

## Combination of a Dinuclear Zn<sup>2+</sup> Complex and a Medium Effect Exerts a 10<sup>12</sup>-Fold Rate Enhancement of Cleavage of an RNA and DNA Model System

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**Abstract:** The catalytic ability of a dinuclear Zn<sup>2+</sup> complex of 1,3-bis-*N*<sub>1</sub>-(1,5,9-triazacyclododecyl)propane (**3**) in promoting the cleavage of an RNA model, 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP, **1**), and a DNA model, methyl *p*-nitrophenyl phosphate (MNPP, **4**), was studied in methanol solution in the presence of added CH<sub>3</sub>O<sup>-</sup> at 25 °C. The di-Zn<sup>2+</sup> complex (Zn<sub>2</sub>:**3**), in the presence of 1 equiv of added methoxide, exhibits a second-order rate constant of  $(2.75 \pm 0.10) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  for the reaction with **1** at <sup>s</sup>pH 9.5, this being 10<sup>8</sup>-fold larger than the *k*<sub>2</sub> value for the CH<sub>3</sub>O<sup>-</sup> promoted reaction (*k*<sub>0CH<sub>3</sub></sub> =  $(2.56 \pm 0.16) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ ). The complex is also active toward the DNA model **4**, exhibiting Michaelis–Menten kinetics with a *K*<sub>M</sub> and *k*<sup>max</sup> of  $0.37 \pm 0.07 \text{ mM}$  and  $(4.1 \pm 0.3) \times 10^{-2} \text{ s}^{-1}$ , respectively. Relative to the background reactions at <sup>s</sup>pH 9.5, Zn<sub>2</sub>:**3** accelerates cleavage of each phosphate diester by a remarkable factor of 10<sup>12</sup>-fold. A kinetic scheme common to both substrates is discussed. The study shows that a simple model system comprising a dinuclear Zn<sup>2+</sup> complex and a medium effect of the alcohol solvent achieves a catalytic reactivity that approaches enzymatic rates and is well beyond anything seen to date in water for the cleavage of these phosphate diesters.

### Introduction

Phosphodiester are important biomolecules for the storage of genetic information due to their great stability.<sup>1–7</sup> While the respective half-times for hydrolysis of RNA<sup>8</sup> and DNA<sup>9</sup> at pH 7 and 25 °C are 110 and up to 100 billion years, Nature provides enzymes that promote the cleavage by up to a factor of 10<sup>15</sup>–10<sup>16</sup>, thus affording some of the most spectacular rate enhancements known. Many of these enzymes have active sites comprising two or more metal ions (usually Zn<sup>2+</sup> and in some cases Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Fe<sup>2+</sup>) as exemplified by phosphodiesterases such as ribonuclease H from HIV reverse transcriptase,<sup>5</sup> 3',5'-exonuclease from DNA polymerase<sup>16</sup>, the P1 nucleases,<sup>7</sup> and phospholipase C.<sup>1–4</sup> Intense research was, and is now, directed at understanding the origins of catalysis of phosphate diester cleavage provided by metal ion systems.<sup>10–13</sup> From earlier work<sup>10–13</sup> and more recent reports<sup>14–20</sup> it is seen

that dinuclear complexes are typically more reactive than their mononuclear counterparts, and there are four main roles by which the dinuclear catalysts are proposed to promote the phosphoryl transfer reactions: (1) by double Lewis acid activation of a phosphate diester through M<sup>2+</sup>–OP(OR)–(OR)O–M<sup>2+</sup> coordination; (2) through bifunctional catalysis whereby the metal ion activates the bound phosphate and delivers a metal-coordinated hydroxide, alkoxide, or oxide that serves as a nucleophile or base; (3) by assisting, in some cases, the departure of the phosphate's leaving group through metal coordination; and (4) as an electrostatic reservoir of (+)-charge to interact favorably with the anionic phosphate to enhance substrate binding and stabilize the transition state for the phosphoryl transfer reaction. As important as all these effects are, there must be an additional factor which has heretofore not been demonstrated experimentally, as none of the Zn<sup>2+</sup>-based

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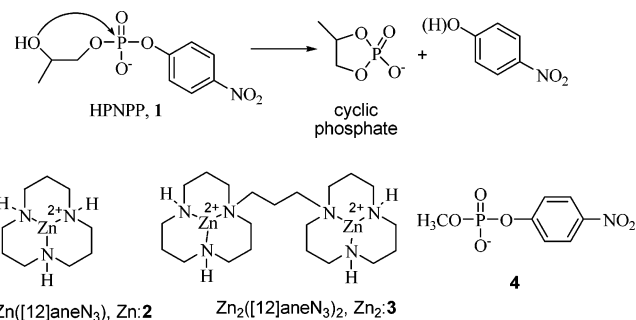
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model systems achieves a catalysis in water that even remotely approaches enzymatic rates or in fact is significantly better than hydroxide reacting with the substrate.<sup>21,22</sup>

It has been stated, in the context of enzymatic binding and catalysis being influenced by short, strong hydrogen bonds, that the “active sites of enzymes are non-aqueous, and the effective dielectric constants resemble those in organic solvents rather than that in water”.<sup>23,24</sup> It is well-known that solvent changes can have important accelerating or decelerating effects on organic transformations depending on the overall solvation of the ground state starting materials and transition states for the rate-limiting steps of the reaction.<sup>25</sup> However, reports of solvent effects on simple reactions of biological interest, particularly metal-ion-catalyzed processes, are relatively sparse. Nevertheless, in selected cases the effects can be large. For example, the decarboxylation<sup>26</sup> of pyruvate promoted by 3,4-dimethylthiazolium ion (a model for thiamine pyrophosphate catalyzed reactions in enzymes) occurs  $10^4$ – $10^5$  faster in ethanol than in water, suggesting that the enhancement arises from the less polar solvent promoting a reaction where the dipolar character of the zwitterionic intermediate is neutralized in the transition state.

It seems to us that metal ion promoted phosphoryl transfers from anionic phosphates would be just the sort of processes where one would expect reduced solvent polarity to have profound effects.<sup>27</sup> A major theme of our recent work involves investigation of the catalytic properties of metal ions in alcohol where the medium produces very strong accelerations of acyl and phosphoryl transfer reactions.<sup>28</sup> We have reported earlier the catalysis of cyclization of the simple anionic phosphate diester model for RNA, namely 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP, **1**), promoted by  $\text{La}^{3+}$  in methanol<sup>29</sup> which

is at least  $3 \times 10^6$ -fold faster than the analogous reaction in water.<sup>30</sup> However,  $\text{La}^{3+}$  is not a biologically relevant ion, and its net charge exceeds that of  $\text{Zn}^{2+}$ . To more closely model the premise that a large part of metal ion catalysis of phosphate diester cleavage within the enzyme active site might arise from reduced dielectric constant medium effects, we report here the catalysis of HPNPP cleavage afforded by the mono- $\text{Zn}^{2+}$  complex of 1,5,9-triazacyclododecane ( $\text{Zn}([\text{12}] \text{janeN}_3)$ , (**Zn:2**)) and a dinuclear  $\text{Zn}^{2+}$  complex of 1,3-bis-*N*<sub>1</sub>-(1,5,9-triazacyclododecyl)propane ( $\text{Zn}_2([\text{12}] \text{janeN}_3)_2$ , (**Zn<sub>2</sub>:3**)). The choice of HPNPP as a model substrate is made on the basis of its availability and widespread prior use, and also because the observable reaction is an intramolecular transesterification resulting in the formation of *p*-nitrophenol(ate) and a cyclic phosphate without incorporation of any water or alcohol from the solvent, rendering the results we obtain in alcohol equally applicable to the mechanistic details as those obtained in water. In addition, we have looked at the reaction of methyl *p*-nitrophenyl phosphate (**4**) promoted by  $\text{Zn}_2\text{:3}$  to compare the reactivity of what might be taken as a DNA model lacking the intramolecular  $\beta$ -OH with that of **1**. As will be shown, the cyclization of **1** in methanol containing 1 mM  $\text{Zn}_2\text{:3}$  is faster than that of any previously reported system in water by several orders of magnitude, proceeding with a  $t_{1/2}$  of 2.5 ms that reflects an acceleration of  $2 \times 10^{12}$  times over the background reaction at a solution  $^s\text{pH}^{31}$  of 9.5.



- (21) A criterion for a bifunctional behavior of the metal bound hydroxide catalyst is that the second-order rate constant for a given process exceeds that of  $\text{HO}^-$  itself. While there are some reports where the dinuclear  $\text{Zn}^{2+}$  or  $\text{Cu}^{2+}$  complexes can be more effective than hydroxide in promoting the hydrolysis of phosphate diesters such as bis(*p*-nitrophenyl) phosphate (Gadja, T.; Düpre, T.; Török, I.; Harmer, J.; Schweiger, A.; Sander, J.; Kuppert, D.; Hegetschweiler, K. *Inorg. Chem.* **2001**, *40*, 4918) or 2',3'-cyclic AMP (Jancsó, A.; Mikkola, S.; Lönnberg, K.; Hegetschweiler, K.; Gadja, T. *Chem.—Eur. J.* **2003**, *9*, 5404) there are few where the complexes effect the cyclization of HPNPP much better than does  $\text{HO}^-$ .
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## Experimental Section

**Materials.** Methanol (99.8% anhydrous), sodium methoxide (0.5 M solution in methanol),  $\text{HClO}_4$  (70% aqueous solution),  $\text{Zn}(\text{CF}_3\text{SO}_3)_2$ , tetrabutylammonium triflate, 1,5,9-triazacyclododecane (**2**), paraoxon (**5**), and sodium diphenyl phosphate were purchased from Aldrich and used as supplied. 2-Hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP, **1**) was prepared by a modification<sup>29</sup> of the prior art.<sup>32</sup> 1,3-Bis-*N*<sub>1</sub>-(1,5,9-triazacyclododecyl)propane (**3**) was synthesized according to the published procedure.<sup>33</sup> Methyl-*p*-nitrophenyl phosphate (MNPP, **4**) was prepared as described.<sup>34</sup> The dinuclear  $(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}] \text{janeN}_3)_2$  complex was prepared as a 2.5 mM solution in methanol by sequential

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addition of aliquots of stock solutions of sodium methoxide, 1,3-bis-*N*<sub>1</sub>-(1,5,9-triazacyclododecyl)propane, and Zn(CF<sub>3</sub>SO<sub>3</sub>)<sub>2</sub> such that the relative amounts were 1:1:2. It has been found that this order of addition is essential for the formation of the complex, and even then its complete formation is achieved only after 40 min (as monitored by the change in catalytic activity). An equimolar mixture of the complexes (CH<sub>3</sub>O<sup>-</sup>):Zn([12]aneN<sub>3</sub>) and Zn([12]aneN<sub>3</sub>) was prepared in situ at a <sup>s</sup>pH equal to the <sup>s</sup>pK<sub>a</sub> in methanol by addition of aliquots of stock solutions of sodium methoxide, 1,5,9-triazacyclododecane, and Zn(CF<sub>3</sub>SO<sub>3</sub>)<sub>2</sub> in a 0.5:1:1 ratio. No effects attributable to the order of addition of the complexes' components were observed in this case.

**Kinetics.** The rates of methanolyse of HPNPP (0.04 mM) catalyzed by the Zn([12]aneN<sub>3</sub>) complex were followed by monitoring the appearance of *p*-nitrophenol at 320 nm at  $2 \times 10^{-4}$  M < [Zn([12]aneN<sub>3</sub>)] <  $5 \times 10^{-3}$  M with varying [OCH<sub>3</sub>]/[Zn<sup>2+</sup>]<sub>t</sub> ratios using a Cary 100 UV-vis spectrophotometer with the cell compartment thermostated at 25.0 ± 0.1 °C. The rates of methanolyse of MNPP (0.04 mM) in methanol were followed by monitoring the appearance of *p*-nitrophenol at 320 nm at varying [(CH<sub>3</sub>O<sup>-</sup>):Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>] with a Cary100 UV-vis spectrophotometer at 25.0 ± 0.1 °C. Reactions were monitored at  $2 \times 10^{-4}$  M < [(CH<sub>3</sub>O<sup>-</sup>):Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>] <  $1.2 \times 10^{-3}$  M. Second-order rate constants for the methoxide reactions of **1** and **4** were taken from our previous work as  $(2.56 \pm 0.16) \times 10^{-3}$  M<sup>-1</sup> s<sup>-1.29</sup> and  $(7.9 \pm 0.6) \times 10^{-7}$  M<sup>-1</sup> s<sup>-1.35</sup> at 25 °C.

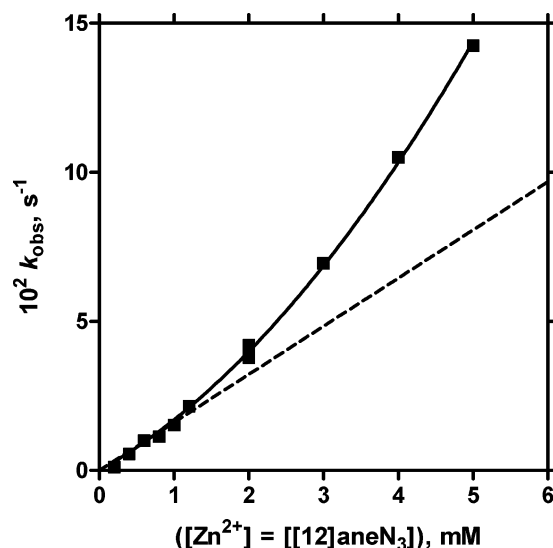
The base dependence of the methanolysis of methyl *p*-nitrophenyl phosphate (MNPP, **4**) catalyzed by Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub> was studied by adding aliquots of stock solutions of sodium methoxide or perchloric acid to the cell containing 0.4 mM of [(CH<sub>3</sub>O<sup>-</sup>):Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>] complex, and the reaction was initiated immediately by the addition of MNPP solution so that the final substrate concentration in the UV cell was  $4 \times 10^{-5}$  M. In general, the reactions followed good first-order kinetics up to about three half-times of methanolysis, and the pseudo-first-order rate constants (*k*<sub>obs</sub>) were determined by nonlinear least-squares (NLLSQ) fitting of the absorbance versus time traces to a standard exponential model.

The rates of the methanolysis of HPNPP (0.04 mM) catalyzed by the Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub> complex were determined using an Applied Photophysics SX-17MV stopped-flow reaction analyzer thermostated at 25.0 °C. Reactions were followed by monitoring the rate of loss of the starting material at 280 nm and appearance of the product *p*-nitrophenol at 320 nm. The study of the base dependence of the methanolysis of HPNPP catalyzed by Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub> was performed by "pH jump" experiments where one syringe of the stopped-flow reaction analyzer contained a 0.4 mM solution of (CH<sub>3</sub>O<sup>-</sup>):Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub> and another one contained a 0.08 mM solution of HPNPP in methanol along with the required amount of perchloric acid or sodium methoxide.

At very low [Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>] (<0.1 mM), the complex is not fully formed. This is evident in the kinetic data in the plots *k*<sub>obs</sub> vs [Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>] (see for example Figure 4) which show a positive intercept of ~0.1 mM in [catalyst]. Due to the complex not being fully formed at low concentrations, we cannot give an exact equation to describe the behavior of the *k*<sub>obs</sub> vs [Zn<sub>2</sub>:**3**] plots. In determining the binding constants, we have chosen an approximate approach which introduces a fitting variable for the intercept (*A*, which is independently fit and generally found to be ~0.1 mM) that is subtracted from the catalyst concentration *vide infra*.

## Results

Shown in Figure 1 is a plot of the observed pseudo-first-order rate constant for the reaction of HPNPP as a function of [Zn([12]aneN<sub>3</sub>)]<sub>t</sub><sup>36</sup> in the presence of 0.5 equiv of CH<sub>3</sub>O<sup>-</sup> to



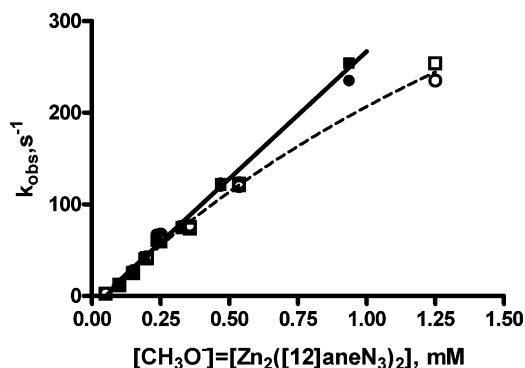
**Figure 1.** A plot of *k*<sub>obs</sub> vs [Zn<sup>2+</sup>] = [[12]aneN<sub>3</sub>] at [OCH<sub>3</sub>]/[Zn<sup>2+</sup>]<sub>t</sub> = 0.5 for the methanolysis of HPNPP ( $4 \times 10^{-5}$  M), <sup>s</sup>pH 9.1 ± 0.1 and *T* = 25 ± 0.1 °C. Dotted line is the linear regression of the initial part of the plot where ([Zn<sup>2+</sup>] = [[12]aneN<sub>3</sub>]) ≤  $1.2 \times 10^{-3}$  M, whereas the solid line is NLLSQ fitted throughout the [Zn<sup>2+</sup>]<sub>total</sub> range to an expression of the form  $k_{\text{obs}} = k_2^{\text{obs}}[[12]\text{aneN}_3] + k_3^{\text{obs}}[[12]\text{aneN}_3]^2$ .

set the <sup>s</sup>pH of the solution at 9.1, the <sup>s</sup>pK<sub>a</sub> of the CH<sub>3</sub>OH + (CH<sub>3</sub>OH):Zn([12]aneN<sub>3</sub>) ⇌ (CH<sub>3</sub>O<sup>-</sup>):Zn([12]aneN<sub>3</sub>) + H<sub>2</sub>O<sup>+</sup>-CH<sub>3</sub> ionization. The plot shows an upward curvature consistent with a process bimolecular in [Zn([12]aneN<sub>3</sub>)]<sub>t</sub> which, when fitted by NLLSQ to the expression  $k_{\text{obs}} = k_2^{\text{obs}}[[12]\text{aneN}_3] + k_3^{\text{obs}}[[12]\text{aneN}_3]^2$  gives  $k_2^{\text{obs}} = 18.9$  M<sup>-1</sup> s<sup>-1</sup> and  $k_3^{\text{obs}} = (1.8 \pm 0.4) \times 10^3$  M<sup>-2</sup> s<sup>-1</sup>. Similar upward curving plots are found when the [OCH<sub>3</sub>]/[Zn<sup>2+</sup>]<sub>t</sub> ratio is 0.3 and 0.7, which is consistent with the participation of both the (CH<sub>3</sub>O<sup>-</sup>):Zn([12]aneN<sub>3</sub>) and (CH<sub>3</sub>OH):Zn([12]aneN<sub>3</sub>) forms of the catalyst. When the ratio is 1.0 and the <sup>s</sup>pH is 11.09, only the (CH<sub>3</sub>O<sup>-</sup>):Zn([12]aneN<sub>3</sub>) form is present and the plot (not shown) is linear with a gradient  $k_2^{\text{obs}} = 16.9 \pm 0.7$  M<sup>-1</sup> s<sup>-1</sup> consistent with a dependence on only one molecule of (CH<sub>3</sub>O<sup>-</sup>):Zn<sup>2+</sup>-containing species. In no case do we observe any evidence of saturation binding of **1** up to a [Zn([12]aneN<sub>3</sub>)]<sub>t</sub> of  $5 \times 10^{-3}$  M, so the *K*<sub>dis</sub> for dissociation of a putative HPNPP:Zn([12]aneN<sub>3</sub>) complex must be at least 5 times higher at  $>2.5 \times 10^{-2}$  M.

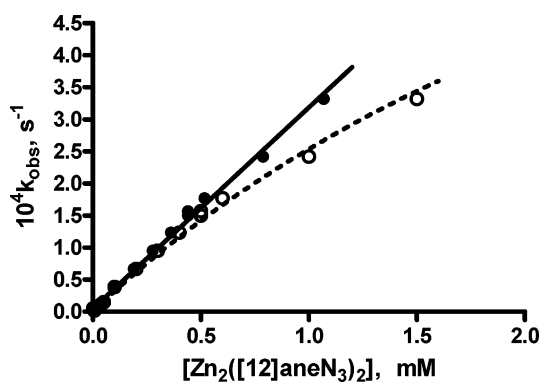
The dinuclear complex Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub><sup>33</sup> with 1 equiv of added methoxide produces a catalyst for HPNPP cleavage that has remarkable activity in methanol. In Figure 2 is a plot of *k*<sub>obs</sub> vs [CH<sub>3</sub>O<sup>-</sup>] = [Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>] determined at a measured <sup>s</sup>pH = 9.5 ± 0.1. The rapidity of the reaction requires it to be monitored by stopped-flow spectrophotometry at λ = 280 nm or 320 nm which are the wavelengths corresponding to the disappearance of HPNPP and appearance of *p*-nitrophenol. On first inspection, the primary data (dashed line) show downward curvature, although this is not due to substrate binding but rather to a specific ion effect of the triflate anions that suppresses the rate (since each Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub> carries with it four <sup>-</sup>OTf counterions). This is confirmed by the data for an independent experiment in which we studied the methanolysis of a weakly binding substrate, paraoxon, **5**. Shown in Figure 3 is a plot of the *k*<sub>obs</sub> for methanolysis of **5** as a function of [(CH<sub>3</sub>O<sup>-</sup>):Zn<sub>2</sub>

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**Figure 2.** A plot of the observed pseudo-first-order rate constants ( $k_{\text{obs}}$ ) for the methanolysis of HPNPP ( $4 \times 10^{-5}$  M) as a function of  $[\text{Zn}_2([\text{12}]\text{aneN}_3)_2]$  in the presence of 1 equiv of added  $\text{CH}_3\text{O}^-$  per complex giving  $\text{pH} = 9.5$ ,  $T = 25 \pm 0.1$  °C. Dotted line is presented as a visual aid directed through all actual data at 280 nm ( $\square$ ) or 320 nm ( $\circ$ ); solid line is a linear fit of the data corrected for inhibition by triflate counterions at 280 nm ( $\blacksquare$ ) or 320 nm ( $\bullet$ ).

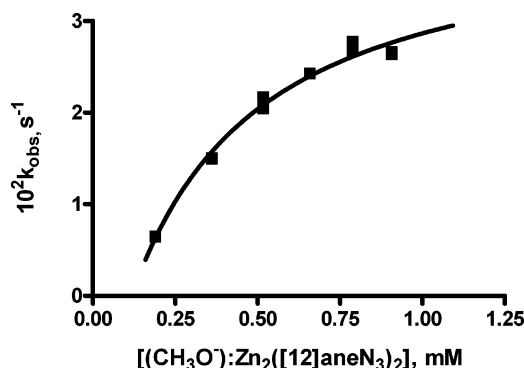


**Figure 3.** A plot of the observed pseudo-first-order rate constants ( $k_{\text{obs}}$ ) for methanolysis of  $2.5 \times 10^{-5}$  M **5** vs  $[\text{Zn}_2([\text{12}]\text{aneN}_3)_2]$  in the presence of 1 equiv of added  $\text{CH}_3\text{O}^-$  per complex,  $\text{pH} = 9.5$ ,  $T = 25 \pm 0.1$  °C. Dotted line is presented as a visual aid directed through all actual data ( $\circ$ ); solid line is a linear fit through the data corrected for inhibition by triflate counterions at ( $\bullet$ ).

$[\text{12}]\text{aneN}_3)_2$  which also exhibits a downward curvature in the same concentration range as does **1**. That a weakly binding substrate (**5**) exhibits the identical curvature in the plot as does the potentially strong binding **1** suggests that the curvature is independent of the substrate and is more likely dependent on the presence of anions that accompany the  $\text{Zn}^{2+}$  complex. Indeed, we have observed that the catalytic activity toward methanolysis of paraoxon of a solution containing 0.4 mM of the  $[(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}]\text{aneN}_3)_2]$  complex does decrease as a function of increasing  $[\text{Bu}_4\text{N}^+(\text{OTf})^-]$ . The kinetic data for this inhibition, when fit to a one-site binding model shown in eq 1, give an inhibition constant of 14.9 mM. This constant can be

$$k_{\text{obs}} = k_{\text{max}} K_{\text{inhib}} / ([\text{Bu}_4\text{NOTf}] + K_{\text{inhib}}) \quad (1)$$

used to calculate the free [catalyst] under the kinetic conditions. When the kinetic data of Figures 2 and 3 are corrected for triflate inhibition by plotting the  $k_{\text{obs}}$  vs free [catalyst], linear correlations are observed, without any evidence for saturation binding of either substrate. The gradient of the linear plot in Figure 3 for paraoxon is  $0.314 \pm 0.006 \text{ M}^{-1} \text{ s}^{-1}$ , while the gradient in Figure 2 is  $(2.75 \pm 0.10) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ; these respectively correspond to the apparent second-order rate



**Figure 4.** A plot of  $k_{\text{obs}}$  for methanolysis of  $4 \times 10^{-5}$  M MNPP (**4**) vs  $[\text{Zn}_2([\text{12}]\text{aneN}_3)_2]$  in the presence of 1 equiv of  $\text{CH}_3\text{O}^-$  per ligand showing a saturation behavior,  $\text{pH} = 9.5$ ,  $T = 25 \pm 0.1$  °C. Line through the data calculated by NLLSQ fits eq 2 giving a binding constant of  $K_{\text{M}} = 0.37$  mM and a maximum rate constant for reaction of the  $\text{MNPP}:(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}]\text{aneN}_3)_2$  complex of  $k^{\text{max}} = (4.1 \pm 0.1) \times 10^{-2} \text{ s}^{-1}$ .

constants for the cleavage of paraoxon and HPNPP promoted by  $(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}]\text{aneN}_3)_2$ .

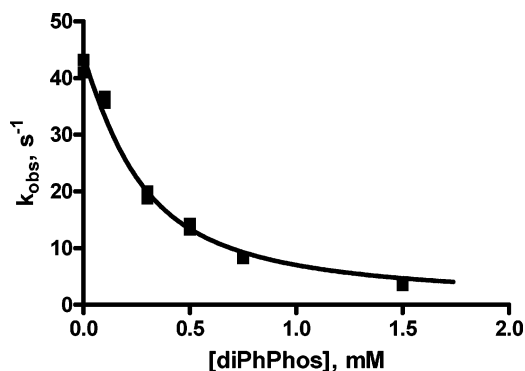
It is generally observed that phosphate diesters only weakly bind to  $\text{Zn}^{2+}$  complexes in water,<sup>37</sup> with the reported  $K_{\text{b}}$  values being  $<0.5 \text{ M}^{-1}$ ; even the trivalent  $\text{La}^{3+}$  binds rather poorly to HPNPP in water ( $73 \text{ M}^{-1}$ ).<sup>30</sup> However our previous results<sup>29</sup> showed that  $\text{La}^{3+}$  binding to HPNPP in methanol was at least  $10^4$  times stronger than that in water, the value being  $>10^6 \text{ M}^{-1}$ . Phosphate diester binding to  $\text{Zn}_2([\text{12}]\text{aneN}_3)_2$  is stronger in methanol than that in water as demonstrated by the kinetic plot in Figure 4 which shows the dependence of  $k_{\text{obs}}$  vs  $[(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}]\text{aneN}_3)_2]$  for the methanolysis of MNPP (**4**). The plot shows clear evidence of saturation binding, even when the  $[(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}]\text{aneN}_3)_2]_{\text{total}}$  data are corrected for the effect of the weaker triflate inhibition. The data are analyzed according to standard Michaelis–Menten kinetics (eq 2) yielding a  $k^{\text{max}}$  of  $(4.1 \pm 0.3) \times 10^{-2} \text{ s}^{-1}$  and a binding constant,  $K_{\text{M}}$ , of  $0.37 \pm 0.07$  mM.

$$k_{\text{obs}} = k^{\text{max}} [\text{cat}] / ([\text{cat}] + K_{\text{M}}) \quad (2)$$

In Figure 4, the X-intercept is clearly greater than zero which results from incomplete formation of the catalyst which is prominently noticed at low concentrations. In eq 2, the [cat] term used for the fitting corresponds to the actual concentration of catalyst which is fitted according to an expression of the form  $[\text{cat}] = ([\text{Zn}^{2+}]_{\text{total}} - A)$ , where  $A$  is an independently fitted parameter generally having the value of  $\sim 0.1$  mM which corresponds to the observable intercept. While we acknowledge this approach is not rigorous, it is the same as what we have used previously to model the  $\text{La}^{3+}$ -catalyzed methanolysis of carboxylate esters and neutral phosphate triesters<sup>28</sup> where only the concentration of a catalytically active  $\text{La}^{3+}$ -containing dimer was taken into account.

Additional evidence for the strong binding of phosphate diesters to  $[(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}]\text{aneN}_3)_2]$  comes from the inhibition of its catalysis of the cyclization of HPNPP in the presence of diphenyl phosphate. Given in Figure 5 is the inhibition plot of  $k_{\text{obs}}$  vs [diphenyl phosphate]; the data for this can be fit to a

(37) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935.



**Figure 5.** An inhibition plot of the variation in  $k_{\text{obs}}$  for the cyclization of HPNPP promoted by  $[\text{Zn}_2([\text{12}] \text{aneN}_3)_2]$  in the presence of 1 equiv of  $\text{CH}_3\text{O}^-$  per ligand, vs [diphenyl phosphate],  $\text{pH} = 9.5$ ,  $T = 25 \pm 0.1$  °C. Line through the data calculated on the basis of eq 3 giving an inhibition constant of  $0.16 \pm 0.03$  mM.

universal binding eq 3<sup>38</sup> that is applicable to both strong and weak binding situations,

$$k_{\text{obs}} = k_{\text{cat}}(1 - (1 + K_d[\text{Lim}] + [\text{Ex}]K_d - X)/(2K_d)/[\text{Lim}]) \quad (3)$$

where [Lim] and [Ex] refer to total concentrations of limiting and excess reagents and X is given in eq 3a.

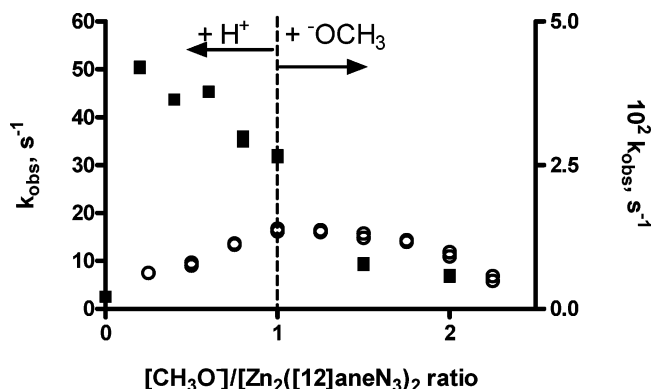
$$X = \{1 + 2K_d[\text{Lim}] + 2[\text{Ex}]K_d + K_d^2[\text{Lim}]^2 - 2K_d^2[\text{Ex}][\text{Lim}] + [\text{Ex}]^2K_d^2\}^{0.5} \quad (3a)$$

The computed inhibition constant for diphenyl phosphate is  $0.16 \pm 0.03$  mM. The demonstration of strong binding of two closely related phosphate diesters to the  $(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}] \text{aneN}_3)_2$  complex seems at odds with the observed linear dependence of the plot of  $k_{\text{obs}}$  for cyclization of HPNPP vs  $[(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}] \text{aneN}_3)_2]$  shown in Figure 2. However, as will be shown later, this is reconciled within the mechanism for the HPNPP reaction for which the rate-limiting step is proposed to be different from that for cleavage of the more slowly reacting phosphate ester **4**. Of note is the fact that the  $k_{\text{obs}}$  vs the  $[\text{CH}_3\text{O}^-]/[\text{Zn}_2([\text{12}] \text{aneN}_3)_2]$  ratio for cyclization of **1** and **4** have very different appearances as shown in Figure 6. The  $\text{Zn}_2([\text{12}] \text{aneN}_3)_2$ -catalyzed methanolysis of **4** exhibits a bell-shaped  $\text{pH}/\text{rate}$  profile as is customary for dinuclear metal catalysts where the most active form is the mono-lyoxy one  $(\text{CH}_3\text{O}^-):\text{Zn}_2([\text{12}] \text{aneN}_3)_2$ , although this is not the behavior of the  $\text{Zn}_2([\text{12}] \text{aneN}_3)_2$ -catalyzed HPNPP cyclization.

## Discussion

**i. Predicted Effect of Reduced Dielectric Constant on Ion–Ion Interactions and Reactions.** It has been noted that, despite numerous efforts to design models for catalyzing the cleavage of phosphate diesters, “none of the several models so far described approach the enormous catalytic efficiency of natural enzymes”.<sup>36</sup> Simple small molecule models will likely not mimic well enzymatic properties that depend on the large size of the enzyme, such as selectivity of substrate binding and reactivity site recognition. However, small molecule catalytic systems may

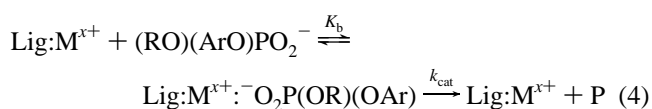
(38) Equation 3 was obtained from the equations for equilibrium binding and for conservation of mass by using the commercially available MAPLE software, Maple V, release 5; Waterloo Maple Inc.: Waterloo, Ontario, Canada.



**Figure 6.** A plot of the observed pseudo-first-order rate constant for the methanolysis of 0.04 mM HPNPP (■, left axis) catalyzed by 0.2 mM  $\text{Zn}_2([\text{12}] \text{aneN}_3)_2$  or 0.04 mM methyl *p*-nitrophenyl phosphate (○, right axis) catalyzed by 0.4 mM  $\text{Zn}_2([\text{12}] \text{aneN}_3)_2$  as a function of the  $[\text{CH}_3\text{O}^-]/[\text{Zn}_2([\text{12}] \text{aneN}_3)_2]$  ratio at  $25 \pm 0.1$  °C. Experiments done by “pH jump” method starting at the methoxide to the  $[\text{CH}_3\text{O}^-]/[\text{Zn}_2([\text{12}] \text{aneN}_3)_2]$  ratio of 1.0 (vertical dashed line,  $\text{pH} = 9.5$ ) and adding acid (left) or base (right).

well approximate the rate enhancements achieved by enzymes for selected reactions. The catalytic deficiencies of currently existing model systems suggest that there are additional factors either overlooked or undiscovered.

For such systems where positively charged  $\text{M}^{x+}$  ions or complexes interact with negatively charged phosphate diesters, the simplest conceivable process given in eq 4 involves a pre-equilibrium binding of the  $\text{M}^{x+}:\text{Lig}$  and  $(\text{RO})_2\text{PO}_2^-$  to form a larger complex from which product formation ensues. If it is true that enzymatic sites are organic-like, with low dielectric constants tailored for enhancing catalytic activity, a venerable hypothesis for the reaction of small molecule cation/anion systems is that enhancing the electrostatic interactions above what they are in water might prove to be an important component of the catalytic reactivity. In fact, these appear to be just the sort of processes expected to experience



large rate accelerations in passing from water to a medium of reduced dielectric constant and lower polarity. Among the organic solvents, the lower alcohols such as methanol are closest to water in terms of structure, H-bonding, and solvation properties and are suitable solvents to investigate low polarity/dielectric constant effects on metal-ion-catalyzed reactions once the control and measurement of  $\text{pH}$  are established. The Debye–Hückel theory for association of spherical ions in a medium of dielectric constant  $D_r$  states that the electrostatic potential energy of interaction between oppositely charged ions is

$$\text{PE} = (z_+e)(z_-e)/(4\pi D_0 D_r r) \quad (5)$$

where  $r$  is the distance between the centers of the ions,  $z_+e$  and  $z_-e$  are their charges in coulombs ( $e$  is the proton charge),  $D_0$  is the permittivity of a vacuum, and  $r$  is the separation of the ions.<sup>39</sup> When the electrostatic attraction energy exceeds the

(39) Levine, I. N. *Physical Chemistry*, 4th ed.; McGraw-Hill, Inc.; U.S.A., 1978; pp 276–281.

thermal kinetic energy of the species in solution (given from its average translational kinetic energy as  $3/2kT$  where  $k$  is the Boltzman constant and  $T$  is the absolute temperature), then the pair of oppositely charged ions is elevated to the status of an “ion pair” and lasts long enough for multiple collisions. A change from water to methanol ( $D_r = 78$  and  $31.5^{40}$ ) increases the potential energy of the attraction for oppositely charged ions by a factor of 2.5. Ignoring specific changes in solvent effects, there is an increase in the calculated the binding constant according to  $\log(K_b^{\text{MeOH}}/K_b^{\text{H}_2\text{O}}) = (-\Delta G_{\text{ROH}} + \Delta G_{\text{H}_2\text{O}})/2.303RT$  for a hypothetical process of  $M^{x+} + A^{y-} \rightleftharpoons M^{x+} \cdot A^{y-}$  where, for every 1 kcal/mol of electrostatic potential energy of binding in water (all of which is expressed in the  $\Delta G_{\text{H}_2\text{O}}$ ), there is an increase of 12.25-fold in the binding constant in passing into methanol.

While increasing the substrate/catalyst binding to generate more of a reactive complex is one possible way of enhancing the reaction rate for a process such as that given in eq 4, it is absolutely required that the catalyst/substrate complex must have a reduced activation energy for the subsequent phosphoryl transfer reaction. This is an expected situation for a metal-catalyzed reaction where there is charge neutralization of the substrate (as in the reaction of a negatively charged phosphate anion) in the rate-limiting transition state for the reaction. Our earlier<sup>28</sup> and more recent studies indicated that metal ion catalysis of the solvolysis of neutral carboxylate esters and activated amides<sup>41</sup> and neutral phosphate, phosphorothioate, phosphonate, and phosphonothioate esters<sup>42</sup> is profoundly accelerated ( $10^6$ – $10^9$ -fold) in methanol relative to water. The main accelerating effects of the solvent change are proposed to be increased preassociation  $M^{x+}/\text{C}=\text{O}$  or  $M^{x+}/\text{P}=\text{O}$  binding and a changed activity of the metal-bound methoxide which is manifested in the actual acyl/phosphoryl transfer subsequent to the binding process. These experimental observations for a strong medium effect on a metal-ion-catalyzed solvolysis do have established theoretical underpinnings. For example, Amis proposed eq 6<sup>43</sup> for a limiting case involving a head-on approach of an ion to a neutral dipolar molecule from electrostatic considerations, as would be the case for metal ion catalysts and neutral  $\text{C}=\text{O}$  or  $\text{P}=\text{O}$  substrates.

$$\ln k'_{D=D} = \ln k'_{D=\infty} + \frac{Z_e \mu}{D_r k T r^2} \quad (6)$$

This expression relates the natural log of the rate constant in a medium of dielectric constant ( $D_r$ ) to the charge on the ion

- (40) Harned, H. S.; Owen, B. B. *In The Physical Chemistry of Electrolytic Solutions*, 3rd ed.; ACS Monograph Series 137; Reinhold Publishing: New York, 1957; p 161.
- (41) (a) Neverov, A. A.; McDonald, T.; Gibson, G.; Brown, R. S. *Can. J. Chem.* **2001**, *79*, 1704. (b) Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2001**, *40*, 3588. (c) Neverov, A. A.; Brown, R. S. *Can. J. Chem.* **2000**, *78*, 1247. (d) Montoya-Pelaez, P. J.; Brown, R. S. *Inorg. Chem.* **2002**, *41*, 309. (e) Neverov, A. A.; Gibson, G.; Brown, R. S. *Inorg. Chem.* **2003**, *42*, 228. (f) Neverov, A. A.; Sunderland, N. E.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 65.
- (42) (a) Tsang, J. S.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 7602. (b) Tsang, J. S. W.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2004**, *2*, 3457. (c) Liu, T.; Neverov, A. A.; Tsang, J. S. W.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 1525. (d) Lu, Z.-L.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 3379. (e) Lewis, R. E.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 4082. (f) Maxwell, C.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 4329. (g) Melnychuk, S. A.; Neverov, A. A.; Brown, R. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1767.
- (43) (a) Amis, E. S. *Anal. Chem.* **1955**, *27*, 1672; (b) Amis, E. S. *J. Chem. Educ.* **1953**, *30*, 351.

( $Z_e$ ), the dipole moment of the molecule ( $\mu$ ), the separation of the ion and head of the dipole ( $r$ ), Boltzman's constant ( $k$ ), and absolute temperature ( $T$ ). It indicates that the rate constant of the reaction for an oppositely charged metal ion and negative dipole head will increase with a reduction in the dielectric constant of the medium. A more general, but complex, treatment has been developed by Landskroener and Laidler<sup>44</sup> to describe the effect of dielectric constant on reaction velocities for ion–dipole, dipole–dipole, and ion–ion reactions. The general approaches used for these treatments are highly simplified since they are really pertinent only in cases where electrostatic interactions on reactions are more important than specific solvation and other effects.<sup>43,44</sup> Admittedly the catalytic processes generated by the association of the metal ions and neutral or anionic  $\text{C}=\text{O}$  or  $\text{P}=\text{O}$  ester substrates are probably more complicated than those analyzed completely through application of the simple form of eq 6, but the general idea that a reduced dielectric constant does lead to a greater interaction energy bringing the reactants together, and enhanced reaction rates, is well rooted in theory. Although dielectric constant effects clearly influence the ion–ion and ion–dipole association, all the ions are generally less solvated in nonaqueous solutions which can result in increased catalytic effects: in reality, the net effect on the catalytic rate is a composite of effects and requires consideration of several facets in order to arrive at a complete explanation. For convenience, we simply combine all these into the term “medium effect”.

**ii. HPNPP Reaction with  $\text{Zn}([\text{12}] \text{janeN}_3)$  in Methanol.** The upward curvature in  $k_{\text{obs}}$  vs  $[\text{Zn}([\text{12}] \text{janeN}_3)]_t$  plots shown in Figure 1 is seen when the  $[\text{OCH}_3^-]/[\text{Zn}^{2+}]_t$  is 0.3, 0.5, or 0.7 but is not seen when the  $[\text{OCH}_3^-]/[\text{Zn}^{2+}]_t$  ratio is 1. This is consistent with the process bimolecular in  $[\text{Zn}([\text{12}] \text{janeN}_3)]_t$  being dependent on both the neutral  $(\text{CH}_3\text{OH})\text{:Zn}([\text{12}] \text{janeN}_3)$  and basic  $(\text{CH}_3\text{O}^-)\text{:Zn}([\text{12}] \text{janeN}_3)$  forms, probably with the former acting to bind the HPNPP and the latter delivering a  $\text{Zn}^{2+}$ -bound methoxide that acts as a base to deprotonate the 2-hydroxypropyl group. A bimolecular process for  $(\text{Zn}([\text{12}] \text{janeN}_3))_t$  catalysis of HPNPP cyclization is not seen in water<sup>36</sup> indicating that the switch to methanol brings on an additional reaction mechanism. The large rate accelerating effect of the medium switch is already evident in these data, since the activity of  $(\text{CH}_3\text{O}^-)\text{:Zn}([\text{12}] \text{janeN}_3)$  in methanol is roughly 1000 times greater than that of  $(\text{HO}^-)\text{:Zn}([\text{12}] \text{janeN}_3)$  in water for which the  $k_2^{\text{obs}}$  for its reaction with HPNPP is reported to be  $0.018 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>36</sup> The activity of  $(\text{CH}_3\text{O}^-)\text{:Zn}([\text{12}] \text{janeN}_3)$  toward **1** is some 6500 times greater than that of  $\text{CH}_3\text{O}^-$  ( $k_2^{\text{MeO}^-} = 2.56 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ )<sup>29</sup> while the aqueous analogue  $(\text{HO}^-)\text{:Zn}([\text{12}] \text{janeN}_3)$  is 3 times less reactive than is  $\text{HO}^-$  in water ( $k_2^{\text{HO}^-} = 0.065 \text{ M}^{-1} \text{ s}^{-1}$ ).<sup>15</sup> This provides significant evidence that a dual catalytic role for the metal ion<sup>21</sup> is more fully realized in a medium of lower polarity.

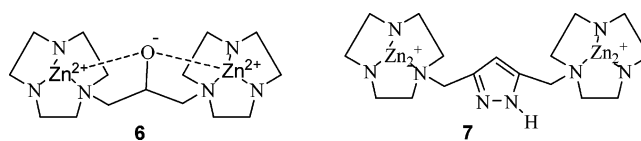
**iii. HPNPP Cyclization and MNPP Methanolysis Promoted by  $\text{Zn}_2([\text{12}] \text{janeN}_3)_2$ .** Tethering the  $\text{CH}_3\text{OH}$  and  $\text{CH}_3\text{O}^-$  forms of the  $\text{Zn}[\text{12}] \text{janeN}_3$  moieties together changes a formally trimolecular process to a bimolecular one with a significant rate enhancement anticipated if the two forms react cooperatively.

- (44) (a) Landskroener, P. A.; Laidler, K. J. *Effects of Solvation of Hydrolysis*. Ph.D. Dissertation, Catholic University of America, Catholic University of America Press: Washington, DC, 1954. (b) Laidler, K. J.; Landskroener, P. A. *Trans. Faraday Soc.* **1956**, *52*, 200.

The dinucleating ligand **3**<sup>45</sup> was prepared, and the catalytic effect of its bis-Zn<sup>2+</sup> complex for hydrolysis of two phosphate diesters was studied by Kim and Lim.<sup>33</sup> We have determined recently the structure of this dinuclear complex by X-ray crystallography<sup>46</sup> showing that the two [12]aneN<sub>3</sub> rings face each other and complex two Zn<sup>2+</sup> ions which are bridged by a single lyoxide anion: also in the unit cell are three triflates for charge neutrality along with a single molecule of methanol. We assume that the lyoxide-bridged form is the one that is present when the catalytically active form is generated in situ through the sequential addition of 1 equiv of **3** and NaOCH<sub>3</sub>, followed by 2 equiv of Zn(OTf)<sub>2</sub>. It is important to note that the in situ formation of the active complex takes about 40–50 min, as judged by the fact that the catalytic activity of the solution continues to rise for that period of time, after which it assumes a constant value for several hours. Such an unusually slow complex formation can be attributed to initial formation of the mononuclear complex of **3**, Zn([12]aneN<sub>3</sub>)<sub>2</sub>, where one Zn<sup>2+</sup> ion is “sandwiched” between two triazamacrocycles within a single ligand molecule as was reported for the bis(triazacyclononyl)propane Zn<sup>2+</sup> complex.<sup>20</sup> Intramolecular dissociation of such a complex and coordination of the second Zn<sup>2+</sup> ion are probably responsible for the relatively slow formation of the thermodynamically stable (CH<sub>3</sub>O<sup>-</sup>):Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>.

As judged by the data in Figure 2, the complex formulated as that above has a remarkable reactivity toward the cyclization of HPNPP. The primary data indicate that at increasing [catalyst] there is a downward curvature in the plot which we have shown is due to inhibitory binding of <sup>-</sup>OTf counterions. Although we have not seen such inhibition of activity of mononuclear Zn-([12]aneN<sub>3</sub>) in this or any previous study of its catalysis of carboxylate or phosphate methanolysis, it is perhaps not surprising that such inhibition exists for the dinuclear complex since it is designed to bind anions like the phosphate diesters and has a higher net positive charge than the mononuclear complexes. When corrected for the inhibition by triflate, a plot of *k*<sub>obs</sub> vs the concentration of free dinuclear complex gives a straight line, the gradient of which is 2.75 × 10<sup>5</sup> M<sup>-1</sup> s<sup>-1</sup>, this being the largest second-order rate constant reported for the cyclization of HPNPP by any catalytic system so-far reported. It is evident that there is a very large cooperative effect for the two metal ions in the dinuclear complex which is brought about by the medium effect in methanol, since the catalyst is 1.1 × 10<sup>8</sup>-fold more reactive toward HPNPP than is methoxide (*k*<sub>2</sub><sup>MeO-</sup> = 2.56 × 10<sup>-3</sup> M<sup>-1</sup>s<sup>-1</sup>).<sup>29</sup> In fact, if we were to compare the reactivity of a 1 mM solution of the catalyst at an operating *s*pH of 9.5, the half time for the catalyzed reaction is 2.5 ms, providing a remarkable acceleration of 2 × 10<sup>12</sup> over the background reaction. Furthermore, the exalted catalytic ability of Zn<sub>2</sub>:**3** is not limited to HPNPP but is also evident for the cleavage of a DNA model, MNPP, as is shown by the saturation kinetic data in Figure 4 which exhibits a *k*<sup>max</sup> value of 4.1 × 10<sup>-2</sup> s<sup>-1</sup> for decomposition of the Zn<sub>2</sub>:**3**:MNPP complex at *s*pH 9.5. Relative to the background methoxide reaction with MNPP (*k*<sub>2</sub><sup>OMe-</sup> = (7.9 ± 0.6) × 10<sup>-7</sup> M<sup>-1</sup>s<sup>-1</sup>) this constitutes an acceleration of 10<sup>12</sup>-fold at that *s*pH. These accelerations

exceed any previously reported catalysis of HPNPP cyclization or phosphate diester cleavage by several orders of magnitude, now approaching the realm of enzymatic catalysis. It is important to emphasize that this sort of catalysis is not seen with dinuclear Zn<sup>2+</sup>-containing catalysts in water, including Zn<sub>2</sub>:**3** in water,<sup>33</sup> and must be a consequence of a medium effect that optimizes substrate binding and catalytic transformation within a substrate/catalyst complex. Several dinucleating complexes<sup>47</sup> of [9]aneN<sub>3</sub> are known, but their Zn<sup>2+</sup> complexes in water are only weakly catalytic toward the hydrolysis of phosphate diesters and toward the cyclization of HPNPP. Richard and Morrow<sup>18</sup> have evaluated several of these and determined that only the di-Zn<sup>2+</sup> complex of 1,3-bis-*N*<sub>1</sub>-(1,3,7-triazacyclononyl)propan-2-ol (**6**) had appreciable activity (120-fold) over its Zn:[9]aneN<sub>3</sub> mononuclear analogue. Several other bis-complexes of the triazacyclononyl system showed only a 3–5-fold larger activity than the mononuclear complex. The



di-Zn<sup>2+</sup> complex **7** is reported<sup>47</sup> to promote the hydrolysis of bis-*p*-nitrophenyl phosphate (BNPP) with observed saturation kinetics at pH 9.2, 35 °C and a *K*<sub>b</sub> and *k*<sub>cat</sub> of 1.2 × 10<sup>-2</sup> M and 2.24 × 10<sup>-6</sup> s<sup>-1</sup> for an overall second-order rate constant of *k*<sub>cat</sub>/*K*<sub>b</sub> = 1.87 × 10<sup>-4</sup> M<sup>-1</sup> s<sup>-1</sup>. However, this value is only 7.5 times that of hydroxide<sup>48</sup> indicating that very little exalted catalysis is evident in water. Finally, Zn<sub>2</sub>:**3** is reported<sup>33</sup> to be no more reactive toward the hydrolysis of BNPP than its mononuclear analogue, indicating there is no cooperativity for this process in water.

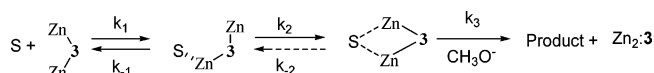
**iv. Mechanisms of Cyclization of HPNPP and Transesterification of Phosphate Diesters Promoted by Zn<sub>2</sub>([12]aneN<sub>3</sub>)<sub>2</sub>.** It is surprising the HPNPP (**1**) and MNPP (**4**), two closely related phosphate diesters in terms of charge, substitution, and size, have such different appearances for the *k*<sub>obs</sub> vs [Zn<sub>2</sub>:**3**] plots as is evidenced by the data in Figures 2 and 4. Of course **1** is far more reactive than **4**, for example, 3200-fold for the methoxide reaction, but it should still require binding to the catalyst to achieve the very large accelerations observed for its cleavage by the dinuclear complex. Diphenyl phosphate also binds to Zn<sub>2</sub>:**3** as is evidenced by its inhibition of the latter's catalysis of the cyclization of HPNPP shown in Figure 5. The inhibition constant for this process is 0.16 ± 0.03 mM which compares favorably with the binding constant of 0.37 ± 0.07 mM determined for MNPP and Zn<sub>2</sub>:**3** from the data in Figure 3, suggesting that the slower-reacting phosphate diesters follow the expected pattern of saturation binding followed by a rate-limiting phosphate cleavage within the complex. As we see no fundamental reason why the faster reacting HPNPP should not bind to Zn<sub>2</sub>:**3** as well as the other phosphates, our simplest explanation of the lack of observed saturation behavior in Figure 2 is that some step preceding the chemical one of cyclization of HPNPP is rate limiting. Shown in Scheme 1 is a working model for catalysis for the binding and reaction of phosphate

(45) Ligand **3** was originally prepared by Weisman, G. R.; Vachon, D. J.; Johnson, V. B.; Gronbeck, D. A. *J. Chem. Soc., Chem. Commun.* **1987**, 886, along with numerous others containing the [12]aneN<sub>3</sub> and [9]aneN<sub>3</sub> binding group.

(46) Lu, Z.-L.; Brown, R. S. To be published.

(47) Vichard, C.; Kaden, T. A. *Inorg. Chim. Acta* **2002**, 337, 173.

(48) The second-order rate constant for OH<sup>-</sup> catalyzed hydrolysis of BNPP at 35 °C is reported to be 2.4 × 10<sup>-4</sup> M<sup>-1</sup> s<sup>-1</sup>.<sup>37</sup>

**Scheme 1.** S=HPNPP or MNPP<sup>a</sup>

<sup>a</sup> Methoxides and solvent omitted for clarity.

diesters promoted by  $\text{Zn}_2\cdot\mathbf{3}$ . The premise is that there are two binding events, a rapid and reversible first binding event, perhaps to form a transient complex with a single  $\text{Zn}^{2+}$  ion in the complex, followed by a rearrangement to form a doubly activated substrate, suggested to involve coordination of both  $\text{Zn}^{2+}$  ions to the phosphate. The latter mode of coordination has been shown to be strongly preferred to optimum metal ion catalysis of the cleavage of phosphate diesters<sup>11</sup> and was also proposed in the  $\text{La}^{3+}$ -catalyzed cleavage of  $(\text{La}^{3+}:\text{HPNPP})_2$ <sup>29</sup>. In the case of the slower reacting **4**, the chemical cleavage step (represented by  $k_3$  and requiring a methoxide probably coordinated to one or both of the metal ions in  $\text{Zn}_2\cdot\mathbf{3}$ ) is relatively slow, so that both the pre-equilibrium steps are established and typical Michaelis–Menten behavior is observed with saturation at higher  $[\text{Zn}_2\cdot\mathbf{3}]$ . On the other hand, with the far more reactive **1** the chemical cyclization step,  $k_3$ , is proposed to be faster than the  $k_{-2}$  step in the concentration range of  $\text{Zn}_2\cdot\mathbf{3}$  used here. In this event, the observed kinetics would be linear in  $[\text{Zn}_2\cdot\mathbf{3}]$  as is the case in Figure 2, with  $k_{\text{obs}} = k_1[\text{Zn}_2\cdot\mathbf{3}]k_2/(k_{-1} + k_2)$ .

Such a mechanism is also consistent with the base dependent behavior demonstrated in Figure 6 for the reaction of **4** and **1** catalyzed by  $\text{Zn}_2\cdot\mathbf{3}$ . The unusual increase in rate constant for the HPNPP reaction that accompanies addition of acid is not consistent with the cyclization step ( $k_3$ ) being rate limiting. The combination of substrate binding to the metal ion and a basic role involving methoxide would necessarily have to maximize when the  $[\text{CH}_3\text{O}^-]/[\text{Zn}_2(\text{[12]aneN}_3)_2]$  ratio is unity as in the case of MNPP. Rather, the increase in rate with added acid is more consistent with a process depending on binding the substrate to a greater amount of a complex devoid of an associated methoxide with its higher net positive charge attracting the negatively charged HPNPP. This requires that the chemical step of methoxide-dependent cyclization would be faster than the rearrangement step throughout most of the plot. However, at some point where the  $\text{pH}$  falls below a critical value and the complex contains little or no methoxide, the rate of the cyclization step drops and becomes rate limiting which accounts for the discontinuity in the plot at the low [methoxide]/[complex] ratio.

The simplified process described in Scheme 2 is consistent with the observed behavior of catalysis for HPNPP and MNPP cleavage promoted by  $\text{Zn}_2\cdot\mathbf{3}$  and introduces another aspect of the medium effect that accelerates the chemical step of HPNPP cleavage to such an extent that it is no longer rate limiting with this substrate. Instead, the reaction now seems to be limited by an aspect of substrate binding, a phenomenon which is often observed in enzymatic systems where the catalytic steps are competitive with the binding steps. While the second-order rate constant of  $2.75 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  for the HPNPP reaction seems

somewhat slow for a binding step involving simple ligand exchange on  $\text{Zn}^{2+}$  (known to be  $10^7$  to  $10^8 \text{ M}^{-1} \text{ s}^{-1}$ ), it is well within the range for more complex ions in which an internal rearrangement or breaking of intramolecular hydrogen bonds occurs, for example, in salicylate ( ${}^-\text{O}_2\text{C}-\text{R}-\text{OH}$  similar to  ${}^-\text{O}_2\text{P}(\text{OR}')\text{O}-\text{R}-\text{OH}$  in the HPNPP anion) binding to  $\text{Zn}^{2+}$  where the reported rate constant for ligand exchange in water is  $1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>49</sup>

## Conclusion

In sum, we have demonstrated herein that the combination of a dinuclear  $\text{Zn}^{2+}$  complex and a medium effect gives a system exhibiting spectacular rate accelerations for the cleavage of both an RNA and a DNA model. While it is well-known that the medium can have a significant effect on reaction rates, we have shown that this is particularly dramatic for metal-catalyzed reactions including those of phosphate diesters in methanol. In promoting HPNPP cleavage, the  $(\text{CH}_3\text{O}^-):\text{Zn}_2(\text{[12]aneN}_3)_2$  complex exhibits a  $k_2$  value of  $2.75 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  that is  $10^8$  times larger than that for the methoxide reaction. At  $\text{pH}$  9.5 a 1 mM solution of the catalyst gives a 2.5 ms half-time that reflects an unprecedented acceleration of  $2 \times 10^{12}$  times the background reaction! The exalted activity is not unique to the HPNPP system, as  $\text{Zn}_2\cdot\mathbf{3}$  accelerates cleavage of the far less reactive MNPP phosphate diester at  $\text{pH}$  9.5 also by a factor of  $10^{12}$ -fold relative to its background reaction. According to our suggested mechanism, the actual chemical step of HPNPP cyclization is even faster, but the reaction is limited by a prior process suggested to be one of a change in the mode of the complex's interaction with HPNPP. The fact that an acceleration approaching the rates seen for enzymatic cleavage of RNA can be realized with such a simple system suggests to us that the more organic nature of the active site of an enzyme with its reduced dielectric constant may play a crucial role in achieving the catalytic rates observed. While we have observed before that the nonbiologically relevant  $\text{La}^{3+}$  ion promotes a similarly large acceleration of HPNPP cleavage in methanol, to our knowledge this is the first demonstration of this concept in a biologically relevant metal-catalyzed reaction.

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