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Dinuclear Zn(II) Complex Promotes Cleavage and Isomerization of 2-Hydroxypropyl Alkyl Phosphates by a Common Cyclic Phosphate Intermediate

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Abstract: The kinetics and cleavage products of 2-hydroxypropyl *p*-nitrophenyl phosphate were determined in methanol containing the di-Zn(II) complex of bis-1,3-N_1,N_1'-(1,5,9-triazacyclododecyl)propane (**4**). Time-dependent ^{1}H NMR spectra of the reaction mixture at $^{s}_{s}$pH 9.8 $\pm$ 0.1 show that the catalytic reaction proceeds via a cyclic phosphate (4-methylethylene phosphate, **2**) that is subsequently cleaved into a kinetic mixture of two isomeric products, 2-hydroxypropyl methyl phosphate (**3**) and 1-hydroxypropan-2-yl methyl phosphate (**3a**), in a 29/71 ratio. In the presence of **4**, the kinetic mixture of **3**/**3a** is transformed into a thermodynamic mixture of 72/28 **3**/**3a**. The time-dependent ^{1}H NMR spectra of **4** and a 22/78 mixture of **3**/**3a** in CD_3OH show that the formation of the thermodynamic mixture occurs on the same time scale as replacement of the P$-$OCH$_3$ group of the **3**/**3a** starting materials with OCD_3. Detailed kinetic studies indicate that the dominant process for loss of the OCH_3 group and equilibration of **3**/**3a** is via a **4**-catalyzed process where each of the isomers cyclizes to methylethylene phosphate (**2**), which subsequently reforms the **3**/**3a** thermodynamic mixture. The k_{cat}^{max} for **4**-catalyzed cyclization of **3** and three other 2-hydroxypropyl *O*-alkyl phosphates (alkyl = CF_3CH_2- (**6a**), CH_2FCH_2- (**6b**), and CH_3CH_2- (**6c**)) has been determined, and the Brønsted plot comprising the log k_{cat}^{max} vs leaving group $^{s}_{s}pK_a$ that includes several previously studied 2-hydroxypropyl aryl phosphates is linear, following the expression log k_{cat}^{max} = (-0.85 ± 0.02) $^{s}_{s}pK_a$ + (12.8 ± 0.4). The β_{lg} value of -0.85 suggests that the catalyzed cleavage of the P$-$OAr/OR bond has progressed to about 45% in the transition state. The combined results are analyzed in terms of two possible processes involving either a concerted reaction leading to the cyclic phosphate **2** from which the thermodynamic mixture of **3**/**3a** is formed or a stepwise one involving a transient phosphorane whose predominant fate is to eliminate methoxide and proceed to **2** rather than partitioning between **3**, **3a**, and **2**.

1. Introduction

In order to understand the mechanism by which metalloenzymes catalyze the cleavage of RNA, intense efforts have been devoted to the study of model systems for the cleavage of phosphodiesters mediated by simple mono- and dinuclear metal ion complexes.[1] The most commonly studied models employ aryloxy leaving groups, as exemplified by 2-hydroxypropyl *p*-nitrophenyl phosphate (**1**), which undergoes cleavage by intramolecular cyclization to form the cyclic phosphate (**2**). While the catalysis of the cyclization step of this type of substrate is well documented,[2,3] far less information is available about the metal-catalyzed opening of **2**,[4] and even less is known about the cyclization of RNA models with alkoxy groups that are closer in pK_a value and structure to biologically relevant substrates.[3,5] Herein we report a study of the cleavage of RNA models with leaving groups whose conjugate acids are substantially less acidic than the phenols customarily investigated. A key part of this study deals with the cleavage of 2-hydroxypropyl methyl phosphate (**3**) promoted by a dinuclear Zn(II) catalyst

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(1) (a) Mancin, F.; Tecillia, P. *New J. Chem.* **2007**, *31*, 800. (b) Weston, J. *Chem. Rev.* **2005**, *105*, 2151. (c) Molenveld, P.; Engbertsen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev.* **2000**, *29*, 75. (d) Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540. (e) Morrow, J. R.; Iranzo, O. *Curr. Opin. Chem. Biol.* **2004**, *8*, 192.

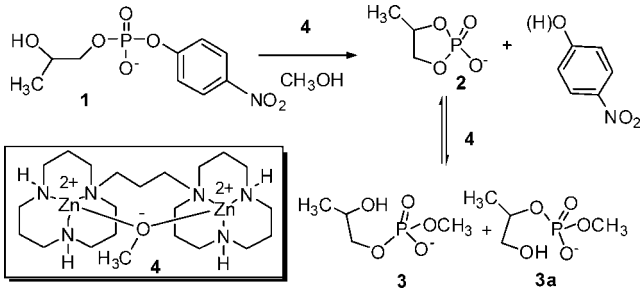
(2) (a) Feng, G.; Mareque-Rivas, J. C.; Williams, N. H. *Chem. Commun.* **2006**, 1845. (b) Mancin, F.; Rampazzo, E.; Tecilla, P.; Tonellato, U. *Eur. J. Chem.* **2004**, 281. (c) Feng, G.; Natale, D.; Prabaharan, R.; Mareque-Rivas, J. C.; Williams, N. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 7056. (d) Yang, M.-Y.; Iranzo, O.; Richard, J. P.; Morrow, J. R. *J. Am. Chem. Soc.* **2005**, *127*, 1064.

(3) (a) Neverov, A. A.; Lu, Z.-L.; Maxwell, C. I.; Mohamed, M. F.; White, C. J.; Tsang, J. S. W.; Brown, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 16398. (b) Lu, Z.-L.; Liu, C. T.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2007**, *129*, 11642. (c) Bunn, S. E.; Liu, C. T.; Lu, Z.-L.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2007**, *129*, 16238. (d) Liu, C. T.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2008**, *130*, 16711.

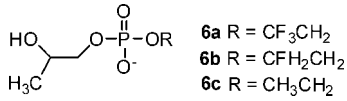
(4) While some of the dinuclear catalysts related to **4** show good activity for the cleavage of **1** in water, they fail to catalyze the opening of **2** in any reasonable time: see ref 2a and Iranzo, O.; Kovalevsky, A. Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 1988.

(5) A di-Zn(II) complex operating in water has recently been shown to catalyze both the isomerization of 3′5′-UpU and its hydrolysis, with the latter being appreciably faster than the former: (a) Linjalahti, H.; Feng, G.; Mareque-Rivas, J. C.; Mikkola, S.; Williams, N. H. *J. Am. Chem. Soc.* **2008**, *130*, 4232.

Scheme 1. Species Involved in the **4**-Catalyzed Decomposition of HPNPP (**1**)



(**4**) that provides remarkable acceleration of the cleavage of phosphate diesters in methanol.[3] As will be shown, the catalytic cleavage and isomerization of **3** proceeds via a mechanism involving rate-limiting formation of cyclic phosphate **2** followed by fast cleavage of this transient species to yield a kinetic mixture of **3** and **3a** which then undergoes equilibration to generate a thermodynamic mixture, Scheme 1. In addition, we report the rate constants for catalyzed cleavage of three other 2-hydroxypropyl alkyl derivatives, **6a**−**c**, in order to extend the previously reported Brønsted plot,[3c] demonstrating that all substrates, with good and poor leaving groups, fall on a single line.



2. Experimental Section

Materials. Methanol-d_3 (D, 99.5%) and -d_4 (D, 99.8%) were obtained from Cambridge Isotope Laboratories, Inc. Methanol (99.8% anhydrous), $Zn(CF_3SO_3)_2$ (98%), sodium methoxide (0.50 M solution in methanol, titrated against N/50 certified standard aqueous HCl solution and found to be 0.49 M), tetrabutylammonium hydroxide (1.0 M solution in methanol), tetrabutylammonium triflate (>99.0%), trifluoroethanol (99%), 2-fluoroethanol (95%), triethylamine (≥99.5%), phosphorus oxychloride (99%), $Ba(OH)_2$, propylene oxide (ReagentPlus, 99%), and Amberlite IR-120H ion-exchange resin (functionalized as sulfonic acid) were purchased from Aldrich and used without further purification. Preparation of all substrates used in this study is described in the Supporting Information, and each of **3**, **3a**, **6a**, **6b**, and **6c** had ^{1}H NMR, ^{31}P NMR, and exact MS spectra consistent with the structure (see the Supporting Information). The sodium salt of 2-hydroxypropyl *p*-nitrophenyl phosphate was available from a previous study.[3c] 1,3-Bis-N_1,N_1'-(1,5,9-triazacyclododecyl)-propane was prepared as described, and the formulation of catalyst **4** followed the method previously described.[3a−c]

Methods. The $_{ss}^{}pK_a$ values in methanol for the alcohol leaving group of **6a** and **3/3a** were obtained from previous work.[6] The $_{ss}^{}pK_a$ values of alcohols **6b** and **6c** were calculated from the linear regression $_{ss}^{}pK_a^{MeOH} = 0.748\ pK_a^{H2O} + 6.53$ as reported previously.[6] For a full description of the kinetic methods and procedures used to determine the observed rate constants (k_{obs}) for the **4**-catalyzed cleavage of substrates **3/3a**, **6a**, **6b**, and **6c**, see the Supporting Information.

3. Results

3.1. 4-Catalyzed Cleavage Process. There are three main observations described below for the **4**-catalyzed cleavage process shown in Scheme 1.

3.1.1. 4-Catalyzed Cleavage of HPNPP (1) Produces 2 and Then a Kinetic Mixture of 3/3a. The **4**-catalyzed cyclization of

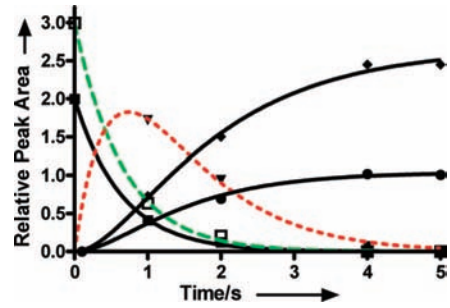


Figure 1. Plot of relative integrated peak areas vs time for methanolysis of **1** (2.81 mM; □ and ■, CH_3 and *o*-phenyl H's at δ 1.165 and 7.435, respectively) and **2** (▼, CH_3, δ 1.355) and the appearance of products **3a** (♦, CH_3, δ 1.285) and **3** (●, CH3, δ 1.185) promoted by 3.13 mM **4** in CH_3OH at $_{ss}^{}pH$ 9.8 ± 0.1 and 25.0 ± 0.1 °C; lines through the data computed on the basis of fits to eq 1 as described in the Supporting Information.

1, followed by observing the opening of **2** to give isomeric diesters **3** and **3a** (Scheme 1), was monitored by 600 MHz ^{1}H NMR using five solutions containing 3.13 mM **4**[3] and 2.81 mM **1** in 1.50 mL of anhydrous CH_3OH at ambient temperature, $_{ss}^{}pH$ 9.8 ± 0.1.[7] Following manual inoculation of a rapidly stirred solution containing **1** with **4**, each sample was quenched manually with 4 equiv of HCl and LiCl in 1 s intervals over the course of 5 s. CH_3OH was then removed, and the solutions were reconstituted with CD_3OD to provide samples for ^{1}H NMR analysis. Shown in Figure 1 is a plot of the time course of the integrated intensities of the ^{1}H NMR signals corresponding to species **1**, **2**, **3**, and **3a**, which was analyzed as follows. The observed first-order rate constant for the disappearance of **1** was determined to be 1.54 ± 0.02 s^{-1} from a fit of the corresponding integrated intensity data to a standard first-order exponential equation. This value was fixed as a constant for the rate of appearance of **2**, and the rate constants for the appearance of **3** and **3a** were determined from NLLSQ fitting of the integrated intensity data to eq 1

$$Y = C(1 + ((1/(k_A - k_B)(k_B \exp(-k_A t) - k_A \exp((k_A - k_B)t))))) \quad (1)$$

where Y is the peak intensity at time t, C refers to the maximum peak intensity for the given signal, k_A is the first-order rate constant for formation of **2**, and k_B is the rate constant for the **4**-catalyzed decomposition of **2** and formation of **3** and **3a**, the average of which is 0.9 ± 0.2 s^{-1}. The individual rate constants for opening of **2** to give **3a**, $k_{2\rightarrow 3a} = 0.64 \pm 0.2\ s^{-1}$, and **3**, $k_{2\rightarrow 3} = 0.26 \pm 0.1\ s^{-1}$, are calculated based on the product ratio of **3** to **3a**.

The observed rate constant for the decomposition of **1** promoted by **4** is much smaller under these experimental conditions than was seen earlier by stopped-flow spectrophotometry.[3a,c] The rate retardation probably results from the way this experiment was conducted with its necessarily higher concentrations of catalyst, substrate, and attendant counterions as well as the poorer mixing and quenching methods associated with the manual additions of the reaction components and quenching solutions. However, the most important part of this experiment

(6) Neverov, A. A.; Sunderland, N. E.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 65. $pK_a^{MeOH} = 0.748\ pK_a^{H2O} + 6.53$.

(7) For the designation of 'pH' in methanol and measurement thereof, see: (a) Gibson, G. T. T.; Neverov, A. A.; Brown, R. S. *Can. J. Chem.* **2003**, *81*, 495. The autoprotolysis constant of methanol is $10^{-16.77}\ M^2$, so neutral $_{ss}^{}pH$ in methanol is 8.4. (b) Bosch, E.; Rived, F.; Rosés, M.; Sales, J. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1953.

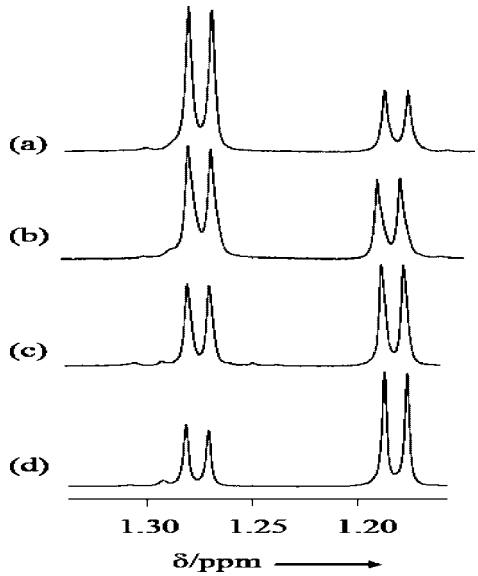
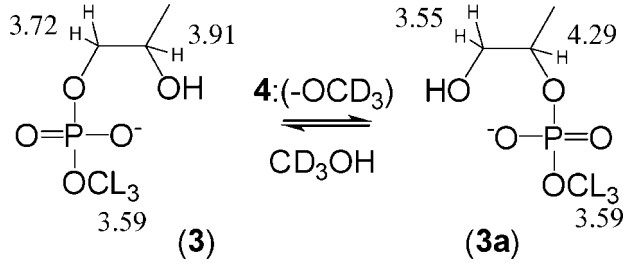


Figure 2. Partial ^{1}H NMR spectra at different times for the interconversion of 2.81 mM total of **3a** and **3** catalyzed by 3.13 mM **4**, determined after (a) 5 s, (b) 1 min, (c) 4 min, and (d) 16 min.

Scheme 2. ^{1}H Chemical Shifts in **3**/**3a** Used for Determining Rate Constants for Equilibration and O−CH_3 to O−CD_3 Transformation (L = H, D)



is the fast and observable conversion of **1** to the transient intermediate **2**, which is converted quickly to **3**/**3a** in an initial ratio of 29/71. The experimental difficulties associated with the quench protocol on this very fast reaction do not appreciably alter this interpretation; nonetheless, the rate constants for decomposition of **2** should be viewed as lower limits for the associated processes.

3.1.2. 4-Catalyzed Transformation of a Kinetic Mixture of 3/3a into a Thermodynamic Mixture. In a second experiment, six solutions containing 3.13 mM **4** and 2.81 mM **1** in 1.50 mL of anhydrous CH_3OH at ambient temperature and $_S^S$pH 9.8 ± 0.1 were prepared and then quenched as above after 5 s, 1 min, 4, 8, 12 and 16 min. Following workup (described above) the 600 MHz ^{1}H NMR spectrum of each sample was obtained. Shown in Figure 2 are four partial spectra displaying the CHC$\underline{H_3}$ doublets for **3** and **3a** at δ 1.185 (d, $^3J_{H-H}$ = 6 Hz) and 1.285 (d, $^3J_{H-H}$ = 6 Hz) that clearly indicate the initially formed **3**/**3a** ratio of 29/71 is kinetically controlled, and upon further exposure to **4** the isomers equilibrate, generating a thermodynamically controlled **3**/**3a** ratio of 72/28.

3.1.3. 4-Catalyzed Isomerization of 3/3a in CD_3OH Occurs with Concurrent Replacement of OCH_3 by OCD_3. The **4**-catalyzed transesterification of an independently synthesized sample of **3**/**3a** ((OCH_3) ratio of 22/78) to form **3** (OCD_3) and **3a** (OCD_3) was monitored by ^{1}H NMR (600 MHz) on a solution comprising 2.45 mM **4** and 2.2 mM authentic **3**/**3a** in 0.51 mL of CD_3OH at 25.0 ± 0.1 °C. The thermodynamic ratio of **3**/**3a**

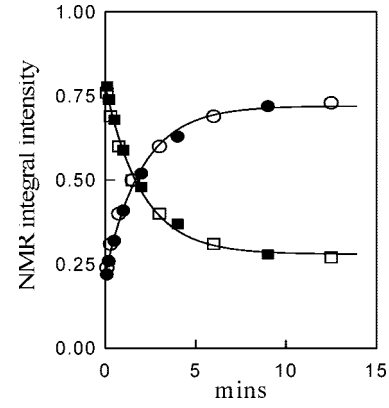
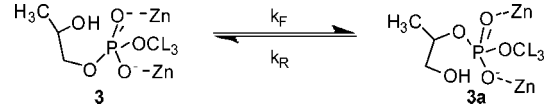


Figure 3. Plot of integrated peak area for isomers **3** (○, ●) and **3a** (□, ■) vs time for the equilibration of a mixture of **3**/**3a** (2.2 mM, having an initial ratio of 22/78) promoted by 2.45 mM **4** in CD_3OH at $_S^S$pH 9.8 ± 0.1 and 25.0 ± 0.1 °C. Signal intensity is normalized to reflect a total of one H. Solid and open symbols represent data for duplicate experiments: lines through the data are from fits to a standard single-exponential equation.

Scheme 3. **4**-Catalyzed Interconversion of **3** and **3a**; Catalyst Represented by the Two Zn Ions



(72/28) was determined following complete equilibration by integrating the signal intensities of the respective methine protons centered at 3.91 and 4.29 ppm (Scheme 2).

Shown in Figure 3 is a plot of the relative intensities of the methine signals for isomers **3a** and **3** versus time which, when fit to a single-exponential model, gives a first-order rate constant of (8.1 ± 0.6) × 10^{-3} s^{-1}. A similar treatment of the δ 3.72 methylene signal intensity vs time data (see the Supporting Information) gives a rate constant of (6.0 ± 0.5) × 10^{-3} s^{-1} which, when averaged with the first methine-based determination, gives (7.0 ± 1.0) × 10^{-3} s^{-1}. The latter, derived for the approach to equilibrium of isomers **3** and **3a**, is the sum of the individual rate constants $k_F + k_R$ depicted in Scheme 3. The ratio of k_F/k_R is defined by the thermodynamic ratio of **3**/**3a** (72/28), and accordingly, k_F and k_R are calculated to be 2.0 × 10^{-3} and 5.0 × 10^{-3} s^{-1}.

The **4**-catalyzed equilibration of isomers **3** and **3a** is accompanied by replacement of the $\underline{CH_3O}$−P group with a $\underline{CD_3O}$−P group derived from the solvent CD_3OH which could be monitored simultaneously with isomerization. The exchangeable OL was kept protium to avoid any potential solvent kinetic isotope effect that could confuse comparison with the previous experiments done in CH_3OH. Shown in Figure 4 is a plot of the combined **3**/**3a** $\mathbf{CH_3}$O−P NMR signal intensity at δ ~3.59 vs time data for the **4**-catalyzed cleavage of the methoxy group from duplicate experiments.

3.2. Analysis of the Methoxy Loss and Isomerization Data. As a starting model, we chose the process given in Scheme 4, where loss of the OCH_3 group occurs only with the formation of cyclic phosphate **2** and the latter subsequently partitions between **3** and **3a** with the introduction of an OCD_3 group from the bulk solvent.

A first analysis of this stepwise process, ignoring the k_3 and k_{-3} terms which will be discussed later, allows us to give detailed rate constants for the interconversion of **3** and **3a** and intermediate **2** as well as relative values for the partitioning of

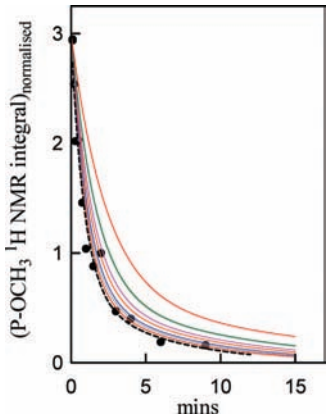
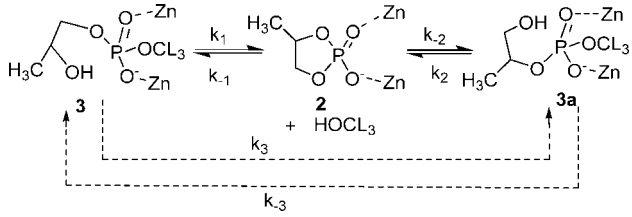


Figure 4. Intensity vs time plot for loss of the P−OCH_3 group at δ 3.59 from an independently prepared mixture of **3/3a** (2.2 mM, having an initial ratio of 22/78) promoted by 2.45 mM **4** in CD_3OH at ^{s}pH 9.8 ± 0.1 and 25.0 ± 0.1 °C; line through the data computed on the basis of fit to eq 5, from which loss of the methoxy group from **3** is given as $k_1 = (3.0 \pm 0.2) \times 10^{-3}$ s^{-1} and loss of the methoxy group from **3a** is given as $k_2 = (1.9 \pm 0.2) \times 10^{-2}$ s^{-1}. See Scheme 4 for definitions of k_1, k_2, and k_3. The seven colored lines are derived from the observed rate of isomerization (from Figure 3) and various ratios of k_1/k_3 (as defined in Scheme 4) at six fixed values: 1, 0.5, 0.4, 0.3, 0.2, 0.1, and 0 (see text and Supporting Information).

Scheme 4. Hypothetical Process for Loss of OCH_3 from **3** and **3a** and Their Conversion to Thermodynamic Mixture; Catalyst Represented by the Two Zn Ions



the latter by the two modes of ring opening. Applying the steady-state approximation to intermediate **2** leads to the rate expression shown in eq 2. The rate constant k_F, for isomerization of **3** into **3a**, is expressed as the product of the forward rate constant, k_1, and the partitioning coefficient $k_{-2}/(k_{-1} + k_{-2})$, eq 3. The k_{-1}/k_{-2} ratio (0.41) is determined from the kinetic product ratio of 29/71 for conversion of **2** into **3** and **3a** as determined from the data in Figure 1. The value of k_F is based on the rate constant for approach to equilibrium of **3** and **3a** and the known ratio of k_R/k_F determined from the thermodynamic ratio of isomers (72/28). According to this analysis k_1 is calculated to be 2.8×10^{-3} s^{-1}, and applying the same procedure k_2 (eq 4) is determined to be 1.7×10^{-2} s^{-1}.

$$d[\mathbf{2}]/dt = [\mathbf{3}]k_1 + [\mathbf{3a}]k_2 - [\mathbf{2}](k_{-1}+k_{-2}) = 0 \qquad (2)$$

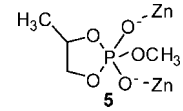
$$k_F = k_1k_{-2}/(k_{-1}+k_{-2}) \qquad (3)$$

$$k_R = k_2k_{-1}/(k_{-1}+k_{-2}) \qquad (4)$$

The dashed line through the data for loss of the OCH_3 group in Figure 4 was generated using eq 5 derived for a two-phase exponential decay normalized to a total of three initial CH_3O−P protons, where F_1 and F_2 define the initial fractions of **3** and **3a** used in the experiment (0.22 and 0.78, respectively). In order to reduce the number of fitted variables in eq 5, the R is introduced as the ratio of k_2/k_1 for rate-limiting formation of the cyclic phosphate (6.3), assuming that the CH_3O−P exchange only occurs via **2**. Fitting the data of Figure 4 to eq 5 leads to computed values for k_1 of $(3.0 \pm 0.2) \times 10^{-3}$ s^{-1} and k_2 of $(1.9 \pm 0.2) \times 10^{-2}$ s^{-1}. These values are experimentally identical to the k_1 and k_2 rate constants determined on the basis of the **3/3a** equilibration.

$$Y = 3(F_1 \exp(-Rk_1t) + F_2 \exp(-k_1t)) \qquad (5)$$

Also shown in Figure 4 are several colored lines that were derived on the basis of a series of numerical fits of the biexponential eq 5 to the process given in Scheme 4 where we introduced an additional pathway for interconversion of **3** and **3a** which does not lead to loss of the methoxy group (k_3 and k_{-3} in Scheme 4). This pathway would encompass other intermediates such as the phosphorane(s) **5** which do not partition to **2**. If isomerization only occurs via **2** (so k_3 and $k_{-3} = 0$), then $k_1 = 2.8 \times 10^{-3}$ s^{-1} and $k_2 = 1.7 \times 10^{-2}$ s^{-1} from the algebraic manipulations of the isomerization data discussed above. If k_3 and k_{-3} are not zero, then loss of the OCH_3 group must become slower than isomerization which would be signified by a shift of the plot in Figure 4 toward the upper right-hand corner. Furthermore, if k_3 and k_{-3} have any prominence, they must be linked by the same ratio as the thermodynamic product ratio (i.e., $k_3:k_{-3} = 28:72$). Inclusion of this hypothetical interconversion pathway encompasses all the mechanisms via phosphoranes (**5**) that isomerize and break down to **3/3a** instead of **2**. Knowing the overall rate of isomerization, the equilibrium position and the partitioning of **2** allows calculation of sets of values for k_1, k_2, and k_3 at various ratios of k_1/k_3 (see Supporting Information). The seven colored lines in Figure 4 are computed under conditions where the k_3/k_1 ratio varies from 1 (outermost red line) to zero (innermost red line), showing that the computed line that best fits the data occurs when there is no intervention of an additional pathway for interconversion of **3** and **3a** that does not generate **2**.



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3.3. Base-Catalyzed Cyclization of HPNPP (1) and Cleavage of 2. The methoxide-promoted cleavage of **2**, which was formed in situ from methoxide-promoted cyclization of **1**, was determined at 25 °C by ^{1}H NMR in CH_3OH containing 0.13 M phosphoric acid (as an internal standard (capillary insert)), 10 mM **1**, and between 0.1 and 0.5 M of tetrabutylammonium hydroxide. The assumption was made that all hydroxide is converted to methoxide under these reaction conditions. The progress of the reactions was assayed by ^{31}P NMR over a period of several days. The ^{31}P NMR signal for **1** decreased rapidly in all reactions and coincided with the formation of a new phosphorus signal assigned to the cyclic phosphate **2** at 16.99 ppm. The slow disappearance of the signal for the cyclic phosphate gave two new phosphorus signals at 2.88 and 2.53 ppm assigned to isomers **3** and **3a**. The ratio of **3/3a** remained constant at ~35/65 over the course of the experiments as well as from run to run carried out at different concentrations of base. The rate data for the disappearance of **2** and formation of **3** and **3a** were fit to standard single exponentials from which the individual rate constants for the various processes at each [NBu_4OMe] could be determined, see Supporting Information. The second-order rate constant for ring opening of **2** is calculated to be $(6.91 \pm 0.8) \times 10^{-7}$ M^{-1} s^{-1} as described in the Supporting Information.

Table 1. Kinetic Constants, k_{obs} and Maximum Rate Constant (k_{cat}^{max}), for Cleavage of Substrates **2**, **3**, and **6a**−**c** Promoted by **4** at $T = 25.0 \pm 0.1$ °C

substrate	$^{s}_{s}pK_a^{LG}$	k_{obs} (s^{-1})	k_{cat}^{max} (s^{-1})[a]	acceleration by catalyst at $^{s}_{s}$pH 9.8[d]
2		(0.9 ± 0.2)	~1.1	1.5×10^{13}
3	18.2[b]	2.8×10^{-3}	3.3×10^{-3}	1.8×10^{12}
3a	18.2[b]	1.7×10^{-2}	2.0×10^{-2}	
6a	15.8[c]	$(2.0 \pm 0.3) \times 10^{-1}$	2.5×10^{-1}	2.13×10^{12}
6b	17.2[c]	$(1.1 \pm 0.1) \times 10^{-2}$	1.4×10^{-2}	1.38×10^{12}
6c	18.5[c]	$(1.4 \pm 0.1) \times 10^{-3}$	1.6×10^{-3}	1.36×10^{12}

[a] k_{cat}^{max} computed based on an average K_M of 9×10^{-5} M as described in the text. [b] $^{s}_{s}pK_a$ for methanol in CH_3OH determined based on the autoprotolysis constant[7] for methanol of $10^{-16.77}$ M^2. [c] Calculated from the linear regression $^{s}_{s}pK_a^{MeOH} = 0.748\ pK_a^{H2O} + 6.53$ as reported in ref 6. [d] Calculated based on the [$^-OCH_3$] existing at $^{s}_{s}$pH 9.8 (1.07×10^{-7} M) and the second-order rate constant for the methoxide-promoted reaction of the phosphates as described previously for 2-hydroxypropyl aryl phosphate diesters incorporating aryloxy leaving groups.[3c]

3.4. 4-Catalyzed Cleavage of Phosphate Diesters 6a−c That Incorporate Poor Leaving Groups. Substrates **6a**, **6b**, and **6c**, bearing leaving groups with conjugate acid $^{s}_{s}pK_a$values in the range of 15.8−18.5, were subjected to the **4**-catalyzed cyclization reaction. The reactions of **6a** and **6b** were monitored by ^{19}F NMR, as described in the Supporting Information. The reaction of substrate **6c** was sufficiently slow to allow in situ monitoring by ^1H NMR in CD_3OD. Substrate **6c** was also subjected to **4**-catalyzed cyclization in CH_3OH, employing the quench protocol and reconstitution in CD_3OD solvent for analysis of the reaction of substrates **6a**, **6b**, **3/3a**, and **1**. The observed rate constant determined in this manner was within error limits of that determined by the in situ monitoring of reaction progress in the deuterated solvent. Determining the solvent kinetic isotope effect in this manner is not rigorous, but it suffices, for our limited purposes, to state that $k_{CH3OH}/k_{CD3OD} \approx 1.0$.

Given in Table 1 are the observed rate constants obtained for reaction of substrates **6a**, **6b**, and **6c**, as well as k_1 for the **4**-catalyzed cyclization of **3**, the determination of which is described above. Also included in the table are the k_{cat}^{max} values corrected for a completely bound substrate calculated under the reasonable assumption that the K_M values for binding of **6a**−**c** and **3** to catalyst **4** are the same as the average experimental dissociation constant found for the **4**:2-hydroxypropyl aryl phosphate complexes (9×10^{-5} M) previously reported.[3c] For example, using the starting concentrations of **3** and **4** given for the equilibration experiment, 84% of the phosphate diester is bound as the Michaelis complex, so the $k_{cat}^{max} = k_1/0.84$ or 3.3×10^{-3} s^{-1}. Shown in Figure 5 is a plot of the log k_{cat}^{max} data vs leaving group $^{s}_{s}pK_a$, which includes the data determined previously for aryloxy leaving groups: this fits a standard linear regression, $\log k_{cat}^{max} = (-0.85 \pm 0.024)\ ^{s}_{s}pK_a + (12.8 \pm 0.4)$.

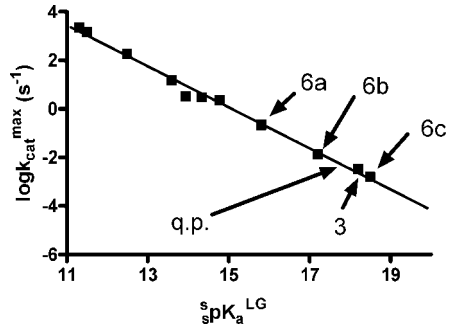

Figure 5. Plot of $\log(k_{cat}^{max})$ vs $^{s}_{s}pK_a$ for **4**-catalyzed cyclization of 2-hydroxypropyl alkyl phosphate diesters and 2-hydroxypropyl aryl phosphate diesters,[3c] which fits a standard linear regression of $\log(k_{cat}^{max}) = (-0.85 \pm 0.024)\ ^{s}_{s}pK_a + (12.8 \pm 0.4)$, $r^2 = 0.9925$ (11 data points). Unlabeled squares = aryloxy leaving groups; q.p.= quasi-symmetrical point located at a $^{s}_{s}pK_a$ of 17.7.

4. Discussion

Central to discussion of any mechanism for cleavage of phosphate diesters such as the presently studied 2-hydroxypropyl aryl and alkyl esters (**1**, **3**, **6a**−**c**) is whether the cyclization reaction is concerted, leading directly to the cyclic phosphate **2**, or stepwise via one or more 5-coordinate phosphorane intermediates (**5** and its associated pseudorotamers) with sufficient stability to be distinguished from a phosphorane-like transition state. Two classical ways to probe the existence of transient intermediates are to determine whether a break exists in a linear free energy relationship such as a Brønsted or Hammett plot or to observe some change in a recovered starting material such as isomerization or isotopic exchange.[8] Although acid-catalyzed hydrolysis of phosphate diesters is accompanied by substrate isomerization, it is generally observed that base-catalyzed hydrolysis of phosphate diesters does not proceed with isomerization of the substrate.[9] This is a probable consequence of the short lifetime of any dianionic phosphorane intermediate whose existence is so fleeting that the pseudorotation[10] required for isomerization is precluded. There is evidence that the components of imidazole/imidazolium buffers can act as general bases for the hydrolysis of UpU dinucleotide and a TTUTT oligomer to promote hydrolysis by a concerted, or near concerted, pathway without observable isomerization of the starting materials. However, imidazolium ion can act as a general acid to promote both the hydrolysis and the isomerization of these substrates.[11] Recent evidence indicates that the base-promoted reactions of more reactive RNA model systems with varying leaving groups being displaced by internal OH nucleophiles probably proceed through stable phosphorane intermediates. For example, the base-catalyzed ring closure of a series of uridine 3′-phosphate diesters with leaving groups spanning a range of 5−17 pK_a units exhibits a break in the Brønsted plot, consistent with the formation of a dianionic phosphorane intermediate, the formation and breakdown of which is rate limiting for good and poor leaving groups, respectively.[12] Interestingly, there is no observed **3′** → **2′** isomerization of the starting material, so the intermediate must not be capable of performing the required pseudorotations.[12]

At least conceptually, it seems reasonable that the same sort of coordinative interactions between a metal ion and phosphate diester that lower the activation energy for P−OR cleavage may provide sufficient stabilization of a dianionic phosphorane to allow an existence long enough to be considered a true intermediate. However, despite intensive investigations showing that metal-ion-containing complexes can catalyze phosphate diester cleavage and hydrolysis,[1−4] only recently were examples

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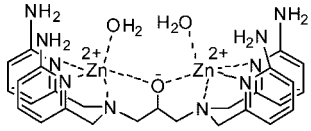
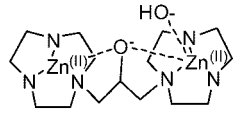
(11) Beckmann, C.; Kirby, A. J.; Kuusela, S.; Tickle, D. C. *J. Chem. Soc., Perkin Trans. 2* **1998**, 573.
(12) Lönnberg, H.; Strömberg, R.; Williams, A. *Org. Biomol. Chem.* **2004**, *2*, 2165.

provided where (1) a dinuclear Co(III) complex was shown, via heavy atom kinetic isotope effect studies, to enact a stepwise cleavage of methyl *p*-nitrophenyl phosphate,[13] (2) a mononuclear Co(III) complex was shown to promote the cleavage of a series of aryl methyl phosphates with an apparent break in the associated Brønsted plot,[14] and (3) a dinuclear Zn(II) complex (**7**) was shown to catalyze isomerization of a phosphate diester,[5] UpU.

Very recently, a heavy atom isotope effect study of the cleavage of labeled **1** mediated by dinuclear Zn(II) complex **8** provided results consistent with a concerted catalyzed cleavage that places considerable negative charge on the departing leaving group. By contrast, the results for the hydroxide-promoted reaction of labeled **1** were consistent with either a stepwise process through a phosphorane intermediate or a concerted associative mechanism with little cleavage of the P−OAr bond in the TS.[15] This is an important finding that provides evidence that the **8**-catalyzed reaction mechanism for cleavage of bound **1** is different from the lyoxide-promoted reaction in terms of charge development on the departing group in the TS. The question of whether the dinuclear Zn(II) complex, **4**, promotes concerted or stepwise cleavage of 2-hydroxypropyl aryl substrates with good leaving groups such as **1** has been addressed through linear free energy investigations in methanol.[3] However, since there were no breaks in the Brønsted plots observed with a variety of aryloxy leaving groups, the reaction was considered to either comprise two steps with rate-limiting formation of a phosphorane followed by fast departure of the aryloxy leaving group or alternatively be a concerted process.



7



8

Herein we probed for the existence of intermediates along the reaction pathway for **4**-catalyzed cleavage of a series of 2-hydroxypropyl alkyl phosphates (**6a**−**c**) to generate an extension of an earlier Brønsted plot[3c] that was based on seven aryloxy leaving groups having pK_a values in methanol from 11.3 to 14.8. In addition, we investigated the **4**-catalyzed equilibration of a kinetically formed mixture of **3** and **3a** into a thermodynamic mixture along with the concomitant loss of the P−OCH_3 group from each isomer. That the Brønsted plot in Figure 5 is linear for both good and poor leaving groups over a range of ∼7.5 $^{s}_{s}$pK_a units gives no observed evidence for a change in rate-limiting step for any of these reactants. A break would be expected for a process going through an intermediate, the formation and breakdown of which could be rate limiting for compounds with different $^{s}_{s}$pK_a values. However, it might be suggested that the compounds studied do not cover a sufficiently high $^{s}_{s}$pK_a range over which the anticipated transition in the plot would occur. The break would occur at the quasi-symmetrical point where the effective pK_a of the leaving group and nucleophile are the same. The effective $^{s}_{s}$pK_a for the ring-opened alcohol(s) from intermediate **5** in forming **3/3a** in Scheme 4 is not known with certainty. However, this can be estimated as ∼17.7 based on the predicted pK_a in water of 14.9 provided for the 2-hydroxypropyl group in **1** by Sánchez-Lombardo and Yatsimirsky[16] and the relationship between the pK_a in water and methanol for aliphatic alcohols.[6] The release of any angle strain generated by the eqO−P−Oax endocyclic angle of ∼90° (where the 5-membered phosphorane ring bridges equatorial and axial positions) would reduce the effective pK_a somewhat, but even without such a correction it can be seen that the quasi-symmetrical point, marked on the plot in Figure 5, lies to the left of the two data for the methoxy and ethoxy derivatives with no evidence for a break in that region. It is therefore plausible to suggest that the **4**-catalyzed reaction is a concerted one, but this interpretation rests on a lack of an anticipated observation of a break in the Brønsted plot close to its high $^{s}_{s}$pK_a end.

It is a fortunate observation that the **4**-catalyzed opening of **2** gives first a 29:71 kinetic mixture of **3**:**3a**, which is then transformed into a 72:28 thermodynamic mixture. The kinetic distribution is controlled by the relative rates of opening of the five-membered ring of the bound substrate following, or concerted with, methoxide attack, but the thermodynamic mixture is controlled by an additional factor involving the relative nucleophilicities and steric demands of the primary and secondary alkoxides (k_2/k_1 in Scheme 4 is 6.1). The fact that when the reaction is monitored in CD_3OH, the P−OCH_3 group is replaced by a P−OCD_3 group concurrently with the equilibration allows us to provide rate constants for both processes in a single experiment. The available data strongly suggest that both the isomerization and the loss of the methoxy group are tied to the formation of the cyclic phosphate **2**. The lines displayed in Figure 4 which are derived from the observed rate of isomerization (from Figure 3) and various ratios of k_1/k_3 (as defined in Scheme 4) provide evidence that the major pathway for isomerization proceeds via **2**. However, the nature of the simulation requires that, as a function of decreasing k_3/k_1 ratio, the lines asymptotically approach the dashed line derived as the best fit one for OCH_3 loss. Given the compression of these lines at low k_3/k_1 ratios, it is not possible to declare with certainty that small amounts of up to 5−10% of isomerization can proceed via a process that does not form **2**.

Despite the above caveats, the general observations are most simply accommodated by the process given in Scheme 4. The mechanism by which **2** is formed cannot be delineated with certainty from the available data, although the Brønsted plot supports a concerted process since there is no observed break. The β_{lg} term for all the aryloxy and alkyl substrates is −0.85 ± 0.02. This value can be compared with the β_{lg} value of −0.72 ± 0.08 found for the k_2^{-OMe} rate constants for methoxide-promoted cyclization of the aryloxy derivatives.[3c] The β_{lg} value for the methoxide reaction, which does not include the data for any of **3** or **6a**−**c** since they are too slow to measure, is slightly higher than but comparable to what is reported for the hydroxide-promoted reaction (−0.54[17] and −0.62[18]). While it

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(16) Sánchez-Lombardo, I.; Yatsimirsky, A. K. *Inorg. Chem.* **2008**, *47*, 2514.

(17) In the study reported in ref 12 the authors reported that the original Brown and Usher data for the hydroxide-promoted cleavage of a series of 2-hydroxypropyl aryl and alky phosphates exhibited some evidence for a break in the Brønsted plot, and for cleavage with good leaving groups the β_{lg} value was −0.54. See: Brown, D. M.; Usher, D. A. *J. Chem. Soc.* **1965**, 6558.

(18) Williams, N. H.; Takasaki, B.; Wall, M.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485.

is not specifically known whether the methoxide reactions involve two steps, this is becoming increasingly likely in water, based on the accumulating evidence that the hydroxide-promoted reactions of 2-hydroxypropyl aryl and alkyl phosphates are likely to proceed via a phosphorane intermediate.[12,15] The extent of breaking of the P−OAr/OR bond in the TS can be measured by the Leffler parameter, α, which is the ratio of the Brønsted β_{lg} for the TS relative to the β_{eq} for equilibrium transfers of the phosphoryl group between oxyanion nucleophiles. In the case of the transfer of the (RO)P(=O)O$^-$ group,[19] the β_{eq} value is −1.74 with the P−OAr/OR oxygen in the starting material having a net effective charge of +0.74 and departing oxyanion having a full negative charge. However, when coordinated to the two Zn(II) ions, the phosphate diester should have an P−OAr/OR charge closer to that of a triester,[20,21] or +0.84.[19] Assuming that the charges and β_{eq} values that have been derived under aqueous conditions can be transcribed accurately to methanol, the Leffler parameter for the **4**-catalyzed reaction, $\alpha = \beta_{lg}/\beta_{eq} = -0.85/-1.84 = 0.45$, suggests that the P−OAr/OR bonding change is ∼45% of the way from starting material to product. This value is consistent with a concerted process or a two-step one, and the similarity of this value and the α of ∼0.41 determined for the CH_3O^--promoted cyclization of the hydroxypropyl aryl phosphates suggests that similar charge shifts are operative for the rate-limiting transition states of both processes.

Aside from the subtleties of the mechanism by which the **4**-catalyzed cyclization of this series of substrates occurs, it is evident that this system is extremely effective in terms of the overall rates of the reaction. Listed in Table 1 are the accelerations provided from the k_{cat}^{max} terms for the fully bound substrates relative to the pseudo-first-order rate constants computed for the methoxide-promoted reactions at $_{s}^{}$pH 9.8, where the catalytic reactions were run. The accelerations for substrates **3** and **6a**−**c** are very big, about 10^{12}-fold, but somewhat uncertain since their k_2^{OMe} second-order rate constants rely on a long extrapolation of the Brønsted relationship log $k_2^{-OMe} = (-0.72 \pm 0.08)\ {}_{s}^{}pK_a + (5.36 \pm 1.08)$ derived for aryloxy-containing substrates.[3c] The acceleration for catalyzed opening of **2** is somewhat larger (1.5×10^{13}) and based on experimentally derived second-order rate constant for the methoxide-promoted opening of $(6.9 \pm 0.8) \times 10^{-7}$ M^{-1} s^{-1} determined as described in the Supporting Information. It is of note that the rate constant for opening of **2** by hydroxide in water does not appear to be known: the closest analogy being the rate constant for opening of ethylene phosphate by hydroxide of $\sim 4.5 \times 10^{-4}$ M^{-1} s^{-1}.[22]

The free energy diagram presented in Figure 6 is constructed for the interconversion of **3**, **3a**, and **2** promoted by **4** based on the various rate and equilibrium constants determined from this study. The scheme applies equally well to a concerted process or ones leading through phosphorane intermediates that partition exclusively between **3** and **2** as well as **3a** and **2**. The free energies of activation for **3** → **2** and **3a** → **2** are those obtained for the k_1 and k_2 steps of Scheme 4 corrected for complete binding by the catalyst (3.3×10^{-3} and 2.0×10^{-2} s^{-1}, respectively), while those chosen for the k_{-1} and k_{-2} steps of Scheme 3 are obtained from the analysis of the data of Figure 1 for the loss of **2** and generation of the kinetic mixture of **3** and **3a**; $k_{2\rightarrow 3a} = k_{-2} = 0.64 \pm 0.2$ s^{-1} and $k_{2\rightarrow 3} = k_{-1} = 0.26 \pm 0.1$ s^{-1}. The free energy diagram predicts that **2** is 2.6 kcal/mol above **3** and 1.9 kcal/mol above **3a**, so that the equilibrium distribution of **3**:**3a**:**2** is ∼71:28:1.

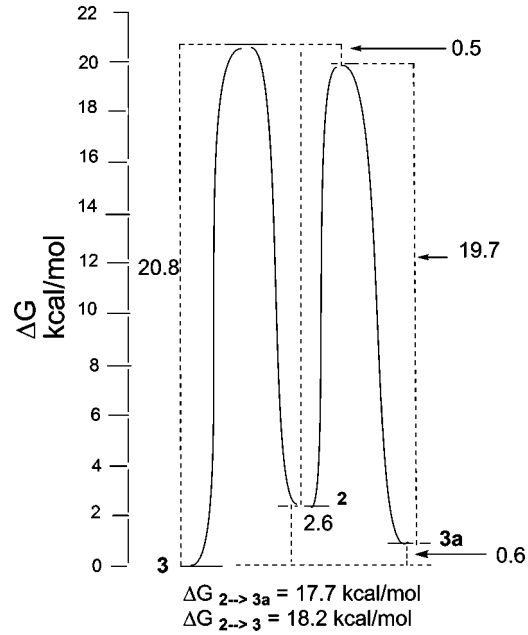

Figure 6. Free energy diagram for the **4**-catalyzed intercoversion of **3** and **3a** via the cyclic phosphate intermediate **2** at $_{s}^{}$pH 9.8. Free energies of activation calculated as $\Delta G^{\ddagger}_{cat} = -RT \ln(k_{cat}^{max}/(kT/h))$ from the Eyring equation where $(kT/h) = 6 \times 10^{12}$ s^{-1} at 298 K.

5. Conclusion

The present study provides convincing evidence that the **4**-catalyzed cleavage of a series of 2-hydroxypropyl aryl and alkyl phosphates proceeds via a process involving intramolecular displacement of the leaving group through a transition state where cleavage of the P---OLG bond has progressed ∼45% of the way from starting material to product **2**. The fact that all the substrates lie on a common Brønsted line indicates that there is no discernible change in the rate-limiting step throughout the series. If phosphorane-like intermediates are formed during the ring-closure process, their lifetimes must be short enough that that they cannot pseudorotate and isomerize to any great extent; otherwise, equilibration of **3** and **3a** would be far faster than loss of the methoxy group. In the alternative, the data are also equally consistent with a concerted process leading to **2.** The catalytic cleavage of **3** is fast when bound to the catalyst, having a $t_{1/2}$ of 3.5 min. The catalytic opening of **2** is even faster, generating **3** and **3a** with rate constants of 0.26 and 0.64 s^{-1} ($t_{1/2}$ = 2.7 and 1.1 s), respectively, which is about 10^{13} faster than the methoxide reaction at $_{s}^{}$pH 9.8. To our knowledge, the only other reports of the kinetics of opening **2** in the presence

(19) Bourne, N.; Williams, A. *J. Org. Chem.* **1984**, *49*, 1200.
(20) On the basis of the changes in the first and second acid dissociation constants of $H_2PO_4^-$ bound between two Co(III) centers, it appears that two metal ions have the effect of a single proton. By extension, a single metal ion is predicted to have the effect of 1/2 a proton. See: Edwards, J. D.; Foong, S.-W.; Sykes, A. G. *J. Chem. Soc., Dalton Trans.* **1973**, 829.
(21) Williams, N. H.; Cheung, W.; Chin, J. *J. Am. Chem. Soc.* **1998**, *120*, 8079.
(22) Kumamoto, J.; Cox, J. R., Jr.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 4858. The rate constant for methoxide reacting with 2 is 650 times slower than the reaction of hydroxide with ethylene phosphate. This is accounted for by a lower polarity medium effect where the alkoxide reaction with an anionic phosphate is 20−50 times slower than the hydroxide reaction in water, as well as a steric effect where the presence of the methyl group in **2** retards the reaction with methoxide.

of metal ions are those concerning a dinuclear Co(III) coordinated **2**,[23] the La^{3+}-promoted opening of **2** in methanol at $^{s}_{s}$pH 5.3,[24] and the opening of **2** in benzene/methanol promoted by a zirconium complex,[25] but none of these is as fast as what we observe here. The results presented here with RNA model substrates having poor leaving groups again supports the idea that the combination of a suitable dinuclear Zn(II) complex and a medium effect can provide manmade systems that are beginning to approach those of real biocatalysts.

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Supporting Information Available: Procedures for synthesizing and purifying **3**, **3a**, and **6a**−**c** and associated spectral data; procedures for kinetic studies of **4**-catalyzed cyclizations of these; procedure for simulated fits of the data in Figure 5 according to eq 5; ^{1}H NMR spectra at various times for the **4**-catalyzed equilibration of **3** and **3a** along with the loss of the P−OCH_3 group in CD_3OH. This material is available free of charge via the Internet at http://pubs.acs.org.

JA900525T

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(24) Tsang, J. S. W.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2003**, *125*, 1559.
(25) Stulz, E.; Leuman, C. *Chem. Commun.* **1999**, 239.