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Demonstration of Prominent Cu(II)-Promoted Leaving Group Stabilization of the Cleavage of a Homologous Set of Phosphate Mono-, Di-, and Triesters in Methanol

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Abstract: A series of phosphate mono-, di-, and triesters with a common leaving group (LG) (2′-(2-phenoxy)-1,10-phenanthroline) was prepared, and the kinetics of decomposition of their Cu(II) complexes was studied in methanol at 25 °C under $_{S}^{S}$pH-controlled conditions. The Cu(II) complexes of 2-[2′-phenanthrolyl]phenyl phosphate (Cu(II):**6**), 2-[2′-phenanthrolyl]phenyl methyl phosphate (Cu(II):**7**), and 2-[2′-phenanthrolyl]phenyl dimethyl phosphate (Cu(II):**8**) are tightly bound, having dissociation constants $K_d \leq 3 \times 10^{-7}$ M, with the Cu(II) being in contact with the departing phenoxide. The $_{S}^{S}$pH/rate profile for cleavage of Cu(II):**6** has a low $_{S}^{S}$pH plateau ($k_o = 6.3 \times 10^{-3}$ s^{-1}), followed by a bell-shaped maximum ($k_{cat}^{max} = 14.7 \pm 0.4$ s^{-1}) dependent on two ionizations with $_{S}^{S}pK_a^2$ and $_{S}^{S}pK_a^3 = 7.8 \pm 0.1$ and 11.8 ± 0.2. The $_{S}^{S}$pH/rate profile for cleavage of Cu(II):**7** has a broad plateau from $_{S}^{S}$pH 3 to $_{S}^{S}$pH 10 followed by a descending wing at higher $_{S}^{S}$pH with a gradient of -2. The $_{S}^{S}$pH/rate profile for cleavage of Cu(II):**8** is sigmoidal with two plateaus ($k_1 = (2.0 \pm 0.2) \times 10^{-5}$ s^{-1}, $k_2 = (1.2 \pm 0.2) \times 10^{-6}$ s^{-1}), connected by an ionization with a $_{S}^{S}pK_a$ of 6.03. Activation parameters are given for the reactions in the plateau regions: all three species show similar $\Delta H^{\ddagger}$ terms of 21.4−21.6 kcal/mol, with major differences in the $\Delta S^{\ddagger}$ terms, which vary from 18 to 2.3 to -7.4 cal/(mol·K) passing from the mono- to di- to triester. Detailed analyses of the kinetics indicate that the reactions involve spontaneous solvent-mediated cleavage of the Cu(II)-coordinated phosphate dianion [Cu(II):**6b**]^0 and phosphate diester monoanion [Cu(II):**7b**]$^+$ and, for the triester, complexes containing Cu(II) and Cu(II):$^-$OCH$_3$ designated as [Cu(II):**8a**]$^{2+}$ and [Cu(II):**8b**]$^+$. Reactions where methoxide is the active nucleophile are not observed. Comparisons of the rates of the decomposition of these species at their $_{S}^{S}$pH maxima in the neutral $_{S}^{S}$pH region with the estimated rates of the background reactions indicate that leaving group assistance provided by the coordinated Cu(II) accelerates the cleavage of the phosphate mono-, di-, and triesters by 10^{14} to 10^{15}, 10^{14}, and 10^5. Detailed Hyperquad 2000 analysis of titration data indicates that phenoxide **9**$^-$ is bound 23 kcal/mol stronger than the phosphate triester **8**. It is the realization of part of this energy in the emerging products resulting from P−O(LG) cleavage that provides the driving force for the catalyzed reactions.

1. Introduction

Phosphate mono-, di-, and triesters have important roles in living systems. Phosphorylation and hydrolysis reactions of phosphate monoesters play vital roles in protein function, energy regulation, metabolism, signal transduction, and many other processes.[1−3] Phosphate diesters are extremely resistant to solvolytic cleavage, making them suitable functionalities for the backbones for DNA and RNA, which are responsible for storing genetic information.[3] Phosphate triesters are not naturally occurring and have no known natural biological function, but they are commercially important as pesticides[4] owing to their toxicity as acetylcholinesterase inhibitors.[5]

Due to the great accelerations required to bring the various phosphate cleavage reactions into a useful time scale for living systems, considerable effort has been expended to understand the mechanistic diversity of phosphoryl transfer reactions mediated by enzymes. In the absence of catalysts, the solvolytic reaction rates of phosphate mono- and diesters with unactivated leaving groups (LGs) are such that the half-time for hydrolysis of a monoester is $\sim 10^{12}$ years, and those for hydrolysis of RNA and DNA under physiological conditions are $\sim$110 and $\sim 10^{8-10}$ years, respectively.[6] However, the rate of phosphoryl transfer reactions in the presence of the most efficient enzymes can be

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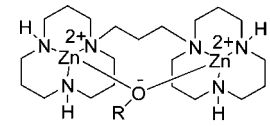
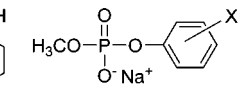
accelerated by 10^{11}- to 10^{12}-fold for phosphate triesters,[7] 10^{15}- to 10^{21}-fold for diesters,[6] and $>10^{17}$-fold for phosphate monoesters.[8]

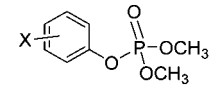
Many enzymes that cleave phosphate esters contain two or more transition-metal ions (Zn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, and Mn^{2+}) in close proximity in their active sites,[3] and their catalytic roles have been discussed at length.[2,3,9] Four main catalytic modes that metalloenzymes are proposed to employ are (1) Lewis acid activation of the substrate via $M^{x+}\cdots O{=}P$ binding, (2) delivery of a metal-bound hydroxide or alkoxide that serves as a nucleophile or a base, (3) electrostatic stabilization of the anionic substrate and nucleophile/base through binding to its +-charged active site and subsequent lowering of the transition-state energy of the reaction,[10] and (4) stabilization of the leaving group through metal ion coordination. Model catalysts have been designed that employ several, but rarely all, of these modes of catalysis in the hopes of achieving the efficiency of enzymatic catalysis in man-made systems.[11,12] Our recent work demonstrated that a simple dinuclear catalyst (**1**) promotes the cleavage of phosphate diesters **2** and **3** in methanol[13] and ethanol[14] with up to 10^{11-13}- and 10^{14-17}-fold rate accelerations over the uncatalyzed processes. That no such acceleration is seen for the cleavage of a phosphate diester promoted by **1** in water[15] indicates that medium effects[16] play a key role in producing large rate enhancements with metal ions, perhaps amplifying weak effects and inducing other modes of catalysis not easily seen in water.



1



2
2b. X = *o*-nitro
2c. X = *o*-methoxycarbonyl



3
3b. X = *o*-methoxycarbonyl

There are surprisingly few, albeit informative, reports documenting LGA promoted by general acids or metal ions even though this should be a vital component of phosphoryl transfer reactions catalyzed by enzymes where the natural substrates contain nonactivated leaving groups. This type of catalysis is expected to be most readily observed for a reaction considered to proceed via a dissociative transition state (TS)[2] with extensive leaving group departure and little nucleophile participation. Consistent with this, significant metal ion-promoted LGA is seen for the alkaline phosphatase-catalyzed cleavage of dianionic phosphate monoesters.[17] Accelerations up to 10^8-fold have been documented for intramolecular general-acid catalysis of the hydrolyses of 8-(dimethylammonium)naphth-1-yl phosphate (**4a**)[18] and salicyl phosphate[19] monoesters relative to the uncatalyzed reactions of these dianions. There are also a handful of examples of LGA for cleavage of phosphate monoesters promoted by a metal ion, notable examples being the Cu(II)-catalyzed cleavages of 2-(4(5)-imidazoyl)phenyl phosphate,[20] 8-quinolyl phosphate,[21] salicyl phosphate,[22] and 2-(1,10-phenanthrolyl) phosphate (**5**)[23] where the metal ion-promoted LGA increases the reactivities of these by 10^4- to 10^8-fold over those of the background reactions.

The hydrolysis of the phosphate diester methyl 8-(dimethylammonium)naphth-1-yl phosphate (**4b**)[24] is accelerated by intramolecular general-acid catalysis as is the cleavage of bis(8-hydroxyquinoline) phosphate.[25] The cleavage of adenosine 3′-alkyl phosphate diesters in the presence of some transition metals and lanthanides is suggested[26] to involve coordination of the departing group to the metal ions, particularly La^{3+}. Our recent work demonstrated an LGA of 10^{12}-fold for the cleavage of diester **2c** promoted by Yb^{3+},[27] as well as an apparent LGA for the dinuclear Zn(II) complex **1** in promoting cleavage of diesters having an *o*-NO_2 or -CO_2Me substituent on the aryloxide leaving groups (**2b,c**).[13c]

For triesters such as diethyl 8-(dimethylammonium)naphth-1-yl phosphate (**4c**),[28] intramolecular general-acid catalysis enhances the attack of water and oxyanion nucleophiles. However, unambiguous examples of metal ion-promoted LGA

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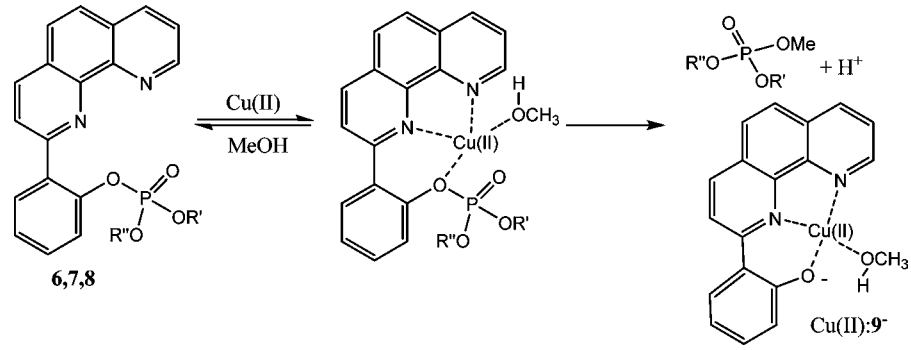
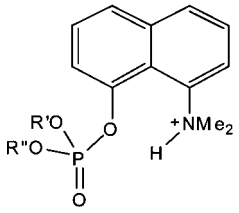
Scheme 1. Cu(II)-Assisted Cleavages of Phosphate Esters **6**−**8** in Methanol[a]



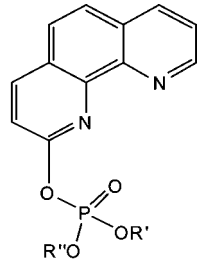
6,7,8

[a] R′ and R″ = H or CH_3.

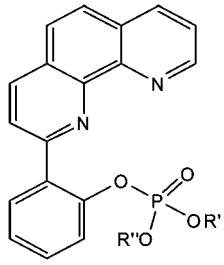
for triesters are exceedingly rare,[29] and the only unambiguous example of which we are aware where metal-assisted LG departure was quantified is that of La^{3+}-promoted methanolysis of triester **3b**, which is cleaved about 60 times faster than triesters without LGA.[30]



4a. OR' = OR" = O^-
4b. OR' = OMe; OR" = O^-
4c. OR' = OR" = OEt



5



6. OR' = OR" = O^-
7. OR' = OMe; OR" = O^-
8. OR' = OR" = OMe

While phosphoryl mono-, di-, and triester systems with the 8-(dimethylamino)-1-naphthyl leaving group[18,24,28] allow one to compare the effectiveness of general-acid assistance of LG departure on the three ester types, there is no analogous set of esters from which one can draw comparisons of the effectiveness of metal ion LGA. In this study we describe the kinetics of Cu(II)-promoted methanolyses of a set of phosphoesters **6**−**8** containing the 2-(2-hydroxyphenyl)-1,10-phenanthroline[31] leaving group, which is known to bind strongly to M^{x+} ions (Scheme 1). The results show that Cu(II)-promoted LGA gives a 10^{14}-fold or more acceleration for the methanolysis of monoester **6** and diester **7** in the neutral $_{S}$pH region. By contrast, the methanolysis of triester **8** in the presence of Cu(II) shows a more moderate, but still appreciable, 10^5-fold rate acceleration at neutrality due to metal ion-promoted LGA.

2. Experimental Section

2.1. Materials. Methanol (99.8%, anhydrous), sodium methoxide (0.50 M in methanol, titrated against N/2 certified standard aqueous HCl solution and found to be 0.50 M), $Cu(CF_3SO_3)_2$ (98%), $Zn(CF_3SO_3)_2$, 2-picoline (98%), 2,6-lutidine (99+%), 2,4,6-collidine (99%), triethylamine (99%), 2,2,6,6-tetramethylpiperidine (99+%), Amberlite IR-120H ion-exchange resin (functionalized as sulfonic acid), sodium carbonate, imidazole (99%), dimethyl chlorophosphate (96%), *p*-nitrophenol (98%), 1,10-phenanthroline (99+%), 2-bromoanisole (97%), lithium (high sodium granule, 99%), $KMnO_4$ (99+%), pyridine hydrochloride (98%), methanol-*d* (99.5 atom % D), NaOH (reagent grade, 97%), and phosphorus oxychloride (99%) were purchased from Aldrich and used as supplied. $HClO_4$ (70% aqueous solution, titrated to be 12.09 M) was obtained from Acros Organics. Lithium chloride and $MnSO_4 \cdot H_2O$ were purchased from Anachemia Chemical Ltd., while 1-methylpiperidine (99%) was obtained from Alfa Aesar and used as supplied. $MgSO_4$ anhydrous and 1-ethylpiperidine (99%) were obtained from Fisher Scientific and TCI America Laboratory Chemicals, respectively. 2-(2-Hydroxyphenyl)-1,10-phenanthroline was prepared following a published procedure.[31] The disodium salt of phosphate monoester **6** was synthesized following a literature method[32] using 2-(2-hydroxyphenyl)-1,10-phenanthroline and $POCl_3$. Both the phosphate diester **7** and the triester **8** were prepared according to a general method[33] with small modifications.[13c] ^{1}H NMR, ^{31}P NMR, and exact MS spectra of phosphates **6**−**8** are consistent with the structures (see the Supporting Information).

2.2. General Methods. ^{1}H NMR and ^{31}P NMR spectra were determined at 400 and 162.04 MHz. $CH_3OH_2^+$ concentrations were determined potentiometrically using a combination glass Fisher Scientific Accumet electrode model no. 13-620-183A calibrated with certified standard aqueous buffers (pH 4.00 and 10.00) as described in previous papers.[34] The $_{S}$pH values in methanol were determined by subtracting a correction constant of −2.24[34] from the electrode readings, and the autoprotolysis constant for methanol was taken to be $10^{-16.77}$ M^2. The $_{S}$pH values for the kinetic experiments were measured at the end of the reactions to avoid the effect of KCl leaching from the electrode. For methanolysis of

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Cu(II):**7** the $^{s}_{s}$pH values measured at the beginning of the reactions were the same as those determined at the end.

2.3. General UV−Vis Kinetics. The Cu(II)-catalyzed methanolyses of **7** and **8** were followed at 414 nm for the appearance of the Cu(II)-bound phenoxide **9** using a UV−vis spectrophotometer with the cell compartment thermostated at 25.0 ± 0.1 °C. The reactions were conducted in the presence of buffers composed of various ratios of $HClO_4$ and amines (2-picoline ($^{s}_{s}$pH 4.8−6.9), 2,6-lutidine ($^{s}_{s}$pH 7.0−7.5), 2,4,6-collidine ($^{s}_{s}$pH 7.8−8.5), *N*-isopropylmorpholine ($^{s}_{s}$pH 8.5−9.0), 1-methylpiperidine ($^{s}_{s}$pH 9.4−9.7), 1-ethylpiperidine ($^{s}_{s}$pH 9.8−10.5), triethylamine ($^{s}_{s}$pH 10.9), and 2,2,6,6-tetramethylpiperidine ($^{s}_{s}$pH 11.0−12.1)) to maintain the $^{s}_{s}$pH in methanol. For reactions at $^{s}_{s}$pH 3 or lower, an appropriate amount of $HClO_4$ was added to achieve the targeted initial [$CH_3OH_2^+$] in solution. A typical kinetic experiment involved preparing a methanol solution of the phosphate substrate (between 0.01 and 0.05 mM) containing excess buffer (0.2−1.0 mM) in a 1 cm path length UV cuvette. The rate constants for the methanolyses of **6** and **7** in the presence of Cu(II) did not exhibit buffer concentration effects (from 0.1 to 20 mM 2-picoline or 2,2,6,6-tetramethylpiperidine buffer), nor was the rate sensitive to the presence of 0.01−20 mM tetrabutylammonium chloride, tetrabutylammonium bromide, or free pyridine. Initiation of the reaction involved addition of an aliquot of Cu(II) stock solution in methanol to the substrate solution in the cuvette to achieve the desired concentrations of the reaction components in a final volume of 2.5 mL. Duplicate kinetics were done, and in cases where the reactions are reasonably fast, the absorbance vs time traces for product appearance were fit to a standard first-order exponential equation to obtain the observed first-order rate constants (k_{obsd}). For very slow reactions, such as those of the Cu(II)-promoted methanolysis of **8** at higher $^{s}_{s}$pH, initial rates of the reactions were obtained by fitting the first 5−10% of the absorbance vs time traces to a linear regression, followed by dividing the initial rates by the expected absorbance change (ΔAbs) if the reaction were to reach 100% completion to get the k_{obsd} constants.

Separate $^{s}_{s}$pH/rate profiles were constructed for the 0.05 mM $Cu(OTf)_2$-catalyzed cleavages of 0.05 mM **7** and 0.05 mM **8** in the presence of 1 mM buffer in methanol. A concentration-dependent study was also conducted with increasing [Cu(II)] and [**7**] in a 1:1 ratio from 0.05 to 0.3 mM in the presence of excess $HClO_4$, such that the $^{s}_{s}$pH was kept at 3.5 ± 0.2. The solvent kinetic isotope experiment for the Cu(II)-assisted breakdown of **7** was carried out as follows. Stock solutions of 5 mM $Cu(OTf)_2$, 7.0 mM substrate **7**, and 50 mM picoline (with 1/2 equiv of added $HClO_4$) buffer in CH_3OD were prepared. Aliquots of these solutions were diluted in UV cells containing either CH_3OH or CH_3OD so that the final concentrations of $Cu(OTf)_2$, **7**, and picoline were 0.05, 0.05, and 1 mM. Duplicate kinetic runs were done, and the $^{s}_{s}$pH values measured after the reactions were found to be 6.2 ± 0.2. A similar experiment was also done for substrate **8** with final concentrations of $Cu(OTf)_2$, **8**, and picoline buffer ($^{s}_{s}$pH 3.5 ± 0.2) of 0.05, 0.05, and 1 mM.

2.4. Stopped-Flow Kinetics. The Cu(II)-promoted cleavage of **6** was monitored at 414 nm for the appearance of product using a stopped-flow apparatus thermostated at 25.0 ± 0.1 °C. One syringe of the stopped-flow analyzer was loaded with 0.02 mM **6** and a 0.4 mM concentration of the desired buffer in methanol. The second syringe was loaded with 0.02 mM $Cu(OTf)_2$ in methanol. When mixed, the final concentrations of $Cu(OTf)_2$, **6**, and buffer were 0.01, 0.01, and 0.2 mM. At least five kinetic runs were recorded at each $^{s}_{s}$pH, and the average values gave the k_{obsd} constants. A concentration-dependent experiment was also conducted by following the reactions of 4×10^{-6} M ≤ [Cu(II):**6**] ≤ 5×10^{-4} M in the presence of a 40-fold excess of 1-methylpiperidine buffer ($^{s}_{s}$pH 10.4 ± 0.2). The effect of increasing [Cu(II)] was investigated by increasing [$Cu(OTf)_2$] from 1×10^{-5} to 7×10^{-5} M while keeping [**6**] constant at 1×10^{-5} M in the presence of 0.2 mM 1-methylpiperidine buffer ($^{s}_{s}$pH 10.2 ± 0.2). A solvent kinetic isotope experiment was done in a fashion similar to that described for substrate **7**, where stock solutions $Cu(OTf)_2$, **6**, and 1-methylpiperidine buffer were 0.5, 5, and 50 mM in CH_3OD. Aliquots of the solutions were diluted into solutions of CH_3OH or CH_3OD to attain the desired concentrations. In this case, one of the syringes of the stopped-flow analyzer was loaded with 0.02 mM **6** and 0.4 mM buffer, and the other syringe contained 0.02 mM $Cu(OTf)_2$. Two separate experiments were conducted with 1-methylpiperidine buffer, setting the measured but uncorrected $^{s}_{s}$pD values at 9.6 ± 0.2 and 10.4 ± 0.2 in methanol. (These uncorrected values were measured at the end of the reactions simply as the electrode readings + 2.24 and have not been corrected for the effect of the deuterated solvent on the electrode reading or on the $^{s}_{s}p$$K_a$ of the buffer. As will be discussed, the actual $^{s}_{s}$pD is less important since this is in the plateau region of the $^{s}_{s}$pH/rate profile.) The average of the first-order rate constants from seven kinetic runs was used for analysis.

2.5. Methanolysis of 4-Nitrophenyl Phosphate (10) at 50 °C. The progress for the methanolysis of 10 mM 4-nitrophenyl phosphate at 50 °C in 20 vol % CD_3OD in CH_3OH was followed using ^{31}P NMR. To six separate standard NMR tubes, each containing 10 mM disodium 4-nitrophenyl phosphate, were added 100 mM NaOMe, 10 mM NaOMe, 10 mM $HClO_4$, 20 mM $HClO_4$, 30 mM $HClO_4$, and 50 mM $HClO_4$. In another sample, no additional base or acid was added to a 10 mM concentration of the phosphate. The seven samples (all with a final volume of 1 mL of solution) were capped and sealed with Parafilm before being incubated in a water bath set at 50.0 ± 0.5 °C. At different times samples were removed from the water bath and ^{31}P NMR spectra were acquired with at least 600 scans on each. ^{31}P NMR spectra of the starting phosphate and monomethyl phosphate in methanol were used as references. All kinetic reactions were followed to a maximum of 15% completion, and for each sample (each concentration of added base or acid) at least triplicate experiments were conducted. The product conversion (%) vs time plots were fit to a linear regression to obtain the rates of the reactions. The rates were converted to first-order rate constants by dividing the conversion (%)/time data by 100%.

2.6. Spectrophotometric Titrations. $^{s}_{s}p$$K_a$ of 9. A 1 cm UV cell was charged with 0.1 mM phenol **9** in 2.5 mL of anhydrous methanol. Small aliquots of a 1.0 M tetrabutylammonium hydroxide in methanol were added to vary its concentration from 0 to 1.0 M in small increments, and the UV−vis spectrum (250−500 nm) of the mixture was collected at 25 °C after each addition of base. The titration was done in duplicate. The absorbance values at 400 nm (appearance of the phenoxide) were corrected for volume change, and the log of the corrected absorbance at 400 nm was plotted against the −(log [H^+]) in solution. The data were fit to the following expression to yield the acid dissociation constant of the phenolic OH group of compound **9**:

$$\log(\mathrm{Abs}) = \log\left(A\left(\frac{K}{K + [\mathrm{H}^+]}\right) + A_0\right) \qquad (1)$$

In eq 1, A is the overall absorbance change expected from converting one species completely into another, A_0 is the initial absorbance when the initial species (phenol **9**) exists at 100%, and

(34) (a) Gibson, G.; Neverov, A. A.; Brown, R. S. *Can. J. Chem.* **2003**, *81*, 495. (b) For the designation of pH in nonaqueous solvents we use the nomenclature recommended by IUPAC: *Compendium of Analytical Nomenclature. Definitive Rules 1997*, 3rd ed.; Blackwell: Oxford, U.K., 1998. The pH meter reading for an aqueous solution determined with an electrode calibrated with aqueous buffers is designated as $^{w}_{w}$pH; if the electrode is calibrated in water and the "pH" of the neat buffered methanol solution then measured, the term $^{s}_{w}$pH is used, and if the electrode is calibrated in the same solvent in which the "pH" reading is made, then the term $^{s}_{s}$pH is used. In methanol $^{s}_{w}$pH−(−2.24) = $^{s}_{s}$pH, and since the autoprotolysis constant of methanol is $10^{-16.77}$, the neutral $^{s}_{s}$pH is 8.4.

K is the dissociation constant of interest. The average $^{s}pK_a$ value of phenol **9** was determined to be 16.16 from duplicate titrations.[35]

2.7. Binding Constant of Cu(II) and Phenol 9. To a 2.5 mL methanol solution containing 0.1 mM phenol **9** and 0.1 mM $Cu(OTf)_2$ were added small aliquots of 0.6 M triflic acid in methanol, and the UV−vis spectrum (220−500 nm) was recorded after each addition. The absorbance values at 450 nm corresponding to the decrease in the complex concentration were corrected for volume changes, and the titration data were analyzed using Hyperquad 2000[36] with a model defined by species **9**, **9**−H^+, Cu(II):**9**, and Cu(II):$\mathbf{9}^-$ (see the Supporting Information). The acid dissociation constants of **9** and **9**−H^+ were fixed to be $10^{-16.16}$ and $10^{-4.73}$ M as determined independently (see the Supporting Information), while the autoprotolysis constant of methanol was taken to be $10^{-16.77}$ M^2.[34] With these inputs, the dissociation constants of Cu(II):$\mathbf{9}^-$ ⇄ Cu(II) + $\mathbf{9}^-$ and Cu(II):**9** ⇄ Cu(II) + $\mathbf{9}^-$ + H^+ were computed as $-[\log(K_d)]$ values of 23.64 ± 0.03 and 24.13 ± 0.01, respectively, from duplicate titrations (see Table 8S, Supporting Information).

2.8. Binding Constant of Cu(II) and 8. To a UV cell containing 7×10^{-5} M **8** and 1 mM collidine buffer (^{s}pH 7.4) in 2.5 mL of methanol were added aliquots of 5.6 mM $Cu(OTf)_2$ stock solution in 5 μL increments. The absorbance at 490 nm after each addition was plotted against [$Cu(OTf)_2$], and the data were fit to a binding equation[37,38] applicable to strong and weak situations.

A more accurate value for the dissociation constant of Cu(II):**8** was acquired as follows. To a UV cell containing a 0.1 mM concentration of the complex in 2.5 mL of anhydrous methanol were added small aliquots of triflic acid in methanol with subsequent determination of the UV−vis spectrum from 220 to 500 nm after each addition. The absorbance values at 263 nm for the reduction in the complex concentration were corrected for volume change and plotted against the −(log [H^+]) of the solution. Data from duplicate titrations were analyzed using Hyperquad 2000 to compute an average dissociation constant (K_d) of 2.8×10^{-7} M.

2.9. Activation Parameters. The kinetic runs at different temperatures for substrates **6** and **7** were done using a thermostated stopped-flow analyzer, and the rate constants determined at each temperature were used to construct Eyring plots. Kinetic runs for substrate **8** at different temperatures were done by UV−vis spectrophotometry and the solution temperatures determineded with a thermometer inserted into the cell at the end of the reaction. First-order rate constants were measured in quadruplicate at six different temperatures ranging from 10 to 35 °C for the Cu(II)-promoted methanolysis of **6** (both at 0.01 mM) in the presence of 0.2 mM 1-methylpiperidine buffer, ^{s}pH 10.4 ± 0.2. Fitting the first-order rate constant vs 1/*T* plot to the Eyring equation gives the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values. For substrate **7**, the activation parameters were determined with 0.05 mM $Cu(OTf)_2$, 0.05 mM **7**, and 0.25 mM $HClO_4$ (^{s}pH 3.6 ± 0.2) and using seven temperatures ranging from 12.0 to 39.8 °C. For substrate **8**, duplicate experiments were conducted with 0.05 mM $Cu(OTf)_2$, 0.05 mM **8**, and 1.0 mM 2-picoline buffer (^{s}pH 3.8 ± 0.2) at seven temperatures ranging from 16.0 to 42.5 °C.

(35) The total DA for conversion of a 0.1 M solution of phenol **9** to phenoxide $\mathbf{9}^-$ was determined to be 0.9 unit at 400 mm from fits of the absorbance vs [$^+NBu_4{}^-OCH_3$] to a standard single ionization model (see the Supporting Information).

(36) Hyperquad 2000 is a computer program that fits titration data into nonlinear expressions containing a series of formation constants (log *β*) for species input to a model: Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739.

(37) $k_{obsd} = k_{cat}(1 + K_B[S] + [cat]K_B - X)/(2K_B)/[S]$, where $X = (1 + 2K_B[S] + 2[cat]K_B + K_B{}^2[S]^2 - 2K_B{}^2[cat][S] + [cat]^2K_B{}^2)^{0.5}$.

(38) A detailed description and examples where the equation has been applied can be found in ref 13.

(39) (a) Bardwell, D.; Black, D.; Jeffery, J. C.; Ward, M. D. *Inorg. Chem.* **1993**, *12*, 1577. (b) Jeffery, J. C.; Moore, C. S. G.; Psillakis, E.; Ward, M. D.; Thornton, P. *Polyhedron* **1995**, *14*, 599.

3. Results

3.1. Cu(II) Complexation with 8 and 9 in Methanol. The X-ray diffraction structure of [$(Cu(II)_2$:$\mathbf{9}^-{}_2$:(μ-$MeCO_2$)][PF_6] shows the Cu(II) bound to **9** in a planar fashion with Cu(II)−N bond distances of ∼2 Å and a Cu(II)−phenoxide−O bond distance of ∼1.9 Å.[31] Similar structures are reported with Ni(II),[31] Pd(II),[39a] and Fe(III)[39b] which indicate that, once bound, the metal ion directly interacts with the phenoxide oxygen. The binding constant for Cu^{2+} and **9** was determined by spectrophotometric titration under acidic conditions (see the Supporting Information, Figure 9S), and the data were analyzed using Hyperquad 2000 to give a K_d of 2.3×10^{-24} M. Ancillary information from the Hyperquad fitting points to a significant drop of the $^{s}pK_a$ of **9** from 16.16 to 0.49 when bound to Cu(II), indicative of a large electrostatic interaction of the phenoxide oxygen of $\mathbf{9}^-$ with the Cu ion.

Triester **8** reacts slowly in the presence of Cu(II) and allows spectrophotometric titration studies. At ^{s}pH 7.4 (1 mM collidine containing 7×10^{-5} M **8** and varying [Cu^{2+}]), the dissociation constant for Cu(II):**8** was found to be $(5.1 \pm 2.4) \times 10^{-7}$ M. A separate study monitoring [Cu(II):**8**] under acidic conditions yields a similar dissociation constant of $(2.8 \pm 0.4) \times 10^{-7}$ M after analysis of the data with Hyperquad 2000 (see the Supporting Information). The reactivities of the Cu(II) complexes of **6** and **7** are too great to allow experimental determination of their dissociation constants, although we assume that these are similar to that of Cu(II):**8** but may have somewhat stronger binding due to electrostatic attraction between the metal ion and anionic phosphates.

3.2. Background Reaction for Methoxide with 8 and 7. The second-order rate constant of $(2.94 \pm 0.09) \times 10^{-3}$ M^{-1} s^{-1} for the CH_3O^--promoted cleavage of **8** was determined under pseudo-first-order conditions at three [$^-OCH_3$] values (see the Supporting Information).

The CH_3O^--promoted methanolysis of **7** is too slow to follow experimentally, so the $^{s}pK_a$ value for the leaving group phenol **9** (16.16), as determined above, was used to estimate the second-order rate constant for the methoxide background reaction of **7**. The Brønsted plot for the methoxide-promoted cleavages of a series of methyl aryl phosphate diesters in methanol[13c] fits the expression $\log(k_2{}^{-OMe}) = (-0.57 \pm 0.06)^{s}pK_a + (0.14 \pm 0.68)$. From this, $k_2{}^{-OMe}$ for methoxide attack on **7** is 8.5×10^{-10} M^{-1} s^{-1} assuming the reactivity of **7** adheres to the same relationship as do those of dimethyl aryl phosphates.

3.3. Methanolysis of 4-Nitrophenyl Phosphate (10). An estimate for the background reaction of **6**, which reacts too slowly to observe, was made from the rate constant for methanolysis of 4-nitrophenyl phosphate (**10**), which exhibits a plateau region between ^{s}pH ≈ 10 and ^{s}pH 14. The cleavage of **10** at 50.0 ± 0.5 °C in methanol was monitored using initial rate techniques at three ^{s}pH values greater than 10.6 (the second $^{s}pK_a$ value of **10** determined by half-neutralization) at various times using ^{31}P NMR (see the Supporting Information), and the average k_{obsd} for the methanolysis of dianionic **10** between ^{s}pH 11.6 and ^{s}pH 14.0 was determined to be 1.4×10^{-7} s^{-1}. This value can be compared with the reported value for cleavage of the dianion of **10** in water of 1.55×10^{-8} s^{-1} at 39 °C,[40] from which a k_{obsd} of 8×10^{-8} s^{-1} is calculated at 50 °C on the basis of the activation parameters in water listed by Guthrie and Jencks.[41] No

(40) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209.

(41) Guthrie, R. D.; Jencks, W. P. *Acc. Chem. Res.* **1989**, *22*, 343.

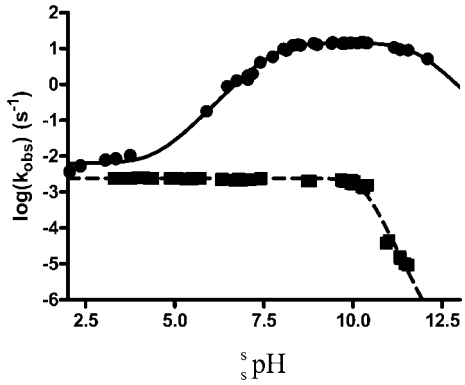


Figure 1. log(k_{obsd}) vs $^{s}_{s}$pH for the Cu(II)-promoted cleavage of **6** (●, 0.01 mM Cu(II) and **6**) in methanol in 0.2 mM buffered solutions and **7** (■, 0.05 mM Cu(II) and **7**) in methanol with 1 mM amine buffer as described in the Experimental Section or excess added $HClO_4$ in the acidic region in methanol at 25 °C. The line through the ● data is computed by an NLLSQ fit to eq 2, giving two macroscopic $^{s}_{s}pK_a^2$ and $^{s}_{s}pK_a^3$ values of 7.8 ± 0.1 and 11.8 ± 0.2, a maximum rate constant (k_{cat}^{max}) of 14.7 ± 0.4 s^{-1}, and k_0 = (6.3 ± 0.4) × 10^{-3} s^{-1} (r^2 = 0.9883). The line through the ■ data is computed by an NLLSQ fit of the data to eq 5 derived for the process in Scheme 4, giving log($^{s}_{s}K_a^2/K_{dim}$) = −20.5 ± 0.2 and k_{cat} = 0.0024 ± 0.0001 s^{-1} (r^2 = 0.9798).

methanolysis product (methyl phosphate) was observed for the cleavage of 3-nitrophenyl phosphate ($^{s}_{s}pK_a$ = 12.41 for 3-nitrophenol in methanol)[13c] after incubation at 50 °C in methanol for 300 h. Assuming the limit of detectability of the product would be 5%, this corresponds to an upper limit for the decomposition rate constant of 1 × 10^{-8} s^{-1}. An upper limit for the Brønsted β value of ∼−1.0 can be computed from these two data, and while there is a large error in the number, it is similar to the values of −1.23 and −1.11 obtained for the cleavage of the dianions of monoesters in water[42] and in *tert*-amyl alcohol,[43] indicating that sensitivity to the substituent is not greatly affected by these sorts of hydroxylic solvents.

Because the Brønsted slopes for the cleavage of the dianions are not very different in water, methanol, and *tert*-amyl alcohol, one may estimate a rate constant for the spontaneous cleavage of **6** in methanol on the basis of the above k_{obsd} for **10** and the difference in $^{s}_{s}pK_a$ values for the two leaving groups (11.18[13] and 16.16). We assume the β_{lg} value in methanol has limits of −1.23 and −1.0. An approximate k_{obsd} for the spontaneous methanolysis of the dianion of **6** at 50 °C is computed as $k_{obsd}^{\mathbf{6}} = 10^{-(\beta(^{s}_{s}pK_a^{4\text{-nitrophenol}} - ^{s}_{s}pK_a^{\mathbf{9}}) - \log(k_{obsd}^{4\text{-nitrophenol}}))} = 10^{-(\beta(11.18-16.16)-(-6.85))}$. The rate constant spans a 13-fold range of 1.1 × 10^{-13} to 1.5 × 10^{-15} s^{-1} if β = −1.23 or −1.0, respectively, but despite the ambiguity, this is an exceedingly slow reaction, and such an error will not greatly affect the analysis.

3.4. Cu(II)-Promoted Methanolysis of 6. The $^{s}_{s}$pH/rate profile given in Figure 1 for the Cu(II)-catalyzed cleavage of **6** in methanol (● symbols) can be fit to eq 2 derived for a model given in Scheme 2 with two ionizable groups having $^{s}_{s}pK_a$ values of 7.8 and 11.8, a maximum rate constant (k_{cat}^{max}) of 14.7 ± 0.4 s^{-1} in the plateau region, and a low $^{s}_{s}$pH plateau of (6.3 ± 0.4) × 10^{-3} s^{-1}. The k_{cat}^{max} value in the neutral $^{s}_{s}$pH region corresponds to a $t_{1/2}$ = 50 ms for catalyzed cleavage of **6**.

Scheme 2. Kinetic Scheme for the $^{s}_{s}$pH Dependence of the Cleavage of Cu(II):**6** in Methanol Having a Reactive Form with Maximum Activity in the $^{s}_{s}$pH Region between the $^{s}_{s}pK_a$ Values of the Two Ionizable Groups

$$[\text{Cu(II)}:\mathbf{6}]^{+1} \underset{}{\overset{^{s}_{s}K_a^2}{\rightleftharpoons}} [\text{Cu(II)}:\mathbf{6}]^{0} + H^+ \overset{^{s}_{s}K_a^3}{\rightleftharpoons} [\text{Cu(II)}:\mathbf{6}]^{-1} + H^+$$

$$[\text{Cu(II)}:\mathbf{6}]^{+1} \xrightarrow{k_0} P \qquad [\text{Cu(II)}:\mathbf{6}]^{0} \xrightarrow{k_{cat}^{max}} P$$

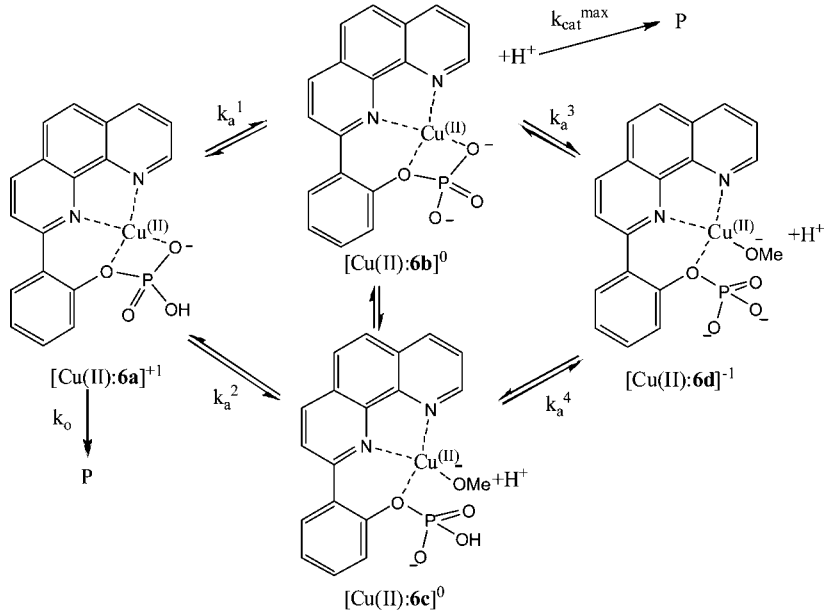
$$k_{obsd} = \left(\frac{k_{cat}^{max}\,{}^{s}_{s}K_a^2}{{}^{s}_{s}K_a^2 + [H^+]}\right)\left(\frac{[H^+]}{{}^{s}_{s}K_a^3 + [H^+]}\right) + k_0\left(\frac{[H^+]}{{}^{s}_{s}K_a^2 + [H^+]}\right) \quad (2)$$

The deuterium kinetic isotope effect was determined at two measured estimated values of "$^{s}_{s}$pD", 10.4 ± 0.2 and 9.6 ± 0.2, in the plateau region where the rate is independent of $^{s}_{s}$pH (0.2 mM 1-methylpiperidine buffer). The $^{s}_{s}$pD values were simply those from the reading of the electrode made in the deuterated solvent plus the added correction factor of 2.24 for passing from water to methanol. The actual $^{s}_{s}$pD values measured in the plateau region are only approximate but are less important than demonstrating that the rate constant obtained at two different $^{s}_{s}$pD values is invariant. This proves to be the case since the k_{MeOH}/k_{MeOD} value determined at $^{s}_{s}$pD 9.6 is (14.7 ± 0.3 s^{-1})/(14.3 ± 0.4 s^{-1}) = 1.03 ± 0.04 and at $^{s}_{s}$pD 10.4 is (15.9 ± 0.6 s^{-1}/14.7 ± 0.4 s^{-1}) = 0.95 ± 0.05.

In Scheme 3 is an expanded version of Scheme 2 where the species [Cu(II):**6a**]$^+$ formed at lower $^{s}_{s}$pH through binding of $\mathbf{6}^-$ to Cu^{2+} undergoes two possible microscopic ionizations[44] to yield two formally neutral complexes, [Cu(II):**6b**]^0 and [Cu(II):**6c**]^0. Chemical intuition suggests that [Cu(II):**6b**]^0, drawn as having a P−O^-···Cu(II) coordination, should be the more active of the two since both nonbridging oxyanions on P can facilitate the departure of the leaving group with assistance of an electropositive Cu(II). A second set of microscopic ionizations convert the neutral complex into a less active or inactive anionic complex, [Cu(II):**6d**]$^-$. It is known that the simple phenanthroline:Cu(II):$^-OCH_3$ complex dimerizes to produce (phenanthroline:Cu(II):$^-OMe)_2$ at low concentrations in methanol,[45] so rate vs complex concentration experiments were conducted in the plateau region at $^{s}_{s}$pH 10.5 where the dominant species are the two [Cu(II):**6**]^0 forms given in Scheme 3. The data in Figure 2 follow a square root dependence on [[Cu(II):**6**]^0] which stems from the equilibrium formation of an inactive dimer as in eq 2. Such monomer/dimer equilibrium behavior is treated by NLLSQ fitting of the rate vs concentration data to eq 4[45] derived for the process in eq 3 where the complex is completely formed at all concentrations used, and the active monomer [Cu(II):**6**]^0 which decomposes with a rate constant k_{cat} is in equilibrium (with dissociation constant K_{dis}) with an inactive dimer, [Cu(II):**6**]$^0_2$. Because it is the rate, not the rate constant, used for the *y* axis in Figure 2, k_{cat} has the units (Abs/s)/M. A k_{cat} value of ∼1.3 × 10^5 (Abs/s)/M is estimated from the rate constants and the changes in absorbance obtained at low [Cu(II):**6**] where the majority species in solution is the monomer. Fixing this k_{cat}, the Figure 2 data were fit to eq 3 to give K_{dis} = (1.12 ± 0.03) × 10^{-5} M.

$$P \xleftarrow[k_{cat}]{} 2[\text{Cu(II)}:\mathbf{6}]^0 \underset{K_{dis}}{\rightleftharpoons} [\text{Cu(II)}:\mathbf{6}]^0_2 \quad (3)$$

Scheme 3. Possible Microscopic Ionizations for Cu(II):**6** Species[a]



[a] Counterions omitted for simplicity.

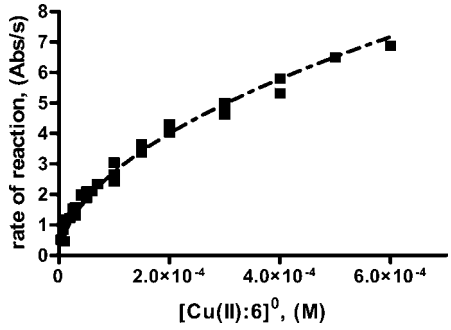


Figure 2. Rate of the reaction (Abs/s) vs [[Cu(II):**6**]^0] for the cleavage of **6** monitored at 414 nm in the presence of 8 mM 1-methylpiperidine buffer (^s_spH 10.4 ± 0.2) at $T = 25$ °C. The dotted line through the data is computed from a fit of the data to eq 3 having a fixed k_{cat} of 1.3×10^5 (Abs/s)/M as described in the text. The computed K_{dis} is $(1.12 \pm 0.03) \times 10^{-5}$ M with $r^2 = 0.9784$.

$$\text{rate} = \{k_{cat}K_{dis}(\sqrt{(1 + 8[[\text{Cu(II)}:\mathbf{6}]^0])/K_{dis}} - 1)/4\} \quad (4)$$

3.5. Cu(II)-Catalyzed Methanolysis of 7. Also shown in Figure 1 is the ^s_spH/rate profile for the Cu(II)-promoted cleavage of **7** under buffered conditions in methanol which exhibits a broad ^s_spH-insensitive region from ^s_spH 3.3 to ^s_spH 10. At higher ^s_spH the plot has a gradient of −2, suggesting that two methoxides are responsible for forming an inactive dimeric entity. In the proposed mechanism the most reactive species is [Cu(II):**7b**]$^+$ shown in Scheme 4 with the monoanionic phosphate bound to the metal ion or its kinetic equivalent where the phosphate is not bound (not shown). A fit of the kinetic data to the expression in eq 5 gives the line through the ■ data in Figure 1, $\log(^s_sK_a^2/K_{dim}) = -20.5 \pm 0.2$, and a k_{cat} of 0.0024 ± 0.0001 s^{-1}. A solvent kinetic isotope study at a measured ^s_spD of 6.2 ± 0.2 at the midpoint of the plateau in the ^s_spH/rate profile (Figure 1) gives $k_{MeOH}/k_{MeOD} = 1.01 \pm 0.04$ ($k_{MeOH} = (2.37 \pm 0.03) \times 10^{-3}$ s^{-1}; $k_{MeOD} = (2.35 \pm 0.05) \times 10^{-3}$ s^{-1}).

$$\log(k_{obsd}) = \log(k_{cat}) + \log\left(\frac{[H^+]^2}{^s_sK_a^2/K_{dim} + [H^+]^2}\right) \quad (5)$$

Finally, a concentration dependence experiment was conducted by monitoring the rate constant for the solvolytic cleavage of **7** as a function of increasing [Cu(II):**7**] in the plateau region of the ^s_spH/rate profile in Figure 1 by adding excess $HClO_4$ to keep the ^s_spH at 3.5 ± 0.2. Unlike the case for Cu(II):**6**, increasing [Cu(II):**7**] from 0.05 to 0.3 mM does not affect the rate constant of the reaction (see the Supporting Information). This is in agreement with the monomeric [Cu(II):**7b**]$^+$ being the only significant species in solution from ^s_spH 3.3 to ^s_spH 10.

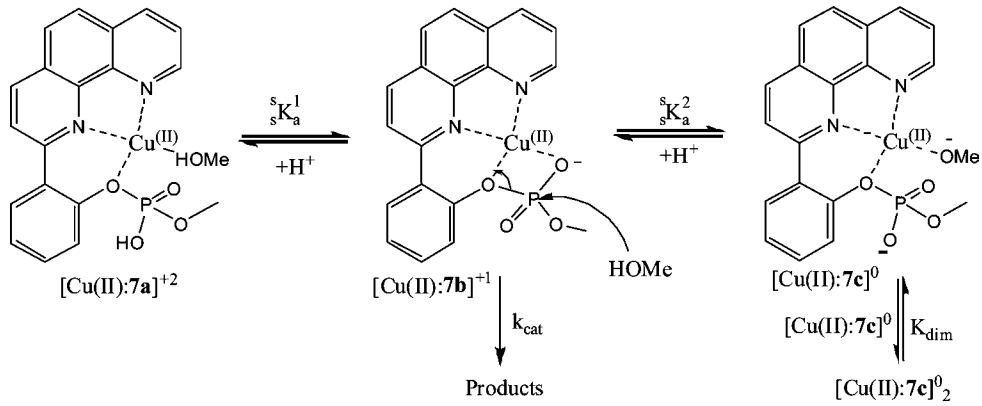
3.6. Cu(II)-Catalyzed Methanolysis of 8. Inspection of the ^s_spH/rate profile for the Cu(II)-catalyzed methanolysis of **8** given in Figure 3 reveals two reactive species connected by a single ionization. The data in Figure 3 are fit to eq 6 derived for the process in Scheme 5 with two different complexes, each cleaving with a different rate constant (k_1 or k_2), interconnected by an acid dissociation $^s_sK_a$ of 6.03. These reactive species are designated as [Cu(II):**8a**]$^{2+}$ at ^s_spH < 5.5 with $k_1 = (2.0 \pm 0.2) \times 10^{-5}$ s^{-1} and [Cu(II):**8b**]$^+$ at ^s_spH > 7.5 with $k_2 = (1.2 \pm 0.2) \times 10^{-6}$ s^{-1}. A solvent kinetic isotope study at ^s_spH 3.5 ± 0.2 in the plateau region of Figure 3, where the majority of the species in solution are [Cu(II):**8a**]$^{2+}$, gives $k_1^{MeOH} = (2.03 \pm 0.04) \times 10^{-5}$ s^{-1}, $k_1^{MeOD} = (9.3 \pm 0.2) \times 10^{-6}$ s^{-1}, and $k_1^{MeOH}/k_1^{MeOD} = 2.2 \pm 0.1$ for the phosphoryl transfer reaction to solvent.

$$\log(k_{obsd}) = \log\left(k_1\frac{[H^+]}{^s_sK_a + [H^+]} + k_2\frac{^s_sK_a}{^s_sK_a + [H^+]}\right) \quad (6)$$

3.7. Activation Parameters. To probe further the nature of the Cu(II)-catalyzed reactions of these phosphate esters, the

(42) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415.
(43) Hoff, R. H.; Hengge, A. C. *J. Org. Chem.* **1998**, *63*, 6680.
(44) For the ionization process of the dibasic acid shown in Scheme 3, the macroscopic ionization constants K_a^1 and K_a^2 are given in terms of the microscopic ionization constants as $K_a^1 = k_a^1 + k_a^2$, $1/K_a^2 = 1/k_a^3 + 1/k_a^4$, and $K_a^1K_a^2 = k_a^1k_a^3 = k_a^2k_a^4$: Cookson, R. F. *Chem. Rev.* **1974**, *74*, 5.
(45) Neverov, A. A.; Brown, R. S. *Org. Biomol. Chem.* **2004**, *2*, 2245.

Scheme 4. Proposed Scheme for the Reaction of Different $^{s}_{s}$pH-Dependent Cu(II):**7** Species[a]



[a] Counterions omitted for simplicity.

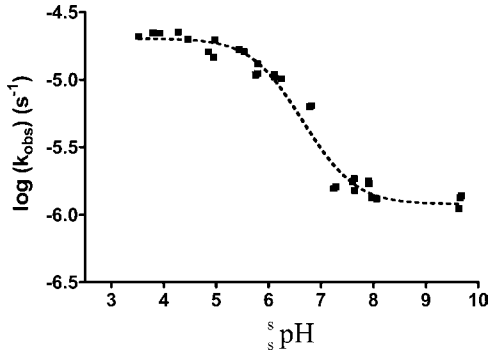
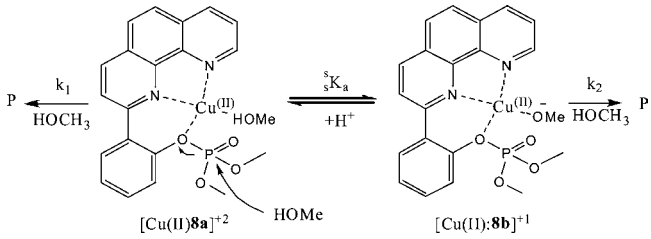


Figure 3. log(k_{obsd}) vs $^{s}_{s}$pH for the Cu(II)-promoted cleavage of **8** (0.05 mM Cu(II) and **8**) with 1 mM amine buffer or excess added $HClO_4$ in the acidic region, $T = 25$ °C. The dotted line is obtained from the fit of the data to eq 6 derived for the process of Scheme 5, giving computed $k_1 = (2.0 \pm 0.2) \times 10^{-5}$ s^{-1}, $k_2 = (1.2 \pm 0.2) \times 10^{-6}$ s^{-1}, and $K_a = (9.4 \pm 2.0) \times 10^{-7}$ ($^{s}_{s}pK_a = 6.03$) ($r^2 = 0.9643$).

Scheme 5. Proposed Mechanism for the Formation of Different Cu(II):**8** Species[a]



[a] Counterions omitted for simplicity.

activation parameters were determined in the plateau regions of the plots given in Figures 1 and 3. The activation data are given in Table 1, and the Eyring plots are presented in Figure 4 (for raw experimental data see the Supporting Information). The lines in Figure 4 are essentially parallel, indicative of very similar $\Delta H^{\ddagger}$ values for cleavage of each complex, while the $\Delta S^{\ddagger}$ values differ by about 26 cal/(mol·K), passing from negative for the triester to positive for the monoester. The values from the solvent kinetic isotope experiments are also included in Table 1 for comparison.

Table 1. Activation Parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ at 25 °C) and the k_{MeOH}/k_{MeOD} Values for the Cu(II)-Assisted Cleavages of Phosphates **6**−**8** in the Plateau Region of the Respective $^{s}_{s}$pH/Rate Profiles

phosphate complex[a]	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ [cal/(mol·K)]	$\Delta G^{\ddagger}$(25 °C) (kcal/mol)	k_{MeOH}/k_{MeOD}
[Cu(II):**6**]^{0} [b]	21.4 ± 0.7	18 ± 2	16.0	0.95 ± 0.05
[Cu(II):**7**]$^{+}$ [c]	21.6 ± 0.4	2.3 ± 1.1	20.9	1.01 ± 0.04
[Cu(II):**8**]$^{2+}$ [d]	21.6 ± 0.5	−7.4 ± 1.7	23.8	2.2 ± 0.1

[a] The activation parameters for substrate **8** are computed at standard-state conditions with 1 M nucleophile. This allows for comparison with the values obtained for substrates **6** and **7**. [b] Activation parameters determined at $^{s}_{s}$pH 10.4 ± 0.2. [c] Activation parameters determined at $^{s}_{s}$pH 3.6 ± 0.2. [d] Activation parameters determined at $^{s}_{s}$pH 3.8 ± 0.2.

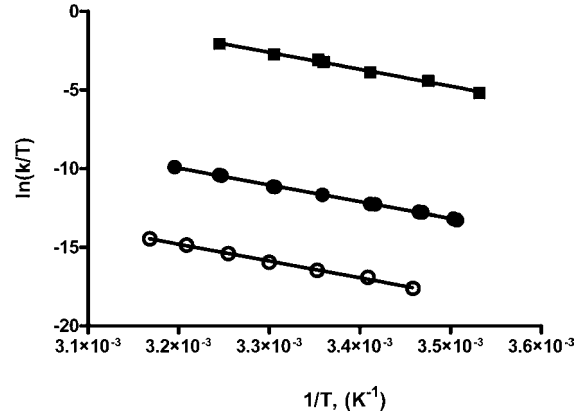


Figure 4. Eyring plots of ln(k/T) vs $1/T$ for the methanolysis of phosphate esters: (■) 0.01 mM Cu(II):**6**, 0.2 mM 1-methylpiperidine buffer, $^{s}_{s}$pH 10.4 ± 0.2; (●) 0.05 mM Cu(II):**7**, 0.25 mM $HClO_4$, $^{s}_{s}$pH 3.6 ± 0.2; (○) 0.05 mM Cu(II):**8**, 1.0 mM 2-picoline buffer, $^{s}_{s}$pH 3.8 ± 0.2. Fits of the data to the Eyring equation give the activation parameters presented in Table 1.

4. Discussion

The concept of, and requirement for, LGA of the cleavage of phosphate esters mediated by enzymes is widely acknowledged,[9b,46] particularly where the leaving group is poor. However, LGA is difficult to demonstrate in small-molecule chemistry[13,20−23,26,27,29,30,47] due to the limitations imposed by size and the synthetic difficulties in precisely positioning the metal ions for assisting LG departure. Demonstration of metal ion-promoted LGA in small-molecule turnover catalysts is a challenge yet to be met in any substantial way unless

(46) (a) Morrow, J. *Comments Inorg. Chem.* **2008**, *29*, 169. (b) Catrina, I.; O'Brien, P. J.; Purcell, J.; Nikolic-Hughes, I.; Zalatan, J. G.; Hengge, A. C.; Herschlag, D. *J. Am. Chem. Soc.* **2007**, *129*, 5760. (c) Admiraal, S. J.; Herschlag, D. *Chem. Biol.* **1995**, *2*, 729. (cc) Cleland, W. W.; Hengge, A. C. *Chem. Rev.* **2006**, *106*, 3252.

Table 2. Rate Constants for the Cleavage of Phosphates **6**−**8** and Their Cu(II) Complexes

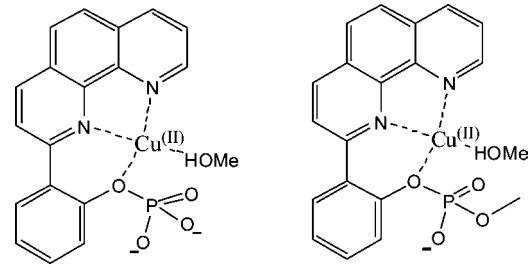
reactant	k_{obsd}[a] (s^{-1})	k_2^{-OMe} (M^{-1} s^{-1})[b]	k_s[c] (s^{-1})	acceleration ($k_{obsd}/k_{background}$)
[Cu(II):**6b**]^0	14.7 ± 0.4[b] (200)[c]			$(1-14) \times 10^{13}$ [d,g]
[Cu(II):**6a**]$^+$	$(6.3 \pm 0.4) \times 10^{-3}$ [d]			
[Cu(II):**7b**]$^+$	$(2.5 \pm 0.1) \times 10^{-3}$ [b]			7.1×10^{14} [b,h]
[Cu(II):**8a**]$^{2+}$	$(2.0 \pm 0.2) \times 10^{-5}$ [b]			4.0×10^{9} [b,i]
[Cu(II):**8b**]$^+$	$(1.2 \pm 0.2) \times 10^{-6}$ [b]			1.0×10^{5} [b,j]
6			$(1.1-15) \times 10^{-13}$	
7		8.5×10^{-10} [e]		
8		$(2.9 \pm 0.1) \times 10^{-3}$ [f]		

[a] k_{obsd} indicates the spontaneous rate constant for decomposition of the Cu(II) complexes at the indicated ^s_spH values in footnotes g−i. [b] $T = 25$ °C. [c] $T = 50$ °C. [d] ^s_spH < 2.5. [e] Determined from application of the $^s_s pK_a$ of 16.16 for the 2-(2′-phenanthrolyl)phenol leaving group (**9**) to $\log(k_2^{-OMe}) = (-0.57 \pm 0.06)^s_s pK_a + (0.14 \pm 0.68)$.[13c] [f] Experimentally determined as described in the Supporting Information. [g] Determined by comparing the spontaneous rate of decomposition of the complex at ^s_spH 10.4 with the projected rate of cleavage of the dianion of **6** as computed in section 3.3. [h] Determined by comparing the k_{obsd} for cleavage of [Cu(II):**7b**]$^+$ at neutral ^s_spH 8.38 with that calculated for the methoxide-promoted reaction of **7** from k_2^{-OMe}. [i] Determined by comparing the k_{obsd} for cleavage of [Cu(II):**8a**]$^{2+}$ at neutral ^s_spH 5.0 with that calculated for the methoxide-promoted reaction of **8** from the observed rate constant $k_2^{-OMe} = (2.94 \pm 0.09) \times 10^{-3}$ M^{-1} s^{-1}. [j] Determined by comparing the k_{obsd} for cleavage of [Cu(II):**8b**]$^+$ at neutral ^s_spH 8.38 with that calculated for the methoxide-promoted reaction of **8** from the observed rate constant $k_2^{-OMe} = (2.94 \pm 0.09) \times 10^{-3}$ M^{-1} s^{-1}.

the substrates contain specially positioned auxiliary groups that transiently coordinate to the metal center during cleavage.[48]

In this proof-of-concept study we have employed a strategy where a departing phenoxy group is connected to an *o*-phenanthroline ligand that firmly positions a Cu(II) ion within ~1.9 Å of the departing oxygen in a series of mono-, di-, and triesters, Cu(II):**6**, Cu(II):**7**, and Cu(II):**8**. We note that the $^s_s pK_a$ of 16.16 for phenol **9** suggests this is not a particularly activated leaving group, lying between CF_3CH_2OH ($^s_s pK_a = 15.8$) and CFH_2CH_2OH ($^s_s pK_a = 17.2$).[49] The unusually high $^s_s pK_a$ value (relative to those of normal phenols (the $^s_s pK_a$ of phenol is 14.3)) possibly results from some steric hindrance to solvation of the phenoxy O^- imposed by the bulky 2-phenanthrolyl group, but this same feature ideally sets up the tight coordination environment for Cu(II). Each of **6**−**8** binds a Cu(II) ion sufficiently strongly that the complexes are fully formed at all concentrations and ^s_spH values by in situ addition of 1 equiv each of the metal ion and ligand. Once bound, these are not turnover catalysts, but rather molecular complexes that cleave quickly by a unimolecular reaction, but the principle of LGA is amply demonstrated and can be quantified because the ^s_spH/rate profiles in Figures 1 and 3 show plateau regions that correspond to a solvent-mediated decomposition of fairly well-defined species. The ground-state forms of [Cu(II):**6b**]^0 and [Cu(II):**7b**]$^-$ in Schemes 3 and 4 are depicted having a nonbridging oxyanion of the mono- and diester coordinated to the Cu(II). This is based on a marked difference in the UV/vis spectrum of Cu(II):**7** where the $P-O^-$ can coordinate relative to that of Cu(II):**8** where such coordination is unlikely (see the Supporting Information) along with computational data that show the PO^- groups of the monoester and diester are capable of coordinating to the Cu(II) as in [Cu(II):**6b**]^0 and [Cu(II):**7b**]$^-$ (see the Supporting Information). In the case of the monoester, the phosphoryl-bound form is computed to be more stable than the uncoordinated structure [Cu(II):**6b′**]^0 by 5.3 kcal/mol (see the Supporting Information). In any event, we have no information on which of these two kinetically equivalent forms of the mono- and diester is the preferred reactive species. Such a distinction, while of fundamental interest, does not change the conclusions of the study.

Of relevance to the question of LGA is how much acceleration one can expect from positioning the Cu(II) metal ion close to the departing phenoxy oxygen of the leaving group in the three complexes investigated here. In principle, the catalysis is evaluated in terms of the energy of binding the Cu(II) of the TS for each of the cleavage reactions relative to the TS for the uncatalyzed reaction. Hyperquad 2000 analysis of the titration data (section 3.1) indicates that the dissociation constants of Cu(II):**9** and Cu(II):**9**$^-$ are 1.1×10^{-8} M and 2.3×10^{-24} M which translate into free energies of binding of 10.8 and 32.2 kcal/mol at 25 °C (see the calculation based on the Hyperquad-determined β_3 and β_4 equilibrium values in the Supporting Information). The 21.3 kcal/mol difference in these values can be taken as the additional energy of binding attributable to a fully bound phenoxide O^-, a portion of which is realized in the TS for cleavage of the P−O(LG) bond. Not all of this needs be realized in a given case, but the magnitude of the stabilization should be directly proportional to the extent of ArO···P bond cleavage as measured by the Leffler index α, which is given as the ratio of the Brønsted β_{lg} for the P···OAr cleavage reaction relative to the β_{eq} for equilibrium transfer of phosphoryl groups between oxyanion nucleophiles.[50]



[Cu(II):**6b′**]^0 [Cu(II):**7b′**]$^{+1}$

(47) (a) Bone, R.; Frank, L.; Springer, J. P.; Atack, J. R. *Biochemistry* **1994**, *33*, 9468. (b) Tsubouchi, A.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 11614. (c) Tsubouchi, A.; Bruice, T. C. *J. Am. Chem. Soc.* **1995**, *117*, 7399. (d) Tibbits, T. T.; Xu, X.; Kantrowitz, E. R. *Protein Sci.* **1994**, *3*, 2005. (e) Rishavy, M. A.; Hengge, A. C.; Cleland, W. W. *Bioorg. Chem.* **2000**, *28*, 283. (f) Dempcy, R. O.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 4511. (g) Bruice, T. C.; Tsubouchi, A.; Dempcy, R. O.; Olson, L. P. *J. Am. Chem. Soc.* **1996**, *118*, 9867. (h) Souza, B. S.; Brandão, T. A. S.; Orth, E. S.; Roma, A. C.; Longo, R. L.; Bunton, C. A.; Nome, F. *J. Org. Chem.* **2009**, *74*, 1042.

(48) While the Yb^{3+}- and **1**-promoted cleavage of methyl *o*-carbomethoxyaryl phosphate diesters[13c,27] **2b,c** and La^{3+}-promoted cleavage of triesters[30] **3b** can exhibit turnover, binding of the lanthanide-metal ions to the departing phenoxy group is very strong so that product inhibition occurs.

(49) Tsang, W.-Y.; Edwards, D. R.; Melnychuk, S. A.; Liu, C. T.; Neverov, A. A.; Williams, N. H.; Brown, R. S. *J. Am. Chem. Soc.* **2009**, *131*, 4158.

(50) (a) Thea, S.; Williams, A. *Chem. Soc. Rev.* **1986**, *15*, 125. (b) Williams, A. *Acc. Chem. Res.* **1984**, *17*, 425. (c) Williams, A. *Concerted Organic and Bio-organic Mechanisms*; CRC Press: Boca Raton, FL, 2000; pp 161−181.

A compendium of the various kinetic rate constants for cleavage of the three complexes is presented in Table 2 to allow easy comparison. Below we deal with Cu(II)-promoted cleavage of esters **6**−**8** in the context of extant relevant data for the background solvolytic cleavages of mono-, di-, and triesters, particularly the solvent-mediated cleavages, when such data are available.

4.1. Monoester. Kirby and Varvoglis[42] showed that phosphate monoesters with good LGs (e.g., 2,4-dinitrophenol, pK_a = 4.07) react faster through the dianionic form than the monoanion via a "dissociative-like" transition state with the P−O(LG) bond being almost completely broken ($\beta_{lg} = -1.23$).[42] However, as the LG becomes poorer, the monoanion reacts faster due to a rate-enhancing protonation of the departing oxygen, either through the formation of a zwitterionic $(^-O)_2P-O(H^+)-LG$ species prior to the rate-limiting chemical step or through an intramolecular proton transfer process with an intervening water molecule. Because of the reduced charge on the now-protonated phenoxy oxygen in the TS, the latter reaction is less sensitive to the nature of the leaving group ($\beta_{lg} = -0.27$).[42] Subsequently, Hengge and Hoff determined that the solvolytic cleavage of 4-nitrophenyl phosphate dianion in *tert*-butyl alcohol is about 2000 times faster than that of the monoanion in *tert*-butyl alcohol.[43] They attributed the increased difficulty in the proton transfer process for the monoanion to a higher pK_a value of the phosphate group in *tert*-butyl alcohol coupled with a less favorable four-membered transition state for the intramolecular proton transfer process in alcohol, contrasting the more favorable six-membered arrangement in water.

In the present work in methanol we find that **6**, when coordinated to Cu(II) as [Cu(II):**6**]^0, reacts rapidly ($\sim$15 s^{-1}) in the plateau region of the $_s^s$pH/rate profile given in Figure 1. The plateau stems from spontaneous, solvent-mediated decomposition of dianionic phosphate complexed to Cu^{2+} as [Cu(II):**6**]^0 where the nonbridging phosphoryl oxygens (one perhaps coordinated to the Cu(II)) partake in pushing the Cu(II)-complexed 2′-(2-phenoxy)-1,10-phenanthroline LG away from an emerging metaphosphate-like unit with little nucleophilic assistance from solvent methanol. This is consistent with the $\Delta S^{\ddagger}$ of 18 ± 2 cal/(mol·K) and a solvent kinetic isotope effect of unity, indicating little change in the participating solvent CH_3O-L force constant during the solvent capture of the emerging PO_3^- unit. On the other hand, the Figure 1 data show a plateau at $_s^s$pH < 2.5 where the monoprotonated complex of the monoester [Cu(II):**6a**]$^+$ reacts with a $_s^s$pH-independent rate constant similar to that of the diester [Cu(II):**7b**]$^+$. The data show that the extra phosphoryl oxyanion created by deprotonation enhances the spontaneous decomposition of [Cu(II):**6**]^0 by $\sim$2500-fold.

In methanol, which is structurally more similar to water than *tert*-butyl alcohol and has an intermediate dielectric constant (ε_r = 80.2, 32.7, and 12.5 for water, methanol, and *tert*-butyl alcohol, respectively),[51] we find that the decompositions of 4-nitrophenyl phosphate mono- and dianions at 50 °C have approximately equal rate constants of 1.4 × 10^{-7} s^{-1} (see the Supporting Information).[52] From this rate constant and the $_s^s$pK_a of 16.16 for the 2′-(2-phenoxy)-1,10-phenanthroline leaving group we estimate (as in the Results) that the spontaneous decomposition of the dianion of **6** should have a rate constant between 1.1 × 10^{-13} and 1.5 × 10^{-12} s^{-1} at 50 °C. Comparison of this rate constant with the 200 s^{-1} rate constant for the decomposition of Cu(II):**6** computed at 50 °C indicates that the Cu(II)-induced LGA is worth about 1.3 × 10^{14} to 1.8 × 10^{15} times or $\sim$19.3−20.8 kcal/mol in free energy terms.[53] Although we do not have reliable activation parameters for the cleavage of **6** in methanol, this LGA should be somewhat higher if the comparison is made at 25 °C. All these numbers must be regarded as estimates given the uncertainties in the rates of the background reactions due to the long extrapolations required, but this will not change the following analysis substantially. Using the above free energy data as limits for the rate acceleration for the Cu(II)-catalyzed vs uncatalyzed reactions, and the 21.3 kcal/mol for the differential binding of the phenolate leaving group to Cu(II) relative to its binding to the 2′-(2-phenoxy)-1,10-phenanthroline unit in the starting material, one can compute a Leffler index of greater than 90% P···OAr cleavage in the TS. Interestingly, the Leffler index for uncatalyzed cleavage of phosphate monoesters in water is reported to be[42] −1.23/−1.37 = 90%, suggesting that the strong leaving group catalysis we see here does not alter the amount of cleavage of the departing group to any great extent.

4.2. Diester. Kirby and Younas[54] showed that the aqueous cleavage of phosphate diesters at 100 °C is an exceedingly slow process that is highly dependent on the LG basicity. The reaction with a reasonably good leaving group, 4-nitrophenoxy, involves hydroxide attack on the monoanion down to pH ≈ 5 with a short, but discernible, plateau region indicative of a water reaction, flanked by a hydronium ion-catalyzed process. The water reaction was observable with leaving groups as good as, or better than, 4-nitrophenoxy but not with poorer ones such as phenoxy or 4-methoxyphenoxy. The methanolytic cleavage of aryl methyl phosphate diesters at 25 °C has been reported,[13c] but the only observed reaction was methoxide attack on the monoanion under the reported conditions. On the basis of the $_s^s$pK_a of 16.16 for 2-(2′-phenanthrolyl)phenol (**9**), the predicted k_2^{-OMe} for methoxide attack on diester **7** is 8.5 × 10^{-10} M^{-1} s^{-1} assuming that this leaving group falls on the Brønsted relationship described for methoxide attack on methyl aryl phosphate diesters.[13c,55]

The long plateau between $_s^s$pH 3 and $_s^s$pH 10 given in Figure 1 is attributable to the spontaneous cleavage of [Cu(II):**7b**]$^+$ (see Scheme 4) having a single nonbridging phosphate oxyanion. The data given in Table 1 for this process indicate a lower $\Delta S^{\ddagger}$ (2.3 ± 1.1) than is the case for decomposition of [Cu(II):**6b**]^0 as well as a solvent kinetic isotope effect of 1.0, indicating little nucleophilic participation of the solvent in the rate-limiting step. This process has a rate constant of (2.5 ± 0.1) × 10^{-3} s^{-1} ($t_{1/2}$ = 4.6 min) and at the neutral $_s^s$pH of 8.38 is about 7 × 10^{14}

(51) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman Scientific & Technical with John Wiley & Sons, Inc.: Harlow, U.K., New York, 1989; pp 1442−1443.

(52) While 4-nitrophenyl phosphate monoanion and dianion react at 39 °C with rate constants of 1.07 ×10^{-6} and 1.55 × 10^{-8} s^{-1}, respectively, the reported rate constants for 2-chloro-4-nitrophenyl phosphate at 39 °C are 2.43 × 10^{-7} and 2.26 × 10^{-7} s^{-1}, respectively.[42]

(53) Computed as 130 > $G^{\ddagger}_{cat} = -RT\ln(k_{cat}/k_{background})$ from the Eyring equation at 298 K (k_{cat} = 15 s^{-1} and $k_{background}$ = 1.1 × 10^{-13} to 1.5 × 10^{-12} s^{-1}).

(54) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 510.

(55) Given that the reactions of phosphate diesters are exceedingly slow, the only estimate that we have for the reaction of **7** at neutral pH is that obtained by the presumed fit to the Brønsted relationship, but this is probably too low an estimate on the basis of the data we have with methoxide attack on dimethyl aryl triesters at 25 °C where the Brønsted relationship is log $k_2^{-OMe} = (-0.51 \pm 0.04)\,_s^s pK_a^{LG} + (4.68 \pm 0.56)$.[30a] The predicted rate constant for methoxide attack of **8** is 2.7 × 10^{-4} M^{-1} s^{-1}, which is 10 times less than the experimental rate constant of 2.94 × 10^{-3} M^{-1} s^{-1} determined here.

faster than the estimated methoxide reaction for cleavage of **7**. Evaluation of the energetics of the Cu(II)-induced LGA is complicated by the fact that the catalyzed reaction appears to proceed by a solvent-mediated mechanism while such a process is probably not present for cleavage of uncomplexed **7** given the high $^{s}pK_a$ for the leaving group.

4.3. Triester. Kirby and Khan[56] have reported that the cleavage of dialkyl aryl phosphate triesters (actually 2-(aryloxy)-2-oxo-1,3,2-dioxaphosphorinanes) having good leaving groups in water at 39 °C is subject to HO^--, H_3O^+-, and solvent-promoted reactions. For the least reactive esters (such as the 4-nitrophenyl derivative) the base and acid wings account for the hydrolysis over almost all the pH range except for a small deviation at ~pH 4 that may result from a water reaction. We reported that the methoxide-promoted reactions of a series of dimethyl aryl phosphates at 25 °C exhibit a Brønsted β_{lg} value of -0.51 ± 0.04[30a] and have no evidence for spontaneous reactions of triesters in methanol. The k_2^{-OMe} for the cleavage of **8** determined here ($(2.94 \pm 0.09) \times 10^{-3}$ M^{-1} s^{-1}) gives a computed pseudo-first-order rate constant for the background reaction of 1.2×10^{-11} s^{-1} at neutral ^{s}pH 8.38 and 5×10^{-15} s^{-1} at ^{s}pH 5 assuming the methoxide reaction dominates at both ^{s}pH values. Comparing these with the rate constants obtained from the Figure 3 data for the decomposition of [Cu(II):**8a**]$^{2+}$ and [Cu(II):**8a**]$^{+}$ in the two plateau regions indicates that Cu(II) complexation gives accelerations of 1×10^5 at ^{s}pH 8.38 and 4×10^9 at ^{s}pH 5. The calculated acceleration at lower ^{s}pH is larger than at neutrality mainly because the background base-promoted reaction decreases linearly with [$^-OCH_3$]. The data rule out an active role for the Cu(II)-coordinated methoxide as a nucleophile or general base since this would imply that the methoxide form should react faster than the one without, contrary to what is observed. The favored reaction at ^{s}pH < 5 involves spontaneous decomposition of [Cu(II):**8a**]$^{2+}$, which reacts about 20 times faster than [Cu(II):**8b**]$^{+}$ due to the greater Lewis acidity of the Cu(II) in the former. The decomposition of [Cu(II):**8b**]$^{+}$ must involve a larger participation of methanol as a nucleophile than with the diester and monoester, possibly with the assistance of a second methanol to aid in displacing the Cu(II)-coordinated LG as judged by the solvent kinetic isotope effect of 2.2 and the negative $\Delta S^{\ddagger}$ of -7.4 ± 1.7, consistent with some restriction of the degrees of freedom of the reacting species.

4.4. General Considerations Based on Activation Parameters and Solvent Kinetic Isotope Effects. The data in Table 1 suggest that the major differences in the activation free energies of the decomposition of [Cu(II):**6**]^0, [Cu(II):**7**]$^+$, and [Cu(II):**8**]$^{2+}$ stem not from enthalpy differences, but from entropy differences which can be analyzed in terms of the tightness or looseness of the transition states for the cleavage processes. In Figure 5 is a More O'Ferrall−Jencks diagram for the solvolytic cleavage of the mono-, di-, and triesters. For simple, noncatalyzed solvolysis reactions, the reaction coordinate profiles for the three sets of esters follow the lower curve (monoester), central diagonal (diester), and upper curve (triester), with the TSs being marked with "‡".[2] Binding of Cu(II) (designated in red) to the O(LG) component of the lower left and upper left corners should stabilize these corners by similar amounts, assumed for all esters to be on the order of 8.9 kcal/mol from the dissociation constant of Cu(II):**8** of 3×10^{-7} M. However, Cu(II) binding to the $^-$O(LG) (**9**$^-$) is determined by potentiometric titration to be very much stronger as indicated by $K_d = 10^{-23.64}$ M. Thus, the entire

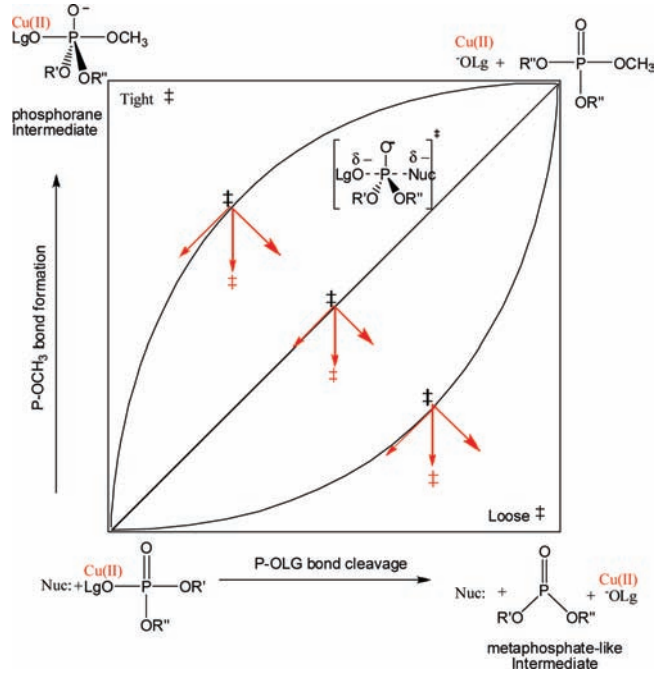


Figure 5. Simplified More O'Ferrall−Jencks diagram illustrating the energy surface for three general phosphoryl transfer reactions where the decomposition of a phosphate monoester is believed to have a loose transition state which becomes an increasingly "tighter" TS for phosphate diesters and triesters.[2] The three TSs for the uncatalyzed reactions of tri-, di-, and monoesters are shown as black ‡ symbols. Binding of the (red) Cu(II) to substrates **6**−**8** moves each TS toward the starting material in the Hammond sense and toward the metaphosphate-like corner in the anti-Hammond sense, leading to a new TS (red ‡ symbols) for each of the catalyzed reactions with a similar amount of P−O(LG) cleavage as in the uncatalyzed reaction, but significantly less CH_3O−P bond development.

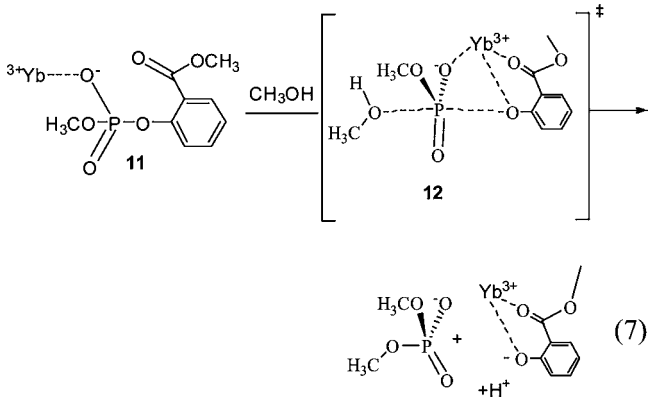
right-hand side of the diagram is pulled down by ~32.2 kcal/mol. The net effect of Cu(II) binding to all the corner species is to shift the TS positions (red arrows in Figure 5) toward the starting material along the reaction coordinate in the Hammond sense, and in the anti-Hammond sense toward the dissociative metaphosphate-like corner. Thus, the predicted three new transition states (indicated with "‡" in Figure 5) have little perturbation of the extent of P−O(LG) cleavage, but have significantly less nucleophilic $CH_3(H)O\cdots P$ bond development.

4.4.1. Monoester. Activation entropies of 3.5 cal/(mol·K)[40] and 24.5 cal/(mol·K)[43] are reported for the solvolysis of 4-nitrophenyl phosphate dianion in water and *tert*-butyl alcohol. Considerable evidence[2] indicates that PO_3^- transfer from phosphate monoesters involves concerted processes in water with a small nucleophilic participation of solvent through a loose TS situated in the lower right corner of the diagram in Figure 5. In the weaker nucleophilic solvent, *tert*-butyl alcohol, the $\Delta S^{\ddagger}$ and observed production of racemic *tert*-butyl phosphate from chiral [^{16}O,^{17}O,^{18}O]-*p*-nitrophenyl phosphate supports a two-step mechanism with a rate-limiting dissociation to form a freely diffusing metaphosphate intermediate which is subsequently captured by solvent. The large positive $\Delta S^{\ddagger}$ of 18 cal/(mol·K) for [Cu(II):**6**]^0 obtained at ^{s}pH 3.8 is consistent with a considerably looser TS than is generally the case for decomposition of phosphate monoester dianions in water and can be compared with the 14.1 cal/(mol·K) that is seen for cleavage of 8-(dimethylammonium)naphth-1-yl phosphate which involves strong intramolecular H-bonding in the starting material followed by general-acid assistance to the leaving group departure.[18] The Cu(II)-mediated LGA moves the TS for decompo-

(56) Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1970**, 1172.

sition of the coordinated dianion further toward the bottom side of the diagram with less participation of the nucleophilic solvent and perhaps closer to the point where the process becomes a stepwise one.

4.4.2. Diester. The neutral hydrolysis of bis(2,4-dinitrophenyl) phosphate monoanion in water has a $\Delta H^{\ddagger} = 19$ kcal/mol and $\Delta S^{\ddagger}$ of -25.5 cal/(mol·K),[54] which is 30 entropy units lower than that for decomposition of the corresponding monoester dianion. This, coupled with the fact that there is a significant solvent deuterium isotope effect $k_H/k_D = 1.55$ at 39 °C and 1.45 at 100 °C, whereas k_H/k_D is unity for the monoester dianion,[40] is consistent with the notion that the diester reacts by a bimolecular mechanism with some involvement of a molecule of water (assisted by a second) in the TS.[54] The decomposition of a phosphate diester subject to intramolecular H-bonding LGA still has a negative $\Delta S^{\ddagger}$ of -12.0 cal/(mol·K) in the neutral pH region.[24] The solvent-promoted methanolysis of the Yb^{3+}:phosphate diester complex **11** exhibits a 10^{12}-fold acceleration relative to the uncatalyzed reaction as well as a k_H/k_D of 1.10 ± 0.15, a $\Delta H^{\ddagger}$ of 16.1 kcal/mol, and a $\Delta S^{\ddagger}$ of -15.0 cal/(mol·K). These data support a reaction (eq 7) proceeding via a looser TS (**12**) having less involvement of a nucleophilic $CH_3O(H)$ relative to the solvent reaction alone.[27] The spontaneous decomposition of [Cu(II):**7**]$^+$ has a $\Delta S^{\ddagger}$ of 2.3 cal/(mol·K), which is more positive than what has been observed to date for the purely solvolytic and LG-assisted cleavages of phosphate diesters and closer to what was observed for the hydrolysis of phosphate monoester monoanions[40,42] (typically -0.6 to -6.0 entropy units for the monoanions of methyl, phenyl, 4-nitrophenyl, and 2,4-dinitrophenyl phosphate), which are considered to proceed through loose dissociative-like transition states subject to LGA by protonation of the departing group. Thus, whereas the solvolytic reactions of phosphate diesters are generally considered[2] to be concerted ones having a reaction coordinate close to the diagonal of the diagram shown in Figure 5, the LGA brought about by the Cu(II) coordination to **7** shifts the new, looser TS closer toward the bottom side of the figure with little change in the extent of cleavage of the P−O(LG) bond and less development of the $CH_3O\cdots P$ bond.



(7)

4.4.3. Triester. Khan and Kirby[56] studied the hydrolysis of dialkyl aryl phosphate triesters (2-(aryloxy)-2-oxo-1,3,2-dioxaphosphorinanes) in water at 39 °C and found that those with leaving groups better than 4-nitrophenoxy have pH/rate profiles comprising hydroxide and hydronium ion wings with a plateau region below pH 7, the breadth of which increases with decreasing pK_a of the leaving phenoxy group. In the case of the 2,4-dinitrophenoxy leaving group, the spontaneous hydrolysis is characterized by a very low entropy of -35.6 cal/(mol·K) and a significant solvent deuterium kinetic isotope effect $k_H/k_D = 2.0$, both data being consistent with a process where an attacking water on P is assisted by a second water acting as a general base. Depending on the leaving group and the nature of the attacking oxynucleophile, there appears to be a continuum of mechanisms from concerted displacement of the leaving group to a stepwise reaction for cyclic six-membered phosphate diesters.[56,57] However, the bulk of the linear free energy[58] and heavy atom kinetic isotope[59] evidence concerning phosphoryl transfer from acyclic diesters to oxygen acceptors indicates that the reactions are concerted.

As far as we are aware, there are no solvolytic data pertaining to acyclic phosphate triesters (or even cyclic ones with poorer leaving groups than 4-nitrophenoxy) that support a water- or solvent-mediated reaction, and it is likely that the high $^{s}_{s}pK_a$ of **9** precludes triester **8** from reacting with the poorly nucleophilic methanol solvent. Hence, a comparison between the $\Delta S^{\ddagger}$ and solvent kinetic isotope data for those reactions with good leaving groups in water and what we observe here for the decomposition of [Cu(II):**8**]$^{2+}$ in methanol cannot be made unless we are allowed to use Khan and Kirby's data[56] for the 2-(aryloxy)-2-oxo-1,3,2-dioxaphosphorinanes. The reaction of [Cu(II):**8**]$^{2+}$ at $^{s}_{s}$pH 3.8 gives a $\Delta S^{\ddagger}$ of -7.4 entropy units and a solvent kinetic isotope effect $k_H/k_D = 2.2$. The latter effect supports a direct nucleophilic attack by methanol possibly with general-base assistance by a second alcohol, and the entropy term, being at least 20 units higher than the value for the 2,4-dinitrophenyl derivative above, suggests that there is a considerably looser TS for the Cu(II)-assisted process probably due to decreased formation of the developing $P-OCH_3$ bond.

5. Conclusions

As part of a continuing effort[13,27,30] to investigate the magnitudes and mechanisms of catalysis resulting from metal ion-promoted LGA, the reactions of the Cu(II) complexes of substrates **6**−**8** were investigated. In this system, the coordinated Cu(II) is bound tightly by the phenanthroline moiety[60] and positioned to directly interact with the bridging phenoxy oxygen as in Scheme 1. Each complex decomposes by one or more solvent-mediated pathways through relatively well-defined species. The most active form of the monoester is [Cu(II):**6b**]^0, where both of the nonbridging phosphoryl oxygens are deprotonated, and is 2500 times more reactive than [Cu(II):**6a**]$^+$, where one of the nonbridging oxygens is protonated. The active forms for the diester and triester in the neutral $^{s}_{s}$pH plateau region are [Cu(II):**7b**]$^+$, [Cu(II):**8a**]$^{2+}$, and [Cu(II):**8b**]$^+$, where the diester has a negatively charged phosphoryl oxygen and the triester has, respectively, Cu(II):methanol coordination ($^{s}_{s}$pH < 5.5) or a Cu(II)-bound methoxide ($^{s}_{s}$pH > 7). The process of transforming Cu(II):**6**,**7**,**8** into Cu(II):**9**$^-$ releases ∼23.3 kcal/mol (8.9−32.2 kcal/mol on the basis of the experimental binding constants for the triester and phenoxide), and the endothermicity of progressively greater P···O(LG) bond cleavage will be offset

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(60) The stability constant reported for Cu(II) + phenanthroline ⇄ Cu(II):phenanthroline is log $K = 9.20$ in water: Sillén, L. G., Martell, A. E. *Spec. Publ.—Chem. Soc.* **1964**, 664−665; **1971**, Suppl. No. 1, 676.

by a larger transition-state binding attributable to the emerging Cu(II):$^{-}$O(LG) interaction. It is interesting that the $\Delta H^{\ddagger}$ values for the reactions of all three coordinated esters are essentially the same and the differences in the reaction free energies are dictated solely by the $\Delta S^{\ddagger}$ values, which span 25 entropy units, passing from +18 to +2.4 to −7.4 cal/(mol·K) for mono-, di-, and triester. The data support the interpretation that Cu(II)-promoted LGA loosens the transition states for the cleavage reactions by reducing both the extent of solvent involvement in forming the CH_3O-P bond and restriction of the degrees of methanols of solvation, with little change in the extent of cleavage of the P−O(LG) bond.

There has been much interest in whether enzyme and man-made catalysts of phosphoryl transfer employ the same or altered transition states as the solution-based reactions,[61] and evidence consistent with the same transition states[61a,b] or different transition states[61,61c−f] in the catalyzed as uncatalyzed reactions exists. In the present study, the available data indicate that, for the triester **8** and diester **7**, the Cu(II)-promoted LGA brings on a solvent-mediated reaction that replaces the lyoxide reaction: now a weakly nucleophilic solvent (methanol) is sufficient to displace the coordinated leaving group. This is to be expected from extant data provided by Kirby et al. demonstrating that the β_{nuc} for attack of oxynucleophiles on phosphate triesters drops as the leaving group becomes better,[56] and the rate constants for water attack on phosphate diesters[54] and the dianions of monoesters[42] increase as the phenoxide leaving groups get better. In the present case the pK_a of **9** drops from 16.16 to 0.49 when fully coordinated to Cu(II), which now provides a very good leaving group capable of being displaced by the weakly nucleophilic solvent. This is reminiscent of the discussion and predictions put forth by Jencks and Kirby about the nucleophilic cleavage of 4-nitrophenyl phosphate dianion by nitrogen-containing nucleophiles where the β_{nuc} was 0.13.[40] They suggested that increasing the reactivity of nucleophilic groups in the active site of an enzyme that cleaves phosphate monoester dianions "will not, in itself, make a large contribution to the rate enhancement brought about by enzymatic catalysis of reactions of phosphate dianions. More effective catalysis might be brought about by the enzyme by enhancing the leaving ability of the leaving group, by reduction of the activation energy through distortion or compression of the substrate(s), or by bringing about a change in the mechanism of the reaction, perhaps by metal ion catalysis."

Chin and co-workers have presented an insightful account[62] detailing the magnitudes of the accelerating effects brought on by metal ions for each mode of catalysis to reach the rates of the enzymatic cleavages of phosphate esters and have concluded that LG assistance giving a 10^6-fold acceleration would be required to cleave natural substrates with poor leaving groups. Previously we have demonstrated that simple systems comprising a metal-containing catalyst and a medium effect brought on by the light alcohols can provide rate accelerations of up to 10^{17}-fold for the cleavage of phosphate triesters[30b,9a] and diesters[9a] by capitalizing on the first three of the four main effects by which metal ions can accelerate the cleavage of phosphate esters. What we have shown here is that a simple man-made system taking advantage of a close positioning of a metal ion and the bridging phenoxy oxygen departing from phosphate mono-, di-, and triesters as well as a medium effect provided by methanol solvent is easily capable of providing 10^{12}- to 10^{15}-fold acceleration of the cleavage reactions for the mono- and diesters and 10^5 to 10^9-fold for the triester (depending on the comparison pH) relative to a background reaction at the same ^{s}pH.

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Supporting Information Available: Figures giving kinetic data as a function of ^{s}pH, text describing compound synthesis and spectral data, details of Cu(II) binding studies and titration studies with **9** and **8**, kinetic studies of 4-nitrophenyl phosphate, computational details for phosphoryl- and methanol-bound forms of [Cu(II):**6**]^0 and [Cu(II):**7**]$^+$, and optimized computed structures. This material is available free of charge via the Internet at http://pubs.acs.org.

JA910111Q

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