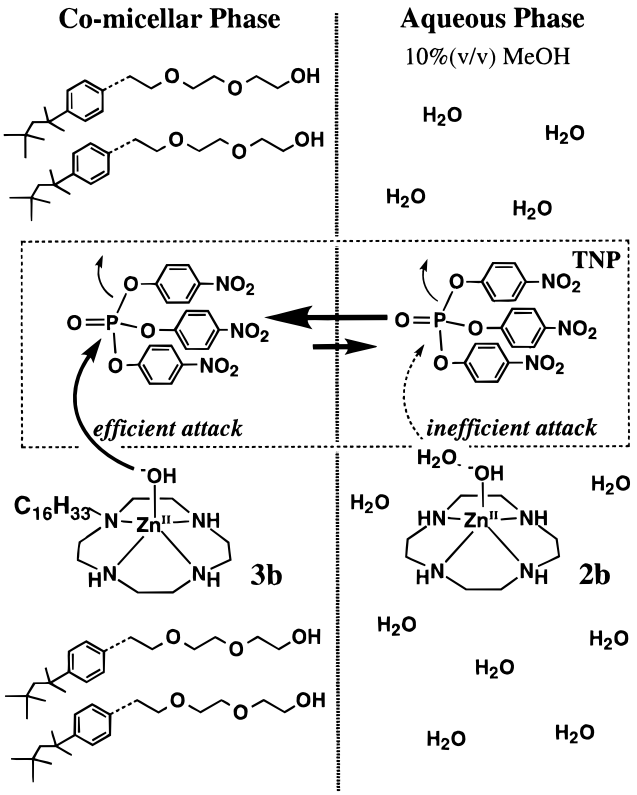
For reference, the NA hydrolysis catalyzed by Zn^{II}−cyclen **2b** has been determined in the presence of 10 mM Triton X-100 by the same method in 10% (v/v) CH_3CN aqueous solution (pH 9.2 with 20 mM CHES buffer) with $I = 0.10$ ($NaNO_3$) at 25 °C. The rate constant of $(4.6 \pm 0.2) \times 10^{-2}$ M^{-1} s^{-1} is smaller than the reported one (0.10 M^{-1} s^{-1})[2g] for the same reaction in the absence of Triton X-100. Provided that only the NA partitioned in aqueous phase was subjected to attack by the hydrophilic **2b**, it can be estimated that ca. 50% of NA is partitioned in the micellar phase, since the k_{NA} value in the presence of Triton X-100 was decreased by half. The comparison of these k_{NA} values [5.0 ± 0.2 vs $(4.6 \pm 0.2) \times 10^{-2}$ M^{-1} s^{-1}] clearly shows the lipophilic cyclen complex **3b** as being a 109 times better catalyst in a micellar system. In micellar phase, the lipophilicity of both the substrate NA and catalyst **3b** would contribute to stabilization of the supramolecular assembly, leading to more probable collisions between them. This interpretation seemed more valid for the hydrolysis of a more lipophilic substrate phosphotriester **6**.

Hydrolysis of Tris(4-nitrophenyl) Phosphate (TNP) 6 by 3. Because TNP has insufficient solubility in 10 mM Triton X-100 aqueous solution, we used MeOH as a cosolvent. The critical micelle concentration of Triton X-100 was determined to be 0.15 ± 0.05 mM in 10% (v/v) MeOH aqueous solution at 25 °C with $I = 0.10$ ($NaNO_3$). The phosphotriester **6** (5−20 μM) was hydrolyzed by **3** (20−200 μM) to yield 4-nitrophenolate and bis(4-nitrophenyl) phosphate BNP^- (**5**) at pH range of 6.9−10.5. The 4-nitrophenolate releasing reaction followed excellent first-order kinetics for more than 5 half-lives. The subsequent hydrolysis of **5** to nitrophenyl phosphate (NP^{2-}) was far slower, see below.

The second-order dependence of the TNP hydrolysis rate catalyzed by **3** fits to the kinetic eq 10. A plot of the observed rate constants k_{obsd} as a function of the pH using 0.1 mM **3** and 10 μM TNP gave a similar sigmoidal curve as found in the earlier NA hydrolysis with an inflection point at pH 8.3 (see Figure 6). Therefore, the same species **3b** is concluded to react with NA (**4**) and TNP (**6**). The second-order rate constant k_{TNP}

(16) Recently, we discovered that phosphate P−O bond cleavage is promoted by an alkoxide bridged dizinc(II) complex with a hydroxyl octaazacryptand. In this case, weakly coordinating secondary nitrogen attacks the substrate phosphomonoester, while the bridging alkoxide O^- anion between two zinc(II) ions has no nucleophilic activity, which is somewhat similar to our dimeric complex **17**: Koike, T.; Inoue, M.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 3091−3099.

Scheme 4



(see eq 11) was extremely large $(1.1 \pm 0.1) \times 10^3$ M^{-1} s^{-1} at 25 °C and pH 10.2 with $I = 0.10$ ($NaNO_3$).

$$v_{obsd} = k_{obsd}[\text{total zinc(II) complex}][\text{TNP}] \quad (10)$$

$$= k_{TNP}[\mathbf{3b}][\text{TNP}] \quad (11)$$

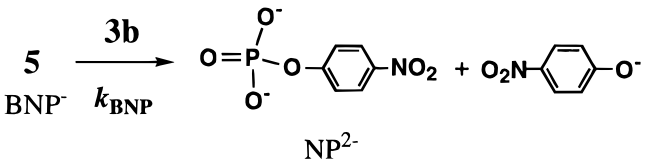
Under the same conditions, the TNP hydrolysis rate constants with **2b** in the absence and presence of 10 mM Triton X-100 were 3.8 ± 0.2 M^{-1} s^{-1} and $(9.0 \pm 0.3) \times 10^{-2}$ M^{-1} s^{-1}, respectively. The hydrophilic complex **2** mostly staying in aqueous phase would not attack the TNP partitioned in comicellar phase. We consider that most of the TNP (>97%) was partitioned in the micelles, since the k_{TNP} value decreased to 1/40 of its former value in the presence of micelles. The larger distribution of TNP in the comicellar phase as compared to 4-nitrophenyl acetate (NA) would be due to the larger lipophilicity, as suggested by the lower solubility of TNP in 10 mM Triton X-100 aqueous solution. The observed rate enhancement of 290 times by **3b** in the presence of 10 mM Triton X-100 over equimolar **2b** in the absence of Triton X-100 may arise from multiple effects as follows: (a) TNP is lipophilic and tends to go into the micelle, where the lipophilic nucleophile **3b** awaits to attack. Thus, the supramolecular assembly of **3b** and TNP leads to the efficient intramolecular type of hydrolysis. (b) The poor aquation both on **3b** and TNP in the comicellar phase makes the access of TNP easy to the metal center (see Scheme 4). Thus, **3** in Triton X-100 micelle may be a good mimic of the hydrophobic active center of zinc(II) containing organophosphorus hydrolase.[4,5]

There are two limiting mechanistic possibilities for the phosphate hydrolysis with the metal-bound OH^- species: (i) an intermolecular nucleophilic attack of metal-bound OH^- on the phosphorus atom, and (ii) an intramolecular nucleophilic attack of metal-bound OH^- on a bound phosphorus atom (coordinated to the metal ion through the phosphoryl oxygen). Previously, we concluded from a kinetic study of TNP hydrolysis catalyzed by various $Zn^{II}-OH^-$ species that the latter intramolecular nucleophilic mechanism is less likely for the TNP hydrolysis catalyzed by five coordinate cyclen$-Zn^{II}-OH^-$ **2b** than by four coordinate [12]ane$N_3-Zn^{II}-OH^-$ **1b**.[2b] This is thought to be due to the greater bulk and larger coordination number of cyclen over [12]aneN_3, preventing the access of phosphorus to the metal center. The large rate enhancement of TNP hydrolysis by hexadecylcyclen$-Zn^{II}-OH^-$ **3b** in the micellar system is thus explained by an intramolecular type of reaction due to the formation of supramolecular assembly.

Among the past metal-bound OH^- complexes, Menger's micellar copper(II) complex **7b** seemed to be the most effective catalyst for hydrolysis of phosphotriesters and for detoxification of nerve gases such as **9**.[6] The second-order rate constant (k_{NDP}) for the hydrolysis of 4-nitrophenyl diphenyl phosphate (**8**) by **7b** is 39 M^{-1} s^{-1} at 25 °C and pH 8.3. For the same reaction, the metal-free OH^- ion and zinc(II)-bound OH^- complex **12b** similarly act as catalysts, which showed the second-order rate constants of 2.8×10^{-2} and 0.28 M^{-1} s^{-1}, respectively, in 50% (v/v) CH_3CN aqueous solution at 25 °C.[9] The relative nucleophilicity indexes of **7b** and **12b** with respect to OH^- may be estimated to be 1360 [= (k_{NDP} for **7b**)/(k_{NDP} for OH^-)] and 10 [= (k_{NDP} for **12b**)/(k_{NDP} for OH^-)]. Although the substrate is a slightly different TNP **6**, a similar nucleophilicity index for **3b** with 10 mM Triton X-100 is calculated to be 103, using the second-order rate constants of $(1.1 \pm 0.1) \times 10^3$ M^{-1} s^{-1} for **3b** and 10.7 M^{-1} s^{-1} for metal-free OH^- ion.[2a] Thus, zinc(II) complex **3b** seems to be a very active nucleophile in hydrolysis of phosphotriesters (and probably for detoxification of nerve gases).

Hydrolysis of Bis(4-nitrophenyl) Phosphate (BNP^-) 5 with 3. The lipophilic zinc(II) complex **3** (0.25−1.0 mM) with 10 mM Triton X-100 also showed phosphodiesterase activity in BNP^- hydrolysis at 35 °C with a similar kinetic behavior as shown in the NA and TNP hydrolysis (see Scheme 5). The rate−pH profile curve disclosed an inflection point at pH ca. 8.3 with 1.0 mM **3**, indicating the kinetically active species again to be the zinc(II)-bound OH^- complex **3b** (see Experimental Section). The second-order rate constant k_{BNP} (see eq 12) for the hydrolysis of BNP^- (**5**) promoted by **3b** was $(4.3 \pm 0.4) \times 10^{-4}$ M^{-1} s^{-1} at 35 °C and pH 10.2 (20 mM CAPS buffer) with $I = 0.10$ ($NaNO_3$). The k_{BNP} value for **3b** with 10 mM Triton X-100 is only ca. 20 times larger than the reported k_{BNP} value for **2b** (2.1×10^{-5} M^{-1} s^{-1})[2a] under the same conditions. The rate enhancement (i.e., 20 times) is not as dramatic as for the more lipophilic TNP (290 times) and NA (50 times).

Scheme 5



$$v_{obsd} = k_{BNP}[\mathbf{3b}][BNP^-] \quad (12)$$

Inhibition of the TNP Hydrolysis by Imides and SCN^-. On the basis of the mechanistic study of the well-known inhibition of carbonic anhydrase (a zinc enzyme) by aromatic sulfonamides or anions such as SCN^-,[2d,17] we earlier discovered that aromatic sulfonamides, imide-containing derivatives[18] [thymine, uracil, succinimide (**22**), etc.], and inorganic anions

(17) *Carbonic Anhydrase*; Botrè, F., Gros, G., Storey, B. T., Eds.; VCH: New York, 1991.

(18) (a) Shionoya, M.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1993**, *115*, 6730−6737. (b) Shionoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 3848−3859. (c) Shionoya, M.; Sugiyama, M.; Kimura, E. *J. Chem. Soc., Chem. Commun.* **1994**, 1747−1748.

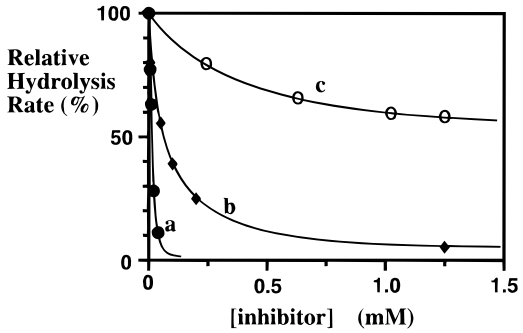
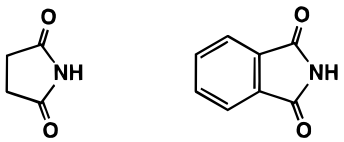


Figure 7. Relative TNP hydrolysis rate catalyzed by **3** (20 μM) and Triton X-100 (10 mM) as a function of the concentration of inhibitor in 10% (v/v) MeOH aqueous solution at 25 °C and pH 9.2 (20 mM CHES buffer) with $I = 0.10$ ($NaNO_3$): (a) for phthalimide **23** (5, 10, 20, and 40 μM); (b) for succinimide **22** (10, 50, 100, 200, and 1250 μM); (c) for NaSCN (0.25, 0.63, 1.00, and 1.25 mM).

are also good inhibitors in our zinc enzyme model study with **1** and **2**.[2] For instance, **22** dissociates at physiological pH (despite its pK_a ca. 10) to form a stable N^--Zn^{II} coordination bond to occupy the catalytic site $Zn^{II}-OH^-$ and thus inhibit the hydrolysis of benzylpenicillin catalyzed by **2b**.[2g] In the present TNP hydrolysis study, we also have observed strong inhibition by **22**, phthalimide (**23**), and thiocyanate (see Figure 7). This fact supports our conclusion that the reactive species is $Zn^{II}-OH^-$. A special remark is about **23**, which was not soluble enough in aqueous solution and thus was not studied for its inhibition of **2**-catalyzed esters or β-lactam hydrolysis.[2a,g] Now in the presence of 10 mM Triton X-100, **23** became soluble in 10% MeOH aqueous solution to allow studies in the TNP hydrolysis kinetics. The experiments with those inhibitors were conducted with **3** (20 μM) and TNP (10 μM) at 25 °C and pH 9.2 (20 mM CHES buffer) with $I = 0.10$ ($NaNO_3$). The results with various concentrations of those inhibitors are shown in Figure 7. Between **22** and **23**, **23** is a stronger inhibitor, as compared by the lower 50% inhibition concentration (ca. 60 vs <15 μM).



22 **23**

Conclusions

A lipophilic hexadecylcyclen zinc(II) complex **3** (ZnL) has been synthesized and fully characterized. It was demonstrated that in comicellar solution with a neutral surfactant Triton X-100 (10 mM), **3** remains stable (i.e., undissociated) above pH 5 and forms the discrete (nonaggregated) zinc(II) complex $(ZnL)_2OH$ **17** at near-neutral pH and $ZnL-OH^-$ **3b** at alkaline pH (see distribution diagram Figure 2). Only the Zn^{II}-bound OH^- species **3b** was found to be the nucleophilic catalyst in the comicelle for hydrolysis of 4-nitrophenyl acetate (NA) **4**, phosphotriester (TNP) **6**, and phosphodiester monoanion (BNP^-) **5**. The kinetic behavior of **3** in the comicelle has been well-defined, which makes the basic reaction mode by lipophilic metal complexes in micelle much clearer. The catalytic activity of **3** is extremely efficient for lipophilic substrates such as TNP **6**, due to favorable formation of supramolecular assembly in the micelle. However, **3** is not so outstanding as a catalyst for more hydrophilic substrates such as anionic BNP^-. The present study suggests that **3** in the presence of the surfactant may be an extremely promising candidate for disposal of nerve gases such as Soman.

Experimental Section

General Information. All reagents and solvents used were purchased at the highest commercial quality and used without further purification. The Good's buffer (Dojindo) and a neutral surfactant Triton X-100 (Nakarai) were commercial available: MOPS [3-(*N*-morpholino)propanesulfonic acid, pK_a = 7.2 at 20 °C], EPPS [4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid, pK_a = 8.0], CHES [2-(cyclohexylamino)ethanesulfonic acid, pK_a = 9.5], CAPS [3-cyclohexylamino-1-propanesulfonic acid, pK_a = 10.4], and Triton X-100 (polyethylene glycol *p*-octylphenyl ether, the average number of the ethylene group is 9.5). 4-Nitrophenyl acetate was recrystallized from dry diethyl ether. Tris(4-nitrophenyl) phosphate was recrystallized from ethyl acetate. Sodium bis(4-nitrophenyl) phosphate was crystallized from an aqueous solution of bis(4-nitrophenyl) phosphoric acid and equimolar NaOH. Tetrahydrofuran (THF) was distilled over $LiAlH_4$. Acetonitrile (CH_3CN) was distilled over calcium hydride and stored in the dark. All aqueous solutions were prepared using deionized and distilled water.

The kinetic study was carried out using a Hitachi U-3500 spectrophotometer equipped with a thermoelectric cell temperature controller (±0.5 °C). IR spectra were recorded on a Shimadzu FTIR-4200 at 25 °C. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a JEOL JNM LA500 spectrometer at 45.0 ± 0.1 °C. Tetramethylsilane (Merck) was used as internal reference. Melting points were determined by using a Yanako micro melting apparatus without any corrections. Elemental analysis was performed on a Yanako CHN Corder MT-3. Thin-layer (TLC) and silica gel column chromatographies were carried out on Merck Art. 5554 (silica gel) TLC plate and Wacogel C-300 (silica gel), respectively. Critical micelle concentration was determined by the vertical plate method using a Shimadzu ST-1 surface tensometer at 25.0 ± 0.5 °C.

Synthesis of 1-Hexadecyl-1,4,7,10-tetraazacyclododecane Trihydrochloride (15·3HCl). A solution of 1-bromohexadecane (5.6 g, 18.3 mmol) was added dropwise to a DMF solution (80 mL) of 2,6-dioxo-1,4,7,10-tetraazacyclodecane (**13**)[10] (4.6 g, 23.0 mmol) at room temperature over 1 h. After the reaction mixture had been heated at 50 °C for 1 day, the solvent was evaporated. The residue was dissolved in an aqueous solution (50 mL) of 1 M NaOH, and then the solution was extracted with CH_2Cl_2 (50 mL × 30). After the combined organic layers had been dried over anhydrous Na_2SO_4, the solvent was evaporated. The oily residue was crystallized from CH_2Cl_2/hexane to give 2,6-dioxo-10-hexadecyl-1,4,7,10-tetraazacyclododecane (**14**) as colorless needles (3.7 g, 47% yield): mp 124 °C; TLC (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 10:1:0.1) R_f = 0.67. IR (KBr pellet): 3409, 2923, 2849, 1657, 1532, 1466, 1132, 1051, 721 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 6.9 Hz, CH_3), 1.20−1.35 (28H, CCH_2C), 1.50 (2H, br, $NCCH_2C$), 2.18 (1H, br, NH), 2.55 (2H, t, J = 6.4 Hz, NCH_2C), 2.58 (4H, t, J = 5.6 Hz, NCH_2C), 3.32 (4H, ab-q, J = 5.6 and 11.2 Hz, NCH_2), 3.37 (4H, s, NCH_2CO), 7.40 (2H, br, CONH). The ^{13}C NMR assignments were obtained by 1D (^1H and ^{13}C) and 2D (NOESY and HMBC) NMR experiments in $CDCl_3$: δ 14.1 (C-16′), 22.7 (C-15′), 27.3 (C-2′), 27.6, 29.4, 29.6, 29.66, 29.68, 29.70, 29.72, 29.73, 32.0 (C-14′), 36.6 (C-8,12), 51.6 (C-9,11), 54.0 (C-1′), 55.6 (C-3,5), 170.8 (C-2,6).

The dioxo macrocycle **14** (3.0 g, 7.1 mmol) was added slowly to a dry THF solution (100 mL) of 1 M BH_3-THF complex at 0 °C. The solution was stirred at room temperature for 1 h and then heated at 60 °C for 1 week. After decomposition of the excess amount of the hydroborane complex with water at 0 °C, the solvent was evaporated. The residue was dissolved in 6 M aqueous HCl (50 mL), and then the solution was heated at 80 °C for 12 h. After evaporation of the solvent, the residue was dissolved in H_2O (50 mL). The solution pH was adjusted to 12 with 10 M NaOH, and then the aqueous layer was extracted with CH_2Cl_2 (50 mL × 15). After the combined organic layers had been dried over anhydrous Na_2SO_4, the solvent was evaporated. The oily residue was crystallized from 6 M aqueous HCl/CH_3CN to give 1-hexadecyl-1,4,7,10-tetraazacyclododecane trihydrochloride (**15**·3HCl·2H_2O) as colorless needles (1.7 g, 45% yield): dec 242 °C; TLC (eluent; CH_2Cl_2/MeOH/28% aqueous NH_3 = 5:2:0.1) R_f

= 0.25. IR (KBr pellet) 3447, 3287, 3019, 2955, 2921, 2851, 2780, 2361, 1559, 1541, 1470, 1449, 1410, 1084, 1061, 720 cm^{-1}. Anal. ($C_{24}H_{59}N_4O_2Cl_3$) C, H, N: calcd, 53.0, 11.1, 10.1; found, 53.2, 11.0, 10.3. The $CDCl_3$ solution of the acid-free ligand **15** for NMR experiment was prepared by extraction with $CDCl_3$ from a suspension of **15** in D_2O (pD = 12). The ^1H NMR assignments were obtained by 1D and 2D (COSY and NOESY) NMR experiments in $CDCl_3$: δ 0.88 (3H, t, J = 7.0 Hz, CH_3), 1.22−1.33 (28H, m, CH_2), 1.47 (2H, m, H_2C-2′), 2.41 (2H, t, J = 7.3 Hz, H_2C-1′), 2.52 (4H, t, J = 5.2 Hz, H_2C-2,12), 2.56 (4H, t, J = 5.2 Hz, H_2C-5,9), 2.62 (4H, t, J = 5.2 Hz, H_2C-3,11), 2.78 (4H, t, J = 5.2 Hz, H_2C-6,8). ^{13}C NMR ($CDCl_3$): δ 14.1, 22.7, 27.4, 27.6, 29.4, 29.6, 29.69, 29.74, 32.0, 45.4, 46.3, 47.2, 51.9, 54.8.

Synthesis of 1-Hexadecyl-1,4,7,10-tetraazacyclododecane Zinc(II) Complex [3·$(ClO_4)_2$]. The trihydrochloride salt of **15** (0.45 g, 0.83 mmol) was dissolved in alkaline aqueous solution (50 mL, pH ca. 12), and then the solution was extracted with CH_2Cl_2 (30 mL × 5). After the combined organic layers had been dried over anhydrous Na_2SO_4, the solvent was evaporated to obtain acid-free ligand **15** as a colorless viscose oil. To the MeOH solution (20 mL) of **15** was added $Zn(ClO_4)_2$·$6H_2O$ (0.40 g, 1.1 mmol). The solution was gradually concentrated to obtain colorless needles as diperchlorate salts **3**·$(ClO_4)_2$ in 52% yield: TLC (eluent; MeOH/10% aqueous NH_4Br = 5:1) R_f = 0.45; IR (KBr pellet) 3476, 2921, 2851, 1559, 1541, 1470, 1449, 1146, 1092, 721, 627 cm^{-1}. Anal. ($C_{24}H_{54}N_4O_9Cl_2Zn$) C, H, N: calcd, 42.2, 8.1, 8.1; found, 42.5, 8.0, 8.3. ^1H NMR ($CDCl_3$): δ 0.88 (3 H, t, J = 6.9 Hz, CH_3), 1.20−1.35 (28H, m, CH_2), 1.52 (2H, br, H_2C-2′), 1.58 (3H, br, NH), 2.75−2.90 (10H, m, NCH_2 and H_2C-1′), 3.00−3.18 (8H, m, NCH_2). ^{13}C NMR ($CDCl_3$): δ 14.1, 22.1, 22.7, 27.5, 29.4, 29.66, 29.67, 29.69, 29.71, 29.75, 29.8, 32.0, 43.1, 44.4, 45.3, 49.8, 53.9.

Potentiometric pH Titration. The pH-meter (Horiba F-16) and electrode system (a pH−glass electrode and a double-junction reference electrode) was daily calibrated as follows:[16] An aqueous solution (50 mL) containing 4.00 mM HCl and 96 mM $NaNO_3$ (I = 0.10) was prepared under an argon atmosphere (>99.999% purity) at 25.0 ± 0.1 °C, and then the first pH value (pH_1) is read. After 4.0 mL of 0.10 M NaOH (>99% purity) is added to the acidic solution, the second pH value (pH_2) is read. The theoretical pH values corresponding to pH_1 and pH_2 are calculated to be pH_1' = 2.481 and pH_2' = 11.447, respectively, using K_w (= a_{H^+}·a_{OH^-}) = $10^{-14.00}$, K_w' ([H^+][OH^-]) = $10^{-13.79}$, and f_{H^+} (a_{H^+}/H^+) = 0.825. The correct pH values (pH = −log a_{H^+}) can be obtained using the following equations: $a = (pH_2' - pH_1')/(pH_2 - pH_1)$; $b = pH_2' - a \times pH_2$; pH = a(pH-meter reading) + b.

All test solutions (50 mL) were sonicated for more than 15 min at room temperature and kept under an argon atmosphere at 25.0 ± 0.1 °C. The potentiometric pH titrations of hexadecylcyclen trihydrochloric acid salt (**15**·3HCl) (1 mM) were carried out under comicelle conditions with 10 mM Triton X-100 in the presence or absence of equimolar $ZnSO_4$ with I = 0.10 ($NaNO_3$), and at least three independent titrations were performed. Four protonation constants ($K_n' = [H_nL]/[H_{n-1}L][H^+]$) of **15** (L), zinc(II) complexation constant [K(ZnL)′ = [ZnL]/[L][Zn^{II}]], and deprotonation constant of the Zn^{II}-bound water [$K_a' = [ZnL{-}OH^-][H^+]/[ZnL]$) were determined by means of the pH−titration program BEST.[19] All σ pH fit values defined in the program are smaller than 0.01. The species distribution values against pH (= −log[H^+] + 0.084) was obtained using the program SPE.[19] The mixed-constants K_n = $[H_nL]/[H_{n-1}L]a_{H^+}$ (M^{-1}) and K_a = $[ZnL{-}OH^-]a_{H^+}/[ZnL]$ (M) are derived from K_n' and K_a' using $[H^+] = a_{H^+}/f_{H^+}$.

Kinetics Procedure for Hydrolysis of 4-Nitrophenyl Acetate in Micellar Solution. All test solutions for the following kinetic study were sonicated for more than 15 min under an argon atmosphere at room temperature. The hydrolysis of 4-nitrophenyl acetate (NA) catalyzed by $Zn^{II}{-}OH^-$ species was followed by the increase in 400-nm absorption of released 4-nitrophenolate (log ϵ = 4.34) in aqueous solution at 25.0 ± 0.5 °C. Buffer solutions containing 2.0 mM Good's buffer (MOPS, pH 6.9 and 7.3; EPPS, pH 7.9 and 8.3; CHES, pH 9.0 and 9.5; CAPS, pH 10.2 and 10.5) were used unless otherwise noted, and the ionic strength was adjusted to 0.10 with $NaNO_3$. For the determination of NA hydrolysis rate, the following typical procedure was employed. After a dry CH_3CN solution of 10 mM NA (3, 7.5, and 15 μL) had been rapidly injected in the buffered solution (3 mL) of **3** (0.20, 0.50, and 1.0 mM) in the presence of 10 mM Triton X-100, the UV absorption increase was recorded immediately. Background NA hydrolysis in the absence of catalyst was determined under the same conditions. The pseudo-first-order rate constants k'_{NA} (s^{-1}) were calculated from the decay (from 10 s after the NA injection) by an initial slope method for the slower reaction ($t_{1/2} \geq$ 600 s) and a log plot method for the faster reaction ($t_{1/2}$ < 600 s). The values of k'_{NA}/[total zinc(II) complex] gave the second-order rate constants k_{obsd} (M^{-1} s^{-1}). To check if the NA hydrolysis was recycling (i.e., catalytic), we followed the NA hydrolysis rate until 80% completion with NA (0.5, 1.0, and 2.0 mM) and **3** (0.1 and 0.2 mM) at pH 9.2 (20 mM CHES buffer) using an absorption increase at 458 nm (log ϵ 3.2) under the same comicellar conditions except with 10% (v/v) CH_3CN as a cosolvent, where the reaction followed excellent first-order kinetics. All kinetic experiments including those described in the following paragraph were run in triplicate, and the obtained rate constants were reproducible to ±10%.

Kinetics Procedure for Hydrolysis of Tris(4-nitrophenyl) Phosphate in Micellar Solution. The hydrolysis rate of tris(4-nitrophenyl) phosphate (TNP) catalyzed by $Zn^{II}{-}OH^-$ species in 10% (v/v) MeOH aqueous solution in the presence of 10 mM Triton X-100 was followed by increase in 400-nm absorption of released 4-nitrophenolate (log ϵ 4.30) at 25.0 ± 0.5 °C. Buffer solutions containing 20 mM Good's buffer (MOPS, pH 6.9 and 7.3; EPPS, pH 7.9 and 8.3; CHES, pH 9.0 and 9.5; CAPS, pH 10.2) were used, and the ionic strength was adjusted to 0.10 with $NaNO_3$ (ca. 90 mM). For the hydrolysis rate determination, the following typical procedure was employed: After rapid injection of a dry THF solution (15 μL) of TNP (1.0, 2.0, and 4.0 mM) in a buffer solution (3 mL) of **3** (20, 50, 100, and 200 μM) (reference experiment did not contain **3**), the UV absorption increase was recorded immediately and then followed until ≥5$t_{1/2}$. Background TNP hydrolysis in the absence of catalyst was determined under the same conditions. The product determination was followed by ^1H NMR in 10% D_2O solution, as previously described for **2b**-catalyzed TNP hydrolysis.[2b] Succeeding hydrolysis of the produced bis(4-nitrophenyl) phosphate was too slow to be detected under the same conditions. The pseudo-first-order rate constants k'_{TNP} (s^{-1}) for TNP hydrolysis were estimated by a log plot method (all correlation coefficient > 0.98), and then the observed second-order rate constants k_{obsd} (M^{-1} s^{-1}) were obtained to be k'_{TNP}/[total zinc(II) complex].

Kinetics Procedure for Hydrolysis of Bis(4-nitrophenyl) Phosphate in Micellar Solution. The hydrolysis rate of bis(4-nitrophenyl) phosphate (BNP^-) catalyzed by $Zn^{II}{-}OH^-$ species in the presence of 10 mM Triton X-100 was measured by an initial slope method [following the increase in 400-nm absorption of the released 4-nitrophenolate (log ϵ 4.29)] at 35.0 ± 0.5 °C. Buffer solutions containing 20 mM Good's buffer (MOPS, pH 7.3; EPPS, pH 8.1; CHES, pH 8.9 and 9.3; CAPS, pH 10.2) were used, and the ionic strength was adjusted to 0.10 with $NaNO_3$ (ca. 90 mM). After BNP^- (5 and 10 mM) and **3** (0.25, 0.5, and 1.0 mM) have been mixed in the buffer solution, the UV absorption increase was recorded immediately and then followed until ca. 1% formation of 4-nitrophenolate. The second-order rate constants k_{BNP} (M^{-1} s^{-1}) were estimated from the decay slope by the same method as described for **3b**-catalyzed NA hydrolysis. The obtained $k_{BNP} \times 10^4$ values are 4.3 ± 0.4 at pH 10.2, 4.2 ± 0.3 at pH 9.3, 3.8 ± 0.3 at pH 8.9, 1.8 ± 0.2 at pH 8.1, and 0.30 ± 0.02 at pH 7.3.

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