La³⁺-Catalyzed Methanolysis of Hydroxypropyl-*p*-nitrophenyl Phosphate as a Model for the RNA Transesterification Reaction

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Abstract: The methanolysis of hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP, **1**) promoted by La(OTf)$_3$ under buffered conditions was studied in methanol as a function of ^s_spH at 25 °C. ^{31}P NMR studies at −90 °C indicate that there are at least three La/**1** complexes formed at ^s_spH $\approx$ 5.3 of 1:1, 2:2, and 1:2 stoichiometry. Kinetic studies of the observed pseudo-first-order rate constants for the methanolysis of **1** as a function of [La³⁺] at 4.5 < ^s_spH < 10.5 indicate there are two general ^s_spH regimes. In the low ^s_spH regime between 4.5 and 7.6, the plots of k_{obs} versus [La³⁺] exhibit saturation behavior with very strong 1:1 binding, with a plateau rate constant that depends on [OCH$_3^-$]. The catalytically productive species is shown to be a 2:2 complex of La³⁺ and **1**, where the phosphate is proposed to be doubly activated, thereby promoting the methoxide reaction by some 4.6×10^{10}-fold. In the high ^s_spH regime from 7.9 to 10.5, 1:1, 2:2, and 2:1 La³⁺/**1** complexes are formed with the La³⁺ coordinated in the form of [La³⁺(OCH$_3^-$)]$_{1,2}$. Throughout this ^s_spH regime at high [La³⁺], a saturation complex, (La³⁺OCH$_3^-$)$_2$/**1**, is formed that spontaneously decomposes with a rate constant of $(5-10) \times 10^{-3}$ s^{-1}, leading to an acceleration of 10^9-fold at ^s_spH 8.0.

Introduction

Many enzymes that promote the hydrolysis of phosphate esters have active sites containing two or more metal ions.[1] These include enzymes cleaving phosphate monoesters[1] (alkaline phosphatase, inositol phosphatase, and purple acid phosphatase) and those cleaving phosphate diesters[2] such as the RNAse from HIV reverse transcriptase,[3a] 3′−5′ exonuclease from DNA polymerase I,[3b] and P1 nuclease.[3c] Because of the obvious biological relevance pertaining to the storage of genetic information in DNA and RNA, much attention has been focused on the hydrolysis of phosphate diesters mediated by metal ions.[4] Among the metal ions, lanthanides exhibit some of the most dramatic accelerations, particularly under basic conditions as their metal-hydroxo forms. As impressive as some of the rate accelerations are, in the cases of studies with metal ions alone in aqueous solution, complete mechanistic evaluation is precluded by the fact that, above the pK_a's of metal−aquo complexes, formation of precipitates or gels of poor definition occurs.[5] In some cases, the problem has been ameliorated by complexing the metal ions to stabilizing ligands,[4n] including cyclodextrins,[4i] and macrocycles,[4h,k] or by inserting them into micelles,[4e] but even so, homogeneity problems usually make it difficult to study the reaction under highly basic conditions.

Recently, we have shown that La³⁺ is soluble in methanol throughout the ^s_spH region where ionization of the La³⁺(CH$_3$-OH)$_n$ occurs, thus making it possible to study the metal-ion-promoted methanolysis of acetylimidazole and its ligand exchange inert complex (NH$_3$)$_3$Co^{III}−AcIm,[6] various unactivated esters,[7] and three aryl phosphate diesters.[8,9] Because of the solubility characteristics of these metal ions in methanol, it is

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possible to greatly expand the concentration and $_s^s pH$ range under which the kinetics can be studied, thus solving some of the problems inherent in analogous water studies. In the present study, we have investigated the La^{3+}-promoted methanolysis of an RNA model, hydroxypropyl-*p*-nitrophenyl phosphate (**1**, HPNPP).[10] As the reaction proceeds by intramolecular cyclization to form the cyclic intermediate (**2**), the actual process is a transesterification and thus serves as an interesting counterpart to the reaction that occurs in aqueous solution, for which numerous studies of its one- or two-metal-ion-catalyzed transesterification have been reported.[4f,11,12]

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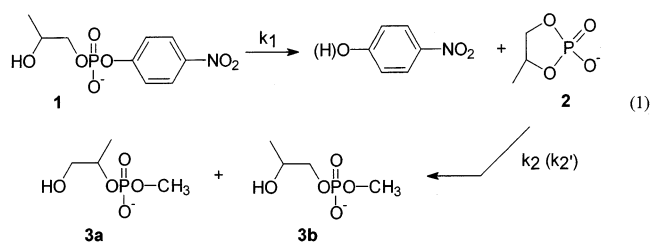
As will be shown, the methanolysis of HPNPP can be studied as a function of $[La^{3+}]$ from $\sim 4.5 < _s^s pH < 10.5$ and proceeds through two main pathways involving $(La^{3+}/1)_2$ that reacts with external methoxide and, at high $_s^s pH$, a species formulated as $(La^{3+}OCH_3^-)_2/1$ which spontaneously decomposes with a rate constant of $\sim(5-20) \times 10^{-3} \ s^{-1}$. For these two pathways, the metal ions promote the methanolysis reactions by $\sim 4.6 \times 10^{10}$-fold and 1×10^9-fold relative to the uncatalyzed reactions.

Experimental Section

Materials. Methanol (99.8% anhydrous), sodium methoxide (0.5 M solution in methanol), $La(CF_3SO_3)_3$, and disodium *p*-nitrophenyl phosphate hexahydrate were purchased from Aldrich and used without any further purification. $HClO_4$ (70% aqueous solution) was purchased from BDH.

Methods. ^{1}H NMR and ^{31}P NMR spectra were determined at 400 and 161.97 MHz using a Bruker Avance-400 NMR spectrometer. ^{31}P NMR spectra were referenced to an external standard of 70% phosphoric acid in water, and upfield chemical shifts are negative.

The $CH_3OH_2^+$ concentration was determined using a radiometer Vit 90 autotitrator, equipped with a radiometer GK2322 combination (glass/

calomel) electrode calibrated with Fisher certified standard aqueous buffers (pH = 4.00 and 10.00) as described in our recent papers.[6−9] Values of $_s^s pH$[13] were calculated by adding a correction constant of 2.24 to the experimental meter reading as reported by Bosch et al.[14] The $_s^s pK_a$ values of buffers used for the present kinetic studies were obtained from the literature[14] or measured at half neutralization of the bases with 70% $HClO_4$ in MeOH.

Synthesis of the Sodium Salt of 2-Hydroxylpropyl-4-nitrophenyl Phosphate (1). The corresponding barium salt was prepared in 44% yield by a slight modification of the reported procedure[10] and subsequently converted into the sodium salt with all water being doubly distilled (see Supporting Information). ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 (2H, d, Ar*H*, *J* = 8 Hz), 7.40 (2H, d, Ar*H*, *J* = 8 Hz), 3.89 (H, m, C*H*), 3.81 (2H, m, C*H*₂), 1.13 (3H, d, C*H*₃, *J* = 6 Hz). ^{31}P NMR (161.97 MHz, CD_3OD, 25 °C) δ −4.27.

Kinetics. The rates of methanolyses of **1** in buffered anhydrous methanol solutions were followed by monitoring the appearance of *p*-nitrophenol at 328 nm or the complex of *p*-nitrophenolate with La^{3+} at 350 nm at varying $[La(OTf)_3]$ with an OLIS modified Cary 17 UV−vis spectrophotometer at 25.0 ± 0.1 °C for $_s^s pH$ values lower than 8.60. Reactions were monitored at $8 \times 10^{-6} \ M < [La^{3+}] < 4 \times 10^{-3}$ M and were initiated by the addition of an aliquot of 2×10^{-3} M stock solution of **1** in anhydrous MeOH to 2.5 mL of the buffered reaction mixture. Final substrate concentration in the UV cell was 2×10^{-5} M, unless otherwise specified. In general, the reactions followed good first-order kinetics up to about 3−4 half-times of methanolysis, and the pseudo-first-order rate constants (k_{obs}) for the methanolysis of **1** were determined by an NLLSQ fitting of the absorbance versus time traces to a standard exponential model.

Kinetic measurements of the methanolysis of **1** in buffered solutions with $_s^s pH$ values higher than 8.60 were determined using an Applied Photophysics SX-17MV stopped-flow reaction analyzer at 25.0 ± 0.1 °C. A 4×10^{-5} M stock solution of **1** in buffer was prepared for one drive syringe, while the other contained the same buffer with $1.6 \times 10^{-5} \ M < [La^{3+}] < 8 \times 10^{-3}$ M.

Buffers were prepared from *N,N*-dimethylaniline ($_s^s pK_a$ = 5.00), 2,6-lutidine ($_s^s pK_a$ = 6.70), *N*-methylimidazole ($_s^s pK_a$ = 7.60), *N*-ethylmorpholine ($_s^s pK_a$ = 8.60), trimethylamine ($_s^s pK_a$ = 9.80), and triethylamine ($_s^s pK_a$ = 10.78) in anhydrous methanol neutralized with 70% $HClO_4$ to set the final $_s^s pH$. Because of the fact that added counterions can ion pair with La^{3+} ions and affect its speciation in solution, ionic strength was controlled through neutralization of the buffer. The total [buffer] varied between 7×10^{-3} M and 1.4×10^{-2} M, and the buffers were partially neutralized with $HClO_4$ to keep the $[ClO_4^-]$ at a low but constant value of 3.5×10^{-3} M which leads to a reasonably constant ionic strength in solution. With $[La^{3+}] > 5 \times 10^{-4}$ M at $_s^s pH$ values between 7.0 and 8.6, the La^{3+} was partially neutralized by the addition of an appropriate amount of NaOMe to help control the $_s^s pH$ at the desired value. $_s^s pH$ measurements were performed before and after each experiment, and in all cases, the values were consistent to within 0.1 units.

Tables 1S−16S, Supporting Information, contain the observed pseudo-first-order rate constants as a function of $[La^{3+}]$ at the various $_s^s pH$ values.

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NMR Studies of 1 in the Presence of La(OTf)$_3$ in d_4-Methanol. The methanolysis of 5×10^{-3} M **1** in unbuffered d_4-MeOH was investigated by ^{1}H NMR at 25 °C in the presence of 5×10^{-3} M La-(OTf)$_3$ which generates a solution ^s_spH ≈ 5.3. The reaction was followed by monitoring the disappearance of the starting material as well as the appearance of the signals ascribed to the five-membered cyclic intermediate (**2**) and p-nitrophenol to obtain k_1 and the hydroxy-propyl methyl phosphate products **3a** and **3b** to obtain k_2 and monitoring the relative product concentrations. The integrated intensities for these species as a function of time are given in Table 17S, Supporting Information. The k_1 and k_2 values were calculated by fitting the integrated NMR intensities to an A → B → C model to obtain average values for k_1 and k_2 of $(6.8 \pm 1.3) \times 10^{-4}$ s^{-1} and $(1.4 \pm 0.3) \times 10^{-4}$ s^{-1}, respectively. Products **3a** and **3b** were formed in a ratio of 75:25, and the k_2 value is the sum of the pseudo-first-order rate constants for the formation of both which equals the rate constant for the depletion of **2**.

The ^{31}P NMR spectra of the two fully reacted hydroxypropyl methyl phosphate products **3a** and **3b** prepared in the presence of equimolar La^{3+} as above were recorded at temperatures of −90 °C, −68 °C, −25 °C, 25 °C, and 55 °C and show tow broadened signals at δ −2.0 ppm and δ −8.8 ppm at lower temperatures which gradually change intensities as the temperature is increased. The chemical shifts of the **3a,b** isomers are very similar,[12b] and the signals for the individual isomers cannot be resolved; the observed peaks must contain the signals for both complexed isomers. The peak height intensity ratios of the δ −2.0 ppm and δ −8.8 ppm peaks are approximately 3:1 at −90 °C, 3:2 at −68 °C, and ∼1:3 at −25 °C with the peaks at the latter temperature being considerably broadened. At 25 °C, no discernible signal is seen.

Finally, to gain some idea of the nature of the complexation of **2** by La^{3+}, ^{31}P NMR spectra of 5×10^{-3} M **1** in CD$_3$OD were obtained as a function of added La(OTf)$_3$ at −90 °C, the ratios of [La^{3+}]/[**1**] being 0.1, 0.25, 0.5, and 1.0.

Results and Discussion

I. La^{3+} Binding to 1 Determined by ^{31}P and ^{1}H NMR Spectra. When no La^{3+} is added, the ^{31}P NMR spectrum of **1** at 25 °C shows major (∼95) and minor (∼5) peaks attributable to 2-hydroxy-1-phosphate and 1-hydroxy-2-phosphate isomers of **1** at −4.27 and −4.74 ppm which, when cooled to −90 °C, appear at −4.6 and −5.0 ppm. The methanolysis of 5.0×10^{-3} and 8.1×10^{-3} M **1** and equimolar La(OTf)$_3$ in anhydrous MeOH containing 12% (by volume) d_4-MeOH was studied by ^{31}P NMR at −90 °C, −68 °C, −25 °C, 0 °C, and 25 °C. The spectrum with 1 equiv of added La^{3+} at −90 °C exhibits prominent peaks at δ −8.9 ppm and −15.9 ppm, the peak heights of which vary with temperature: the ratios were 2:1 at −90 °C and 1:1 at −68 °C, but at −25 °C the signal at δ −8.9 ppm is essentially absent. With further increases in temperature, the signal at δ −15.9 ppm progressively broadens until there is no prominent signal at 25 °C. The La^{3+}/**1** mixture was allowed to react overnight at room temperature, after which the spectrum of the La^{3+}-complexed products **3a** and **3b** at −90 °C depicts peaks at δ −2.0 and −8.8 ppm with intensities about 2:1. The intensity ratio varies as temperature increases: ∼3:2 at −68 °C and 1:3 at −25 °C, suggestive of a shift in equilibrium concentration for the product species as a function of temperature. Above −25 °C, the peak at δ −2.0 ppm is no longer visible, and the peak at δ −8.8 ppm becomes progressively wider at 0 and 25 °C, suggestive of some exchange phenomenon.

The ^{31}P NMR spectrum of 5×10^{-3} M **1** in CD$_3$OD was obtained as a function of added La(OTf)$_3$ at −90 °C, the ratios of [La^{3+}]/[**1**] being 0.1, 0.25, 0.5, and 1.0. The addition of 0.1 equiv of La(OTf)$_3$ produces little change in the spectrum, but at 0.25 equiv of La^{3+} four peaks are observed at about δ −4.6, −9.1, −13.5, and −15.5 ppm, the most prominent being that at δ −4.6 for unbound **1**. The three latter peaks are considerably broadened such that exact peak location and integration are not possible. At 0.5 equiv of La^{3+}, the −4.6 peak is nearly gone and replaced by three broadened signals at about δ −9, −13.5, and −15.5 ppm. At a [La^{3+}]/[**1**] ratio of 1.0, two signals are observed at δ −8.9 ppm and −15.9 ppm, the intensities of which are 2:1 at −90 °C and 1:1 at −68 °C.

On the basis of the above, we can propose that there are at least three La^{3+}/**1** complexes in solution, the relative amounts of which are dependent on the La^{3+}/**1** ratios, similar to what is observed in our earlier study of the effect of La^{3+} binding to diphenyl phosphate in methanol.[8] Since all free **1** is eliminated at a La^{3+}/**1** ratio of 0.5, the three observed signals are attributed to complexes **4**−**6**, which are in slow exchange at −90 °C.
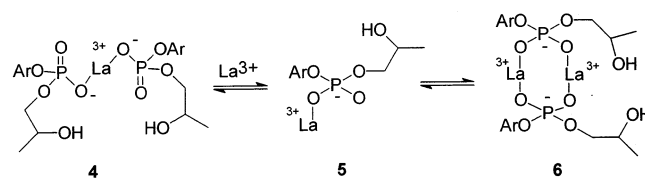

At a 1:1 ratio, where there are only two prominent ^{31}P NMR signals at δ −8.9 and −15.5, we suggest that the 1:1 and 2:2 complexes dominate. While at this time we are not sure which complex is assigned to a given chemical shift, the approximate 6.5 ppm separation for the ^{31}P signals is consistent with the known chemical shift differences of bridging phosphates relative to monodentate-coordinated phosphates in related Co^{III} complexes.[11g,15] At room temperature, all species are in dynamic equilibrium on the NMR time scale.

II. Preliminary ^{1}H NMR Kinetics of Methanolysis of 1 Catalyzed by La^{3+} at ^s_spH ≈ 5.3. The methanolysis of 5×10^{-3} M **1** and equimolar La(OTf)$_3$ in unbuffered d_4-MeOH was investigated by ^{1}H NMR at 25 °C, ^s_spH ≈ 5.3. Shown in Figure 1 is a plot of the integrated intensities of signals attributed to **1**, **2**, p-nitrophenol, and **3a** as a function of time. (See Table 17S, Supporting Information, for tabulation of the original integrated intensity data). Analysis of the intensity versus time data according to a model appropriate for **1** → **2** → **3a,b** gives pseudo-first-order rate constants $k_1 = (6.8 \pm 1.3) \times 10^{-4}$ s^{-1} and $k_2 = (1.4 \pm 0.3) \times 10^{-4}$ s^{-1}. Analysis of the product mixture indicates that the ratio of products **3a** and **3b** is about 75:25.

III. Kinetic Studies. a. Methanolysis of 1 in the Absence of La^{3+}. Stulz and Leuman[12b] have investigated the solvolytic activity of HPNPP promoted by a zirconium complex in a 1:1 benzene−methanol solution at 28 °C and reported rate constants of 2.93×10^{-3} M^{-1} s^{-1} and 1.97×10^{-3} M^{-1} s^{-1} for the attack of CH$_3$O$^-$ on **1** and **2** in the absence of metal ion. In our studies, the methoxide reaction of **1** in pure methanol was monitored by UV-visible kinetics by observing the rate of appearance of p-nitrophenoxide at methoxide concentrations of 9.8×10^{-3} and 2.0×10^{-2} M, the second-order rate constant being (2.56

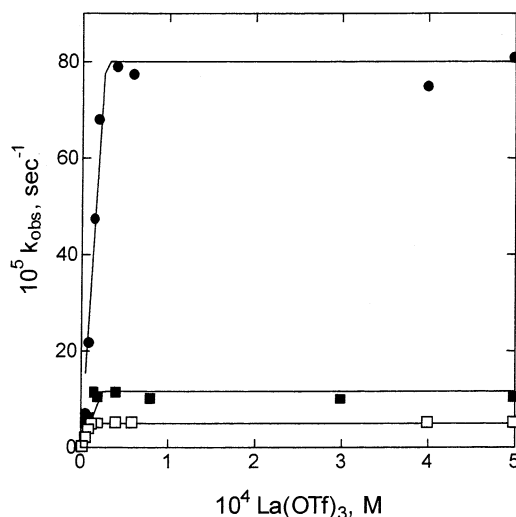(15) Wahnon, D.; Lebuis, A.-M.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2412.
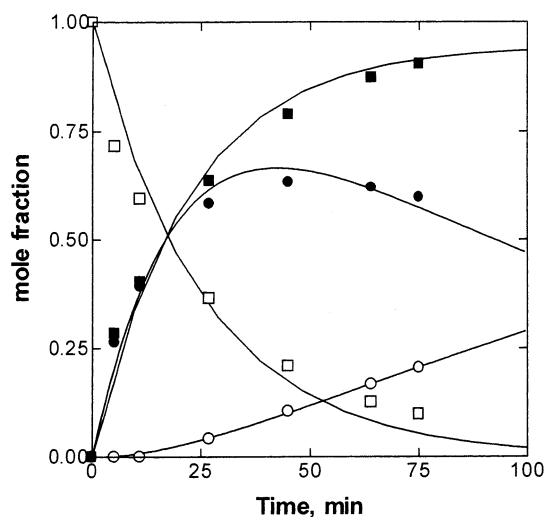
Figure 1. Plot of the progress of a reaction of 5×10^{-3} M **1** with equimolar La(OTf)$_3$ in d_4-methanol at 25 °C monitored by ^{1}H NMR at $_s^s$pH $\approx$ 5.3 showing the disappearance of **1** ($\square$) and the appearance of **2** ($\bullet$), *p*-nitrophenol ($\blacksquare$), and **3a** ($\circ$).
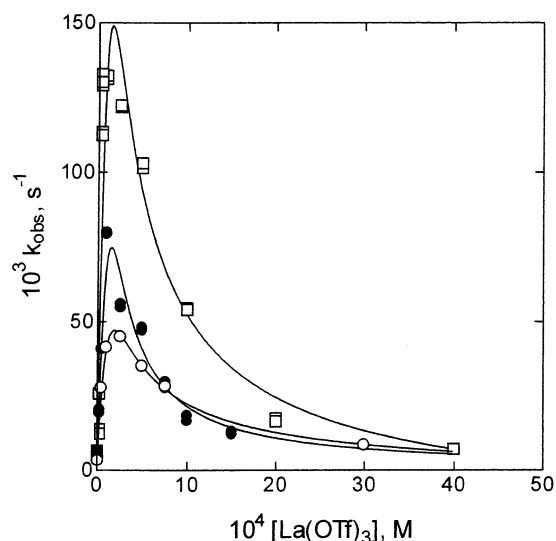

Figure 2. Plots of k_{obs} vs [La(OTf)$_3$] for the transesterification of **1** (2.02×10^{-5} M) in the low $_s^s$pH regime at three values, $T = 25$ °C. $_s^s$pH = 5.0 ($\square$), 5.6 ($\blacksquare$), and 6.4 ($\bullet$). Lines through the data computed from fits to eq 5.

$\pm$ 0.16) $\times 10^{-3}$ M^{-1} s^{-1}. The second-order rate constant for the initial transesterification step is some 3000-fold larger than that for the attack of methoxide on methyl-*p*-nitrophenyl phosphate (($7.9 \pm 0.6) \times 10^{-7}$ M^{-1} s^{-1} at 25 °C),[8] attesting to the effectiveness of the intramolecular displacement in HPNPP to form the cyclic ester **2**. From the known autoprotolysis constant of methanol[14] $K = 10^{-16.77}$, pseudo-first-order rate constants for **1** $\rightarrow$ **2** and **2** $\rightarrow$ **3a,b** of 8.7×10^{-15} s^{-1} and 6.7×10^{-15} s^{-1} can be computed at $_s^s$pH = 5.3,[16] assuming that the methoxide reaction dominates. By way of comparison, the La^{3+}-catalyzed conversion of **1** $\rightarrow$ **2** at $_s^s$pH = 5.3 determined by ^{1}H NMR above gives an acceleration of about 7.8×10^{10}-fold, while the La^{3+}-catalyzed conversion of **2** $\rightarrow$ **3a,b** is accelerated by 2×10^{10}-fold.

b. La^{3+}-Promoted Transesterification of 1. The data presented to this point do not give an indication of the mechanism by which the rate enhancements are achieved, but it is noteworthy that these are among the highest reported accelerations for any metal-ion-promoted transesterification of HPNPP. To gain more information about the mechanistic details, we conducted extensive studies of the effect of [La^{3+}] on k_{obs} for the transesterification of **1** at $4.5 < {}_s^s$pH < 10.6. Individual rate constants for these are deposited as Tables 1S–16S, Supporting Information.

The general appearance of the k_{obs} versus [La^{3+}] plots falls into two categories, depending upon the $_s^s$pH. In the low $_s^s$pH regime between 4.5 and 7.6, the plots, as typified in Figure 2, show a steep rise with increasing [La^{3+}] followed by a plateau region indicative of a saturation phenomenon. In the second, or high $_s^s$pH regime from 7.9 to 10, the plots, as typified in Figure 3, show a linear rise with increasing [La^{3+}] and then a decrease asymptotically approaching a limiting value of about $(5-10) \times 10^{-3}$ s^{-1}. The overall behavior is reminiscent of what we have seen before with La^{3+}-promoted methanolysis of diphenyl phosphate (DPP) and methyl-*p*-nitrophenyl phosphate[8]
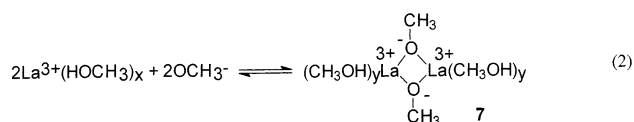
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(16) The value 6.7×10^{-15} s^{-1} is computed on the basis of the methoxide rate constant 1.97×10^{-3} M^{-1} s^{-1} for the opening of **2** in a 1:1 benzene–methanol solution at 28 °C presented by Stulz and Leuman.[12b]

Figure 3. Plots of k_{obs} vs [La^{3+}] for the transesterification of **1** in the high $_s^s$pH regime, $T = 25$ °C, [**1**] = 2.02×10^{-5} M. $_s^s$pH = 8.6 ($\circ$), 8.9 ($\bullet$), and 9.8 ($\square$). Lines are calculated from fits of the data to eq 7.

and is consistent with the change in the speciation of La^{3+} as a function of $_s^s$pH,[7] both of which are briefly reviewed below prior to discussing the HPNPP situation.

Titration of La(OTf)$_3$ in methanol reveals an apparent $_s^s$pK_a of 7.4–7.8 consuming 1 equiv of CH$_3$O$^-$ per La^{3+}, depending on the initial concentration, which is tied to the formation of (La^{3+})$_2$(OCH$_3^-$)$_2$ (**7**), as in eq 2. In the presence of one-half an

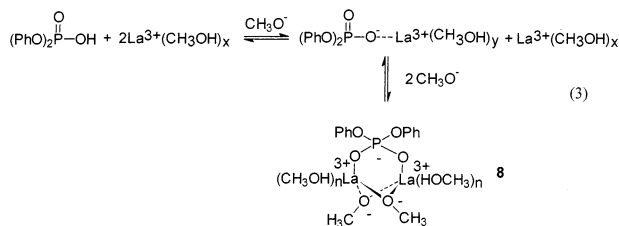$$2La^{3+}(HOCH_3)_x + 2OCH_3^- \rightleftharpoons (CH_3OH)_yLa \overset{\overset{\displaystyle CH_3}{\underset{\displaystyle |}{O}}}{\underset{\underset{\displaystyle CH_3}{\underset{\displaystyle |}{O^-}}}{}} La(CH_3OH)_y \quad (2)$$

equiv of (PhO)$_2$PO$_2$H, the titration reveals two events, the first consuming 0.5 equiv of CH$_3$O$^-$ with an apparent $_s^s$pK_a of 3.0 ± 0.1 corresponding to the deprotonation of a La^{3+}-bound DPP, and the second consuming 1 equiv of CH$_3$O$^-$ with an apparent $_s^s$pK_a of 7.8 tied to the formation of a DPP-bridged La^{3+} dimer with two associated methoxides (**8**), as shown in eq 3. These

Table 1. Rate Constants (k_{cat}) for the Reaction of $La^{3+}/\mathbf{1}$ Computed by Fits of the k_{obs} vs $[La^{3+}]$ Data (Table 1S–10S, Supporting Information) in the Low ^s_spH Regime to Eq 5

^s_spH	k_{cat} (s^{-1})
4.6	3.10×10^{-5}
5.0	5.03×10^{-5}
5.6	1.17×10^{-4}
6.4	8.01×10^{-4}
7.0	5.20×10^{-3}
7.6	1.90×10^{-2}

observations are directly relevant to the situation with **1** in both the low and high ^s_spH regimes.

$$(PhO)_2\overset{\displaystyle O}{\underset{}{P}}\!-\!OH + 2La^{3+}(CH_3OH)_x \rightleftharpoons \underset{CH_3O^-}{} (PhO)_2\overset{\displaystyle O}{\underset{}{P}}\!-\!O^-\!\!-\!\!-La^{3+}(CH_3OH)_y + La^{3+}(CH_3OH)_x$$

$$\Updownarrow 2\,CH_3O^- \qquad (3)$$

$$\underset{\mathbf{8}}{(CH_3OH)_nLa^{3+}\cdots La^{3+}(HOCH_3)_n}$$

b. La^{3+}-Promoted Transesterification of 1 in the Low ^s_spH Regime. The plots shown in Figure 2 indicate the saturation binding of **1** by La^{3+} throughout the low ^s_spH regime and suggest the mechanism shown in eq 4.[17] The substrate is

$$\mathbf{1} + La^{3+} \overset{K}{\rightleftharpoons} \overset{k_{cat}}{\underset{[OCH_3]}{\longrightarrow}} \mathbf{2}{:}La^{3+} \qquad (4)$$
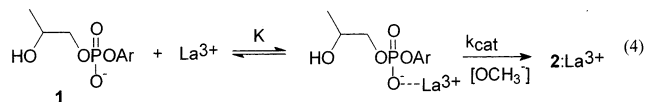
very tightly bound, so much so that the k_{obs} versus $[La^{3+}]$ data cannot be treated by simple Michaelis–Menton kinetics, but rather as a tight binding process (eq 5)[18]

$$k_{obs} = k_{cat}(1 + K[\mathbf{1}] + [La^{3+}]KX)/[\mathbf{1}](2K) \qquad (5)$$

where K refers to the association constant to form the $La^{3+}/\mathbf{1}$ complex, $[\mathbf{1}]$ and $[La^{3+}]$ refer to total concentrations, and X is given as

$$X = \{1 + 2K[\mathbf{1}] + 2[La^{3+}]K + K^2[\mathbf{1}]^2 - 2K^2[La^{3+}][\mathbf{1}] + [La^{3+}]^2K^2\}^{0.5}$$

Listed in Table 1 are the computed values for k_{cat} in the ^s_spH regime between 4.5 and 7.6. Because of the tight $La^{3+}/\mathbf{1}$ binding, exact values for the association constant cannot be reliably calculated from our data, other than to set a lower limit for K of 10^6 M^{-1}. Since the association constant of La^{3+} with **1** is markedly larger than that with diphenyl phosphate or methyl-p-nitrophenyl phosphate,[8] it is likely that the intramolecular OH group of **1** aids in the binding, although we do not explicitly show this in any proposed structure. The binding in methanol is at least 10^4-fold larger than the reported value of 73 M^{-1} for La^{3+} and **1** in aqueous solution at pH 6.85,[4f] a probable
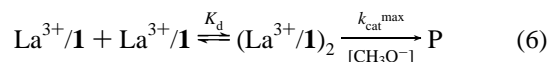
(17) The low ^s_spH data are treated through the range $0 < [La^{3+}]\ 5 \times 10^{-4}$ M. Further increases in the $[La^{3+}]$ produce slight increases in the k_{obs} values, as can be seen in the tables in the Supporting Information, suggesting involvement of some additional La^{3+} derived process, but we have not included this in the discussion because it is not a prominent effect.

(18) Equation 3 was obtained from the equations for equilibrium binding and for conservation of mass by using the commercially available MAPLE software. *Maple V,* release 5; Waterloo Maple Inc.: Waterloo, Ontario, Canada.
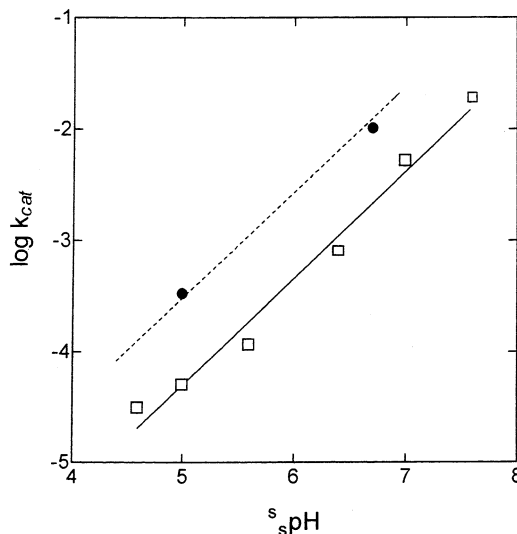
Figure 4. Plot of the computed log k_{cat} values listed in Table 1 for the methoxide induced-cyclization of $\mathbf{1}/La^{3+}$ as a function of ^s_spH at 25 °C, slope = 0.96 ± 0.07, $k_{cat}^{OMe} = 2.65 \times 10^7$ M^{-1} s^{-1}. Also shown as ● are two data points determined at ^s_spH 5.0 and 6.7 representing the methoxide-induced cyclization of the $(La^{3+}/\mathbf{1})_2$ dimer under conditions identified in the caption for Figure 5.

consequence of the reduced dielectric constant of the alcoholic medium (31.5 at 25 °C)[19] which strongly favors ion association.

Given in Figure 4 is a linear plot (slope = 0.96 ± 0.07) of the k_{cat} values between ^s_spH 4.5 and ^s_spH 7.6 which yields a value of $k_{cat}^{OMe} = 2.65 \times 10^7$ M^{-1} s^{-1} for the second-order rate constant for the OCH_3^--promoted cyclization of La^{3+}-coordinated **1**. However, the mechanism is actually more complicated and proceeds predominantly through dimers formulated simply as $(La^{3+})_2/(\mathbf{1})_2$, and at the $[\mathbf{1}]$ of 2.02×10^{-5} M used to collect the data from ^s_spH 4.5–7, we show below that only about 20% of the bound material is in the form of dimers. Shown in Figure 5 are plots of the observed pseudo-first-order rate constants for the reaction as a function of equal concentrations of La^{3+} and **1** at ^s_spH 5.0 and 6.7. These are conditions where substrate is completely bound by metal, and the fact that the rate constants increase with $[La^{3+} = \mathbf{1}]$ and eventually reach a plateau requires a saturation binding process of two $La^{3+}/\mathbf{1}$ monomers to give a reactive dimer as given in eq 6.

$$La^{3+}/\mathbf{1} + La^{3+}/\mathbf{1} \overset{K_d}{\rightleftharpoons} (La^{3+}/\mathbf{1})_2 \overset{k_{cat}^{max}}{\underset{[CH_3O^-]}{\longrightarrow}} P \qquad (6)$$

Fitting of the k_{obs} versus $[La^{3+}/\mathbf{1}]$ data in Table 2 to a standard one-site binding model[20] gives values for the dimerization constants (K_d) and maximum catalytic rate constants (k_{cat}^{max}) $(9.1 \pm 0.5) \times 10^3$ M^{-1} and $(3.30 \pm 0.07) \times 10^{-4}$ s^{-1} at ^s_spH 5.0 and $(7.1 \pm 0.9) \times 10^3$ M^{-1} and $(1.02 \pm 0.06) \times 10^{-2}$ s^{-1} at ^s_spH 6.7, the rate constants being placed in Figure 4 as ●. Interestingly, the calculated saturation curves for the dimer

(19) Harned, H. S.; Owen, B. B. *The Physical Chemistry of Electrolytic Solutions,* 3rd ed.; ACS Monograph Series 137; Reinhold Publishing: New York, 1957; p 161.

(20) At first glance, the process described in eq 6 is bimolecular in $[La^{3+}/\mathbf{1}]$, but the kinetics strictly adhere to a first-order process for the loss of starting material. However, the subsequent observation that the products of the reaction actually catalyze the decomposition of the starting material allows us to treat the kinetics at each ^s_spH according to a simple one-site binding model: $k_{obs} = k_{cat}^{max}[La^{3+}/\mathbf{1}]_{init}/(K_d + [La^{3+}/\mathbf{1}]_{init})$.
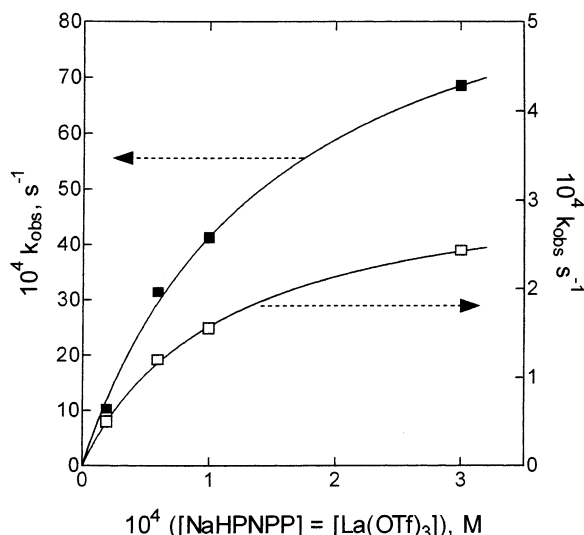
Figure 5. Plot of the observed pseudo-first-order rate constant for the methanolysis of equimolar [La^{3+}] = [**1**] at 25 °C at ^s_spH 5.0 (*N,N*-dimethylaniline buffer, □, right axis) and ^s_spH 6.7 (2,6-lutidine buffer, ■, left axis). Lines through the data are computed from fits to a standard one-site binding model.[20]

Table 2. Pseudo-First-Order Rate Constants for the La^{3+}-Catalyzed Methanolysis of **1** at ^s_spH 5.0 and 6.7 under Conditions of Varying [La^{3+} = **1**], T = 25 °C

[La^{3+} = 1] (M)	k_{obs} (s^{-1}), ^s_spH = 5.0	k_{obs} (s^{-1}), ^s_spH = 6.7
2.0×10^{-5}	4.97×10^{-5}	1.03×10^{-3}
6.0×10^{-5}	1.20×10^{-4}	3.14×10^{-3}
1.0×10^{-4}	1.56×10^{-4}	4.12×10^{-3}
3.0×10^{-4}	2.43×10^{-4}	6.85×10^{-3}

indicate that at very low concentrations of La^{3+}/**1**, the rate constant becomes negligible which indicates that the monomeric form is far less reactive (at least 100-fold) than the dimer. The fact that the binding constant does not change with ^s_spH indicates the dimerization does not require a methoxide, but since the k_{cat}^{max} value increases linearly with ^s_spH, the cyclization of the phosphate within the dimer requires an external methoxide. We can calculate the second-order rate constant for the methoxide-induced cyclization within the dimer, $k_{cat}^{max\ OMe}$ = 1.18×10^8 M^{-1} s^{-1}. Relative to the simple methoxide-promoted cyclization of **1** (($2.6 \pm 0.2) \times 10^{-3}$ M^{-1} s^{-1}), the dimer provides an acceleration of 4.6×10^{10}-fold in the low ^s_spH regime. It is interesting that the acceleration in methanol is 3 million-fold larger than that for the La^{3+}-promoted cyclization of bound **1** in water, for which a rate enhancement of 14 000-fold over the simple hydroxide-promoted cyclization has been reported,[4f] but in the latter case, dimers are probably not involved.[4f] This points to a significant solvent effect of methanol favoring the formation of reactive dimers with two metal ions, the dinuclear metal ion core being a known motif employed by nature in promoting such reactions in the active sites of enzymes. The likely mode of the interaction within the dimers is through double activation[4b,11d,g,k,n,o] of the phosphates as in **9**, Scheme 1, where the actual catalytic role of the second phosphate in the dimer is to act as a template in stabilizing the dinuclear La^{3+} core. Each dimer releases 2 equiv of *p*-nitrophenol in sequential steps, but the kinetics adhere to a first-order process, so the second methanolysis must be at least as fast as the first.

Scheme 1
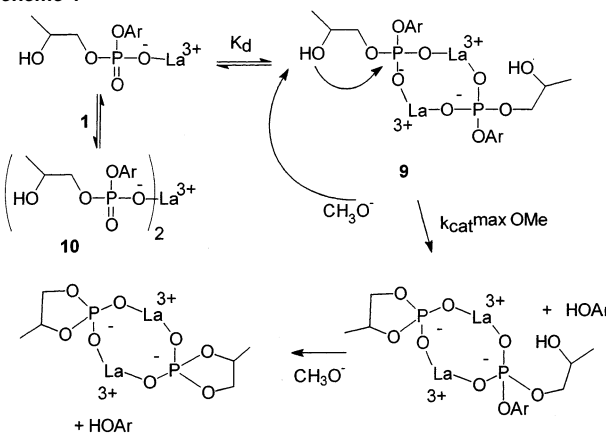
Table 3. Observed Pseudo-First-Order Rate Constants for the La^{3+}-Catalyzed Methanolysis Obtained for Four Separate Additions of [**1**] (2.02×10^{-5}M) to a Single Cell at 25 °C, ^s_spH 5.0, [Dimethylaniline Buffer] = 7×10^{-3} M

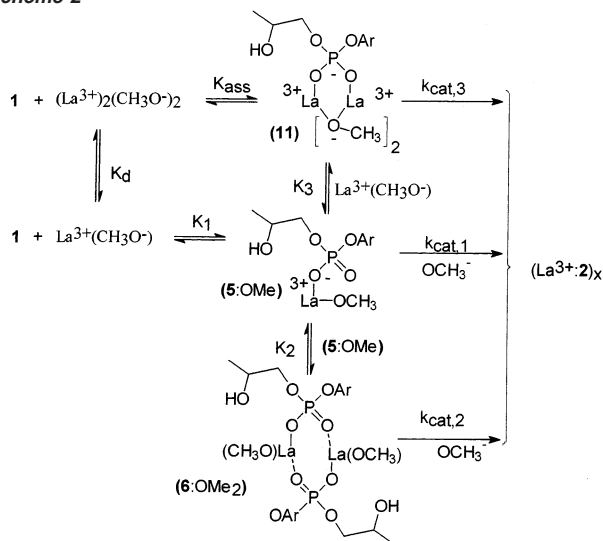| cycle	k_{obs}, s$^{-1\ a}$	k_{obs}, s$^{-1\ b}$	k_{obs}, s$^{-1\ c}$
1	6.04×10^{-5}	5.19×10^{-5}	6.20×10^{-5}
2	7.39×10^{-5}	7.90×10^{-5}	7.88×10^{-5}
3	9.38×10^{-5}	1.11×10^{-4}	1.07×10^{-4}
4	1.04×10^{-4}	1.39×10^{-4}	1.30×10^{-4}

^{a} Initial added [La^{3+}] = 0 M and each cycle initiated by the addition of 2.02×10^{-5} M each of **1** and La(OT)$_3$. ^{b} In the presence of 2×10^{-4} M La(OTf)$_3$. Kinetics determined on sample containing four separate additions of 2.02×10^{-5} M **1**. ^{c} In the presence of 1×10^{-3} M La(OTf)$_3$. Kinetics determined on sample containing four separate additions of 2.02×10^{-5} M **1**.

Other phosphates, including the products of the reaction (**3a,b**), can also act to stabilize the formation of dinuclear dimers. A repetitive kinetic experiment was performed at ^s_spH 5.0 where 2.02×10^{-5} M in each of **1** and La^{3+} were allowed to react to completion, followed by subsequent additions of the same amounts of starting reagents over the course of 4 days and the determination of the k_{obs} value for each cycle (see Table 3). Interestingly, the k_{obs} value for each step in the cycle is larger than the preceding one, indicating that the product complexes actually promote the transesterification of La^{3+}/**1** undoubtedly through the formation of (La^{3+}/**3a,b**)(La^{3+}/**1**) mixed complexes. This is not an effect attributable to increasing [La^{3+}] alone, as is confirmed by repeating the cycling experiment at two higher but constant [La^{3+}] of 2×10^{-4} and 1×10^{-3} M (see Table 3). The above clearly demonstrates an interesting example of product catalysis of a reaction that extends over several days and also indicates that the activity of the La^{3+} is preserved in methanol for a significant time, contrary to the situation in water.

While the catalytic mechanism in the low ^s_spH regime can be explained by the presence of 1:1 and 2:2 La^{3+}/**1** species, we have some kinetic evidence that 1:2 complexes are also present. Given in Table 4 are pseudo-first-order rate constants for the methanolysis of **1** at three different [La^{3+}] as a function of increasing [**1**] at ^s_spH 5.0. When the k_{obs} versus [**1**] data are plotted (not shown), there is a saturation decrease in the rate constant at [**1**] > [La^{3+}], indicative of the formation of a 1:2 La^{3+}/**1** complex formulated as **10** in Scheme 1. The exact reason for the decrease in k_{obs} when the 1:2 complex is formed could be attributed to a complex mixture of effects having to do with decreasing the net (+) charge on the 1:2 complex and inhibiting the formation of bis-La^{3+}-coordinated doubly activated phos-

Table 4. Pseudo-First-Order Rate Constants for the Methanolysis of **1** at Fixed [La(OTf)$_3$] and Varying [**1**], ^s_spH 5.0, *N,N*-dimethylaniline Buffer, *T* = 25 °C

[**1**] (M) (×10^{-5})	k_{obs} (s^{-1}) (×10^{-5}) [La^{3+}] = 5 × 10^{-6} M	k_{obs} (s^{-1}) (×10^{-5}) [La^{3+}] = 1 × 10^{-5} M	k_{obs} (s^{-1}) (×10^{-5}) [La^{3+}] = 2 × 10^{-5} M
1.2	3.36	4.88	5.31
2.0	2.22	3.71	4.96
8.0	1.89	3.20	4.41

Scheme 2 ^{a}

^{a} Solvation of La^{3+} omitted for clarity.

phates, but this was not investigated. Nevertheless, all the above kinetic data provide confirmatory evidence for the ^{31}P NMR studies that demonstrate the existence of three La^{3+} complexes of **1** in solution at low ^s_spH.

c. La^{3+}-Promoted Transesterification of 1 in the High ^s_spH Regime. At ^s_spH values higher than 7.9, a different behavior is observed in the plots of k_{obs} versus [La^{3+}], three such plots at ^s_spH 8.6, 8.9, and 9.8 being shown in Figure 3. In the high ^s_spH regime, the La^{3+} is associated with methoxide in a La^{3+}(OCH$_3^-$) monomer ⇌ (La^{3+}(OCH$_3^-$))$_2$ dimer equilibrium and these species are likely to be the ones responsible for the binding of **1**. The appearance of the plot clearly requires at least two pathways that are, respectively, first and second first-order in La^{3+} as well as a limiting saturation behavior of the di-La^{3+}-coordinated form. This behavior is very reminiscent of what we observed before with the La^{3+}-catalyzed methanolysis of methyl-*p*-nitrophenyl phosphate.[8] Shown in Scheme 2 is a simplified process which contains both monomer and dimer pathways, as well as a pathway involving the 2:2 complex discussed previously, but this time with all the La^{3+} being associated with methoxide. There are too many rate and equilibrium constants to be independently derived by fits to the limited number of data at any given ^s_spH value. We have, however, treated the data according to the simplified process given previously for the La^{3+}-catalyzed methanolysis of methyl-*p*-nitrophenyl phosphate[8] as in eq 7

$$k_{obs} = (2k_1 K_{ass}((1 + 8[La^{3+}]_t/K_d)^{0.5} - 1) + $$
$$k_{cat,3}(4[La^{3+}]_t/K_d - (1 + 8[La^{3+}]_t/K_d)^{0.5} + 1))/$$
$$(8K_{ass}/K_d + 4[La^3]_t/K_d - (1 + 8[La^{3+}]_t/K_d)^{0.5} + 1) \quad (7)$$

Table 5. Calculated Apparent Second-Order Rate Constants ($k_{1:1}$ + $k_{2:2}$) for the Bimolecular Reaction of **1** with La^{3+} in the High ^s_spH Regime Obtained from the Slope of the Initial Straight Line Portions of the k_{obs} vs [La^{3+}] Plots in Figure 4 and the Data in Tables 11S−14S, Supporting Information

^s_spH	$k_{1:1}$ + $k_{2:2}$ (M^{-1} s^{-1})
7.9	228
8.6	537
8.9	800
9.8	3500
10.5	3000

where k_1 refers to a composite second-order rate constant incorporating all the kinetic and equilibrium terms leading from **1** + La^{3+}(CH$_3$O$^-$) to product, namely K_1, K_2, $k_{cat,1}$, and $k_{cat,2}$. Even though these fits are reasonable, as evidenced by the lines through the data in Figure 3, most of the computed constants are heavily correlated and the only one which can be defined uniquely is $k_{cat,3}$, which refers to the spontaneous decomposition of the (La^{3+})$_2$(OCH$_3^-$)$_2$/**1** complex formulated as **11** in Scheme 2. The computed $k_{cat,3}$ terms all approach the same value of about 5 × 10^{-3} to 1 × 10^{-2} s^{-1} irrespective of ^s_spH.

The composite rate constant (k_1 from eq 7) for the reaction of the (La^{3+}/**1**/OMe) and (La^{3+}/**1**/OMe)$_2$ species (**5**/OMe and **6**/OMe$_2$) in Scheme 2 can be graphically evaluated at each ^s_spH as the slope of the initial straight line part of the plots in Figure 3 (data in Tables 11S to 14S, Supporting Information) and are compiled in Table 5. The k_1 composite rate constant determined in this way is an apparent second-order rate constant that incorporates the binding constants and rate constants listed previously. Although the actual processes for binding must be closely related to what is observed in the low ^s_spH regime, saturation binding for the monomeric La^{3+}/**1**/OMe form is not observed because of the intervention of the (La^{3+})$_2$(OCH$_3^-$)$_2$/**1** complex (**11** in Scheme 2). Interestingly, the k_1 composite rate constant does increase with ^s_spH up to 9.8, but not in a first-order way. This probably reflects a situation similar to what we observed earlier with methyl-*p*-nitrophenyl phosphate[8] and explained in terms of a counterbalancing effect attributable to the four individual rate and equilibrium constants that respond in different ways to La^{3+}-associated OCH$_3^-$.

More interesting is fact that the reaction of the di-La^{3+} complex **11** is independent of ^s_spH throughout the region from 7.9 to 10.5, $k_{cat,3}$ being (5−10) × 10^{-3} s^{-1}. This complex probably employs double activation of the phosphate bridged between two La^{3+} ions which are also bridged by two methoxides. The purpose of the methoxides is twofold: first to stabilize the dinuclear La^{3+} core and second to act as internal general bases to deprotonate the intramolecular OH group of bound HPNPP.

A kinetically equivalent possibility would involve a deprotonated HO group of the HPNPP stabilized by virtue of its coordination to the metal ion, but if this were bridging the two La^{3+} ions, it is unlikely that it would be sufficiently nucleophilic to attack the phosphate. The spontaneous decomposition of **11** is quite effective, being some (1−2) × 10^9-fold accelerated relative to the methoxide-promoted cyclization of **1** at ^s_spH 8.

Conclusions

In the previously mentioned, we have shown that the use of buffered methanol allows for the investigation of the kinetics

of the La^{3+}-promoted cyclization of **1** over an extended range of $[La^{3+}]$ and $[OCH_3^-]$ such that two main catalytic pathways can be discerned. Several species have been identified for which the $La^{3+}/\mathbf{1}$ ratio is 1:2, 1:1, 2:2, and 2:1. The kinetic behavior as a function of $_s^s pH$ shows there are two general $_s^s pH$ regimes.

(i) In the low $_s^s pH$ regime from 4.6 to 7.6, the plots of k_{obs} versus $[La^{3+}]$ exhibit saturation kinetics with a very strong 1:1 binding of La^{3+} and **1**. Most of the catalysis is shown to arise from the formation of a 2:2 complex, formulated as **9** with a phosphate proposed to be doubly activated by virtue of its coordination with two La^{3+} ions, which is then attacked by external methoxide to form cyclized **2**. Throughout the low $_s^s pH$ regime, the methoxide-promoted cyclization of the phosphates in the $(La^{3+}/\mathbf{1})_2$ has a large rate constant of 1.18×10^8 M^{-1} s^{-1} and is accelerated by 4.6×10^{10}-fold relative to the simple methoxide reaction on **1** which is not bound to a metal ion.

(ii) In the high $_s^s pH$ regime from 7.9 to 10.5, the reaction becomes more complicated and involves 1:1, 2:2, and 2:1 $La^{3+}/\mathbf{1}$ complexes associated with metal-bound methoxide. The 2:1 complex, formulated as **11**, spontaneously reacts throughout this $_s^s pH$ regime with a $k_{cat,3}$ rate constant of $(5-10) \times 10^{-3}$ s^{-1}, providing an acceleration of $(1-2) \times 10^9$-fold at $_s^s pH$ 8.0. It is proposed that one of the two methoxides associated with **11** acts as a general base to intramolecularly deprotonate

the hydroxyl of coordinated HPNPP which then cyclizes to La^{3+}-bound **2**.

(iii) The catalyzed methanolysis of HPNPP is most effective when the phosphate is doubly activated by two La^{3+} ions. It is apparent that anions such as OCH_3^- and $(RO)_2PO_2^-$ can act as bridges to coordinate La^{3+} and act as templates to hold the catalytic ensemble together. Potentially, it might be possible to design other bridges which are nonionic to stabilize the dinuclear La^{3+} core, but this is not an absolute necessity, since these dimers spontaneously form in methanol solution above the first $_s^s pK_a$ 7.8.

Supporting Information Available: Synthesis of HPNPP, Tables 1S–16S. Observed pseudo-first-order rate constants for La^{3+}-catalyzed methanolysis of **1** under various conditions of $_s^s pH$ and Table 17S. ^1H NMR study of transesterification of 5×10^{-3} M each of $La(OTf)_3$ and NaHPNPP in d_4-MeOH at 25 °C, $_s^s pH \approx 5.3$ (9 pages, print/PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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