On the question of stepwise *vs.* concerted cleavage of RNA models promoted by a synthetic dinuclear Zn(II) complex in methanol: implementation of a noncleavable phosphonate probe†

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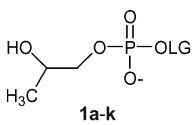
To address the question of concerted *versus* a stepwise reaction mechanisms for the cyclization of the 2-hydroxypropyl aryl and alkyl RNA models (**1a–k**) promoted by dinuclear Zn(II) complex (**4**) at $^{s}_{s}$pH 9.8 and 25 °C, the non-cleavable *O*-hydroxypropyl phenylphosphonate analogues **6a** and **6b** were subjected to the catalytic reaction in methanol. These phosphonates did not undergo isomerization in the study, the only observable methanolysis reaction being release of 1,2-propanediol and the formation of *O*-methyl phenylphosphonate. The observed first order rate constants for methanolysis promoted by **4** are $k_{obs}^{6a} = (1.47 \pm 0.09) \times 10^{-4}$ s^{-1} and $k_{obs}^{6b} = (2.08 \pm 0.09) \times 10^{-6}$ s^{-1}, respectively. The rates of methanolysis of a series of *O*-aryl phenylphosphonates (**8a–f**) in the presence of increasing [**4**] were analyzed to provide binding constants, K_b, and the catalytic rate constant, k_{cat}^{max}, for the unimolecular decomposition of the **8** : **4** Michaelis complex. A Brønsted plot of the log (k_{cat}^{max}) *vs.* $^{s}_{s}pK_a^{phenol}$ (acidity constant of the conjugate acid of the leaving group in methanol) was fitted to a linear regression of log $k_{cat}^{max} = (-0.80 \pm 0.07)^{s}_{s}pK_a + (10.2 \pm 1.0)$ which includes the datum for **6a**. The datum for **6b**, which reacts ~70–fold slower, falls significantly below the linear correlation. The data provide additional evidence consistent with a concerted cyclization of RNA models **1a–k** promoted by **4**.

Introduction

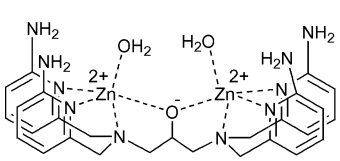
The phosphate diester linkages present in the biological polymers of RNA and DNA are highly resistant to cleavage as demonstrated by the estimated $t_{1/2}$ for hydrolysis at neutral pH and 25 °C of 110[1] and 30 million years.[2] Numerous metallo-enzymes including paroxonase,[3] alkaline phosphatase,[4] phospholipase C[5] and P1 Nuclease[6] that catalyze the hydrolysis of neutral and anionic phosphate esters have active sites containing a dinuclear Zn(II) core. These are among Nature's most proficient catalysts since they accelerate the hydrolysis of their phosphate ester substrates by as much as 10^{17}.[7]

The cleavages of phosphate diesters of general structure **1** promoted by di-Zn(II) complexes (**2–4**) have been actively studied as model systems for metallo-RNase enzymatic cleavage of RNA.[8,9,10] Of current interest is whether the cleavage reactions proceed in a concerted fashion or through a 5-membered dianionic phosphorane intermediate.[11,12] For simple RNA models like **1** and uridine-5′-phosphodiesters, LFE relationships[13] demonstrated that the base catalyzed cleavages of the latter, and in all probability the former, proceed *via* stepwise processes. It is a current question whether enzymes and/or small molecule catalysts that cleave phosphate diesters select for transition states that are altered relative to those of the uncatalyzed reaction.[12] A recent study indicates that the hydrolysis of 3′5′-UpU promoted by **2** generates cleavage product along with some isomerized 2′5′-UpU, providing the first evidence that a dinuclear complex at near neutral pH can stabilize a dianionic phosphorane intermediate enough to allow pseudorotation and reversion to isomerized starting material.[11] Conversely, complex **3** was recently shown, by heavy atom kinetic isotope effect (kie) studies, to promote the cleavage of **1a** by a concerted process.[12]



1a-k

LG = a) 4-nitrophenyl, b) 4-nitro-3-methylphenyl, c) 3-nitrophenyl, d) 4-chlorophenyl, e) 3-methoxyphenyl, f) phenyl, g) 4-methoxyphenyl, h) trifluoroethyl, i) 2-fluoroethyl, j) methyl, k) ethyl



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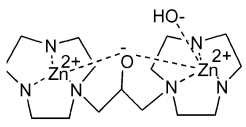

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We reported that the cleavage of a series of 2-hydroxypropyl aryl and alkyl phosphates **1a–k** was promoted by complex **4** in methanol[14] and ethanol.[15] The Brønsted plot of the catalyzed process in CH_3OH was linear for the **1a–k** series spanning a $^{s}_{s}pK_a^{phenol}$ range from 11.3 to 18.5, with a fit of log(k_{cat}^{max}) = (–0.85 ± 0.024)$^{s}_{s}pK_a^{phenol}$ + (12.8 ± 0.4), $r^2 = 0.9925$.[14b] We also observed that a synthetic mixture of **1j** and **1j′** (initial ratio 22 : 78) isomerizes in

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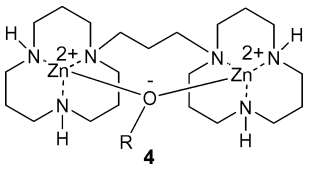
† Electronic supplementary information (ESI) available: Synthetic procedures for the preparation of **6a**, **6b** and **8a–f** as well as analytical data (^{1}H, ^{31}P NMR and HRMS). Partial ^{1}H NMR spectra for the **4** promoted methanolysis of **6b**. Description of kinetic procedures and tables of observed rate constants. See DOI: 10.1039/b918310h

the presence of **4** to generate a thermodynamic ratio of 72 : 28 and that this process proceeds through cyclic intermediate **5** involving cleavage of P-OCH_3 that is faster than, or equal to, the rate of isomerization (Scheme 1). Insofar as these studies failed to detect the intermediacy of a 5-coordinate phosphorane, each is consistent with the reaction proceeding *via* a concerted cyclization to form the complexed cyclic phosphate **5**.



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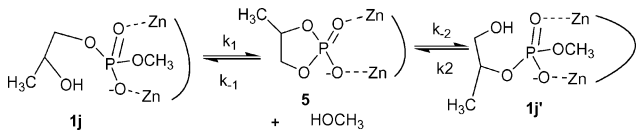
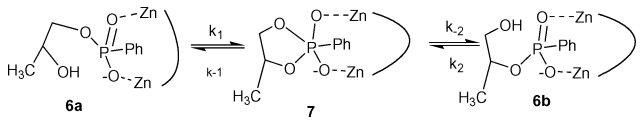


1j **5** **1j'**

Scheme 1 Isomerization of **1j** and **1j′** promoted by catalyst **4** depicting the cyclic intermediate **5**.

Since failure to detect does not disprove the existence of a fleeting intermediate, we sought an additional test that adds information as to whether the **4**-promoted cyclizations are stepwise or concerted. We disclose results of experiments where phosphonates **6a** and **6b**, that do not possess a suitable leaving group and thus cannot form a cyclic ester, were subjected to reaction in the presence of **4** in methanol. The observation that **6a** and **6b** undergo interconversion would lend strong support for the existence of phosphorane intermediate(s) of general structure **7** (Scheme 2) and by implication a phosphorane intermediate in the **4**-promoted cyclization of substrates **1**.



6a **7** **6b**

Scheme 2 Putative isomerization of **6a** and **6b** promoted by catalyst **4** depicting the hypothetical intermediate **7**.

Experimental

Materials

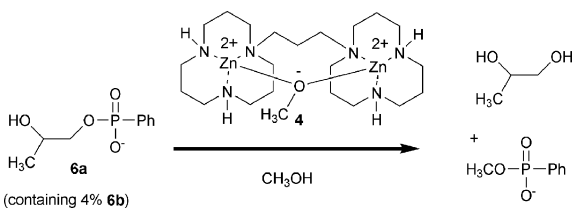
Methanol (99.8% anhydrous), sodium methoxide (0.5 mol dm^{-3} solution in methanol) and $Zn(CF_3SO_3)_2$ were purchased from Aldrich and used as supplied. 1,3-Bis-N1-(1,5,9-triazacyclododecyl)propane was synthesized according to the published procedure.[16] The dinuclear (CH_3O^-):$Zn(II)_2$ complex **4** was prepared as a 2.5 mmol dm^{-3} solution in methanol by sequential addition of aliquots of stock solutions of sodium methoxide, 1,3-bis-N1-(1,5,9-triazacyclododecyl)propane and $Zn(CF_3SO_3)_2$ such that the relative amounts were 1 : 1 : 2. This order of addition is essential: complete formation of **4** requires ~ 40 min (as monitored by optimization of catalytic activity).[14] Preparation of *O*-aryl phenylphosphonates **8a–f** and **6a**, **6b** is described in the Supporting Information.†

Kinetics studies (substrates 6a and 6b). A 2.43×10^{-3} mol dm^{-3} mixture of phosphonate **6a** (containing 4% **6b**) was reacted with 2.44×10^{-3} mol dm^{-3} of catalyst **4** in anhydrous methanol at ambient temperature, ^{s}pH 9.8 ± 0.1. Each of three reactions was quenched after 5 min, 1 h and 24 h by the addition of 10×10^{-3} mol dm^{-3} HCl and 10×10^{-3} mol dm^{-3} LiCl. The volatiles were removed under reduced pressure and the reaction components were reconstituted in CD_3OD for ^{1}H NMR analysis. To sharpen further the signal intensities in the ^{1}H NMR spectrum, an additional aliquot of LiCl was added to the NMR samples, bringing the total concentration of chloride ion to 0.14 mol dm^{-3}. An identical procedure was used to study the reaction of isomerically pure **6b**.

Kinetic studies (substrates 8a–f). The rates of methanolysis for *O*-aryl phenylphosphonates **8a–f** (5×10^{-5} mol dm^{-3}) promoted by **4** were determined with an Applied Photophysics SX-17MV stopped-flow reaction analyzer at ^{s}pH 9.8 ± 0.1, 25.0 °C. Reactions were monitored for the appearance of the product phenols at 320 (**8a**), 340 (**8b**), 284 (**8c**), 280 (**8d**), 292 (**8e**), 276 (**8f**) nm. Plots of k_{obs} *versus* $[\mathbf{4}]_{free}$ were fit to eqn (2) from which the unimolecular rate constants for decomposition of the **8**:**4** complex, k_{cat}^{max}, and the binding constants K_b could be determined. For the definition of $[\mathbf{4}]_{free}$ see footnote.‡

Results

Three separate experiments were run in which a mixture of phosphonates **6a** and **6b** (2.43×10^{-3} mol dm^{-3}, isomeric ratio of 96 : 4) were reacted in the presence of 2.44×10^{-3} mol dm^{-3} **4** in CH_3OH at room temperature, ^{s}pH 9.8 ± 0.1. The three reactions were terminated after 5 min, 1 h and 24 h using a quench and solvent removal/reconstitution protocol identical to that previously employed by us[14] in studies of the **4**-catalyzed cyclization of a series of 2-hydroxypropyl alkyl phosphate diesters. In Fig. 1 are the partial ^{1}H NMR spectra (600 MHz) of the reconstituted reaction contents that indicate the $CH_3CH<$ doublet of **6a** at δ 1.078 ppm (3H, d, $J = 6.60$ Hz) is converted into one centered at δ 1.119 ppm (3H, d, $J = 6.60$ Hz). This was independently identified as belonging to the $CH_3CH<$ group of 1,2-propanediol resulting from cleavage of **6a** according to the process shown in eqn (1). The observed rate constant for cleavage of **6a** is $k_{obs}^{6a} = (1.47 \pm 0.09) \times 10^{-4}$ s^{-1} which is the average of the two rate constants 1.38×10^{-4} s^{-1} and 1.56×10^{-4} s^{-1}, determined from the signal intensities of starting material and product for the spectra obtained after 5 min and 1 h. Substrate **6b** was prepared in isomerically pure form (Supporting Information†) and subjected to **4**-catalyzed methanolysis. As before, the reactions were quenched at 5 min, 1 h and 24 h. Integration of the signal intensities corresponding to 1,2-propanediol and the methyl substituent of **6b** (δ 1.085 ppm (3H, d, $J = 6.6$ Hz)) at 60 min and 24 h leads to a calculated $k_{obs}^{6b} = (2.08 \pm 0.09) \times 10^{-6}$ s^{-1}. Shown in Fig. 2 are the partial ^{1}H NMR spectra obtained following reaction of isomer **6b**.



6a (containing 4% **6b**) **4** CH_3OH (1)

‡ Previously we have established that triflate ion is an inhibitor of the catalysis afforded by the dinuclear Zn(II) complex with an inhibition constant of 14.9 mmol dm^{-3} from which one can determine the free catalyst concentration or $[4]_{free}$ at any [triflate]. See ref. 14.

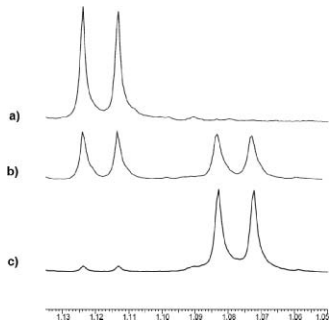


Fig. 1 Partial ^{1}H NMR (600 MHz, CD_3OD, 25 °C) spectra obtained following the **4**-catalyzed methanolysis of a mixture of **6a** and **6b** (2.43×10^{-3} mol dm^{-3}, isomeric ratio of 96 : 4) showing the conversion of starting material into 1,2-propanediol after a) 24 h; b) 60 min and c) 5 min. The $CH_3CH<$ doublets of **6a** and 1,2-propanediol appear at δ 1.078 ppm (3H, d, $J = 6.60$ Hz) and δ 1.119 ppm (3H, d, $J = 6.60$ Hz), respectively.

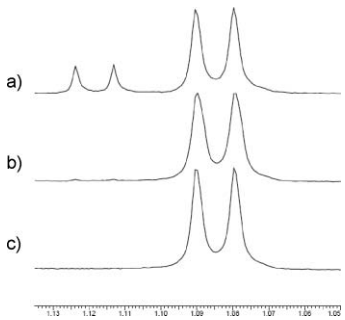
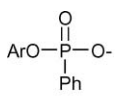


Fig. 2 Partial ^{1}H NMR (600 MHz, CD_3OD, 25 °C) spectra obtained following the **4**-catalyzed methanolysis of **6b** (2.43×10^{-3} mol dm^{-3}) showing the conversion of starting material into 1,2-propanediol after a) 24 h; b) 60 min and c) 5 min. The $CH_3CH<$ doublet of **6b** appears at δ 1.085 ppm (3H, d, $J = 6.6$ Hz).

Phosphonates **8a–f** were prepared by standard means (Supporting Information†) and subjected to **4**-catalyzed cleavage at 25 °C, and ^s_spH 9.8 ± 0.1. Shown in Fig. 3 is a representative plot of k_{obs} *versus* $[\mathbf{4}]_{free}$ for the catalyzed methanolysis of **8a**. All saturation plots showed strong substrate binding and a slight x-intercept.[14] These data were analyzed by fitting to a strong binding eqn (2)[14,15] to give the binding constants (K_b) and k_{cat}^{max} values for the cleavage of the P-OAr group from the **8** : **4** complex listed in Table 1. In Fig. 4 is the Brønsted plot of log k_{cat}^{max} *vs.* $^s_s pK_a^{phenol}$ which gives a best fit of log $k_{cat}^{max} = (-0.80 \pm 0.07)^s_s pK_a^{phenol} + (10.2 \pm 1.0)$. The fit includes the **6a** data; **6b** reacts 70-fold slower and its cleavage rate constant falls below the line.



8a-f

Ar = a) 4-nitrophenyl
b) 3-nitrophenyl
c) 4-chlorophenyl
d) phenyl
e) 4-methoxyphenyl
f) 2,4-dimethylphenyl

$$k_{obs} = k_{cat}^{max}(1 + K_{bind}[S] + [\mathbf{4}]_{free}K_b - X)/(2K_b)/[S] \quad (2)$$

where:

$$X = (1 + 2K_b[S] + 2[Cat]K_b + K_b^2[S]^2 - 2K_b^2[Cat][S] + [Cat]^2K_b^2)^{0.5}$$

Table 1 Catalytic rate constants k_{cat}^{max} and K_b determined for the **4**-catalyzed cleavage of phosphonates **8a–f** and **6a**,**b**

Substrate	$^s_s pK_a^{phenol}$	k_{cat}^{max}/s^{-1} [a]	$K_b/dm^3\ mol^{-1}$ [a]
6a	17.17[b]	(1.75×10^{-4})[c]	—
6b	17.70[b]	(2.48×10^{-6})[c]	—
8a	11.30	5.29 ± 0.1	$(2.8 \pm 0.2) \times 10^3$
8b	12.41	3.09 ± 0.09	$(5.1 \pm 0.6) \times 10^4$
8c	13.59	0.48 ± 0.01	$(1.1 \pm 0.1) \times 10^4$
8d	14.33	$(9.2 \pm 0.2) \times 10^{-2}$	$(4.6 \pm 1.2) \times 10^4$
8e	14.77	$(4.5 \pm 0.2) \times 10^{-2}$	$(2.3 \pm 0.8) \times 10^4$
8f	15.04	$(1.1 \pm 0.1) \times 10^{-2}$	$(2.0 \pm 1.5) \times 10^4$

[a] Determined from fit of k_{obs} *versus* $[\mathbf{4}]_{free}$ data to eqn (2). [b] For the calculation of $^s_s pK_a^{6a}$ and $^s_s pK_a^{6b}$ see Supporting Information. [c] k_{cat}^{max} computed assuming that the K_b for these two substrates can be approximated by the average K_b of 2.6×10^4 dm^3 mol^{-1} determined for **8a–f** as discussed in the main text.

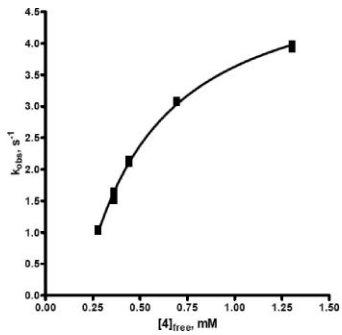


Fig. 3 Plot of k_{obs} *versus* $[\mathbf{4}]_{free}$ for the catalyzed methanolysis of **8a** (5×10^{-5} mol dm^{-3}) determined from the rate of appearance of product phenol at 320 nm, ^s_spH 9.8 ± 0.1 and 25 °C. Fitting of the data to the expression given in eqn (2) gives $k_{cat}^{max} = 5.29 \pm 0.1$ s^{-1} and $K_b = (2.80 \pm 0.17) \times 10^3$ dm^3 mol^{-1}, and an intercept $(A) = (0.186 \pm 0.006) \times 10^{-3}$ mol dm^{-3}.

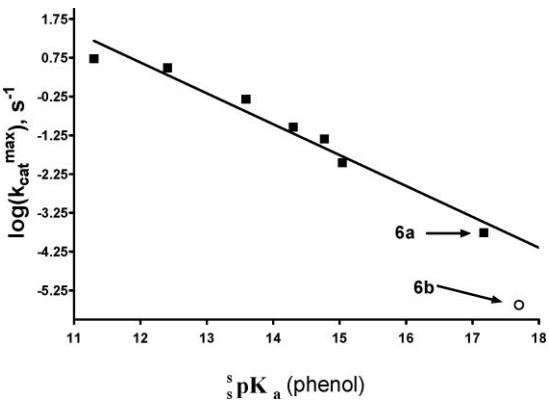


Fig. 4 Brønsted plot of the log (k_{cat}^{max}) *vs.* $^s_s pK_a$ (phenol) for the **4** catalyzed cleavage of phosphonates **8a–f** and **6a**. Fit of the data to a standard linear regression provides log $k_{cat}^{max} = (-0.80 \pm 0.07)^s_s pK_a^{phenol} + (10.2 \pm 1.0)$. Data for cleavage of diol from **6a** and **6b** are indicated by arrows, and their $^s_s pK_a$ values were assessed as given in the Supporting Information.†

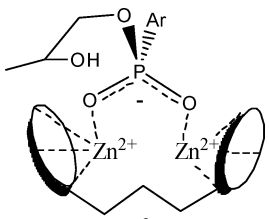
Discussion

(a) General considerations

The methanolysis of **6a** (containing 4% of isomer **6b**) promoted by catalyst **4**, monitored over a 24 h time period, revealed that the 2-hydroxypropyl substituent exclusively cleaves to produce

1,2-propanediol and *O*-methyl phenylphosphonate, eqn (1). The analogous cleavage of the 1-hydroxypropan-2-yl group from isomerically pure **6b** is also effected by **4**, albeit about 70-times slower than for **6a**, $k_{obs}^{6a} = (1.47 \pm 0.09) \times 10^{-4}$ s^{-1} and $k_{obs}^{6b} = (2.08 \pm 0.09) \times 10^{-6}$ s^{-1}, respectively. No interconversion of **6a** and **6b** was detected in either reaction mixture. It is essential to investigate the reactions of both isomers, since a finding of no isomeric interconversion during investigation of only one starting material could be due to a fortuitous choice of the thermodynamically most stable one.

The methanolytic cleavage of a series of *O*-aryl phenylphosphonates **8a–f** promoted by dinuclear catalyst **4** was studied at $_{s}^{s}$pH 9.8 and 25 °C. The K_b values (Table 1) determined for the series vary from 0.3 to 5.1×10^4 dm^3 mol^{-1} in no particular order with an average binding constant of $(2.7 \pm 1.9) \times 10^4$ dm^3 mol^{-1}. The binding constants determined for the **4**-catalyzed cleavage of *O*-aryl phenylphosphonates are similar to, and perhaps slightly larger than those previously determined by us for the reaction of methyl aryl phosphate diesters,[17] suggesting that replacement of the P-OMe substituent of a phosphate diester with a C_6H_5 group to form the phosphonate has a minor effect on the relative stability of the **4**:substrate complex. This observation is in line with a proposed mode of binding illustrated in **9** where the actual ligand is ear-muff like when bound to the two Zn(II) ions and accommodates the bridging substrate $>PO_2^-$ group. The unbound RO-P, ArO-P and/or R-P groups are perpendicular to the plane defined by the two Zn(II) ions and thus point away from the catalytic pocket being relatively free from steric interactions with the azacyclic ligand.[18]



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The k_{cat}^{max} values for the unimolecular decomposition of the **4**:**8a–f** Michaelis complexes span a factor of ~500. Direct comparison can be made between the k_{cat}^{max} terms determined for **8a–e** and a series of methoxy aryl phosphate diesters[17] containing identical leaving groups which indicates that the phosphonates react some 130–200 times faster than the diester containing the same leaving group. The k_{cat}^{max} values for **6a** and **6b** can be calculated based on the reasonable assumption that their binding constants with catalyst **4** are the same as the average binding constant determined for substrates **8a–f** ($K_b^{ave} = 2.7 \times 10^4$ dm^3 mol^{-1}). Accordingly one computes that substrates **6a** and **6b** were each 84% bound[19] to **4** under the experimental conditions and so $k_{cat}^{max} = k_{obs}^{6}/0.84$ (Table 1).

The Brønsted plot of log k_{cat}^{max} *vs.* $_{s}^{s}pK_a^{phenol}$ (Fig. 4) fits a regression of log $k_{cat}^{max} = (-0.80 \pm 0.07)\ _{s}^{s}pK_a + (10.2 \pm 1.0)$. The fit includes the datum for **6a** but not that for **6b**, since the latter reacts 70 times slower and its datum falls below the line. A factor of 2.5 between the k_{obs}^{6a} and k_{obs}^{6b}, is due to the $\Delta_{s}^{s}pK_a^{phenol}$ (17.17 for **6a** and 17.70 for **6b** see Supporting Information†), while the remaining ~30-fold is suggested to result from the secondary/primary alcohol substitution pattern.

The hydrolysis of *O*-aryl methylphosphonates is subject to base catalysis with a reported β^{LG} of –0.69,[20] and their hydrolysis, when doubly coordinated to an exchange inert dinuclear Co(III) complex, is proposed to be concerted on the basis of kinetic isotope effect data ($^{18}k_{nuc}$ and $^{18}k_{lg}$).[20] In that instance a β^{LG} of –1.12 was reported which is larger than that determined here for the **4**-promoted methanolysis of **8a–f** and **6a** ($\beta^{LG} = -0.80$). It is difficult to determine if the respective Brønsted slopes determined here and in ref. 20 indicate a different degree of P-OAr bond cleavage in consideration of the fact that neither is normalized by a known β^{EQ} value in water or methanol nor are the effects of substitution of a P-CH_3 for P-Ph group known.

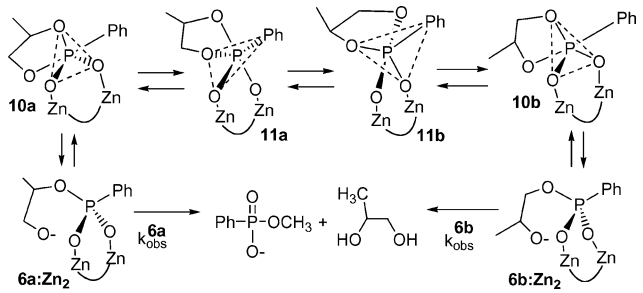
(b) Mechanistic implications for the cleavage of 2-hydroxypropyl alkyl and aryl phosphate diesters

The **4**-catalyzed cyclization of 2-hydroxypropyl alkyl and aryl phosphate diesters **1a–k** in methanol is a highly efficient reaction which is accelerated by at least 10^8 relative to the corresponding CH_3O^- promoted reaction. The catalytic reactions were shown to exhibit transition state stabilization in excess of 20 kcal mol^{-1} relative to the base-promoted reaction.[14] A combination of the large rate acceleration of the catalytic reaction and the fact that substrates of general structure **1** are widely used in mechanistic studies as models for RNA cleavage[8–15,18] has prompted detailed study of the reaction mechanism for this transformation. Two classical ways to probe the existence of transient intermediates are to determine if a break exists in a LFE relationship such as a Brønsted or Hammett plot, or to observe some change in a recovered starting material such as isomerization or positional isotopic exchange.[21] The absence of a break in the Brønsted plot determined for the **4**-catalyzed cyclization for the entire series of **1a–k** spanning $_{s}^{s}pK_a^{phenol}$ values from 11.3 to 18.5, was considered to support a concerted cyclization reaction.[14b] However, as pointed out in the original report, this assertion rests on the lack of an anticipated observation of a break in the Brønsted plot at the quasi-symmetrical point (where $_{s}^{s}pK_a^{Nu} = {}_{s}^{s}pK_a^{LG} = 17.7$) which is close to its high pK_a end.

Experiments also show that a synthetic mixture of **1j**/**1j′** (see Scheme 1, initial ratio of 22/78) undergoes fast equilibration in the presence of equimolar **4** to generate a 72/28 thermodynamic mixture. The pseudo-first order rate constant for the approach to equilibrium is $(7.0 \pm 1.0) \times 10^{-3}$ s^{-1} ($t_{1/2} = 99$ s). Positional isotope exchange studies in CD_3OH indicate that both **1j** and **1j′** lose OCL_3 on the same timescale as the isomerization occurs. Taken together, these results indicate that isomerization of **1j**/**1j′** occurs through a cyclic phosphate intermediate (**5**) with concomitant loss of OCL_3 as in Scheme 1. The data are consistent with concerted cleavage of the P-OLG bond although not uniquely so. Additional intermediates formed along the reaction coordinate, such as 5-coordinate phosphoranes, could be formed, but these must exclusively break down to **5** and thus do not play any extensive role in the direct **1j** to **1j′** isomerization process.[14b]

In this work we have used a third test, employing poorly reactive phosphonates **6a** and **6b**, to attempt to distinguish between concerted and step-wise mechanisms for the **4**-catalyzed cyclization of **1a–k**.[22] Such a test has been used by Lönneberg and co-workers[23] and Mikkola and Williams[11] to study isomerization of phosphoryl groups between 2′- and 3′- positions of ribose

rings. In these and the present cases, a concerted process which requires an in-line attack through a trigonal bipyramidal TS with leaving and entering groups at the apical position cannot occur, leaving the only possible pathway to be a two step one which would be clearly evident if isomerization of **6a**/**6b** could be demonstrated. Phosphonates are generally at least 10 times more susceptible to nucleophilic attack than their corresponding phosphates[24,25] (130–300 fold faster in this work with aryl phenylphosphonates *vs.* methoxy aryl phosphates *vide supra*) and **4**-catalyzed intramolecular attack of the 2-hydroxypropyl oxygen on the central P of phosphate diesters is 1000 to 5000 times faster than direct nucleophilic attack of coordinated methoxide.[14–17,18] Thus, it is difficult to envision that a similar attack cannot occur in phosphonates **6a**/**6b** to give a 5-coordinate intermediate, although isomerization of these species *via* a cyclic ester as was seen with the diesters **1j**/**1j′** is precluded. A proposed mechanism by which phosphonates **6a** and **6b** might undergo isomerization is *via* a suite of pseudo-rotating phosphoranes **10a**,**b** and **11a**,**b** as in Scheme 3.



Scheme 3 Postulated process by which isomerization of **6a** and **6b** might occur. Equatorial substituents on phosphoranes identified by dashed triangle.

From X-ray crystallographic structure considerations of the Zn(II)$_2$[14a] and Cu(II)$_2$[18b] complexes of the 1,3-bis-N1-(1,5,9-triazacyclododecyl)propane ligand, intramolecular attack of the hydroxypropyl anion on a doubly Zn(II)-coordinated **6a** or **6b** can occur most easily perpendicular to the plane defined by the Zn(II)-O$^-$-P–O$^-$-Zn(II) moiety and in line with the P-Ph bond. This gives phosphoranes **10a** or **10b** in which the attacking group and the phenyl substituent occupy apical positions while the two O$^-$-Zn(II) units occupy equatorial positions. Interconversion of **10a** to **10b** requires two pseudorotations to form **11a** and then **11b** in which each of the oxygens of the 5-membered cycle is in turn apical to an apical O$^-$Zn(II) group, violating one of the Westheimer rules.[26] The final formation of the isomerized product (**6b** from **6a** or *vice versa*) from the process proposed in Scheme 3 requires a third pseudorotation placing the Ph and departing oxyanion in apical positions.

An alternative explanation, less likely in our opinion for this system due to the restrictive geometry of the complex, deems that the initial attack of the hydroxypropyl oxyanion occurs *anti* to one of the O$^-$-Zn(II) units, meaning that **6a** would directly give **11b** and **6b** would give **11a**. These would have to interconvert to effect isomerization again violating a Westheimer rule.

Despite the above considerations, **6a**/**6b** isomerization is not observed and the only reaction products detected were those of the cleavage of the *O*-alkyl substituents, namely 1,2-propanediol and *O*-methyl phenylphosphonate. This latter reaction does not involve the intramolecular cyclization, and thus should occur independent of whether the 2-hydroxypropyl group is present. The fact that the k_{cat}^{max} for cleavage of the leaving group from **6a** lies on the line of the Brønsted plot constructed for the *O*-aryl phenylphosphonates (**8**) suggests that all these phenylphosphonate esters react by a common mechanism that probably involves a concerted attack of Zn(II) coordinated methoxide on the P. This is similar to one of the mechanisms that was proposed for cleavage of aryl methyl phosphate diester DNA models by **4**.[17] That the rate constant for the cleavage of **6b** lies considerably below this line likely results from a steric effect in the displacement of a secondary alcoholate relative to a primary one.[14b]

Conclusions

An observation that **6a** ↔ **6b** isomerization occurred during the slow cleavage of these phosphonates would provide powerful evidence for the existence of 5-coordinate phosphorane intermediates, and thus lend credence to the possibility that such intermediates could occur during the cleavage of phosphate diesters **1a–k**. However, no observed isomerization does not provide as convincing evidence for the absence of phosphorane intermediates. Such intermediates could be formed but their lifetime is too short, or they are too conformationally restricted when bound to the catalyst, to allow the pseudorotations required for isomerization. It was noted,[11] in a recent report of a man-made catalyst promoting the isomerization of 3′5′-UpU to 2′5′-UpU concurrent with cleavage of the former, that "enzymes or ribozymes that catalyze RNA cleavage do not appear to catalyze isomerization as well, but presumably in these cases, the confines of the active site even more firmly direct the reaction toward cleavage if similar phosphoranes are involved".

On the other hand, the lack of isomerization of **6a**/**6b** and the previous data[14b] are in full accord with the simplest explanation of a concerted process for the catalytic cleavage of **1a–k**.

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