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Study on the Transesterification of Methyl Aryl Phosphorothioates in Methanol Promoted by Cd(II), Mn(II), and a Synthetic Pd(II) Complex

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Methanol solutions containing Cd(II), Mn(II), and a palladacycle, (dimethanol *bis*(*N,N*-dimethylbenzylamine-2C, N)palladium(II) (**3**), are shown to promote the methanolytic transesterification of *O*-methyl *O*-4-nitrophenyl phosphorothioate (**2b**) at 25 °C with impressive rate accelerations of 10^6-10^{11} over the background methoxide promoted reaction. A detailed mechanistic investigation of the methanolytic cleavage of **2a**−**d** having various leaving group aryl substitutions, and particularly the 4-nitrophenyl derivative (**2b**), catalyzed by Pd-complex **3** is presented. Plots of k_{obs} versus palladacycle [**3**] demonstrate strong saturation binding to form **2b**:**3**. Numerical fits of the kinetic data to a universal binding equation provide binding constants, K_b, and first order catalytic rate constants for the methanolysis reaction of the **2b**:**3** complex (k_{cat}) which, when corrected for buffer effects, give corrected (k_{cat}^{corr}) rate constants. A sigmoidal shaped plot of log(k_{cat}^{corr}) versus ${}^{s}_{s}$pH (in methanol) for the cleavage of **2b** displays a broad ${}^{s}_{s}$pH independent region from $5.6 \leq {}^{s}_{s}\text{pH} \leq \sim 10$ with a $k^{minimum} = (1.45 \pm 0.24) \times 10^{-2}\ \text{s}^{-1}$ and a [lyoxide] dependent wing plateauing above a kinetically determined ${}^{s}_{s}\text{p}K_a$ of 12.71 ± 0.17 to give a $k^{maximum} = 7.1 \pm 1.7\ \text{s}^{-1}$. Brønsted plots were constructed for reaction of **2a**−**d** at ${}^{s}_{s}$pH 8.7 and 14.1, corresponding to reaction in the midpoints of the low and high ${}^{s}_{s}$pH plateaus. The Brønsted coefficients (β^{LG}) are computed as -0.01 ± 0.03 and -0.86 ± 0.004 at low and high ${}^{s}_{s}$pH, respectively. In the low ${}^{s}_{s}$pH plateau, and under conditions of saturating **3**, a solvent deuterium kinetic isotope effect of $k^H/k^D = 1.17 \pm 0.08$ is observed; activation parameters ($\Delta H^{\ddagger}_{Pd} = 14.0 \pm 0.6$ kcal/mol and $\Delta S^{\ddagger}_{Pd} = -20 \pm 2$ cal/mol·K) were obtained for the **3**-catalyzed cleavage reaction of **2b**. Possible mechanisms are discussed for the reactions catalyzed by **3** at low and high ${}^{s}_{s}$pH. This catalytic system is shown to promote the methanolytic cleavage of *O,O*-dimethyl phosphorothioate in CD_3OD, producing $(CD_3O)_2P{=}O(S^-)$ with a half time for reaction of 34 min.

1. Introduction

Phosphorothioate esters (**1**) are analogues of phosphate esters in which one or more of the non-bridging oxygen atoms have been substituted with sulfur. Neutral phosphorothioate triesters (**1a**) such as fenitrothion (*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate and parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate) are important in agriculture where they have seen widespread use as pesticides.[1]

Phosphorothioate diesters (**1b**) and monoesters (**1c**) have been employed as mechanistic probes for enzyme catalyzed phosphoryl transfer reactions to deduce transition state structure[2] and sites of metal ion coordination.[2d,3] Phosphorothioate modification of synthetic oligonucleotides confers increased stability toward nuclease activity[4] leading to a number of useful applications in molecular biology.[5] Antisense oligonucleotide therapies incorporating phosphorothioate internucleotide linkages have shown antiviral activity toward HIV and Hepatitis B.[6] The Dnd gene cluster present in *S. lividans* and other bacterial genomes promotes the sequence specific and stereoselective substitution of a

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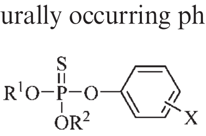
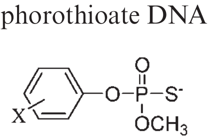
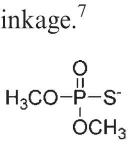
non-bridging phosphoryl oxygen with sulfur to form a naturally occurring phosphorothioate DNA linkage.[7]



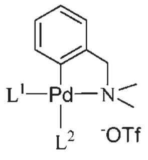


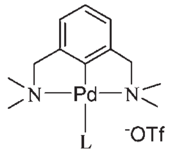


1a-c:
a) $R^1=R^2=R$
b) $R^1=H$; $R^2=R$
c) $R^1=R^2=H$

2a-d
a) 2-Cl,4-nitro
b) 4-nitro
c) 4-chloro
d) H

2e





3: $L^1 = L^2 =$ MeOH **4**: L = MeOH

Considerable effort has been devoted to studying solvolytic reactions of phosphate esters, and a number of comprehensive reviews exist.[8] By contrast, much less attention has been directed toward the related chemistry of phosphorothioates. Transfer of the dimethoxy phosphorothioyl group between oxyanion nucleophiles is best described as an A_ND_N mechanism consistent with the linear free energy data for attack of substituted phenoxides on fenitrothion in water.[9] Heavy atom kinetic isotope effects indicate a tighter, more associative transition state for the reaction of parathion (**1a**, $R^1 = R^2 =$ Et; X = 4-NO_2) relative to that of a corresponding phosphate triester.[10] Dianionic phosphorothioate monoesters with aryloxy leaving groups are thought to react by a highly dissociative $D_N + A_N$ mechanism. This proposal is consistent with the large negative β^{LG} of -1.1 determined for the hydrolysis of aryl phosphorothioate dianions,[11] a $\Delta S^{\ddagger}$ of $+29$ cal/mol·K for reaction of the 4-nitrophenyl phosphorothioate dianion[12] and the racemization observed in the hydrolysis of chiral 4-nitrophenyl [^{16}O,^{18}O]phosphorothioate.[13] The transition state for hydrolysis of the diester *O*-ethyl *O*-4-nitrophenyl phosphorothioate (**1b**, $R^1 =$ Et; $R^2 =$ H; X = 4-NO_2) is likely to be more associative than that of a monoester but more dissociative than that of a triester. Phosphorothioate analogues of uridyl(3′,5′)uridine containing a non-bridging phosphoryl-sulfur undergo a complex series of pH dependent reactions involving cyclization to 2′,3′-cyclic phosphorothioates in competition with desulfurization and isomerization.[14]

We are aware of only one report describing attempts to promote the solvolysis of a phosphorothioate monoester with metal ions, where Mg^{2+} and Cd^{2+} were shown to inhibit the rate of hydrolysis of *O*-4-nitrophenyl phosphorothioate.[12] Improved metal ion catalysis was achieved with Zn(II), Cd(II), and Gd(III) for the transesterification of uridyl(3′,5′)uridine containing a phosphorothioate diester linkage where rate accelerations for cyclization of up to 3600 relative to the background reaction at pH 5.6 and 363 K were reported.[15] Several metallacycle Pd and Pt complexes have been developed for promoting the decomposition of phosphorothioate triesters in water.[16] Reports emanating from our lab have described efficient palladacycle (**3**), Zn(II)-complex, and Cu(II)-complex systems for the catalytic cleavage of neutral phosphorothioate pesticides in alcohols such as methanol where rate accelerations of $> 10^9$ over the background reaction were achieved.[17] The methanolytic cleavage of a series of *O, O*-dimethyl *O*-aryl phosphorothioates (**1a**, $R_1 = R_2 = CH_3$) promoted by palladacycle **3** has been extensively investigated and shown to proceed via a multistep pathway with rate limiting ligand exchange on the Pd by substrates with good leaving groups, switching to rate-limiting breakdown of a Pd-bound 5-coordinate phosphorane (Pd:$^{-}$S(CH_3O)$_3$P(OAr)) with substrates having poor leaving groups.[17d]

Some efforts have been made to quantify the effects on reactivity of oxygen for sulfur exchange for the cleavage of phosphorothioates relative to phosphates. The thio effect is defined as the ratio of rate constants for reaction of a phosphate ester relative to that of the corresponding phosphorothioate ester (k^O/k^S). Hengge has determined the activation parameters for hydrolysis of a contiguous series phosphorothioate and phosphate mono-, di-, and triesters, all having a common 4-nitrophenoxy leaving group.[12,18] Thio effects at 298 K of 12.6 can be calculated for hydrolysis of *O,O*-diethyl *O*-4-nitrophenyl phosphate and *O,O*-diethyl *O*-4-nitrophenyl phosphorothioate, 3.3 for *O*-ethyl *O*-4-nitrophenyl phosphate and *O*-ethyl *O*-4-nitrophenyl phosphorothioate and 0.13 for the dianions of *O*-4-nitrophenyl phosphate and *O*-4-nitrophenyl phosphorothioate. By this analysis, the calculated thio effects for simple hydrolysis are small, being less than an order of magnitude for all three classes of ester, but thio effects determined for cleavage reactions of phosphate and phosphorothioate esters catalyzed by enzymes and ribozymes employing metal ion cofactors are often very much larger. For example the nucleotide phosphodiesterase from *Xanthomonas axonopodis* (NPP) exhibits a thio effect of 48 for hydrolysis of *O*-methyl *O*-4-nitrophenyl phosphate/*O*-methyl *O*-4-nitrophenyl phosphorothioate.[2f] The Ca(II)-dependent cleavage of S_p-1,2-dipalmitoyl-*sn*-glycero-3-(1-thiophospho-1-D-*myo*-inositol)phosphatidylinositol catalyzed by a phosphatidylinositol phospholipase C from *Streptomyces antibioticus* displays a uniquely large thio effect of $\sim 10^8$.[2d] This particular thio effect is diminished by a factor of 400 by substituting the Ca(II) cofactor with the soft thiophilic metal

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ion Cd(II). The disparity in the magnitudes of observed thio effects determined for solution reactions relative to enzymatic transformations suggests that intimate interactions between the metal ions and the non-bridging oxygen and sulfur atoms play a pivotal role in catalysis. Despite this interesting apparent consequence of Hard/Soft interactions[19] in the enzymatic reactions, it is surprising that so few studies of metal ion promoted solvolytic reactions of phosphorothioate mono- and diesters have been undertaken in an effort to establish a mechanistic framework for the interpretation of thio effect data in enzyme catalyzed phosphoryl- and phosphorothioyl transfer. It is this subject that forms the basis for the following report.

Herein we report that the soft metal ions[19] Cd(II), Mn(II), and Pd(II) in palladacycle **3** are all effective promoters of the methanolytic cleavage of *O*-methyl *O*-4-nitrophenyl phosphorothioates (**2a**−**d**). The reactions studied are models for the transesterification of the phosphorothioate diester substrates often employed in mechanistic studies of enzyme catalyzed phosphororyl transfer including the NPP catalyzed phosphorylation of an active site threonine (Thr90)[2f,20] and the *Tetrahymena* group I ribozyme catalyzed nucleotidyl transfer to an external guanosine (CCCUCUA + G_{OH} → CCCUCU + GA).[3a] A detailed mechanistic study is presented for the cleavage reactions of **2** catalyzed by [Pd(II)(*C*,*N*-dimethylbenzylamine)] (**3**).·It is also shown that dimethyl phosphorothioate ($(CH_3O)_2P{=}O(S^-)$), which possesses a more biologically relevant alkoxy leaving group (${}^{s}_{s}pK_a^{LG} = 18.2$) is also a competent substrate for catalytic cleavage promoted by **3**, giving a 34 min $t_{1/2}$ for replacement of the CH_3O group with a CD_3O group.

2. Experimental Section

A description of the materials used in this study is found in the Supporting Information. The $CH_3OH_2^+$ concentrations were determined potentiometrically using a combination glass electrode (Radiometer model XC100-111-120-161) calibrated with certified standard aqueous buffers (pH = 4.00 and 10.00) as described previously.[21] The ${}^{s}_{s}pH$ values in methanol were obtained by subtracting a correction constant of −2.24[21] from the electrode readings, and the autoprotolysis constant for methanol was taken to be $10^{-16.77}$ M^2, meaning that neutral ${}^{s}_{s}pH$ in methanol is 8.38. The ${}^{s}_{s}pK_a$ values of the substituted phenols in methanol used in this study can be found in a previous report.[22] Kinetic data were obtained by monitoring reaction progress using standard UV−vis spectrophotometry. In all cases, the reactions displayed excellent first-order kinetic traces with complete release of the expected amount of product, and no evidence of significant product inhibition.[23] Detailed descriptions of the kinetic methods appear in the Supporting Information.

Table 1. Second Order Rate Constants, k_2^{cat}, for the Methanolysis of **2b** Promoted by NBu_4OMe, $Cd(ClO_4)_2$, $Mn(OTf)_2$, and **3** at 25 °C and the Computed Rate Accelerations Provided by the Metal Ions

metal system	${}^{s}_{s}pH^{a}$	k_2^{cat} $(M^{-1}\ s^{-1})^{b}$	catalytic rate acceleration [c]
NBu_4OMe		$(2.3 \pm 0.2) \times 10^{-7}$	
$Cd(ClO_4)_2$	10.0	0.56 ± 0.02	2×10^{6}
$Mn(OTf)_2$	9.6	0.35 ± 0.1	1.5×10^{6}
3	11.2	$(5.5 \pm 1.7) \times 10^{4}$	2×10^{11}

[a] ${}^{s}_{s}pH$ set by the addition of one-half equiv of NBu_4OMe relative to $[M(II)]_{total}$ and measured following reaction. [b] The second order rate constants, k_2, were determined from plots of k_{obs} versus $[M(II)]_{total}$ as described in the Supporting Information. [c] Catalytic rate acceleration determined as the ratio of second order rate constants for the metal promoted reaction of **2b** to that promoted by NBu_4OMe: (k_2^{metal}/k_2^{OMe}).

3. Results

a. Methanolysis of 2b Promoted by Methoxide and Metal Ion Systems, Cd(II), Mn(II), and 3. Phosphorothioate **2b** (9×10^{-5} M) was subjected to base-promoted methanolysis at 25 °C under pseudo-first order conditions of excess NBu_4OMe (10−80 mM). Observed rate constants were determined by the method of initial rates using UV−vis spectrophotometry monitoring the production of 4-nitrophenolate at 399 nm, and the second order rate constant, k_2^{cat}, was computed as the slope of the linear plot of k_{obs} versus $[NBu_4OMe]$, see Table 1. The effect of metal ions on the rate of **2b** cleavage was determined in methanol containing $Cd(ClO_4)_2$, $Mn(OTf)_2$, and **3** at 25 °C. The solution ${}^{s}_{s}pH$ values for this series of reactions were buffered at the ${}^{s}_{s}pK_a$ of the first ionization of the $M(II)(HOCH_3)$ by the addition of one-half equiv of NBu_4OMe relative to $[M(II)]_{total}$. Observed rate constants were determined by fits of the absorbance versus time traces to a standard first order exponential equation. Second order rate constants (k_2^{cat}) were determined from the plots of k_{obs} versus $[M(II)]_{total}$ as described in the Supporting Information. The computed second order constants and catalytic rate accelerations appear in Table 1.

b. ^{31}P NMR of 3 and Sodium *O*,*O*-Dimethyl Phosphorothioate (2e) in Methanol. The ^{31}P NMR spectrum (CH_3OH, 202 MHz, referenced to 70% phosphoric acid) obtained on an unlocked solution of neutral methanol containing **3** (3.0 mM) and **2e** (1.5 mM) at 298 K displays signals at δ 54.0 and 28.2 ppm as shown in Figure 1. Obtaining accurate integrals is difficult because of a combination of a low signal-to-noise ratio and partial broadening of the peaks; nonetheless an approximate ratio of 1:1 is indicated.

c. UV−vis Titration of 3 with 2e in Methanol. Aliquots of **2e** (1.8×10^{-5} M) were added to a non-buffered solution of methanol containing **3** and the UV−visible spectrum recorded from 400 to 250 nm at 25 °C. The change in absorbance at λ = 325 nm was recorded as a function of [**2e**] as shown in Figure 2. The data were fit to

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(23) The observation that good first order kinetics are observed for the full Abs versus time profile and that there is full release of product indicates that there is no appreciable inhibition by product. This stems from the fact that the product is a monoanionic phosphorothioate diester as is the starting material, and both should have very similar binding constants to the catalyst. The observation of saturation kinetics indicates that the starting material and catalyst are in equilibrium, and so the catalyst must also be in equilibrium binding with the product. If the establishment of equilibrium is fast, then the total available pool of catalyst does not change during the course of the reaction, so product inhibition will not be observed. On the other hand, if the product binds far tighter than the starting material, which would be the case if this were a hydrolysis process and the product was a phosphorothioate mono ester, product inhibition in the form of burst kinetics, followed by a subsequent step dependent on catalyst turnover would be observed if the [catalyst] < [substrate]. If [catalyst] > [substrate], then product inhibition would not be seen since the observed process is a unimolecular decomposition of a fully formed substrate:catalyst complex.

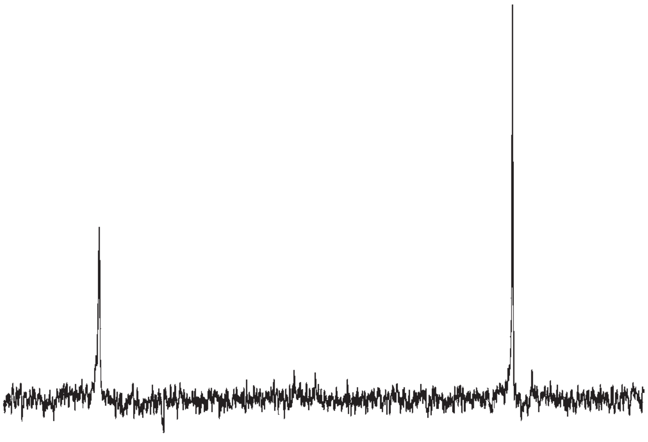

Figure 1. ^{31}P NMR spectrum (CH_3OH, 202 MHz, referenced to 70% phosphoric acid) of a neutral methanol solution containing **3** (3.0 mM) and **2e** (1.5 mM) obtained at 298 K displaying two peaks at δ 54.0 and 28.2 ppm. The two peaks integrate in an approximate ratio of 1:1.

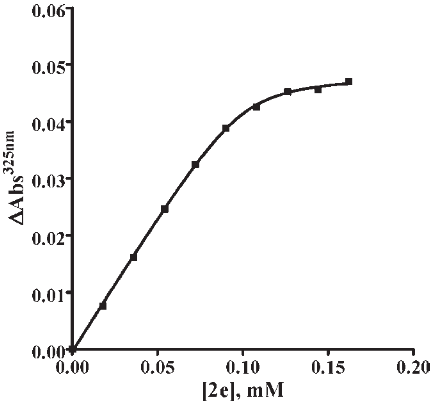

Figure 2. Plot of $\Delta Abs^{325\ nm}$ versus [**2e**] determined for the spectrophotometric titration of **3** at 25 °C. Fit of the data to eq 1 provides K_b = $(4.2 \pm 1.3) \times 10^5\ M^{-1}$.

eq 1[24] to give a binding constant, K_b, of $(4.2 \pm 1.3) \times 10^5\ M^{-1}$.

$$\Delta Abs_{obs} = \Delta Abs_{max}(1 + K_b[\mathbf{3}] + [\mathbf{2e}]K_b - X)/(2K_b)/[\mathbf{3}] \tag{1}$$

where

$$X = (1+2K_b[\mathbf{3}]+2[\mathbf{2e}]K_b+K_b{}^2[\mathbf{3}]^2 - 2K_b{}^2[\mathbf{2e}][\mathbf{3}]+[\mathbf{2e}]^2K_b{}^2)^{0.5}$$

d. $^{s}_{s}$pH/Rate Profile for the 3-Catalyzed Cleavage of 2b. Substrate **2b** (2×10^{-5} M) was subjected to **3**-catalyzed methanolysis at 25 °C, $5.06 \le {}^{s}_{s}pH \le 14.95$. It was determined early in the study that the use of bulky amines (NEt_3, ^{i}Pr-morpholine, 2,2,6,6-tetramethylpiperidine, etc) to control reaction $^{s}_{s}$pH led to complex effects consistent with buffer inhibition, and under certain conditions, buffer catalysis. Accordingly, we turned to the use of a buffering system comprising $La_2(^{-}OMe)_x$; where x = 1−5, to maintain a constant hydronium ion concentration over the range $7.06 \le {}^{s}_{s}pH \le 11.35$. ($La^{3+}$ gives a small catalysis of the reaction; this is a hard metal ion, and the reaction is subject to profound catalysis only by soft metal ions, (vide infra)). For the series of reactions run at $^{s}_{s}$pH 5.06, $Yb(OTf)_3$ was substituted for $La(OTf)_3$ owing to the lower $^{s}_{s}pK_a$ of the Yb^{3+}:($HOCH_3$) complex.[21] An excess of NaOMe was simply added to the reactions run at $^{s}_{s}$pH 14.07 and 14.77 to maintain [H^+].

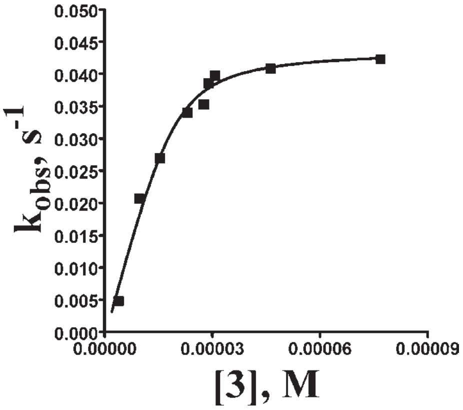

Figure 3. Plot of k_{obs} versus [**3**] for the methanolysis of **2b** (2×10^{-5} M) determined at [$La(OTf)_3$] = [NaOMe] = 2.0 mM, $^{s}_{s}$pH 8.80 and 25 °C. Fitting of the data to the expression giving in eq 2 gives $k_{cat} = (4.39 \pm 0.21) \times 10^{-2}\ s^{-1}$ and $K_b = (5.2 \pm 2.4) \times 10^5\ M^{-1}$.

$$k_{obs} = k_{cat}(1 + K_B[S] + [Cat]K_B - X)/(2K_B)/[S] \tag{2}$$

where

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

Plots of k_{obs} versus [**3**] display saturation binding as shown in Figure 3, and the data can be fit to eq 2[24] to provide a binding constant, K_b, and catalytic rate constant, k_{cat}. Under conditions of saturating [**3**] there is a linear dependence of the reaction rate on added [La^{3+}], Figure 4. The second order rate constants for this buffer catalysis, $k_2{}^{La}$, calculated as the gradients of the plots of k_{obs} versus [La^{3+}] are given in Table 2. Also included in Table 2 are the **2b**:**3** binding constants, K_b, and the corrected catalytic rate constants for the cleavage reaction of this complex, k_{cat}^{corr}, determined by extrapolation of the plots of k_{obs} versus [La^{3+}] to zero. Buffer catalysis was not observed for reactions run at $^{s}_{s}$pH 5.06 in the presence of Yb^{3+}. Control experiments performed in the absence of **3** indicate that the cleavage of **2b** is promoted by La^{3+} alone, albeit slowly. For example the cleavage of **2b** in a methanol solution containing 1.7−6.7 mM $La(OTf)_3$ at 25 °C and $^{s}_{s}$pH 8.4 provides an average observed rate constant of $(1.3 \pm 0.2) \times 10^{-5}\ s^{-1}$ that is independent of metal ion concentration over the concentration range investigated. Shown in Figure 5 is a plot of log(k_{cat}^{corr}) versus $^{s}_{s}$pH which, when fit to eq 3, provides a low $^{s}_{s}$pH plateau of $k^{minimum} = (1.45 \pm 0.24) \times 10^{-2}\ s^{-1}$ and a high $^{s}_{s}$pH plateau of $k^{maximum} = 7.1 \pm 1.7\ s^{-1}$ connected via a kinetic $^{s}_{s}pK_a$ of 12.71 ± 0.17.

$$\log(k_{cat}^{corr}) = \log(k^{maximum}(K_a/([H^+] + K_a)) + k^{minimum}) \tag{3}$$

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[24] Equations 1 and 2 were 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.

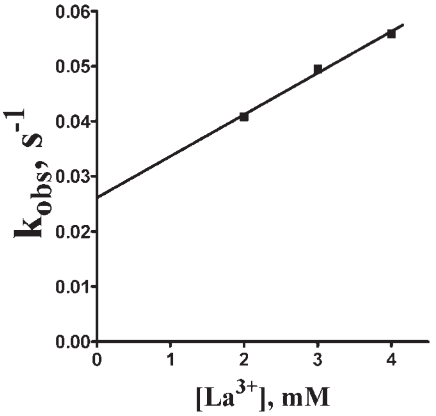

Figure 4. Plot of k_{obs} versus [La^{3+}] for the methanolysis of **2b** (2×10^{-5} M) determined at [**3**] = 5×10^{-5} M, ${}_{s}^{s}\mathrm{pH}$ 8.80 and 25 °C. Fitting of the data to a linear regression provides $k_2^{La} = 7.55 \pm 0.64$ M^{-1} s^{-1} and $k_{cat}^{corr} = (2.61 \pm 0.20) \times 10^{-2}$ s^{-1}, $r^2 = 0.9929$.

Table 2. Kinetic Constants k_{cat}^{corr}, K_b, and k_2^{La} for the Methanolysis of **2b** Catalyzed by **3** ($5.60 \leq {}_{s}^{s}\mathrm{pH} \leq 14.95$, $T = 25$ °C)

${}_{s}^{s}\mathrm{pH}$	k_2^{La} (M^{-1} s^{-1})^a	k_{cat}^{corr} (s^{-1})^b	K_b (M^{-1})^b
5.60	*c*	$(1.23 \pm 0.07) \times 10^{-2}$	$(4.7 \pm 2.2) \times 10^{4}$
7.06	*c*	$(1.02 \pm 0.05) \times 10^{-2}$	$(8.0 \pm 2.3) \times 10^{4}$
7.65	2.87 ± 0.12	$(1.41 \pm 0.05) \times 10^{-2}$	$(9.8 \pm 0.3) \times 10^{4}$
8.80	7.6 ± 0.6	$(2.6 \pm 0.2) \times 10^{-2}$	$(5.2 \pm 2.4) \times 10^{5}$
10.38	1.0 ± 0.2	$(4.56 \pm 0.09) \times 10^{-2}$	$(1.8 \pm 1.0) \times 10^{5}$
11.35	14.8 ± 1.7	$(3.3 \pm 0.3) \times 10^{-1}$	$(9.2 \pm 1.6) \times 10^{3}$
14.07	*c*	5.4 ± 0.2	$(6.6 \pm 1.3) \times 10^{4}$
14.95	*c*	8.9 ± 1.8	$(5.6 \pm 2.5) \times 10^{3}$

[a] k_2^{La} determined from the slope of the plots of k_{obs} versus [La^{3+}]. [b] k_{cat} and K_b determined by fits of the k_{obs} versus [**3**] data to eq 2, and the reported k_{cat}^{corr} is calculated by extrapolation to [buffer] = 0 as described in the text. [c] Where La^{3+}-catalysis was not observed, the reported k_{cat}^{corr} corresponds to the computed k^{cat} determined directly from eq 2.

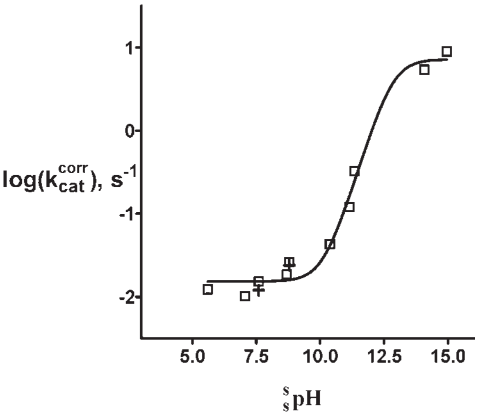

Figure 5. Plot of log(k_{cat}^{corr}) versus ${}_{s}^{s}\mathrm{pH}$ for the methanolysis of **2b** (2×10^{-5} M) determined at 25 °C catalyzed by **3** (□) where a fit of the data to eq 3 provides $k^{maximum} = 7.1 \pm 1.7$ s^{-1}, $k^{minimum} = (1.45 \pm 0.24) \times 10^{-2}$ s^{-1}, and a kinetic ${}_{s}^{s}\mathrm{p}K_a$ of 12.7 ± 0.2. The k_{cat}^{corr} data (+) obtained in CD_3OD at [La^{3+}]/[NaOMe] = 2 and 1 appear in the Figure at ${}_{s}^{s}\mathrm{pH}$ 7.65 and 8.80, respectively.

Table 3. Kinetic Parameters for the Methanolysis of Phosphorothioates **2a–d** Catalyzed by **3** at $T = 25$ °C

substrate	${}_{s}^{s}\mathrm{pH}$	(${}_{s}^{s}\mathrm{p}K_a^{LG}$)	k_{cat}^{corr} (s^{-1})^a	K_b (M^{-1})^a
2a	7.65	9.35	$(2.4 \pm 0.2) \times 10^{-2}$	$(9.8 \pm 5.0) \times 10^{4}$
2b	7.65	11.18	$(1.85 \pm 0.02) \times 10^{-2}$	$(4.4 \pm 2.0) \times 10^{4}$
2c	7.65	13.6	$(2.2 \pm 0.2) \times 10^{-2}$	$(8.0 \pm 5.4) \times 10^{4}$
2c	14.1^b	13.6	$(4.44 \pm 0.03) \times 10^{-2}$	$(5.9 \pm 1.0) \times 10^{5}$
2d	7.65	14.3	$(1.59 \pm 0.07) \times 10^{-2}$	$(4.2 \pm 3.5) \times 10^{5}$
2d	14.1^b	14.3	$(1.0 \pm 0.2) \times 10^{-2}$	$(1.8 \pm 1.0) \times 10^{4}$

[a] k_{cat} and K_b data determined from fits of the k_{obs} versus [**3**] data to eq 1, and the k_{cat}^{corr} data in the table are corrected for buffer catalysis unless otherwise noted. [b] Reactions run at ${}_{s}^{s}\mathrm{pH}$ 14.1 were performed in the absence of La^{3+}, and so the k_{cat}^{corr} values listed in the table represent k_{cat}.

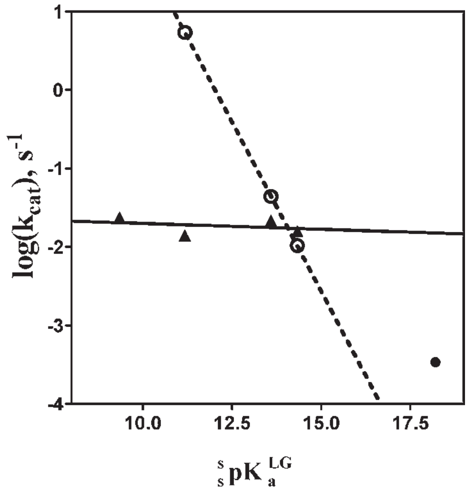

Figure 6. (a) Brønsted plot of log(k_{cat}^{corr}) versus ${}_{s}^{s}\mathrm{p}K_a^{LG}$(solid line, ▲) determined for the **3**-catalyzed methanolysis of **2a–d** at 25 °C, ${}_{s}^{s}\mathrm{pH}$ 7.65. Linear regression gives log(k_{cat}^{corr}) = $(-0.01 \pm 0.03){}_{s}^{s}\mathrm{p}K_a^{LG} - (1.55 \pm 0.40)$ ($r^2 = 0.094$, 4 data). (b) Brønsted plot of log(k_{cat}) versus ${}_{s}^{s}\mathrm{p}K_a^{LG}$ (dashed line, ○) determined for the **3**-catalyzed methanolysis of **2b,c,d** at 25 °C, ${}_{s}^{s}\mathrm{pH}$ 14.1 where a linear regression of gives log(k_{cat}) = $(-0.86 \pm 0.01)\,{}_{s}^{s}\mathrm{p}K_a^{LG} + (10.39 \pm 0.05)$, 3 data ($r^2 = 1.000$, 3 data). (c) Catalytic rate constant (●) for the **3**-catalyzed cleavage of *O,O*-dimethyl phosphorothioate (**2e**) as described in the text, (Results section i below).

e. Linear Free Energy Correlation for the 3-Catalyzed Cleavage of Phosphorothioates 2a–d. Plots of k_{obs} versus [**3**] were determined for the catalyzed methanolysis of **2a–d** (5×10^{-5} M) at 25 °C buffered at ${}_{s}^{s}\mathrm{pH}$ 7.65 with 2 mM $La(OTf)_3$ and 1 mM NaOMe. The plots of k_{obs} versus [**3**] for these substrates showed strong saturation binding and were fit to eq 2 to determine the catalytic constants k_{cat} and K_b. The k_{cat}^{corr} values listed in Table 3 reflect correction of the k_{cat} terms by extrapolation to zero buffer concentration. Substrates **2b,c,d** were also subjected to **3**-catalyzed methanolysis at the higher ${}_{s}^{s}\mathrm{pH}$ of 14.1, 25 °C. The catalytic constants for **2c** and **2d** appear in Table 3 whereas the data for **2b** appear in Table 2. In Figure 6 is a Brønsted plot of log(k_{cat}^{corr}) versus ${}_{s}^{s}\mathrm{p}K_a^{LG}$ for reaction of substrates **2a–d** at ${}_{s}^{s}\mathrm{pH}$ 7.65 which, when fit to a standard linear regression, provides log(k_{cat}^{corr}) = $(-0.01 \pm 0.03){}_{s}^{s}\mathrm{p}K_a^{LG} - (1.5 \pm 0.4)$. Also included in Figure 6 is the limited Brønsted plot determined for the **3**-catalyzed reaction of **2b,c,d** at ${}_{s}^{s}\mathrm{pH}$ 14.1 which fits a linear regression of log(k_{cat}) = $(-0.86 \pm 0.01)\,{}_{s}^{s}\mathrm{p}K_a^{LG} + (10.39 \pm 0.05)$.

f. Activation Parameters for the 3-Catalyzed Cleavage of 2b. The catalyzed methanolysis of substrate **2b** (5×10^{-5} M) was carried out at ${}_{s}^{s}\mathrm{pH}$ 8.8 in the presence of **3** = 1.2×10^{-4} M and [La^{3+}] = [NaOMe] = 1.68, 3.36, and 5.04 mM over a temperature range spanning 10–43 °C. Under these reaction conditions it is assumed that **2b** is fully bound to **3** and therefore the observed rate constants are interpreted as k_{cat}. The k_2^{La} and k_{cat}^{corr} data were obtained as the gradients and *y*-intercepts, respectively, in the plots of k_{obs} (or k_{cat}) versus [La^{3+}]. Fitting the data to the Eyring equation provides $\Delta H^{\ddagger}_{\mathrm{Pd}} = 14.0 \pm 0.6$ kcal/mol and $\Delta S^{\ddagger}_{\mathrm{Pd}} = -20 \pm 2$ cal/mol·K for the **3**-catalyzed cleavage of **2b**. The activation parameters for the corresponding La(III)-catalyzed process are $\Delta H^{\ddagger}_{\mathrm{La}} = 6.4 \pm 0.4$ kcal/mol, $\Delta S^{\ddagger}_{\mathrm{La}} = -33 \pm 2$ cal/mol·K.

g. Solvent Deuterium Kinetic Isotope Effect. Substrate **2b** (4.3×10^{-5} M) was subjected to catalyzed methanolysis in the presence of **3** (0.2 to 1.2×10^{-4} M), 2 mM La^{3+} and 1 mM NaOMe in CD_3OD (99% deuterium content) at 25 °C. A fit of the data to eq 2 provides $K_b = (3.0 \pm 2.2) \times 10^5\ M^{-1}$, $k_{\mathrm{cat}} = (1.87 \pm 0.11) \times 10^{-2}\ s^{-1}$ and after correcting for buffer catalysis $k_{\mathrm{cat}}^{\mathrm{corr}}$ is determined to be $(1.19 \pm 0.06) \times 10^{-2}\ s^{-1}$. Under conditions where [La^{3+}] = [NaOMe] = 2.0 mM, $k_{\mathrm{cat}}^{\mathrm{corr}}$ was determined as $(2.4 \pm 0.3) \times 10^{-2}\ s^{-1}$. The $k_{\mathrm{cat}}^{\mathrm{corr}}$ data appear in Figure 5 overlaid upon the data obtained in CH_3OH at $^{s}_{s}$pH 7.60 and 8.80 under otherwise identical conditions. The solvent deuterium kinetic isotope effect is calculated to be $k^{H}/k^{D} = 1.17 \pm 0.08$.

h. 4-Promoted Methanolysis of 2b. Substrate **2b** (2×10^{-5} M) was subjected to methanolysis promoted by the pincer palladacycle **4** (3×10^{-5} M) at 25 °C, $^{s}_{s}$pH 7.9 ([La^{3+}] = 2.9 mM, [NaOMe] = 2.0 mM). The fit of the absorbance versus time data to a standard first order exponential equation provided an observed rate constant of $1 \times 10^{-5}\ s^{-1}$ which is not significantly different from that obtained in the absence of **4**. The reaction of **2b** was also carried out in the presence of **4** (3×10^{-5} M) at $^{s}_{s}$pH 14.8 maintained with 10 mM NaOMe at 25 °C. These conditions gave an observed rate constant of $3.7 \times 10^{-6}\ s^{-1}$. By way of comparison the second order rate constant for methoxide promoted cleavage of **2b** is $2.3 \times 10^{-7}\ M^{-1}\ s^{-1}$ providing a computed observed rate constant of $2.3 \times 10^{-9}\ s^{-1}$ at $^{s}_{s}$pH 14.8. This represents a $\sim 10^3$-fold rate acceleration over the background methoxide promoted reaction[25] at that $^{s}_{s}$pH; however, this is still some 10^6-fold slower than the reaction catalyzed by **3** under similar reaction conditions.

i. Methanolysis of *O,O*-dimethyl Phosphorothioate (2e) Promoted by 3 in d_4-Methanol. Five separate reaction solutions were prepared containing 1.1×10^{-4} M **3**, 2.0 mM $La(OTf)_3$, and 1.0 mM $NaOCD_3$ in 5 mL of methanol (10% CH_3OH/90% CD_3OD). To each of these was added sodium *O,O*-dimethyl phosphorothioate **2e** (6×10^{-5} M), and the reactions were allowed to progress for a set time period (0−43.5 min) prior to quenching with 50 mM LiCl and 112 mM LiBr (these anions bind tightly to the metal ions in methanol and inhibit catalysis). The quenched reactions were concentrated to dryness under reduced pressure and then reconstituted in CD_3OD for ^1H NMR analysis (CD_3OD, 600 MHz). Reaction progress was determined by integrating the residual CH_3O-P signal (δ 3.66 ppm, d, J = 12 Hz) relative to that of the methylene protons of **3** (δ 3.98 ppm, singlet) which is assumed to remain constant over the course of reaction. Shown in Figure 7 is a stacked plot of the partial ^1H NMR spectra illustrating the loss of the CH_3O-P signal intensity as the group undergoes conversion to CD_3O-P. An average observed rate constant of $(3.4 \pm 0.5) \times 10^{-4}\ s^{-1}$ was calculated from a standard first order exponential equation. Under the conditions of the study it is determined that **2e** is >95% metal bound based on the binding constant determined by spectrophotometric titration ($K_b = 4.2 \times 10^5\ M^{-1}$), and since the NMR experiment looks at the loss of both CH_3O groups, it therefore follows that for the loss of a single methoxy group $k_{\mathrm{cat}} = 2k_{\mathrm{obs}}^{2e}$.[26] The datum for reaction of **2e** that appears in Figure 6 is seen to lie significantly below the data corresponding to reaction of **2a−d**.

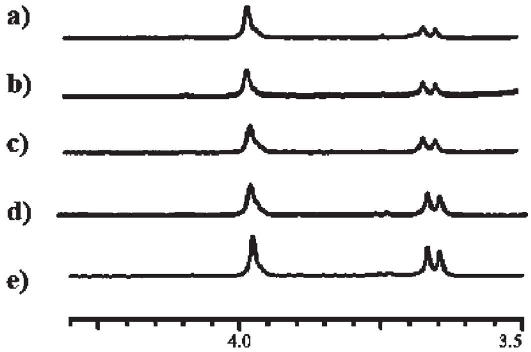

Figure 7. Partial ^1H NMR (CD_3OD, 600 MHz) spectra obtained following reaction of **2e** (6.1×10^{-5} M) catalyzed by **3** (1.06×10^{-4} M) in the presence of 2 mM La^{3+} and 1 mM $NaOCD_3$ employing the described quench protocol at (a) 43.5 min; (b) 26.3 min; (c) 24.3 min; (d) 9.0 min; and (e) 0 min.

4. Discussion

a. Methanolysis of 2b Promoted by Lyoxide and Metal Ions (Cd(II), Mn(II), Pd(II), La(III)). The second order rate constant for methoxide attack on **2b** (Table 1) is the same as that determined for the base promoted hydrolysis of *O*-ethyl *O*-4-nitrophenyl phosphorothioate ($2.1 \times 10^{-7}\ M^{-1}\ s^{-1}$) at the same temperature.[10] It is interesting to compare the rate of CH_3O-promoted cleavage of phosphorothioate **2b** to that of *O*-methyl *O*-4-nitrophenyl phosphate, which has a reported second order rate constant of $7.9 \times 10^{-7}\ M^{-1}\ s^{-1}$.[22] Thus, the thio effect, defined as the ratio of rate constants for reaction of the all oxygen phosphate diester to that of the phosphorothioate diester (k^{O}/k^{S}), is 3.4 at 25 °C in methanol which compares favorably to the thio effect of 3.3 for the hydrolysis of *O*-ethyl *O*-4-nitrophenyl phosphate and *O*-ethyl *O*-4-nitrophenyl phosphorothioate in water.[18] The thio effects reported here are for reaction at 25 °C, but it is emphasized that these values are necessarily dependent upon the reaction temperature chosen for the comparison owing to the small but significant differences in $\Delta H^{\ddagger}$ expected for solvolytic cleavage of the two classes of substrate.[27]

The solvolytic cleavage of phosphorothioates is efficiently promoted by soft metal ions in methanol as demonstrated

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[25] It must be noted that the value of $3.7 \times 10^{-6}\ s^{-1}$ determined for the pincer complex is an upper limit, and may be at least partially due to trace impurities (<1% of **3** or another catalytically active materials which are not seen in the analysis of the pincer ligand, but contribute to catalysis. Nevertheless, the point of this comparison is to indicate that **4** does not have the required two adjacent sites for catalysis.

[26] Since the observed process corresponds to the loss of both the CH_3O groups, the pseudo-first order rate constant for the loss of a single group would be twice as large, and this is the number we report.

[27] Examination of the activation parameters reported in ref 18 for the base promoted hydrolysis of ethyl 4-nitrophenylphosphate and ethyl 4-nitrophenylphosphorothioate reveal an isokinetic temperature of ~100 °C.

by the cleavage of **2b** in the presence of Cd(II), Mn(II), and palladacycle **3**. The second order rate constants for the cleavage of **2b** at 25 °C in methanol containing Mn(II) and Cd(II) and one-half equivalent of methoxide are similar at 0.35 and 0.56 M^{-1} s^{-1}, but palladacycle **3** is at least 10^5 more efficient for the cleavage of **2b**, having a second order rate constant of 5.5×10^4 M^{-1} s^{-1}. A crude estimate of the effectiveness of the metal ions at promoting the methanolysis of **2b** is arrived at by comparing the rate of the methoxide promoted reaction to those obtained in the presence of metal ions: efficiencies of $\sim 10^6$ are determined for Mn(II) and Cd(II) whereas **3** provides a rate acceleration of $\sim 10^{11}$ (Table 1). Lönnberg et al. have reported that the transesterification of uridylyl-((3′,5′)uridine phosphoromonothioate [3′,5′-Up(s)U] in water containing 5 mM metal ion is accelerated over the background reaction by 410 using Zn(II), 3600 with Cd(II), and 2000 with Gd(III) at pH 5.6 and 363 K.[15] The exceptional catalytic efficiency for the metal promoted cleavage of **2b** in methanol relative to the metal promoted cleavage of 3′,5′-Up(s)U in water is intriguing and suggests a pronounced medium effect provided by the alcohol in stabilizing not only the bound form of the M(II):**2b** complex, but also its ensuing transition state for cleavage of the P−OAr bond.

In the absence of any of the soft metal ion catalysts, a methanol solution containing 1.7−6.7 mM La^{3+} at ${}_{s}^{s}pH$ 8.5 gives a k_{obs} value of 1.3×10^{-5} s^{-1} for the methanolysis of *O*-methyl *O*-4-nitrophenyl phosphorothioate **2b**, this rate constant being experimentally invariant in respect to $[La^{3+}]$. This behavior suggests a process where **2b** undergoes saturation binding to a La^{3+} species followed by unimolecular breakdown of a $La(III)_2(^{-}OCH_3)_x$(**2b**) complex to give product. The closely related *O*-methyl *O*-4-nitrophenyl phosphate undergoes methanolytic cleavage with a rate constant of 7.5×10^{-6} s^{-1} under similar conditions of saturating La^{3+}, 25 °C and ${}_{s}^{s}pH$ 8.5,[28] demonstrating that this hard metal ion possesses no special aptitude to promote the cleavage of the sulfur containing **2b** relative to its all oxygen analogue.

Under conditions of saturating [**3**], where **2b** is fully bound to the palladacycle, the catalytic behavior of La^{3+} is somewhat different in that it provides an acceleration of the cleavage of Pd-bound **2b** that is linear in $[La^{3+}]$ (Figure 4). The second order rate constants for the La^{3+}-promoted process, determined as the gradients of the plots of k_{obs} versus $[La^{3+}]$, vary by only a factor of ∼5 (3 to 15 M^{-1} s^{-1}) over the range $7.65 \leq {}_{s}^{s}pH \leq 11.35$. Under these reaction conditions the speciation of the La^{3+} is dimeric with a ${}_{s}^{s}pH$ dependent number of associated methoxides ($[La_2(^{-}OMe)_x]$; where $x = 1−5$) which are responsible for the observed buffer catalysis consistent with previous studies emanating from this lab.[8c]

On the basis of these results, one can conclude the following: (1) in the absence of Pd the catalysis provided by La^{3+}-ions for the cleavage of **2b** is modest and does not interfere with the **3**-catalyzed reactions studied here; and, (2) the catalytic effect for the decomposition of the complex formed between **2b** and **3** is linear with respect to $[La^{3+}]$ so correcting the catalytic rate constants to zero [buffer] is straightforward.

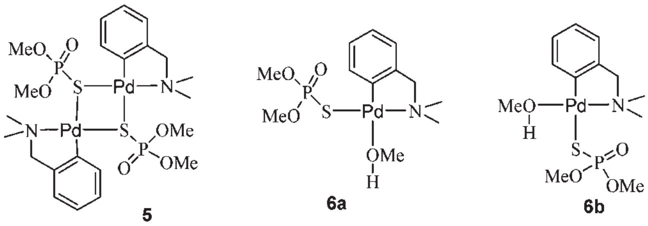

Figure 8. Proposed structures for the complexes formed between **1** and **3e** in neutral methanol as determined by ^{31}P NMR.

b. ^{31}P NMR of a Methanol Solution Containing 3 and 2e. The ^{31}P NMR chemical shift in CD_3OD of sodium *O*, *O*-dimethyl phosphorothioate (**2e**) is 61.3 ppm whereas the spectrum of a neutral methanol solution containing a 2:1 mixture of **3** and **2e** displays peaks at 28 and 54 ppm in the mM range (Figure 1). ^{31}P NMR data are reported for several palladacycle species bearing attached *O,O*-dimethyl phosphorothioate ligands and serve as a guide in the assignment of the peaks displayed in Figure 1. A mixture of the dimeric complexes *cis*-[(*μ*-**2e**)(2-(2-pyridyl)phenyl-C,N)Pd$]_2$ and *trans*-[(*μ*-**2e**)(2-(2-pyridyl)phenyl-C,N)Pd$]_2$ have been characterized by X-ray crystallography and solid-state MAS $^{31}P\{^1H\}$ NMR.[16e] The reported ^{31}P NMR spectrum consists of two peaks at δ 29.3 and 35.9 ppm assigned to the *cis*-isomer and a single peak at 32.9 ppm assigned to the *trans*-isomer. A similar ^{31}P NMR chemical shift at δ 29 ppm is reported for $[Pd(C,N\text{-}(C_6H_4C\text{-}(CH_3){=}NOH)\text{-}2)(\mu\text{-}\mathbf{2e})]_2$ in a mixed acetone-D_2O solvent system.[16d] Assigning the peak at δ 28 ppm in Figure 1 to symmetrical dimeric complex **5** is therefore consistent with literature precedent although it is possible that the actual structure consists of the phosphorothioate ligands binding the two Pd centers with the non-bridging phosphoryl-oxygen and -sulfur to form an eight membered ring chelate. Monomeric complexes of **2e** bound to palladacycle complexes have also been characterized by ^{31}P NMR in acetone-D_2O including [Pd(2-(2-pyridyl)phenyl-C,N)(THF)-(**2e**)] having a peak in THF at 50.5 ppm[16e] and $[Pd(C,N\text{-}(C_6H_4C\text{-}(CH_3){=}NOH)\text{-}2)(\mathbf{2e})(py)]$ displaying a peak at 53.6 ppm.[16d] These chemical shifts are similar to the downfield peak seen in Figure 1 obtained in a methanol solution of **3** and **2e**, and accordingly we propose structures **6a** or **6b** as being consistent with the available NMR data. Phosphorothioate **2e** is suggested to exist as an equilibrium mixture of dimeric complex **5** and monomeric complexes **6** (See Figure 8) under the millimolar concentrations employed in the NMR experiment. However, the concentration of any dimeric complex would be small under the kinetic conditions employed in this study which involve > 10-fold dilution, and we see no kinetic evidence for the intervention of dimers in the cleavage of these phosphorothioates.

c. Binding Affinity of the Palladacycle 3 for Anionic Phosphorothioates. The plots of k_{obs} versus [**3**] for the catalytic cleavage of **2b** at 25 °C display saturation binding over the entire ${}_{s}^{s}pH$ range investigated. The binding constants were determined by fits of the kinetic data to eq 2, and for reactions run in the low ${}_{s}^{s}pH$ domain ($5.6 < {}_{s}^{s}pH < 10.4$) these fall in the broad range of (0.5 to 5×10^5) M^{-1}. The binding constants determined for reaction of **2a**−**d** at ${}_{s}^{s}pH$ 8.7 (Table 3) all fall within this general range and show no obvious trend with respect to the ${}_{s}^{s}pK_a$ of the

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(28) Neverov, A. A.; Brown, R. S. *Inorg. Chem.* **2001**, *40*, 3588.

aryloxy substituent. The binding constants determined from the kinetic plots complement that determined for **2e** by UV−vis titration in neutral methanol where K_b is computed to be 4.2×10^5 M^{-1}. At higher ${}_{s}^{s}$pH there is an apparent trend toward decreased binding affinity suggesting that, at higher concentrations, methoxide is capable of displacing **2b** from **3** (Table 1, 11.35 < ${}_{s}^{s}$pH < 14.95). The binding constants determined for catalyst **3** and **2** are large for a mononuclear metal-containing species and suggest that the soft Pd center has a special affinity for the sulfur-containing anionic phosphorothioates. By way of comparison, it is reported that Zn^{2+} forms a 1:1 complex with uridyl(3′,5′)uridine phosphoromonothioate in water with an estimated K_b of ∼30 M^{-1} at ${}_{s}^{s}$pH 5.6 and 363 K.[15] Stronger binding of $K_b = 293$ M^{-1} is observed for complex formation between the monoester *O*-4-nitrophenyl phosphorothioate and Cd^{2+} at ${}_{s}^{s}$pH 8 in water.[12] It would appear that **3** has an unprecedented affinity for binding to monoanionic phosphorothioates **2** in methanol, probably because of soft metal/soft substrate interactions and increased Coulombic attraction of the oppositely charged species in the reduced dielectric constant medium. The binding between catalyst and the ensuing transition state for P−OAr cleavage must be greater still, otherwise catalytic rate acceleration would not be achieved.

d. ${}_{s}^{s}$pH Rate Profile for the 3-Catalyzed Cleavage of 2b. The k_{cat}^{corr} constants determined as described in the Results section show an interesting ${}_{s}^{s}$pH dependence as shown in Figure 5. There are two ${}_{s}^{s}$pH independent regions having computed first order rate constants of $k^{maximum} = 7.1$ s^{-1} and $k^{minimum} = 1.45 \times 10^{-2}$ s^{-1} that differ by a factor of 500. The kinetic ${}_{s}^{s}$pK_a of 12.7 determined from Figure 5 is ascribed to the ionization of a Pd-bound solvent molecule in accordance with the process [Pd(*C*,*N*-(dimethylbenzylamine)(**2b**)(CH_3OH)] $\longleftrightarrow$ [Pd(*C*,*N*-(dimethylbenzylamine)-(**2b**)(CH_3O^-)] + H^+. The ${}_{s}^{s}$pK_a determined for this complex is significantly higher than the ${}_{s}^{s}$pK_a of 10.8 determined previously for [Pd(*C*,*N*-(dimethylbenzylamine)(pyridine)-(CH_3OH)][17b] which we attribute to the effect of replacing a neutral pyridine ligand with a tightly bound and anionic phosphorothioate **2b**, converting the formally cationic Pd to a neutral species prior to the acid dissociation of the Pd-bound CH_3OH.

e. Linear Free Energy Relationships for 3-Catalyzed Cleavage of Phosphorothioates 2a−d. The k_{cat}^{corr} values for the **3**-catalyzed cleavage of **2a**−**d** at ${}_{s}^{s}$pH 7.65 and 25 °C are relatively invariant despite the ∼5 ${}_{s}^{s}$pH unit difference in the ${}_{s}^{s}$pK_a^{LG} of the aryloxy leaving groups. The plot of log(k_{cat}^{corr}) versus ${}_{s}^{s}$pK_a^{LG} provides a gradient of $\beta^{LG} = -0.01$. Brønsted coefficients such as the β^{LG} determined in the present study are often used to gauge the extent of bond cleavage involving the reporter group and charge development at the transition state (TS) of reactions.[29] The near zero value of β^{LG} for the reaction of **2a**−**d** catalyzed by **3** at ${}_{s}^{s}$pH 7.65 strongly indicates that charge changes on the leaving group oxygen have not occurred to any appreciable extent at the TS and may argue instead for a rate limiting ligand exchange or intramolecular rearrangement occurring prior to the intervention of a chemical step involving cleavage of the P−OAr bond. An alternative interpretation for the low dependence of the reaction rate on ${}_{s}^{s}$pK_a^{LG} might involve a rapid pre-equilibrium followed by a compensatory kinetic step so that the respective Brønsted coefficients are of equal magnitude but opposite sign (e.g., $\beta^1 + \beta^2 \approx 0$). A similar effect has been forwarded for the acid catalyzed hydrolysis of esters[30] and also considered for the [Zn(II):(1,5,9-triazacyclododecane)($^-OCH_3$)] and [$La^{3+}{}_2$($^-OCH_3$)$_2$] promoted cleavages of carboxylate esters.[31]

The datum for catalytic cleavage of *O*,*O*-dimethyl phosphorothioate **2e** is included on the plot of Figure 6 for comparison from which it can be seen that it reacts some ∼44 times slower than the aryloxy phosphorothioates **2a**−**d** at the same ${}_{s}^{s}$pH. Assuming that the methoxy derivative fits the same Brønsted relationship as do the aryloxy derivatives, this observation is suggestive of a change in rate limiting step on going from the activated substrates **2a**−**d** containing aryloxy leaving groups to the less activated alkoxy leaving group of **2e**. A line drawn directly from the data point for reaction of **2d** to **2e** provides an upper limit[32] of −0.4 for the β^{LG} describing the putative descending portion of the Brønsted plot.[33] It is stressed that this is only an upper limit as it assumes the break point of the Brønsted plot occurs at the ${}_{s}^{s}$pK_a^{LG} of the leaving group for **2d**. Nonetheless, the significant dependence of the reaction rate for **2e** on leaving group ${}_{s}^{s}$pK_a^{LG} is consistent with a chemical step involving considerable change in the P−OCH_3 bonding being rate limiting, in contrast to the reaction of **2a**−**d**.

Included in Figure 6 is a Brønsted plot for the **3**-catalyzed cleavage of **2b**−**d** determined at ${}_{s}^{s}$pH 14.1 and 25 °C corresponding to reaction along the high ${}_{s}^{s}$pH plateau. Linear regression of the data provides a β^{LG} of −0.86 indicating that, in contrast to reaction at lower ${}_{s}^{s}$pH, there is now a strong dependence of reaction rate on the ${}_{s}^{s}$pK_a^{LG} of the leaving group. The steepness of the gradient for the Brønsted correlation determined at high ${}_{s}^{s}$pH relative to that at low ${}_{s}^{s}$pH leads to an intersection point at a ${}_{s}^{s}$pK_a of 14.1. The β^{LG} for the methanolysis of *O*-methyl *O*-aryl phosphorothioates is not known, and so no comparison can be made between the extent of P−OAr bond cleavage for the background reaction and that catalyzed by **3**. A more complete analysis of the linear free energy data is complicated by the absence of a known β^{Eq} for the reactions under question.

f. Proposed Reaction Mechanism for the 3-Catalyzed Methanolysis of 2. (f.1). High ${}_{s}^{s}$pH Region above the ${}_{s}^{s}$pK_a of the Palladacycle-Bound $HOCH_3$. In discussing

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[29] (a) Leffler, J. E.; Grunwald, E. *Rates and equilibria of organic reactions*; Wiley: New York, 1963. (b) Williams, A. *Acc. Chem. Res.* **1984**, *17*, 425. (c) Williams, A. *Concerted Organic and Bioorganic Reaction Mechanism*; CRC Press: Boca Raton, FL, 2000.

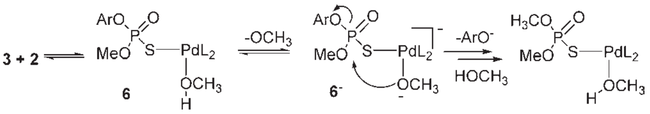
[30] (a) Taft, R. W. Jr. *J. Am. Chem. Soc.*, **1952**, *74*, 2729, 3120; (b) Taft, R. W., Jr. *J. Am. Chem. Soc.* **1952**, *74*, 3120. (c) Taft, R. W., Jr. *J. Am. Chem. Soc.* **1953**, *75*, 4231.

[31] Neverov, A. A.; Sunderland, N. E.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 65.

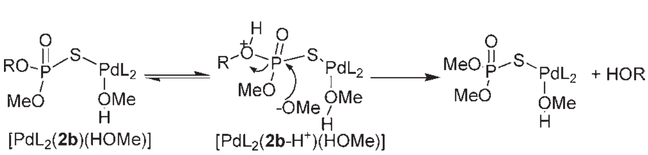
[32] To clarify, the upper limit of $\beta^{LG} = -0.4$ states that the actual value might be significantly more negative ($\beta^{LG} < -0.4$) but be of a larger absolute magnitude overall.

[33] The rate constant for catalytic cleavage of **2e** has not been corrected for buffer effects. It should also be noted that this reaction was carried out in methanol solvent containing 90% deuterium content. The combined effects of buffer catalysis and any isotopic perturbation on the rate constant are not known; however, it is probable that these are minimal considering that buffer effects for **2a-d** never account for more than 40% of the reaction flux and the determined solvent deuterium kinetic isotope effect for **2b** is 1.17.

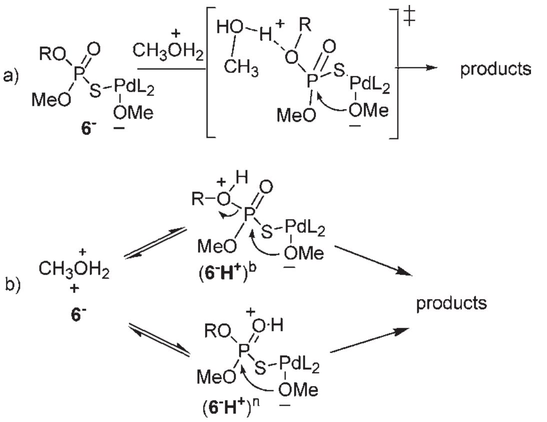
Scheme 1. Proposed Catalytic Cycle for Reaction of Phosphorothioates **2** Promoted by **3** at High $^{s}_{s}$pH



Scheme 2. Possible Catalytic Mechanism for Reaction of Phosphorothioates Promoted by **3** at low $^{s}_{s}$pH



Scheme 3. Alternative Possible Mechanisms Proceeding through Formally Neutral TS for the Reaction of Phosphorothioates Promoted by **3** at Low $^{s}_{s}$pH



the mechanism for the **3**-catalyzed methanolysis of series **2** we first address the reaction at high $^{s}_{s}$pH (Scheme 1). Binding of *O*-methyl *O*-aryl phosphorothioates **2** to **3** generates the ground state complex **6** which undergoes ionization of coordinated methanol to generate **6**$^-$ with a kinetically determined $^{s}_{s}pK_a$ of 12.7. Intramolecular delivery of coordinated methoxide displaces the leaving group in what might be either a concerted displacement or alternatively a two-step process ultimately regenerating **6** containing an *O,O*-dimethyl phosphorothioate ligand. Studies of the hydrolytic cleavage of phosphate diesters,[29b,c] and some phosphorothioate diesters[10,18] suggest concerted processes proceeding by an associative TS with slightly less departure of the aryloxy leaving group in the TS involving the latter phosphorothioates. It is also possible that the metal ion catalyzed methanolyses discussed herein are concerted, bearing in mind the possibility that a (di)anionic phosphorane-like species formed on the reaction pathway between starting material and product might be sufficiently strongly bound by the catalyst that it becomes a true intermediate.[34] As shown in Scheme 1, the nucleophile in the reaction is proposed to be a Pd-bound species and not an external methoxide. This is consistent with the observation that pincer complex **4** at $^{s}_{s}$pH 14.8 catalyzes the reaction of **2b** some 10^6-fold slower than does **3** at the same $^{s}_{s}$pH. The low level of activity determined for **4** points to a requirement for the catalytically competent **3** having two available *cis*-planar coordination sites to accommodate both a substrate and the methoxide nucleophile.

(f.2). Low $^{s}_{s}$pH Region. The rate of reaction of substrate **2b** in the low $^{s}_{s}$pH plateau region from $5.5 \leq {}^{s}_{s}\mathrm{pH} \leq 10$, as shown in Figure 5, is only ~500 times slower than that in the high $^{s}_{s}$pH plateau. A small, normal solvent deuterium kinetic isotope effect of $k^H/k^D = 1.17$ suggestive of the absence of a proton in flight was determined at two points in the low $^{s}_{s}$pH independent plateau. The β^{LG} in this region is -0.01, indicating an insensitivity to the nature of the leaving aryloxy group, and that little charge change has occurred at that group in the TS. A plausible $^{s}_{s}$pH independent reaction mechanism is shown in Scheme 2 involving attack of an external methoxide on a protonated Pd-bound **2b**. This reaction mechanism is similar to that proposed by Kirby and Vargolis some time ago for the decomposition of monoanionic aryl phosphate monoesters and is consistent with the small dependence of reaction rate on leaving group $^{s}_{s}pK_a$.[35] However, considering the rate constant of 1.23×10^{-2} s^{-1} for cleavage of Pd bound **2b** at $^{s}_{s}$pH 5.6 and that $[CH_3O^-] = K^{auto}/[H^+] = 10^{-16.77}/10^{-5.6} = 7 \times 10^{-12}$ M, the second order rate constant for methoxide attack would be 2×10^9 M^{-1} s^{-1}. This value approaches the diffusion limit in methanol (estimated to be 5.2×10^{10} M^{-1} s^{-1})[36] and moreover does not allow for the unfavorable equilibrium between [PdL$_2$(**2b**)(HOMe)] and [PdL$_2$(**2b**-H$^+$)(HOMe)] because the $^{s}_{s}pK_a$ of the latter would be on the order of -3 (vide infra). Furthermore the reaction mechanism shown in Scheme 2 fails to accommodate the poor reactivity seen with pincer complex **4** at low $^{s}_{s}$pH.

Three alternative process, given in Scheme 3, posit that a Pd-bound methoxide attacks a partially or fully protonated Pd-bound **2b**: (a) a general acid promoted cleavage of **6**$^-$ involving a leaving group assistance by lyonium ion; or (b) protonation of the leaving group's bridging oxygen $(\mathbf{6}^-\mathbf{H}^+)^b$, or the non-bridging oxygen of the P═O unit $(\mathbf{6}^-\mathbf{H}^+)^n$. These apparently satisfy the criterion of a $^{s}_{s}$pH neutral reaction, are consistent with a low β^{LG} observed in Figure 6, and do not suffer from a diffusion limited restriction for an attack by external methoxide. Nevertheless, these are almost certainly ruled out by the $^{s}_{s}$pH/rate profile in Figure 5 and considerations of the concentrations of reactive species. We deal first with the general acid process depicted in Scheme 3a, specifically with substrate **2b**, noting that at $^{s}_{s}$pH 9 the $[CH_3OH_2^+]$ is 10^{-9} M, the k_{cat}^{corr} for the decomposition of the **3**:**2b** complex is 1.58×10^{-2} s^{-1} and since $^{s}_{s}pK_a = 12.7$, $[\mathbf{6}^-] = 1/5000\ [\mathbf{6}]_{total}$. Under these conditions, the computed second order rate constant for methoxide attack with general acid catalysis is 7.9×10^{10} M^{-1} s^{-1}, exceeding the diffusion limit, and along with the solvent isotope effect of $k^H/k^D = 1.17 \pm 0.08$, eliminates the general acid process with its proton in flight.

For the processes in Scheme 3b, full protonation of either the bridging or non-bridging oxygens is required, with a subsequent intramolecular attack on the phosphorus.

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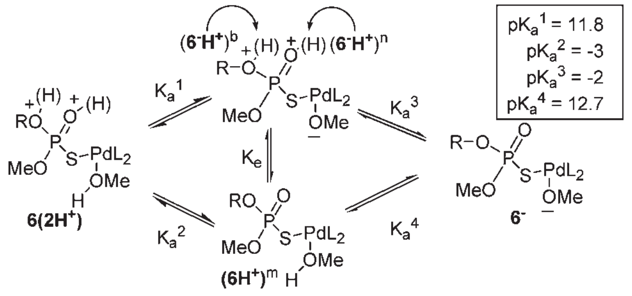
[34] In a recent study of the methanolytic cleavage of a series of neutral phosphorothioate triesters promoted by **3**,[17c] we have presented computational evidence that the catalyzed reaction is multistep, proceeding through a tightly bound phosphorane intermediate, the breakdown of which is rate-limiting for substrates with poor leaving groups.

[35] Kirby, A. J.; Vargolis, A. G. J. *Am. Chem. Soc.* **1967**, *89*, 415.

[36] Chen, R.; Freeman, G. R. *J. Phys. Chem.* **1995**, *99*, 4970.

Scheme 4. Hypothetical Acid Dissociations of the Dibasic Forms of **3**:**2** Complexes **6**(**2H**$^+$) where O-Protonation Can Occur on Either the Bridging (b) or Non-Bridging (n) Oxygens



We consider that the unimolecular process with (**6**$^-$**H**$^+$)^b of Scheme 3b involves an associative TS with concerted Pd-bound methoxide attack and departure of the protonated leaving group. The analogous process with (**6**$^-$**H**$^+$)^n, if concerted, would involve displacement of the *p*-nitrophenoxide anion with or without general assistance by solvent or perhaps by a synchronous translocation of the proton from the P=O unit to the departing group with possible solvent assistance. In the limit, this process could become a two step one having a phosphorane intermediate with subsequent Pd-ligand exchange and phosphorus pseudorotation required to assist departure of the leaving group.

Calculation of the concentration of the (**6H**$^+$)^m, (**6**$^-$**H**$^+$)^b, and (**6**$^-$**H**$^+$)^n intermediates shown in Scheme 3b requires estimation of the microscopic $^{s}_{s}\mathrm{p}K_a$ values for dissociation of the bridging or non-bridging H$^+$ which is difficult. Experimental determination of $K_a{}^1$ and $K_a{}^2$ values (Scheme 4) is not possible because of instability of the Pd-complex at $^{s}_{s}$pH values below 4, but these are crudely estimated as follows (for details of the analysis see Supporting Information). The reported $\mathrm{p}K_a$ values for the protonated phosphonium ions of trimethyl phosphate and dimethyl phosphate in water are −3.6 and −2.6, respectively,[37] and a value of −3 is assumed at the outset to be appropriate for the $K_a{}^2$ constant for ionization of the non-bridging proton of **6**(**2H**$^+$) to form the methanol associated complex (**6H**$^+$)^m. This ignores medium effect differences on the solvation of the species on either side of the equilibrium since these have similar charge types,[38] and any $\mathrm{p}K_a$ variations between phosphates and phosphorothioates, and also assumes that the Pd-complexation of the anionic S of **2b** essentially converts the anionic phosphorothioate into a neutral form.[39] The $^{s}_{s}\mathrm{p}K_a^1$ for ionization of **6**(**2H**$^+$) to form (**6**$^-$**H**$^+$)^n or (**6**$^-$**H**$^+$)^b should be lower than $^{s}_{s}\mathrm{p}K_a^4$ because the bound ligand is formally neutral, and the starting complex is doubly protonated, so $^{s}_{s}\mathrm{p}K_a^1$ is assumed to be ~11.8, halfway between the 12.7 value determined experimentally for ionization of **3**:**2f** and the 10.8 value determined previously for ionization of [Pd(*C*,*N*-(dimethylbenzylamine)(pyridine)(CH_3O*H*)].[17b]

Finally, for ionization of the non-bridging O−H$^+$ from (**6**$^-$**H**$^+$)^n to form **6**$^-$, we assume the $^{s}_{s}\mathrm{p}K_a^3$ is close to that of $^{s}_{s}\mathrm{p}K_a^2$ but raised to ~−2 because of a slight electrostatic stabilization of the (**6**$^-$**H**$^+$)^n. For consistency in the thermodynamic cycle small changes to any of the constants can be accommodated using the relationship $K_a{}^1K_a{}^3 = K_a{}^2K_a{}^4$. The $^{s}_{s}\mathrm{p}K_a^3$ for ionization of the bridging O−H$^+$ from the Pd-bound phenol of (**6**$^-$**H**$^+$)^b to form the corresponding **6**$^-$ is calculated from the thermodynamic cycle where $K_a{}^1K_a{}^3 = K_a{}^2K_a{}^4$. On the basis of these estimates, one can calculate from $K_e = K_a{}^2/K_a{}^1 = [(\mathbf{6H}^+)^m]/[(\mathbf{6}^-\mathbf{H}^+)^n] = 10^{14.8}$. On this basis, the amount of (**6**$^-$**H**$^+$)^n material required to form the products is exceedingly small and insufficient to account for the rate of the reaction. To account for the observed rate constant of 1.5×10^{-2} s^{-1} (**6**$^-$**H**$^+$)^n would have to decompose with a unimolecular rate constant of $(1.5 \times 10^{-2}) \times 10^{14.8}$ or 9.5×10^{12} s^{-1}, a value that approaches the lifetime of a transition state (10^{-13} s) which seems unlikely for such a complicated process involving several bond-making and bond breaking processes. A similar calculation for the pathway involving (**6H**$^+$)^m demonstrates that this too is a non-viable species.[40]

So what accounts for the rapid reaction in the low $^{s}_{s}$pH region? In Scheme 5 is a proposed reaction mechanism consistent with the available data. From $^{s}_{s}$pH 5.6 to 10.5, palladacycle **3** binds substrates **2** with displacement of one methanol to generate the key intermediate **6** (which from the above discussion must exist as (**6H**$^+$)^m as shown in Scheme 4). This is probably an associative process consistent with the generally accepted mechanism for ligand exchange at Pd(II) centers.[41] The lack of a significant β^{LG} for the ArO cleavage reaction in the low $^{s}_{s}$pH region suggests that the rate limiting step cannot have substantial changes in the P−OAr bonding, leading to the simplest conclusion that a rate limiting intramolecular rearrangement of **6** occurs, possibly generating complex **7** or a similarly disposed intermediate, with little perturbation of the P−OAr bonding. A similar binding geometry to that proposed for **7** has been observed for the square planar *bis*-(*O*,*O*-diethyl dithiophosphato)platinum(II) complex as characterized by X-ray crystallography, demonstrating an internal S−P−S bond angle of 102.4°.[42]

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[37] Azéma, L.; Ladame, S.; Lapeyre, C.; Zwick; Lakhdar-Ghazal, A. F. *Spectrochim. Acta, A* **2005**, *62*, 287.

[38] Gibson, G. T. T.; Mohamed, M. F.; Neverov., A. A.; Brown, R. S. *Inorg. Chem.* **2006**, *45*, 7891.

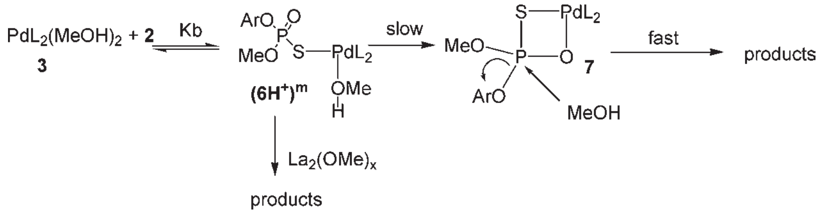
[39] On the basis of the changes in the first and second acid dissociation constants of $H_2PO_4^-$ bound between two Co(III) centers, it appears that two metal ions have the effect of a single proton. By extension, a single metal ion is predicted to have the effect of 1/2 a proton: (a) Edwards, J. D.; Foong, S.-W.; Sykes, A. G. *J. Chem. Soc., Dalton Trans.* **1973**, 829. (b) Williams, N. H.; Cheung, W.; Chin, J. *J. Am. Chem. Soc.* **1998**, *120*, 8079.

[40] For the calculation predicated in Scheme 4, protonation on the bridging oxygen of the aryloxy group should be far more difficult than on the phosphoryl oxygen, making (**6**$^-$**H**$^+$)^b far less stable than (**6**$^-$**H**$^+$)^n. This rests on two findings. The first is that in the study of the dissociation constant of protonated trimethyl phosphate or dimethyl phosphate in water[37] the authors found no spectroscopic evidence for the presence of a protonated methoxy phosphate, meaning that protonation adjacent to the P unit is less favored than P=O protonation. Second, it is known from extensive studies of the equilibrium transfer of the dialkylphosphoryl unit ($(RO)_2P{=}O$) between oxyanionic nucleophiles (ref 29), that the O-Ar oxygen of phosphate tri- and diesters has an effective charge of + 0.83 and +0.74 in water[29b,c] which is assumed to be the same as in methanol, and that for the bridging phenolic oxygen on phorphorothioate triesters is +0.50.[17c] This means that the protonated form of the bridging oxygen of the Pd-bound substrates **2** should be very acidic and have a $^{s}_{s}\mathrm{p}K_a$ much lower that the values of ~ −3 considered here.

[41] (a) Dies, V.; Cuevas, J. V.; Garcia-Herbosa, G.; Aullon, G.; Charmant, J. P. H.; Carbayo, A.; Munoz, A. *Inorg. Chem.* **2007**, *46*, 568. (b) Soldatovic, T.; Shoukry, M.; Puchta, R.; Bugarcic, Z. D.; van Eldik, R. *Eur. J. Inorg. Chem.* **2009**, 2261. (c) Hohmann, H.; Suvachittanont, S.; van Eldik, R. *Inorg. Chim. Acta* **1990**, *177*, 51.

[42] Gianni, M.; Caseri, W. R.; Gramlich, V.; Suter, U. W. *Inorg. Chim. Acta* **2000**, *299*, 199.

Scheme 5. Proposed Mechanism for the Reaction of Phosphorothioates at Low $^{s}_{s}$pH^{a}



^{a} Charges and the associated counterions have been omitted for clarity.

The angle strain imposed by the square planar binding geometry of the Pd(II) center is expected to activate **7** for nucleophilic attack by even weak nucleophiles such as solvent, but the steric and geometric details of such a reaction are opaque. The demonstrated lack of catalytic activity of **4** for the methanolysis of **2b** in the low $^{s}_{s}$pH region provides evidence consistent with the proposed mechanism of Scheme 5. For reaction of **2a−d** it is proposed that rate limiting formation of a doubly activated complex such as **7** is followed by fast chemical cleavage of the aryloxy leaving group. Formation of **7** might be expected to display a Brønsted dependence of ~0 owing to a compensatory effect of increased nucleophilicity for phosphorothioates with high $^{s}_{s}pK_a$ leaving groups and a concomitant decrease in the Lewis acidity of the corresponding Pd(II) center. Activation of **2** for chemical cleavage by bidentate coordination to Pd(II) as in **7** is an energetically uphill process as indicated by the $\Delta H^{\ddagger}_{\mathrm{Pd}}$of 14.0 kcal/mol and $\Delta S^{\ddagger}_{\mathrm{Pd}}$ of −20 cal/mol·K. The negative entropy of activation further suggests that **6** undergoes some restriction of degrees of freedom in the transition state for formation of **7**, possibly resulting from the formation of a strained four-membered ring. The details of the reaction are not obvious, as it could follow a concerted or stepwise process. In the latter case, breakdown of the 5-coordinate phosphorane would be fast for aryloxy leaving groups, but become slow, and possibly rate limiting from poorer leaving groups such as methoxy. The significantly slower symmetrical identity reaction of **2e** relative to **2a−d** is consistent with such a pathway where chemical cleavage from an intermediate has become slow relative to intramolecular rearrangement for displacement of the high $^{s}_{s}pK_a$ methoxy leaving group.

It should be noted that the $^{s}_{s}$pH independence of the reaction determined for **2b** at low $^{s}_{s}$pH (Figure 5) provides no insight to the nature of the nucleophilic species since the rate limiting step is proposed to occur prior to chemical cleavage. However, the possibilities for the nucleophile are narrowed by consideration of the following. Double activation of the phosphorothioate as depicted in Scheme 2 completed the 4-coordination sphere for the Pd, thus suggesting an external nucleophilic mechanism. The k^{min} term of ~0.02 s^{-1} for reaction of **2b** provides a lower limit for the rate of chemical cleavage and considering the [CH_3O^-] at $^{s}_{s}$pH 5.6, the external methoxide attack on complex **7** at least approaches, and in all probability exceeds, the diffusion limit in methanol as discussed above. For these reasons we favor a process involving attack by solvent methanol on a doubly activated complex such as **7** leading to product formation.

The observation that the decomposition of **6** is subject to buffer catalysis by La^{3+} supports the intervention of a new pathway for the La^{3+} reaction at low $^{s}_{s}$pH (Scheme 2). Under the reaction conditions the speciation of the La^{3+} is dimeric with a $^{s}_{s}$pH dependent number of associated methoxides ([$La_2(^{-}OMe)_x$]; where x = 1−5) which are responsible for the observed buffer catalysis. This is consistent with the results obtained for our previous studies involving La^{3+}-promoted cleavage of anionic phosphate diesters,[27] neutral carboxylate, [30] phosphonate,[43] and phosphonothioate [44] esters as well as phosphate triesters. [45] That cleavage of complex **6** is subject to significantly more efficient La^{3+} catalysis than is the cleavage of unbound phosphorothioate **2b** suggests cooperativity between the palladacycle and La^{3+} systems in promoting methanolytic cleavage, but this topic lies outside the scope of the current study.

5. Conclusions

The thiophilic metal ions Cd(II) and Mn(II) are effective promoters for the methanolysis of *O*-methyl *O*-4-nitrophenyl phosphorothioate providing catalytic rate accelerations of ~10^6. These metal ions are often used in the study of enzyme and ribozyme catalyzed phosphoryl transfer in conjunction with thio effect data derived from the reaction of native phosphate and sulfur modified phosphorothioate substrates. It is often assumed in these studies that "metal ion rescue" effects[46] result directly from a synergistic effect of the enzyme/ribozyme acting in concert with the thiophilic metal ions to bind and transform the phosphorothioate substrate. The current study demonstrates that in instances where the "metal ion rescue" experiments result in low levels of returned enzymatic activity, caution should be exercised to ensure that the observable kinetic effects are not attributable to the metal ions acting alone in solution to catalyze phosphothioyl transfer.

Palladacycle **3** is shown to be even more effective than Cd(II) or Mn(II) in promoting the methanolysis of phosphorothioates where rate accelerations[47] of ~10^{10} and 10^{12} relative to the second order methoxide promoted reaction were achieved for the cleavage of **2b** in the low and high $^{s}_{s}$pH domains, respectively. This represents an impressive catalytic efficiency, particularly when one considers that the phosphodiesterase NPP from *X. axonopodis* catalyzes the hydrolysis

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(43) Lewis, R. E.; Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 4082.

(44) Melnychuk, S. A.; Neverov, A. A.; Brown, R. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1767.

(45) Liu, T.; Neverov, A. A.; Tsang, J. S. W.; Brown, R. S. *Org. Biomol. Chem.* **2005**, *3*, 1525.

(46) The use of metal ion rescue experiments is discussed in: Forconi, M.; Herschlag, D. *Methods Enzymol.* **2009**, *468*, 91.

(47) Computed from the ratio of the average apparent second order rate constant for the catalytic reaction (2.9×10^3 M^{-1} s^{-1} computed from the average of rate and K_b data in the low pH 5.6 to 8.8 plateau, and 2.2×10^5 M^{-1} s^{-1} from the average of rate and K_b data in the high pH range in Table 2) versus the second order rate constant for the methoxide reaction (2.3×10^{-7} M^{-1} s^{-1} from Table 1).

of R_p-*O*-methyl *O*-4-nitrophenyl phosphorothioate with a reported acceleration of 10^{13}.[2f] The efficacy of the cleavage of **2b** promoted by **3** relative to the corresponding methoxide reaction in the high ^s_spH plateau can be compared with a value of 1.9 × 10^6 fold determined earlier[17c] for the cleavage of the far more reactive triester *p*-nitrophenyl dimethyl phosphorothioate promoted by **3** in methanol. This points to an interesting phenomenon whereby the same catalyst promotes the reaction of a far less reactive substrate better than it does a reactive substrate. It is also significant that **3** catalyzes the cleavage of *O,O*-dimethyl phosphorothioate (^s_spK_a^{LG} 18.2) in CD_3OD to generate $(CD_3O)_2P{=}O(S^-)$ with a rate constant of $3.4 \times 10^{-4}\ s^{-1}$ at what is essentially neutral ^s_spH. Examples of catalytic systems capable of cleaving alkoxy leaving groups from phosphate diesters are rare making the **3**-promoted cleavage of **2e** all the more noteworthy.[22,48]

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(48) Jagoda, M.; Warzeska, S.; Pritzkow, H.; Wadepohl, H.; Imhof, P.; Smith, J. C.; Krämer, R. *J. Am. Chem. Soc.* **2005**, *127*, 15061.

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Supporting Information Available: Procedures for the preparation of **2a−e** and corresponding analytical data, description of the kinetic methods used, plots of observed rate constants versus [metal] for the cleavage of **2b** in the presence of Cd(II), Mn(II), La(III), plot of observed rate constant versus [$^-$OMe] for the base promoted cleavage of **2b**, tables of rate constants for the **3**-catalyzed cleavage of **2b** as a function of ^s_spH and temperature, tables of rate constants for the **3**-catalyzed cleavage of **2a,c,d**, and methodology for the speciation calculations for the process in Scheme 4 (16 pages). This material is available free of charge via the Internet at http://pubs.acs.org.