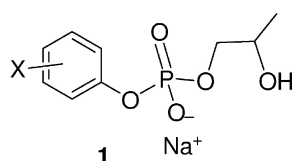


## Biomimetic Cleavage of RNA Models Promoted by a Dinuclear Zn(II) Complex in Ethanol. Greater than 30 kcal/mol Stabilization of the Transition State for Cleavage of a Phosphate Diester

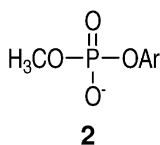
C. Tony Liu, Alexei A. Neverov, and R. Stan Brown

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- 1a. X = 4-NO<sub>2</sub>  
 b. X = 4-NO<sub>2</sub>, 3-CH<sub>3</sub>  
 c. X = 3-NO<sub>2</sub>  
 d. X = 4-Cl  
 e. X = 3-OCH<sub>3</sub>  
 f. X = H,  
 g. X = 4-OCH<sub>3</sub>



- |                                      |                              |
|--------------------------------------|------------------------------|
| a. 2, 4-dinitrophenyl                | h. 2-nitro-4-methoxyphenyl   |
| b. 2-chloro-4-nitrophenyl            | i. 3-nitrophenyl             |
| c. 4-chloro-2-nitrophenyl            | j. 4-chlorophenyl            |
| d. 2-(methoxycarbonyl)-4-nitrophenyl | k. 3-methoxyphenyl           |
| e. 2, 4, 5-trichlorophenyl           | l. 2-(methoxycarbonyl)phenyl |
| f. 4-nitrophenyl                     | m. phenyl                    |
| g. 2-nitrophenyl                     | n. 4-methoxyphenyl           |

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# Biomimetic Cleavage of RNA Models Promoted by a Dinuclear Zn(II) Complex in Ethanol. Greater than 30 kcal/mol Stabilization of the Transition State for Cleavage of a Phosphate Diester

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**Abstract:** The cleavage of a series of seven substituted aryl 2-hydroxypropyl phosphates (**1a–g**) promoted by a dinuclear Zn(II) complex (**3**:Zn(II)<sub>2</sub>(<sup>-</sup>OCH<sub>2</sub>CH<sub>3</sub>) was investigated in ethanol at  $\text{pH } 9.0 \pm 0.2$  and 25 °C. The kinetics for appearance of the product phenols follow very strong saturation behavior for all substrates where the dissociation constant of the bound complex has an upper limit of  $K_m = 3 \times 10^{-7}$  M and the  $k_{\text{cat}}^{\text{max corr.}}$  values (corrected for triflate inhibition) range from 168 to 3 s<sup>-1</sup>. A partial  $\text{pH}/\log k_{\text{cat}}^{\text{max corr.}}$  profile for the **3**:Zn(II)<sub>2</sub>(<sup>-</sup>OCH<sub>2</sub>CH<sub>3</sub>)-catalyzed reaction of **1e** (3-methoxyphenyl 2-hydroxypropyl phosphate) is bell-shaped, plateauing from 7.9–10, and is fit to a two kinetically important ionizations having  $\text{p}K_a$  values of 7.22 and 10.9. The Brønsted plot of  $\log(k_{\text{cat}}^{\text{max corr.}})$  vs the  $\text{p}K_a$  values for the phenols shows a break at about 14.3 with two  $\beta_{\text{lg}}$  values of  $-1.12$  and  $0.0$ . This is analyzed in terms of a change in rate limiting step from cleavage of the phosphate to a conformational change where the binding of the phosphate changes from one P–O----Zn(II) interaction to a Zn(II)----O–P–O---Zn(II) double activation. An energetics calculation comparing the ethoxide promoted cleavage of **1a–g** with the **3**:Zn(II)<sub>2</sub>(<sup>-</sup>OEt) promoted reaction indicates that the complex, **3**:Zn(II)<sub>2</sub>, stabilizes the ethoxide plus substrate transition state for the cleavage of **1a–g** by between 33 and 36 kcal/mol. The origins of the large stabilization are discussed in terms of the effect of the medium on the various rate and equilibrium constants involved.

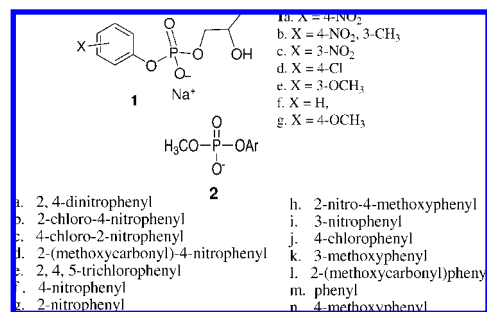
## 1. Introduction

The stability of phosphate diesters toward solvolytic cleavage is vitally tied to the biological roles of DNA and RNA as guardians of genetic information in all living systems. In the absence of a catalyst or an enzyme, phosphate diesters are extremely stable toward solvolytic cleavage but in the presence of phosphodiesterase enzymes rate accelerations of  $10^{15-17}$ -fold<sup>1</sup> are achieved. Many of these enzymes contain metals ions such as Zn<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, and Mn<sup>2+</sup> and the efficiency of such

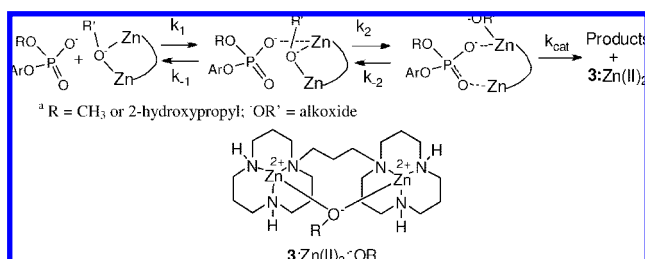
metalloenzymes has, not surprisingly, sparked much effort in constructing simpler catalytic systems for the cleavage of phosphate esters.<sup>2,3</sup>

Recent work from this laboratory<sup>4,5</sup> has focused on the catalytic cleavage of a series of aryl 2-hydroxypropyl phosphates (**1a–g**; simplified models of RNA) and aryl methyl phosphates (**2a–n**; models of DNA) promoted by the dinuclear Zn(II) and Cu(II) complexes of 1,3-bis-*N*<sub>1</sub>,*N*'-(1,5,9-triazacyclododecyl)propane (**3**) in methanol. Detailed mechanistic investigation led to the proposal that the catalyzed cleavage follows a multistep pathway consistent with a minimal process given in Scheme 1 consisting of a bimolecular binding step of the catalyst to the phosphate followed by a rearrangement to

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form the catalytically active species. This is followed by one or more chemical steps that result in the production of the aryloxy leaving group and a corresponding methoxylated

Scheme 1<sup>a</sup>

<sup>a</sup> R = CH<sub>3</sub> or 2-hydroxypropyl; <sup>-</sup>OR' = alkoxide.

phosphate.<sup>4,5</sup> For both of these systems, the synergy created between a highly active dinuclear **3**:Zn(II)<sub>2</sub>(<sup>-</sup>OCH<sub>3</sub>) catalyst and the methanol solvent accelerates the cleavage of phosphate diesters by 10<sup>11–13</sup> times relative to the methoxide promoted background reactions at *s*pH 9.8 and 25 °C. That the rate enhancement far exceeds anything reported for this<sup>6</sup> or related catalytic RNase or DNase models in water<sup>2,3,7</sup> seems to be intimately tied to the reduced dielectric constant/polarity of the medium. Very recent work has shown that a different sort of medium effect provided by 80% DMSO/water confers very large rate accelerations of 2.7 × 10<sup>9</sup> to 4.4 × 10<sup>10</sup> for the Eu(III) and La(III) catalyzed hydrolysis of **1a** at essentially neutral pH in that medium.<sup>8</sup>

It seems possible that a further reduction of polarity/dielectric constant, while still retaining significant hydrogen bonding such as would be occasioned with ethanol, (dielectric constant  $\epsilon = 31.5$  and 24.3 for methanol and ethanol respectively)<sup>9</sup> might lead to even higher catalytic rate enhancements for the cleavage of phosphate diesters by **3**:Zn(II)<sub>2</sub>(<sup>-</sup>OR).<sup>10</sup> Herein we report a comprehensive study of the catalytic cleavage of diesters **1a–g** promoted by **3**:Zn(II)<sub>2</sub> in anhydrous ethanol (99.9%). As will be seen, the kinetic pathways for all substrates proceed with a very strong substrate/catalyst saturation binding followed by a rate-limiting  $k_{\text{cat}}^{\text{max}}$  process which is shown to change from a chemical one for substrates with poor leaving groups, to a conformational change for those with good leaving groups. These are very fast reactions having  $k_{\text{cat}}^{\text{max}}/K_{\text{m}}$  terms 10<sup>12</sup> to 10<sup>14</sup> times larger than the corresponding second order rate constants for the ethoxide catalyzed reactions. Finally, in order to provide a deeper understanding of the catalytic process, we provide energetics calculations to determine the contributions of each of the kinetic and thermodynamic terms toward the acceleration achieved.

## 2. Experimental Section

**2.1. Materials.** Sodium ethoxide (21 wt % solution in denatured ethanol, titrated against N/50 Fisher Certified standard aqueous HCl solution and found to be 2.68 M) and Zn(CF<sub>3</sub>SO<sub>3</sub>)<sub>2</sub>, were purchased from Aldrich and used without further purification. Tetrabutylammonium ethoxide in ethanol (~40%, titrated against 1 N Fisher certified standard aqueous HCl solution and found to be 1.08 ±

**Table 1.** Table of the Wavelengths Used to Monitor the **3**:Zn(II)<sub>2</sub>(<sup>-</sup>OCH<sub>3</sub>CH<sub>3</sub>)-Catalyzed Reactions ( $\lambda_{\text{cat}}$ ) and the Base Promoted Reactions ( $\lambda_{\text{base}}$ ) of Phosphates **1** in Ethanol at 25 °C

phosphate	1a	1b	1c	1d	1e	1f	1g
$\lambda_{\text{cat}}$ (nm)	320	323	340	284	282	280	292
$\lambda_{\text{base}}$ (nm)	401	399	407	305			

0.01 M) was obtained from Fluka. HClO<sub>4</sub> (70% aqueous solution, titrated to be 11.40 M) was purchased from Acros Organics and used as supplied. Anhydrous ethanol was purchased from Commercial Alcohols Inc. and was degassed by bubbling Ar through it for 1 h before storing it under Ar. The degassed ethanol was freshly dispensed prior to each set of kinetic experiments. Freshly dispensed ethanol was kept for a maximum duration of 1 h in an oven-dried, capped Erlenmeyer flask sealed with Parafilm between uses. The [H<sub>2</sub>O] in the freshly dispensed degassed ethanol was found to be 0.028 ± 0.007 M using a Mettler Toledo DL32 Karl Fischer Coulometer, while the [H<sub>2</sub>O] for the ethanol that has been kept in an Erlenmeyer flask for 1 h as described above (and used for experiments) was determined to be 0.029 ± 0.007 M. The sodium salts of aryl 2-hydroxypropyl phosphates (**1a–g**) were prepared and characterized as described earlier.<sup>4</sup> 1,3-Bis-*N*<sub>1</sub>,*N*<sub>1</sub>'-(1,5,9-triazacyclododecyl)propane (**3**) was prepared as described.<sup>5b</sup> The dinuclear **3**:Zn(II)<sub>2</sub>(<sup>-</sup>OCH<sub>2</sub>CH<sub>3</sub>) complexes were prepared as 2.5 mM stock solutions in degassed absolute ethanol by sequential addition of aliquots of stock solutions of sodium ethoxide, 1,3-bis-*N*<sub>1</sub>,*N*<sub>1</sub>'-(1,5,9-triazacyclododecyl)propane, and Zn(CF<sub>3</sub>SO<sub>3</sub>)<sub>2</sub> in stoichiometric ratios of 1:1:2. The complete formation of the active *dizinc* complex is achieved only after 50 min in ethanol (as monitored by the change in catalytic activity over time. This same phenomenon was observed for creation of the active forms of the *di*Zn(II) and *di*Cu(II) catalysts in methanol<sup>4,5</sup>).

**2.2. Methods.** <sup>1</sup>H NMR and <sup>31</sup>P NMR spectra were determined at 400 and 162.04 MHz. The CH<sub>3</sub>OH<sub>2</sub><sup>+</sup> and CH<sub>3</sub>CH<sub>2</sub>OH<sub>2</sub><sup>+</sup> concentrations were determined using a combination glass electrode (Radiometer model XC100-111-120-161) calibrated with Fisher Certified standard aqueous buffers (pH = 4.00 and 10.00) as described in a previous paper.<sup>11</sup> *s*pH values in ethanol were determined by subtracting a correction constant -2.54 from the readings obtained from the electrode, and the autoprotolysis constant of ethanol ( $K_{\text{auto}}$ ) is taken to be 10<sup>-19.1</sup>.<sup>11</sup>

Literature *s*pK<sub>a</sub> values<sup>12</sup> of a series of different substituted phenols in ethanol and the measured half-neutralization *s*pK<sub>a</sub> values of *p*-nitrophenol and 2,4-dinitrophenol (0.5 mM of the phenols and 0.25 mM of NaOCH<sub>2</sub>CH<sub>3</sub> in degassed absolute ethanol), were plotted against the aqueous pK<sub>a</sub> values and found to fit a linear relationship,  $s\text{p}K_{\text{a}}^{\text{EtOH}} = (1.24 \pm 0.01)s\text{p}K_{\text{a}}^{\text{HOH}} + (3.2 \pm 0.1)$  (11 phenols;  $r^2 = 0.9990$ ) This relationship was used to interpolate the *s*pK<sub>a</sub><sup>EtOH</sup> values for the corresponding phenols of **1b,c,g** in ethanol.

**2.3. Kinetics of Transesterification of 1a–g in Ethanol.** The transesterification of phosphates **1a–g** in degassed absolute ethanol were followed by following the rates of the appearance of the corresponding phenolic products by regular and stopped-flow UV/visible spectrophotometry at 25.0 ± 0.1 °C at the wavelengths listed in Table 1.

For the complex-catalyzed reactions, a 2.5 mM stock solution of **3**:Zn(II)<sub>2</sub>(<sup>-</sup>OCH<sub>3</sub>CH<sub>3</sub>) in degassed absolute ethanol was prepared in a capped and sealed (with Parafilm) oven-dried vial under N<sub>2</sub> at ambient temperature 1 h prior to the kinetic experiments to ensure the complete formation of the catalyst complex. This solution was used to prepare solutions of the catalyst with concentrations ranging from 0.02 mM ≤ [**3**:Zn(II)<sub>2</sub>(<sup>-</sup>OCH<sub>2</sub>CH<sub>3</sub>)] ≤ 0.2 mM, which were

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then loaded into one syringe of the stopped-flow reaction analyzer. Solutions of  $(8-16) \times 10^{-5}$  M of substrates **1a-g** in ethanol were loaded into the second syringe. The final concentration of the phosphates was  $(4-8) \times 10^{-5}$  M. At each  $[3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)]$  the pseudo-first-order rate constants ( $k_{\text{obs}}$ ) were evaluated by fitting the UV/vis absorbance vs time traces to a standard exponential model. All reactions were followed to at least three half-times and found to exhibit good first-order rate behavior. At least five kinetic runs were conducted at each  $[3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)]$ , with the reported  $k_{\text{obs}}$  values reported in Supporting Information being the average.

To determine the stability of the catalyst in the reaction medium,  $8 \times 10^{-5}$  M of **1a** in ethanol was loaded into one of the syringes in the stopped-flow reaction analyzer. An ethanol solution containing 0.8 mM of  $3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)$  was loaded into the other syringe. Judging from the rate of appearance of the phenol product at 320 nm at different times over 200 min, there was no decomposition of the catalyst during this time.

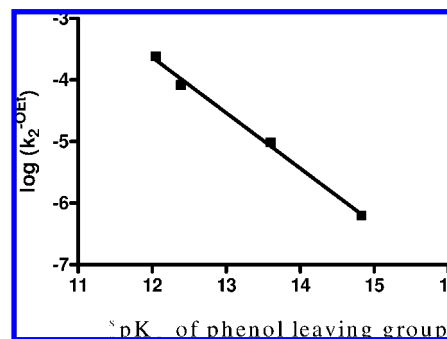
During the above kinetic experiments, the ionic strength was not controlled nor was the  $\text{pH}$  controlled by buffers as we have found that the associated anions of these heavily inhibit the reactions. Thus, the  $\text{pH}$  values were set by the catalytic system itself and were generally found to be  $9.0 \pm 0.2$  in the plateau region of the  $k_{\text{obs}}$  vs  $[3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)]$  plots. However, a  $\text{pH}/\log$  rate constant profile for the cleavage of **1e** catalyzed by  $3:\text{Zn}(\text{II})_2$  in ethanol was conducted in the following manner. First, varying amounts of  $\text{NaOCH}_2\text{CH}_3$  or  $\text{HClO}_4$  stock solutions (5 mM) in ethanol were added to solutions containing  $2 \times 10^{-4}$  M of a preformed  $3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)$  complex in ethanol which had been prepared an hour in advance to allow for complete formation of the catalyst complex. After the introduction of the additional acid or base the mixture was allowed to stand for 30 min to equilibrate (independent experiments showed that this time was optimum to attain the maximum kinetic activity) and then loaded into one of the two syringes of the stopped-flow analyzer while a  $1.6 \times 10^{-4}$  M solution of **1e** in ethanol was loaded into the other. The final concentrations after mixing in the reaction chamber were  $1 \times 10^{-4}$  M of  $3:\text{Zn}(\text{II})_2$  and  $8 \times 10^{-5}$  M of **1e** in ethanol. The  $\text{pH}$  values were measured at the end of the reactions. The  $\text{pH}/\log$  rate constant profile plot given in Figure 5 shows a broad plateau from  $\text{pH}$  7.9–10, with the line through the data being derived from a fit of the solid squares data to a process that depended on two ionizable groups having  $\text{p}K_a$  values of 7.2 and 10.8.

To determine the magnitude and type of  $\text{CF}_3\text{SO}_3^-$  inhibition, a  $4 \times 10^{-5}$  M solution of **1a** was premixed with varying concentrations of tetrabutylammonium triflate so that the final [triflate ion] ranged from  $8 \times 10^{-4} - 4.8 \times 10^{-3}$  M, and the rate of the reaction in the presence of 0.2 mM of  $3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)$  at each [triflate] was monitored in duplicate in ethanol, ( $\text{pH} = 8.54-8.85$ ).

The rates of the ethoxide-catalyzed reactions of **1a-d** ( $(1-2) \times 10^{-4}$  M) were determined by UV/vis spectrophotometry at  $25.0 \pm 0.1$  °C in the presence of tetrabutylammonium ethoxide at various concentrations between 0.004 and 0.4 M in degassed absolute ethanol. The kinetic data were analyzed by the initial rate method in which the first 2–10% of the Abs. vs time traces for appearance of phenolate products were fitted to a linear regression and the so-obtained rates converted to first-order rate constants ( $k_{\text{obs}}$ ) by dividing them by the expected absorbance change if the reaction were to reach 100% completion. The reactions were carried out in duplicate and the plots of the average first-order rate constants ( $k_{\text{obs}}$ ) vs [tetrabutylammonium hydroxide] were fitted to a standard linear regression model to find the second-order rate constants ( $k_2^{-\text{OEt}}$ ).

### 3. Results

**3.1. Ethoxide-Catalyzed Transesterification of 1a-d in Ethanol.** The ethoxide promoted cleavages of **1a-d** in ethanol at  $25.0 \pm 0.1$  °C were studied under pseudo-first-order conditions of excess  $[\text{CH}_3\text{CH}_2\text{O}^-]$  and the kinetics of formation of



**Figure 1.** Brønsted plot of  $\log(k_2^{-\text{OEt}})$  vs the  $\text{p}K_a$  values for the  $3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)$ -catalyzed cleavage of **1a-d** in degassed absolute ethanol at  $25.0 \pm 0.1$  °C. The data fit a standard linear regression of  $k_2^{-\text{OEt}} = (-0.90 \pm 0.04) \text{p}K_a + (7.2 \pm 0.6)$ ;  $r^2 = 0.9955$ .

the phenolate products were monitored by initial rate methods due to the slowness of the reactions. Phosphates **1e-g** react too slowly in base to determine the rate constants accurately in a reasonable time. In Figure 1 is a plot of the second order rate constants ( $k_2^{-\text{OEt}}$ ) for substrates **1a-d** vs the  $\text{p}K_a$  of the corresponding phenols in ethanol (see Supporting Information). These data were fit by linear regression as  $k_2^{-\text{OEt}} = (-0.90 \pm 0.04) \text{p}K_a + (7.2 \pm 0.6)$ ,  $r^2 = 0.9955$ ;  $n = 4$ , which was used to estimate the rate constants for **1e-g** given in Table 2.

**3.2.  $3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)$ -Promoted Transesterification of 1 in Ethanol.** Shown in Figure 2 is a representative plot of the uncorrected  $k_{\text{obs}}$  for reaction of **1a-g** vs total  $[3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)]_t$  put into the solution. The plots for all the substrates follow the same general appearance, in passing from substoichiometric amounts of catalyst to a roughly 2-fold excess of catalyst/substrate. All the plots show an apparent  $x$ -intercept and very strong 1:1 saturation binding superimposed on an inhibition curve that depends upon the increasing  $[\text{OTf}]$  (each equivalent of catalyst brings with it 4 equiv of triflate).

We have previously demonstrated that triflate anion is a competitive inhibitor of the catalysis of phosphate diesters exhibited by  $3:\text{Zn}(\text{II})_2:(\text{OCH}_3)$  with a  $K_i = 14.9$  mM in anhydrous methanol.<sup>5b</sup> In ethanol, the affinity of triflate for the positively charged catalyst is enhanced and analysis of the  $k_{\text{obs}}$  vs  $[\text{OTf}]$  data shown in Figure 3 for the  $3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3)$  promoted reaction of **1a** in ethanol gives an inhibition constant of  $K_i = (0.36 \pm 0.02)$  mM. The analysis of the data is somewhat complicated and requires explanation to account for the appearance of the primary data plot in Figure 2 and the considerable inhibition provided by triflate.

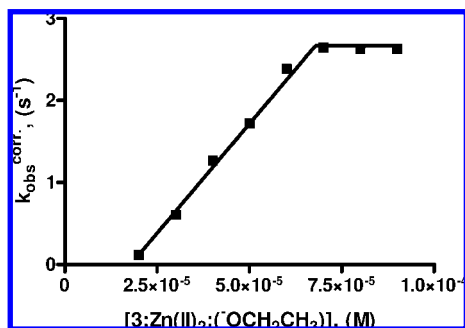
The appearance of the triflate inhibition plot in Figure 3 suggests that this anion is an uncompetitive<sup>13</sup> inhibitor following the simplified process given in Scheme 2 where  $K_i$  refers to the dissociation constant for the triflate inhibited complex,  $(3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3):1:(\text{OTf}))$  in units of mM. The scheme is based on the assumption that the binding of the phosphate is far larger than that of triflate, and that there are two substrate-bound forms of the catalyst, namely  $(3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3):1)_{\text{free}}$ , which leads to the product, and a triflate bound form,  $(3:\text{Zn}(\text{II})_2:(\text{OCH}_2\text{CH}_3):$

(13) Nelson, D. L.; Cox, M. M. *Lehninger Principles of Biochemistry*, 3rd ed.; Worth Publishers: New York, 2000; pp 266–268.

(14) Since the triflate binding is quite strong, the more accurate method for determining the  $K_i$  is to use a variant of the universal binding equation (see Supporting Information). However, using this form lowers the values of the  $k_{\text{obs}}^{\text{corr}}$  by only 4%, which is within the experimental uncertainty, so we have opted to use simplified method of eqs 1 and 2.





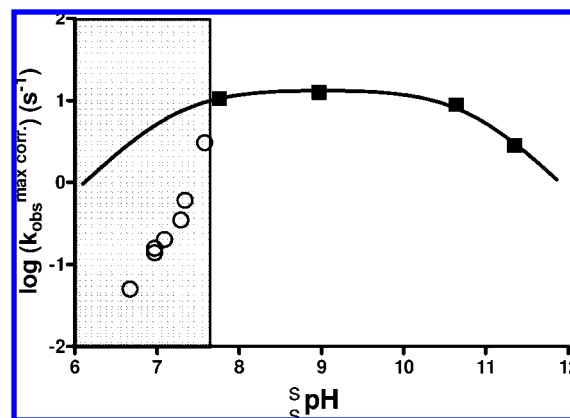


**Figure 4.** Plot of  $k_{\text{obs}}^{\text{corr.}}$  vs  $[3:\text{Zn}(\text{II})_2:(^-)\text{OCH}_2\text{CH}_3]$  for the catalyzed cleavage of **1g** ( $5 \times 10^{-5}$  M) at 292 nm and 25 °C in absolute ethanol. The raw data (Figure 2) was corrected for triflate inhibition using eqs 1 and 2. By fitting the corrected data to eq 3, one gets the line through the data with the maximum rate constant ( $k_{\text{obs}}^{\text{max corr.}}$ ) determined to be  $2.67 \pm 0.06 \text{ s}^{-1}$  and  $A = (1.78 \pm 0.01) \times 10^{-5} \text{ M}$ .

for the  $3:\text{Zn}(\text{II})_2:(^-)\text{OCH}_2\text{CH}_3$ -catalyzed cleavage of each of **1a–g** in ethanol (see Supporting Information) and the kinetic data are fitted to eq 3 to determine the  $k_{\text{cat}}^{\text{max corr.}}$  values (after correcting for triflate inhibition): these are presented in Table 2.

Since the binding of the phosphate to the complex is so strong, accurate values for  $K_B$  cannot be obtained from the data at hand. However an upper limit can be estimated through an iterative procedure where the  $K_B$  value is increased until the goodness of fit maximized. For all substrates, the goodness of the fits did not change when the  $K_B$  value exceeded  $10^{6.5} \text{ M}^{-1}$ , so we have assumed an upper limit for the binding constant for all substrates of  $K_B = 3.2 \times 10^6 \text{ M}^{-1}$  (correspondingly  $K_m = 3.2 \times 10^{-7} \text{ M}$ ).

A  $\text{pH}$  vs  $\log k_{\text{obs}}^{\text{max corr.}}$  profile (Figure 5) was constructed for the reaction of **1e** promoted by  $3:\text{Zn}(\text{II})_2$  as follows. A  $2 \times 10^{-4} \text{ M}$  solution of the catalyst ( $3:\text{Zn}(\text{II})_2:(^-)\text{OCH}_2\text{CH}_3$ ) was prepared in ethanol, as described in the experimental section, and then treated with varying amounts of  $\text{HClO}_4$  or  $\text{NaOEt}$  to vary the  $\text{pH}$ . The mixtures were allowed to equilibrate for 30 min and then mixed by stopped-flow with  $1.6 \times 10^{-4} \text{ M}$  of phosphate **1e**, and the kinetics of the reaction were monitored (final concentrations were half-those in the syringes). At these concentrations all the substrate is bound to catalyst so the observed rate constant corresponds to  $k_{\text{obs}}^{\text{max}}$  which is then numerically corrected for triflate inhibition. Following the reaction, the  $\text{pH}$  values of the mixtures were measured and assumed to be representative of those during the reaction. The  $\text{pH}/\log k_{\text{obs}}^{\text{max corr.}}$  plot of Figure 5 shows a hint of bell-shape with a broad plateau between  $\sim \text{pH}$  7.9 and 10, and when the solid squares data are fit to a two  $\text{pK}_a$  model (Scheme 3, eq 2S in Supporting Information), this gives a computed  $k_{\text{cat}}^{\text{max corr.}}$  of  $13.7 \text{ s}^{-1}$  (compare with the value of  $14.5 \pm 0.3 \text{ s}^{-1}$  in Table 2 determined in a different way) and  $\text{pK}_a^1$  and  $\text{pK}_a^2$  values of 7.2 and 10.8.<sup>16</sup> Notable is the observation that below  $\text{pH}$  7.7, the catalytic activity drops more precipitously than theory predicts, suggesting that the catalyst is not stable once the



**Figure 5.**  $\text{pH}/\log k_{\text{obs}}^{\text{max corr.}}$  profile for the reaction of  $3:\text{Zn}(\text{II})_2:(^-)\text{OCH}_2\text{CH}_3$  and **1e** conducted at 25 °C at respective concentrations of  $1 \times 10^{-4} \text{ M}$  and  $8 \times 10^{-5} \text{ M}$ . The line through the square data points is computed on the basis of the process given in Scheme 3 having a bell-shaped  $\text{pH}/\log k_{\text{obs}}^{\text{max corr.}}$  profile giving two fitted  $\text{pK}_a$  values of  $7.2 \pm 0.4$  and  $10.8 \pm 0.1$ . The shaded data below  $\text{pH}$  7.7 were not used for fitting.

removal of the ethoxide (or its kinetic equivalent) commences. Therefore, none of the data in the shaded area was used for any fits.

## 4. Discussion

**4.1. Ethoxide-Promoted Reactions of Phosphates 1.** The Figure 1 Brønsted plot for the ethoxide-promoted reactions of **1a–e** in ethanol has a  $\beta_{\text{lg}}$  value of  $-0.90 \pm 0.04$  which is slightly larger than the  $-0.72 \pm 0.08^4$  observed for the methoxide-promoted cyclization of **1a–g** in methanol and the hydroxide-promoted cyclization of 2-hydroxypropyl aryl phosphates in water ( $\beta_{\text{lg}}$  value of  $-0.62$ ).<sup>17</sup> There is considerable debate about whether the cleavage of phosphate diesters of type **1** is concerted or stepwise.<sup>18,19</sup> However, a recent study of the  $^-$ OH-promoted cleavage of uridine 3'-phosphate esters in water suggested a stepwise cleavage mechanism with the rate-limiting step with good leaving groups being the cyclization step ( $\beta_{\text{lg}} = -0.52$ ) while that with poor leaving groups being the breakdown of the phosphorane ( $\beta_{\text{lg}} = -1.34$ ).<sup>20</sup> In the latter report<sup>20</sup> it was suggested that the original data for the base promoted cyclization of 2-hydroxypropyl aryl and alkyl phosphates<sup>21</sup> could be reinterpreted as being consistent with a stepwise process involving a five-membered cyclic phosphorane intermediate. The change in the rate-limiting step observed for such stepwise reactions occurs at the quasi-symmetrical point where the  $\text{pK}_a$  of the leaving group (HOR) is approximately the same as the  $\text{pK}_a$  of the nucleophilic 2-hydroxypropyl group. Since the  $\text{pK}_a$  of the corresponding phenol leaving groups of phosphates **1a–d**

(15) 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, 2003.

(16) The first  $\text{pK}_a$  is not well-defined by the data of Figure 5 due to the presumed decomposition of the catalyst. However, an experimentally similar  $\text{pH}$  vs  $\log k_{\text{obs}}$  profile is seen for the cleavage of a series of aryl methyl phosphate esters in ethanol catalyzed by  $3:\text{Zn}(\text{II})_2:(^-)\text{OEt}$   $\text{pK}_a^1 = \sim 7$  and  $\text{pK}_a^2 = 10.8$ ; to be published.

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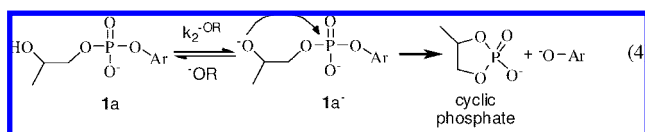
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(22) Sánchez-Lombardo and Yatsimirsky have estimated the  $\text{pK}_a$  of the 2-hydroxy group in **1a** as 14.9 in water. Based on the relationship  $\text{pK}_a^{\text{EtOH}} = (1.24 \pm 0.01)\text{pK}_a^{\text{H}_2\text{O}} + (3.2 \pm 0.1)$  given in the Experimental Section herein, the computed  $\text{pK}_a^{\text{EtOH}}$  is 21.7.

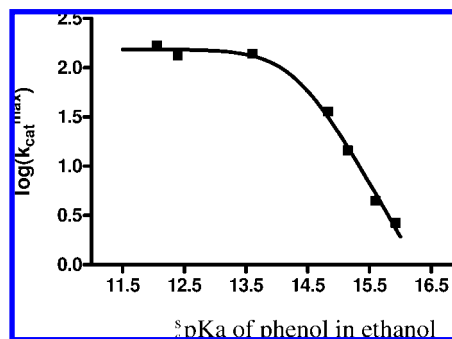
are all lower than the  $\text{p}K_{\text{a}}$  of the 2-hydroxypropyl group (estimated to be about 21.7 in ethanol)<sup>22</sup> the  $k_2^{-\text{OEt}}$  constant for cyclization of all of **1a–g** should all fall on a Brønsted line corresponding to rate-limiting formation of a phosphorane intermediate. Assuming that the  $\beta_{\text{eq}}$  of  $-1.74$  for the transfer of the phosphoryl group between oxyanions in water<sup>23</sup> can be extrapolated to ethanol, the Leffler parameter,  $\alpha = \beta_{\text{lg}}/\beta_{\text{eq}} = 0.52$  for the ethoxide reactions of phosphates **1a–g** in ethanol, suggests that in the transition state for cyclization the P–OAr bonding character progress some 52% of the way from the starting material to phenolate product with the aryloxy oxygen now having a net charge of  $\sim -0.16 = (+0.74 - 0.90)$  in the TS. Should the process really be concerted and proceed via a single TS, the analysis would be essentially the same suggesting that the TS is central, about halfway between starting material and product.

It is an expected consequence of reduced dielectric constant/polarity that the rates of reactions between species of the same charge type are retarded, while those between oppositely charged species are accelerated. For the lyoxide-promoted reaction of **1a** shown in eq 4,  $k_2^{-\text{OEt}}$  is  $2.4 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1}$ ,  $k_2^{-\text{OMe}}$  is  $2.6 \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}$ <sup>24</sup> and that for the hydroxide promoted cyclization is reported to be  $9.9 \times 10^{-2} \text{ M}^{-1}\text{s}^{-1}$ <sup>25</sup> or  $6.5 \times 10^{-2} \text{ M}^{-1}\text{s}^{-1}$ .<sup>26</sup> The 10-fold rate reduction in ethanol relative to methanol is a consequence of the reduced polarity/dielectric constant which opposes the pre-equilibrium formation of the dianionic form (**1a<sup>-</sup>**).



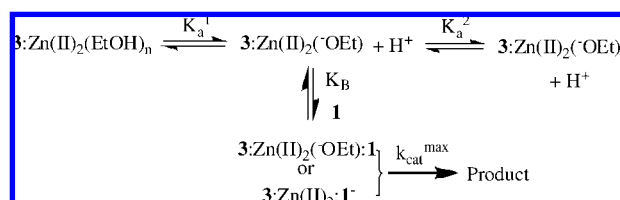
As will be shown later, this same reduced dielectric constant/polarity effect considerably enhances the reactions of substrates **1** when they are bound to the positively charged **3:Zn(II)<sub>2</sub>:(-OCH<sub>2</sub>CH<sub>3</sub>)** catalyst as **3:Zn(II)<sub>2</sub>:(-OCH<sub>2</sub>CH<sub>3</sub>):1** or the kinetic equivalent **3:Zn(II)<sub>2</sub>:1<sup>-</sup>** in ethanol relative to methanol.

**4.2. 3:Zn(II)<sub>2</sub>:(-OCH<sub>2</sub>CH<sub>3</sub>)-Catalyzed Transesterification of 1.** The catalytically active form of the complex in this study is almost certainly stoichiometrically analogous to that found in methanol,<sup>4,5</sup> comprising a 1:2:1 ratio of ligand:Zn(II)<sub>2</sub>:(-OR). While **3:Zn(II)<sub>2</sub>:(-OCH<sub>3</sub>)** and **3:Zn(II)<sub>2</sub>:(-OCH<sub>2</sub>CH<sub>3</sub>)** are stable when prepared as described in the Experimental Section, they form only slowly and so are not amenable to titrimetric studies to provide the thermodynamic  $\text{p}K_{\text{a}}$  values. In past work we have estimated the first  $\text{p}K_{\text{a}}$  by determining the  $\text{pH}$  at half-neutralization immediately following the addition of half an



**Figure 6.** Brønsted plot of  $\log(k_{\text{cat}}^{\text{max corr.}})$  vs the  $\text{p}K_{\text{a}}$  values of the corresponding aryl leaving groups for the **3:Zn(II)<sub>2</sub>:(-OCH<sub>2</sub>CH<sub>3</sub>)**-catalyzed cleavage of **1a–g** in absolute ethanol at 25 °C. The two lines cross at approximately  $\text{p}K_{\text{a}} = 14.3$ . The line was constructed by NLLSQ fitting all the data to an expression  $k_{\text{cat}}^{\text{max corr.}} = k_1 k_2 / (k_{-1} + k_2) = C_1 C_2 10^{(\beta_1 + \beta_2) \text{p}K_{\text{a}}}$  ( $C_{-1} 10^{\beta_1 \text{p}K_{\text{a}}} + C_2 10^{\beta_2 \text{p}K_{\text{a}}}$ ).<sup>27</sup> The two fitted  $\beta$  values are  $-1.12 \pm 0.12$  and  $0.0 \pm 0.1$ .

**Scheme 3.** Proposed Process Depending on Two  $\text{p}K_{\text{a}}$  Values for the Catalyzed Cleavage of **1** Promoted by Complex **3:Zn(II)<sub>2</sub>:(-OCH<sub>2</sub>CH<sub>3</sub>)**



equivalent of HClO<sub>4</sub> to a methanol solution of **3:Zn(II)<sub>2</sub>:(-OCH<sub>3</sub>)**. However, in this study it was found that the partial  $\text{pH}$ /rate profile shown in Figure 5 could be obtained by adding small amounts of HClO<sub>4</sub> or NaOEt to a preformed catalyst, allowing this mixture to equilibrate for some time, and then determining the  $k_{\text{obs}}^{\text{max corr.}}$  for the kinetics of cleavage of substrate **1e** which is fully bound to the complex as **3:Zn(II)<sub>2</sub>:(-OCH<sub>2</sub>CH<sub>3</sub>):1e** or a possible kinetic equivalent, **3:Zn(II)<sub>2</sub>:1e<sup>-</sup>**, where the 2-hydroxy group is deprotonated. The  $\text{pH}$  of the mixtures were measured following determination of their kinetics and were assumed to be representative of that in the reacting solution. The  $\log k_{\text{obs}}^{\text{max corr.}}/\text{pH}$  data in Figure 5 follow an apparent bell-shaped profile with the catalytic activity plateauing between  $\text{pH}$  7.9 and 10, suggesting that it follows the process given in Scheme 3 with two ionizations having  $\text{p}K_{\text{a}}$  values of  $\sim 7.2$  and 10.8 determined from fitting the solid square data of the figure to an appropriate equation (see eq 2S, Supporting Information). The kinetic data (○) obtained in the low  $\text{pH}$  domain do not fit the theoretical model and indicate that once the ethoxide begins to be removed from the complex, the system is unstable, probably dissociating one of the Zn(II) ions with concomitant loss of activity. This is consistent with our experience that indicates one cannot form these complexes in methanol or ethanol without having one equivalent of alkoxide present along with the ligand prior to the addition of the metal ions.

#### 4.2.1. Change in Rate-Limiting Step for the $k_{\text{cat}}^{\text{max corr.}}$

**Term.** The Brønsted plot given in Figure 6 exhibits a sharp downward break in  $\log k_{\text{cat}}^{\text{max corr.}}$  commencing at  $\text{p}K_{\text{a}} \approx 14.3$  suggesting a change in rate-limiting step for the unimolecular term dealing with product formation from some form of catalyst: substrate complex. Unlike the case in methanol,<sup>4</sup> all the kinetic

(23) The extent of breaking of the P–OAr bond in the TS can be measured by the Leffler parameter,  $\alpha$ , which measures the change in the Brønsted  $\beta_{\text{lg}}$  for the TS relative to the  $\beta_{\text{eq}}$  for equilibrium transfers of the phosphoryl group between oxyanion nucleophiles. In the case of the transfer of the (RO)P(=O)O<sup>-</sup> group **18a**, the  $\beta_{\text{eq}}$  value is  $-1.74$  with the O–Ar oxygen in the starting material having a net effective charge of  $+0.74$ . For the cyclization reaction involving attack of the 2-hydroxypropyl oxyanion, the Leffler parameter,  $\alpha$  is given as  $\beta_{\text{lg}}/\beta_{\text{eq}} = 0.52$  suggesting that the P–OAr cleavage is 52% of the way from starting material to product, this assuming that the  $\beta_{\text{eq}}$  determined in water can be transposed into ethanol.

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(25) O'Donoghue, A. M.; Pyun, S. Y.; Yang, M.-Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2006**, *128*, 1615.

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data for the catalyzed cleavages of **1a–g** in ethanol exhibit saturation behavior with very strong binding.

The fact that the  $k_{\text{cat}}^{\text{max corr.}}$  terms for substrates **1a–c** containing good leaving groups are essentially independent of the  $\text{p}K_{\text{a}}$  ( $\beta_{\text{lg}} = \sim 0$ ) indicates that the process that limits the rate for those substrates cannot be dependent on any chemical step where changes in the bonding of the P–OAr linkage is prominent. This is consistent with a nonchemical step such as a rearrangement process ( $k_2$  in Scheme 1) becoming rate-limiting for substrates with good leaving groups (**1a–c**) while the rates of the reactions for substrates with poorer leaving groups (**1d–g**) are limited by some chemical step where there is a large dependence on the leaving group ( $\beta_{\text{lg}} = -1.12$ ).

These data and those previously in methanol<sup>4</sup> can be accommodated within the simplified model for the catalyzed reaction given in Scheme 1 with consideration of the effect of the reduced dielectric constant medium on the first equilibrium constant ( $K_{-1} = k_{-1}/k_1$  in units of M). For the Debye–Hückel model for association of spherical ions in a medium of dielectric constant  $\epsilon_r$ , the electrostatic potential energy of interaction between oppositely charged ions is:

$$P.E. = (z_+e)(z_-e)/(4\pi\epsilon_0\epsilon_r r) \quad (5)$$

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), and  $\epsilon_0$  is the dielectric constant of the solvent in question.<sup>28</sup> In passing from water, to methanol and then ethanol, each 1 kcal/mol of potential energy of attraction increases by a factor of 2.5 and then 3.2, so there is a dramatic effect of reduced dielectric solvent on the binding of ions of opposite charge. That effect will increase the  $k_1$  association rate constant and decrease the  $k_{-1}$  dissociation rate constant, consistent with the observed larger catalyst:substrate binding constant in ethanol relative to methanol. While the dielectric constant has an obvious role in increasing the binding, once the complex is formed the ensuing chemical transformation should be less sensitive to changes in  $\epsilon_r$ , since the reactants are in intimate contact with solvent being excluded.

The solvent effect on the binding steps can be numerically evaluated using an approach similar to what we used before to analyze the change in rate limiting step in the **3:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>)<sub>3</sub>**-catalyzed cleavage of **1a–g** in methanol.<sup>4</sup> In that case, analysis of the kinetic data suggested that the change in rate limiting step resulted from the substrate dependent partitioning of the doubly activated phosphate complex ( $k_{\text{cat}}^{\text{max}}$  vs  $k_{-2}$ ). Although the  $k_{\text{cat}}^{\text{max corr.}}$  values we determined here for catalyzed cleavage of substrates **1c–g** with poorer leaving groups are very close to the analogous values determined in methanol, the observation that all substrates adhere to saturation kinetics in ethanol suggests there are important differences in the two

solvents. The application of Michaelis–Menten kinetics to the process of Scheme 1 where the first step is treated as an equilibrium gives:

$$\text{rate} = \frac{dP}{dt} = \frac{k_{\text{cat}}^{\text{max}}[\mathbf{3:Zn(II)}_2:(\text{OCH}_2\text{CH}_3)]_{\text{free}}[\mathbf{1}]}{K_m + [\mathbf{3:Zn(II)}_2:(\text{OCH}_2\text{CH}_3)]_{\text{free}}} \quad (6)$$

$$K_m = \frac{k_{-1}}{k_1} \cdot \frac{k_{-2} + k_{\text{cat}}^{\text{max}}}{k_2} = K_{-1} \frac{k_{-2}}{k_2} + K_{-1} \frac{k_{\text{cat}}^{\text{max}}}{k_2} \quad (7)$$

The  $K_m$  term can be broken down into two components as defined in eq 7. With substrates **1d–g** having poor leaving groups where  $k_{\text{cat}}^{\text{max}}$  is less than  $k_{-2}$ ,  $K_m$  is approximated as  $K_{-1}(k_{-2}/k_2)$  and has an average value of  $\sim(9 \pm 1) \times 10^{-5}$  M in methanol and, since it is dominated by binding effects, is insensitive to the nature of the leaving group. In ethanol, the analogous data for all the substrates gives an upper limit of  $K_m$  of  $3 \times 10^{-7}$  M, which is a likely consequence of a reduction in the  $K_{-1}$  term pertaining to dissociation of phosphate away from **3:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>)<sub>3</sub>**. The fact that the break in the plot of Figure 6 is at  $k_{\text{cat}}^{\text{max corr.}} \approx 150 \text{ s}^{-1}$  suggests that this is the value for the proposed rearrangement step ( $k_2$ ) to form the proposed doubly activated phosphate by binding to both Zn(II) ions. Using this value it can be calculated that  $K_{-1}k_{-2}$  is  $\sim 4.5 \times 10^{-5} \text{ M} \cdot \text{s}^{-1}$ ; assuming this same  $k_2$  value obtains for the rearrangement step in methanol one calculates that  $K_{-1}k_{-2}$  there would be  $1.4 \times 10^{-2} \text{ M} \cdot \text{s}^{-1}$ .

**4.2.2. The  $\text{p}K_{\text{a}}$  Dependent  $k_{\text{cat}}^{\text{max corr.}}$  Terms.** The  $k_{\text{cat}}^{\text{max corr.}}$  terms for the catalyzed cleavages of the bound substrates **1d–g** adhere to a Brønsted relationship having a  $\beta_{\text{lg}}$  of  $-1.12 \pm 0.12$  which is experimentally the same as was found for the catalyzed cleavage of **1c–g** in methanol ( $-0.97 \pm 0.05$ )<sup>4</sup> and perhaps slightly larger than for the ethoxide promoted cleavage of **1a–d** in ethanol ( $-0.90 \pm 0.04$ ). While the rate-limiting step is either a concerted displacement or formation of a phosphorane intermediate, a weak conclusion can be drawn that there is a slightly more extensive change in the P–OAr bond in the **3:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>)<sub>3</sub>**-catalyzed cleavage relative to the ethoxide promoted cleavage.

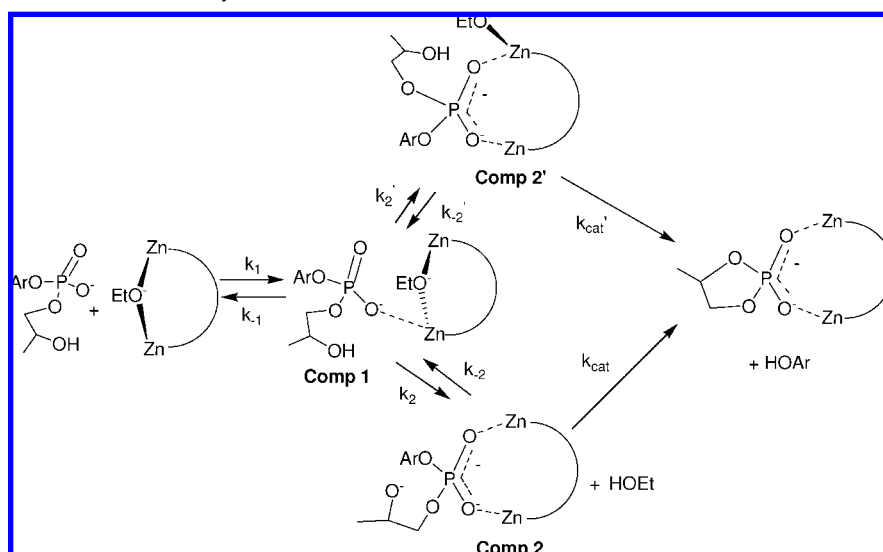
Shown in Scheme 4 is a proposed mechanism slightly expanded from that presented in Scheme 1 for the catalyzed cleavage based on the information gained in ethanol. As a starting point we formulate the essential ethoxide in the **3:Zn(II)<sub>2</sub>** complex as bridging between the two metal ions based on the structural evidence gained with the diZn(II)<sup>4</sup> and diCu(II)<sup>5c</sup> in methanol. Due to the high binding constant between **1** and **3:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>)<sub>3</sub>**, we propose formation of a large equilibrium amount of Comp 1 where there is binding of the phosphate to one of the metal ions with a loosening of the bridging ethoxide. This is followed by rearrangement step(s) where the phosphate becomes doubly activated by binding to both Zn ions. The  $k_2$  step involves simultaneous removal of the coordinated ethoxide and deprotonation of the 2-hydroxypropyl group to yield Comp 2 which can undergo intramolecular cyclization (stepwise or concerted) to give the five-membered cyclic phosphate product.<sup>29</sup> An alternative rearrangement process via  $k_2'$  involves formation of Comp 2' having a coordinated ethoxide (bridging or singly coordinated), which subsequently acts as a general base to assist in the cyclization. A general base process is excluded in the case of a diZn(II) complex promoting cyclization of **1a** in water<sup>30</sup> but is proposed as the viable mechanism for the Ln(III) promoted cleavages of **1a** in 80% DMSO/water,<sup>8</sup> so it is possible that there is a shift in

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(29) The first formed phosphate product is the cyclic five-membered phosphate and the observed product is the phenol/phenoxide of the parent **1**. We have observed that the cyclic phosphate opens up very rapidly ( $t_{1/2} \approx 2 \text{ s}$ , but at least 10-times slower than the reaction of the slowest of our substrates **1**) in the presence of **3:Zn(II)<sub>2</sub>** in methanol to form a kinetic mixture of 2-hydroxypropyl methyl phosphate and its isomeric (1-(hydroxymethyl)ethyl)methyl phosphate in a 30:70 mixture. While we have not checked the situation in ethanol, there is no reason to suspect that the cyclic phosphate will not react rapidly with ethanol in the presence of the catalyst. Tsang, W. Y.; Edwards, D.; Melnychuk, S. A.; Liu, C.; Neverov, A. A.; Brown, R. S. manuscript in preparation.



Scheme 4. Proposed Mechanism for the Catalyzed Reaction in Ethanol<sup>a</sup>

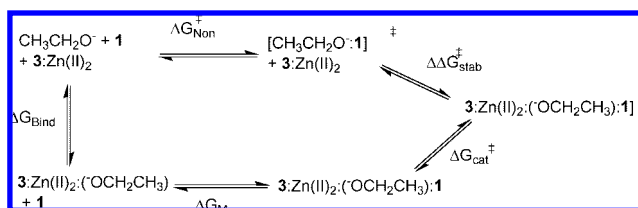
<sup>a</sup> Zn charges omitted for simplicity.

mechanism from specific catalysis to general catalysis brought about by the medium effects that give the fast reactions observed here and previously.<sup>4,5,8</sup> Indeed the available evidence now allows us to rule out a specific base catalyzed process in ethanol where external ethoxide acts as the base to remove the proton from the 2-hydroxy group prior to cyclization. For the **3**:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>CH<sub>3</sub>)-catalyzed cleavage of **1e** at  $\text{pH}$  7.76, the observed  $k_{\text{cat}}^{\text{max corr.}}$  is  $10.6 \text{ s}^{-1}$ . At that  $\text{pH}$ , the free [OEt<sup>-</sup>] is  $4.6 \times 10^{-12} \text{ M}$  ( $K_{\text{auto}}$  of ethanol is  $10^{-19.1}$ ),<sup>11</sup> so the rate constant for cleavage promoted by external OEt<sup>-</sup> would need to be  $2.65 \times 10^{12} \text{ M}^{-1} \text{ s}^{-1}$ , exceeding the diffusion limit in ethanol<sup>31</sup> by a factor of 265.

**4.3. Energetic Considerations for the Catalysis.** In Table 2 are presented ( $k_{\text{cat}}^{\text{max corr.}}/K_{\text{m}})/k_2^{-\text{OEt}}$  values indicating the catalytic reactions in ethanol are  $10^{12}$  to  $10^{14}$  larger than the corresponding ethoxide promoted reactions. Although the experimental  $k_{\text{cat}}^{\text{max}}$  values in ethanol and methanol for substrates **1c–g** are close to each other, the ( $k_{\text{cat}}^{\text{max}}/K_{\text{m}})/k_2^{-\text{OMe}}$  ratios for these substrates in methanol vary from about  $4 \times 10^8$  to  $4 \times 10^9$ .<sup>4</sup> The kinetic data clearly indicate the apparent  $10^4$  larger activity in ethanol stems from respective 10 to 100-fold and at least 100-fold reductions in both the  $k_2^{-\text{OR}}$  terms and  $K_{\text{m}}$  terms relative to their values in methanol. An alternative comparison that gives apparently spectacular accelerations relates the  $k_{\text{cat}}^{\text{max corr.}}$  values to the presumed ethoxide reaction at the  $\text{pH}$  where the catalyzed reactions were conducted. The [OEt<sup>-</sup>] at  $\text{pH}$  9.0 is  $\sim 10^{-10} \text{ M}$ , so that for **1c–g**, the catalytic acceleration would be  $10^{17}$ -fold!<sup>32</sup> By this measure, the catalytic acceleration in ethanol is  $10^5$  larger than in methanol, but this stems from three main factors, including the 10 to 100-times less reactivity of the alkoxide reaction in ethanol, the decreased autoprotolysis constant of ethanol relative to methanol ( $10^{-16.77}$ ) and the lower working  $\text{pH}$  (9.0 vs 9.8).

The more thermodynamically correct method to evaluate the catalytic efficacy of enzyme- or synthetic catalyst-promoted

Scheme 5



reactions computes the free energy of binding of the catalyst to the transition state of the presumed lyoxide promoted reaction.<sup>3,33,34</sup> Following the procedures we used to analyze the **3**:Zn(II)<sub>2</sub>:(OCH<sub>3</sub>) promoted cleavages of **1a–g** and **2a–n** in methanol,<sup>4,5</sup> we consider in Scheme 5 a cycle encompassing the **3**:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>CH<sub>3</sub>) and ethoxide reactions for **1a–g** in ethanol: the definitions of the terms are given in ref 4. Equation 8<sup>35</sup> provides the calculated free energy of binding of the catalyst to the ethoxide:substrate complex ( $\Delta\Delta G_{\text{stab}}^{\ddagger}$ ), that is, [**3**:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>CH<sub>3</sub>):1]<sup>‡</sup> and [CH<sub>3</sub>CH<sub>2</sub>O<sup>-</sup>:1]<sup>‡</sup>.<sup>4,5</sup>

$$\Delta\Delta G_{\text{stab}}^{\ddagger} = (\Delta G_{\text{Bind}} - \Delta G_{\text{M}} + \Delta G_{\text{cat}}^{\ddagger}) - \Delta G_{\text{Non}}^{\ddagger} = -RT \ln \left[ \frac{(k_{\text{cat}}/K_{\text{m}})(K_{\text{a}}/K_{\text{auto}})}{k_2^{-\text{OEt}}} \right] \quad (8)$$

The  $k_{\text{cat}}^{\text{max corr.}}$  and  $k_2^{-\text{OEt}}$  values are from Table 1 with the  $k_2^{-\text{OEt}}$  values for the less reactive substrates **1e–g** being extrapolated from the Brønsted plot in Figure 1. The upper limit for the  $K_{\text{m}}$  values for all substrates was taken to be  $10^{-6.5} \text{ M}$  and the  $\text{p}K_{\text{a}}$  for the formation of **3**:Zn(II)<sub>2</sub>:(OCH<sub>2</sub>CH<sub>3</sub>) from **3**:Zn(II)<sub>2</sub>:(HOCH<sub>2</sub>CH<sub>3</sub>) was taken to be 7.2 from the fitting of the data shown in Figure 5. While there may be some error in the latter two numbers, this does not affect the general picture

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(34) For applications of this to phosphate cleavage and other reactions see: Yatsimirsky, A. K. *Coord. Chem. Rev.* **2005**, 249, 1997.

(35) Equation 8 is a correct form of the eq 5 in the original methanol manuscript<sup>4</sup> where a typographical error appeared in the expression ( $\Delta\Delta G_{\text{stab}}^{\ddagger} = (\Delta G_{\text{Bind}} + \Delta G_{\text{M}} + \Delta G_{\text{cat}}^{\ddagger}) - \Delta G_{\text{Non}}^{\ddagger}$ ) placing a (+)-sign in front of the  $\Delta G_{\text{M}}$  term. Since  $K_{\text{M}}$  refers to the dissociation constant for the Michaelis complex, and we are interested in the binding energy of catalyst and **1**, the correct form of the equation should be  $-\Delta G_{\text{M}}$ .

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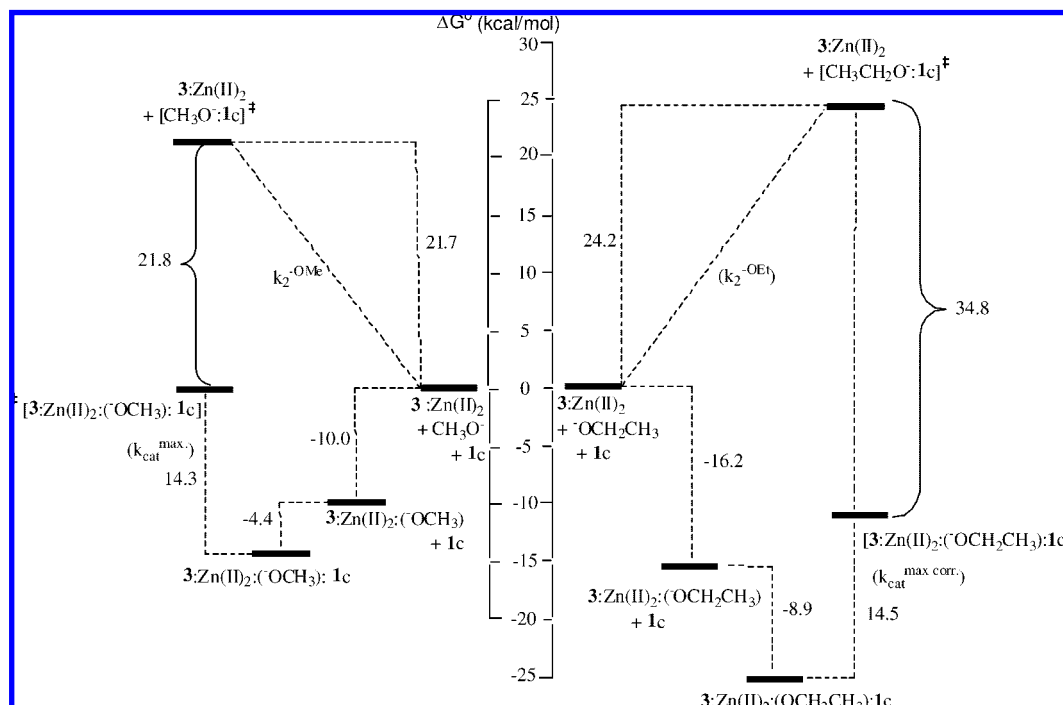
(31) Schwarz, H. A.; Gill, P. A. *J. Phys. Chem.* **1977**, 81, 22.

(32) Since we know that the activity of **3**:Zn(II)<sub>2</sub>:(OEt) is maintained down to  $\text{pH}$  7.9, the acceleration relative to the base promoted reaction at that  $\text{pH}$  would be over  $10^{18}$ -fold.

**Table 3.** Tabulation of the  $(k_{\text{cat}}^{\text{max}}/K_m)(\S K_a/K_{\text{auto}})$  Constants and the Computed Free Energies for the Formation of Catalytic Complexes ( $\Delta G_{\text{Bind}} - \Delta G_m$ ), the Free Energies of Activation for  $k_{\text{cat}}^{\text{max corr.}}$  ( $\Delta G_{\text{cat}}^{\ddagger}$ ), and the Free Energies of Stabilization of the Ethoxide Transition State through Binding to  $3:\text{Zn}(\text{II})_2$  ( $\Delta\Delta G_{\text{stab}}^{\ddagger}$ )<sup>a</sup> for the Catalyzed Reaction of Substrates **1a–g** at 25 °C in Ethanol

substrate	$(k_{\text{cat}}^{\text{max corr.}}/K_m)(\S K_a/K_{\text{auto}})$ ( $\text{M}^{-2}\text{s}^{-1}$ ) <sup>b,c</sup>	$\Delta G_{\text{Bind}} - \Delta G_m$ (kcal/mol) <sup>d</sup>	$\Delta G_{\text{cat}}^{\ddagger}$ (kcal/mol) <sup>e</sup>	$\Delta G_{\text{Non}}^{\ddagger}$ (kcal/mol) <sup>e</sup>	$\Delta\Delta G_{\text{stab}}^{\ddagger}$ (kcal/mol)
<b>1a</b>	$4.2 \times 10^{20}$	-25.1	14.4	22.3	-33.0
<b>1b</b>	$3.3 \times 10^{20}$	-25.1	14.5	23.0	-33.6
<b>1c</b>	$3.5 \times 10^{20}$	-25.1	14.5	24.2	-34.8
<b>1d</b>	$9.0 \times 10^{19}$	-25.1	15.3	25.9	-36.5
<b>1e</b>	$3.6 \times 10^{19}$	-25.1	15.8	26.2	-35.5
<b>1f</b>	$1.1 \times 10^{19}$	-25.1	16.5	26.8	-35.4
<b>1g</b>	$6.7 \times 10^{18}$	-25.1	16.8	27.1	-35.4

<sup>a</sup>  $\Delta\Delta G_{\text{stab}}^{\ddagger}$  computed from application of kinetic and equilibrium constants to eq 8. <sup>b</sup>  $(k_{\text{cat}}^{\text{max corr.}}/K_m)/k_2^{-\text{OEt}}$  values from Table 1 where the  $K_m$  values for **1a–g** are assumed to have an upper limit of  $10^{-6.5}$  M. <sup>c</sup>  $\S K_a$  of  $10^{-7.22}$  determined from the fit of the data in Figure 5 corresponding to the first ionization in Scheme 3;  $K_{\text{auto}} = 10^{-19.1}$ ;  $\S K_a/K_{\text{auto}} = 7.94 \times 10^{11}$  and corresponds to the binding constant of  $-\text{OEt}$  and  $3:\text{Zn}(\text{II})_2$ . <sup>d</sup> Computed as  $(\Delta G_{\text{Bind}} - \Delta G_m) = -RT \ln(\S K_a/K_{\text{auto}}/K_m)$ . <sup>e</sup> Computed from  $\Delta G_{\text{cat}}^{\ddagger} = -RT \ln(k_{\text{cat}}^{\text{max corr.}}/(kT/h))$  or  $\Delta G_{\text{Non}}^{\ddagger} = -RT \ln(k_2^{-\text{OEt}}/(kT/h))$  from the Eyring equation where  $(kT/h) = 6 \times 10^{12} \text{ s}^{-1}$  at 298 K.



**Figure 7.** Comparison of the activation energy diagram for the  $3:\text{Zn}(\text{II})_2:(-\text{OR})$  and  $\text{RO}^-$ -catalyzed cleavages of **1c** in ethanol and methanol at standard state of 1 M and 25 °C showing the calculated energies of binding the alkoxide by  $3:\text{Zn}(\text{II})_2$ , of binding of **1c** to  $3:\text{Zn}(\text{II})_2:(-\text{OR})$  and the calculated activation energies associated with  $k_{\text{cat}}^{\text{max corr.}}$  and  $k_2^{-\text{OR}}$ . Methanolysis data taken from ref 4.

greatly except for a small numerical uncertainty of the  $\Delta G$  terms that depend on those constants. In Table 3 are given the  $(k_{\text{cat}}^{\text{max corr.}}/K_m)(\S K_a/K_{\text{auto}})$  and the computed  $\Delta\Delta G_{\text{stab}}^{\ddagger}$  values for the catalyzed reactions of **1a–g**.

The data in ethanol invite comparison with those determined earlier for the catalyzed reactions in methanol<sup>4</sup> and shown in Figure 7 are the free energy data for the cleavage of **1c** in both solvents at a standard state of 1 M and 25 °C. The most striking feature is that the  $\Delta\Delta G_{\text{stab}}^{\ddagger}$  in ethanol (ranging from -33 to -36.5 kcal/mol) is very large in absolute terms and some 11–13 kcal/mol more negative than for the same substrate in methanol. Figure 7 visually informs us that the main differences in the  $\Delta G$  values in the two solvents arise from three terms: a much stronger binding in ethanol of alkoxide to  $3:\text{Zn}(\text{II})_2$  and of **1c** to  $3:\text{Zn}(\text{II})_2:(-\text{OR})$  as well as the ~65-fold slower reaction of alkoxide in ethanol than in methanol.

The first two terms place the three reaction components into a 10–11 kcal/mol deeper thermodynamic well when fully bound in ethanol, while the third raises the alkoxide reaction's transition

state by about 2.5 kcal/mol. However, the surprising aspect is that the  $k_{\text{cat}}^{\text{max corr.}}$  term is about the same in both solvents, the  $\Delta G_{\text{cat}}^{\ddagger}$  being 14.5 and 14.3 kcal/mol. It is also very interesting that the free energy of the  $(3:\text{Zn}(\text{II})_2:(-\text{OEt})\text{I})^{\ddagger}$  is lower than the ground-state for the uncomplexed reaction partners by 10–11 kcal/mol in ethanol while in methanol the corresponding TS is roughly isoenergetic with the free reaction partners.

It is worthwhile to attempt to compare the accelerations for the reactions achieved by this catalytic system in ethanol with what is achievable by phosphodiesterase enzymes, bearing in mind that the medium, substrates and, in particular, the leaving groups are different. The cleavage of 3',5'-UpA is reported to have a first order rate constant at pH 6,  $T = 25$  °C of  $5 \times 10^{-9} \text{ s}^{-1}$ <sup>36</sup> while a rate constant of  $2.2 \times 10^{-11} \text{ s}^{-1}$  was observed for the cleavage of 3',5'-ApG moiety inside in a strand of deoxynucleotides at 23 °C and pH 6.<sup>37</sup> Since it is probable that

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both these processes are specific base catalyzed throughout the accessible pH regions,<sup>38</sup> respective second order rate constants for the base catalyzed process of  $5 \times 10^{-1}$  and  $2.2 \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}$  are calculated which can be compared with the reported value of  $2 \times 10^{-3} \text{ M}^{-1}\text{s}^{-1}$  cleavage of UpU.<sup>39,40</sup> Since enzymes that promote the cleavage of RNA type phosphodiester typically have  $k_{\text{cat}}/K_{\text{M}}$  values of  $10^6$  to  $10^8 \text{ M}^{-1}\text{s}^{-1}$ ,<sup>1d</sup> the computed acceleration as measured by  $(k_{\text{cat}}/K_{\text{M}})/k_2^{-\text{OH}}$  for the enzyme catalyzed cleavage of RNA would be  $\sim 10^7$  to  $10^{11}$ . In this study, accelerations of  $1-2 \times 10^{14}$  are seen for substrates **1d-g** where the  $k_{\text{cat}}^{\text{max corr.}}$  term specifically refers to the chemical cleavage step of the bound substrate.

## 5. Conclusions

The catalytic acceleration for the cleavage of substrates **1** by a dinuclear Zn(II) catalyst in methanol and ethanol far exceeds anything so far reported for metal ion containing catalysts in aqueous solution.<sup>2</sup> It is notable that the reaction in question is not a hydrolytic process in any case, but rather an intramolecular cyclization so the importance of the solvent as a nucleophile is not relevant. It has been stated that the effective dielectric constants in enzyme active sites resemble those of organic solvents rather than water.<sup>41,42</sup> In the present case, reductions in dielectric constant such as what happens when one proceeds from water, to methanol and then ethanol, seem to be a particularly effective strategy for accelerating the rate of metal catalyzed acyl and phosphoryl transfer reactions.<sup>43</sup> The dinuclear catalyst (**3**:Zn(II)<sub>2</sub>) in water is reported<sup>6</sup> to be poor, and in fact

no more effective in promoting the hydrolysis of bis-*p*-nitrophenyl phosphate than the Zn(II) complex of 1,5,9-triazacyclododecane, so the strong activities seen in methanol and ethanol for phosphate diester cleavage<sup>4,5</sup> point to a synergistic interaction between the catalyst and medium. It is an essential, but not exclusive, requirement that the catalyst must readily recruit the reaction partners (**3**:Zn(II)<sub>2</sub> + **1** + <sup>-</sup>OR) into a reactive complex and clearly the reduced polarity medium enhances these interactions between oppositely charged components. However, simple binding of the reaction partners cannot lead to rate accelerations unless there is a greater binding of the transition state for the catalyzed reaction. Indeed this binding amounts to 33–36 kcal/mol in while that in methanol is 21–23 kcal/mol, the difference in the two solvents being largely dependent on the far stronger binding of the anionic reactants by the positively charged dinuclear complex in ethanol. What is interesting is the fact that the  $k_{\text{cat}}^{\text{max}}$  terms are very similar in ethanol and methanol for the substrates where this term relates to a chemical cleavage step. Perhaps this results from the fact that once bound by the catalyst, the polar groups such as the metal ions and transforming phosphate are at the interior of the catalyst:substrate complex and so not subject to significant solvent effects.

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**Supporting Information Available:** Tables of pseudo-first-order rate constants for reactions of **1a-g** with **3**:Zn(II)<sub>2</sub>:(<sup>-</sup>OCH<sub>2</sub>CH<sub>3</sub>) or ethoxide in ethanol; plots of  $k_{\text{cat}}^{\text{max corr.}}$  vs the [**3**:Zn(II)<sub>2</sub>:(<sup>-</sup>OCH<sub>2</sub>CH<sub>3</sub>)] for the catalyzed reactions of **1a-g** in ethanol, mathematical treatments for determining the triflate inhibition constants and for the fitting of the Figure 5 data to a two  $\text{p}K_{\text{a}}$  model. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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