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Recent Studies of Nucleophilic, General-Acid, and Metal Ion Catalysis of Phosphate Diester Hydrolysis

ANDREI BLASKÓ AND THOMAS C. BRUICE*

Department of Chemistry, University of California, Santa Barbara, California 93106

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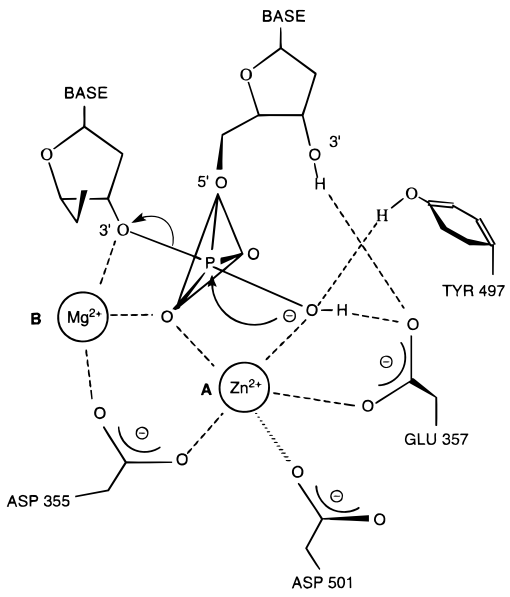
Introduction

The stability of DNA phosphodiester bonds is important in the preservation of genetic information. The half-life for the hydrolysis of dialkyl phosphate esters at neutrality far exceeds the life span of a human.[1] In stark contrast to phosphodiesters, the hydrolysis of phosphomonoesters (by P−O bond breaking) takes place by a rather facile elimination reaction involving the monoanion {RO−P(O)-(OH)(O^-)}, while phosphotriesters readily undergo hydrolysis by nucleophilic catalysis or nucleophilic attack of lyate species.[2] Removal of the negative charge of RO−(PO_2^-)−OR′ by conversion to a triester, metal ion ligation,[3] or by protonation increases the rate of nucleophilic attack at P by $\geq 10^4-10^5$.

The Biological Systems. The catalytic cleavage of DNA and RNA is rapid in the presence of enzymes, many of which are activated by metal ions. A mechanism involving two metal ions was proposed by Beese and Steitz for the 3′−5′ exonuclease reaction of the Klenow fragment of *Escherichia coli* DNA polymerase I (Chart 1).[4] This has become a very popular proposal and has been applied to a number of phosphotransfer enzymes (examples being *E. coli* alkaline phosphatase,[5] phospholipase C from *Bacillus cereus*,[6] RNase H from HIV reverse transcriptase,[7] and hammerhead ribozyme[8]). In many cases[9,10] the presence of two metal ions in an active site is an artifact of the high concentration of metal ion used to soak the crystals prior to structure analysis and the use of Mn^{2+} rather than Mg^{2+} to locate metal binding sites (different specificities of binding). The involvement of two metal ions has most definitely been eliminated for RNase H from HIV reverse transcriptase.[9,11] Several possible modes of action for metal-promoted phosphate ester hydrolysis have been discussed.[12]

Chart 1



Approaches To Mimic the Biological Systems. Ligands capable of complexing two metals have been reported to catalyze the transesterification of dinucleotides by cooperation of both metal ions.[13] Lanthanum ion and its complexes have attracted attention as catalysts for phosphate ester hydrolysis in this[14] and many other laboratories[15] because of the high efficiency of La^{3+} catalysis. Other rare earth metals, in particular Ce(IV), significantly catalyze the hydrolysis of DNA phosphodiester bonds, while Th^{4+} markedly accelerate the hydrolysis of phosphodiester bonds of dCpC in acidic aqueous solution.[16] Studies of putative two metal ion catalysis of phosphate ester hydrolysis by use of mono- and binucleating ligands complexed with Ni^{2+}, Cu^{2+}, and Zn^{2+} have been reported.[17]

The purpose of this contribution is not to provide a comprehensive review, but to indicate the interesting areas of research and inquiry based on our recent studies on the catalysis of phosphate diester hydrolysis by one and two functional group catalysis and by one, two, and three metal ion catalysis. A 10^{13} rate enhancement is presented.

One Functional Group Catalysis of Phosphate Diester Hydrolysis

The pK_a values of the phenolic groups on 6- and 8-hydroxyquinoline are the closest of the hydroxyquinoline

Andrei Blaskó was born in 1952 in Baia-Mare, Romania, and obtained his M.Sc. (1976) and Ph.D. (1988) in Chemical Engineering from the Technical University of Iasi, Romania. His expertise is in physical organic/bioorganic chemistry and molecular biology of putative antigene/antisense agents. Prior to becoming a Research Scientist at Roche Bioscience, Palo Alto, CA, Dr. Blaskó completed two postdoctoral positions at the University of California, Santa Barbara, with Professors Thomas C. Bruice and Clifford A. Bunton. He has carried out collaborative research with scientists from Italy, Spain, Brazil, and Chile and has published over 60 scientific papers.

Thomas C. Bruice. After dropping out of school in the eleventh year (1943) to serve in the military, Thomas C. Bruice attended the University of Southern California (B.S. 1950; Ph.D., 1954) and was a National Research Council postdoctoral at UCLA. Prior to joining the faculty of the University of California at Santa Barbara in 1964, Professor Bruice held faculty positions at Yale, Johns Hopkins, and Cornell Universities. Inventor of the term Bioorganic Chemistry, he has contributed importantly to many areas of mechanistic chemistry dealing with problems important in biochemistry. His accomplishments have been recognized by a number of prestigious awards.

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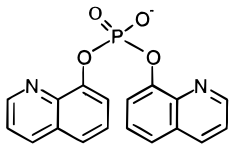
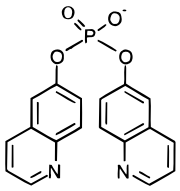
isomers.[18] Thus, 8-hydroxyquinoline and 6-hydroxyquinoline should be comparable as leaving groups in the hydrolysis of bis(8-hydroxyquinoline) phosphate (**I**) and bis(6-hydroxyquinoline) phosphate (**II**) diesters. The pH−



I



II

rate profile for hydrolysis of **I** between pHs 1 and 8 is "bell shaped" (Figure 1),[19] typically seen in the hydrolysis of monophosphate esters[20] but not in the hydrolysis of phosphate diesters. The "bell" is bound between the two pK_a values of the quinolinic amines of **I** with the maximum velocity of hydrolysis occurring approximately midway between these two amine pK_a values. These general observations are in accord with the spontaneous or water-catalyzed hydrolysis of the **IH** species (Scheme 1).

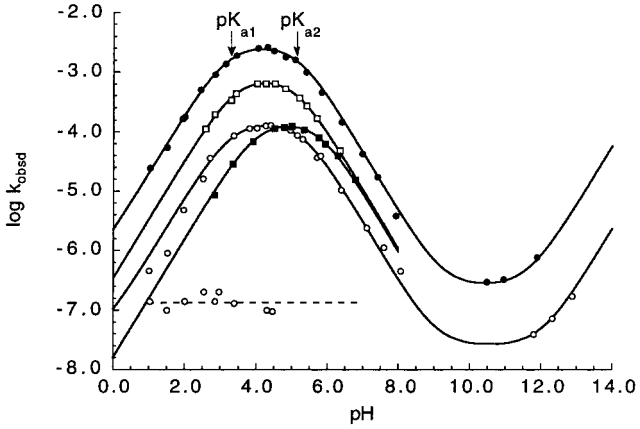
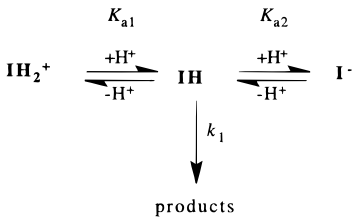
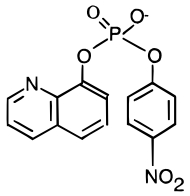


FIGURE 1. Dependence of the pseudo-first-order rate constants (k_{obsd} s^{-1}) on pH for the hydrolysis of bis(8-hydroxyquinoline) phosphate in the absence of added metal ions (μ = 1.0) at 60 °C (●), 45 °C (□), 30 °C (○), and 30 °C in D_2O solvent (■). The dashed line indicates a mean for the values of k_{obsd} for bis(6-hydroxyquinoline) phosphate.

Scheme 1



A useful analogy to the hydrolysis of bis(8-hydroxyquinoline) phosphate is the hydrolysis of 4-nitrophenyl quinolin-8-yl phosphate (**III**). The rate constants for the spontaneous or water-catalyzed hydrolysis of **III** (3.5×10^{-4} s^{-1}) and **I** (1.5×10^{-4} s^{-1}) are comparable as expected from the similar pK_a values for 4-nitrophenol

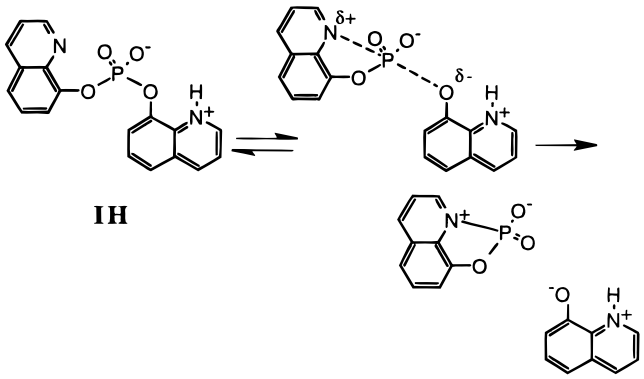


III

(pK_a = 7.15) and *N*-protonated 8-hydroxyquinoline (pK_a = 6.81).[15] The favorable comparison of pK_a values for *N*-protonated 6- and 8-hydroxyquinolines (pK_a = 7.15 and 6.81) would lead one to believe that they would be comparable as leaving groups. The reality is different. The hydrolysis of bis(6-hydroxyquinoline) phosphate (**II**) is much slower than the hydrolysis of bis(8-hydroxyquinoline) phosphate (**I**) at all pH values studied.[19] Also, the values of the pseudo-first-order rate constants for hydrolysis of **II** do not exhibit the "bell-shaped" pH dependence characteristic of the hydrolysis of **I**. At pH 4.5 the values for the rate constants for hydrolysis of **I** and **II** are maximally separated. At this pH, the pseudo-first-order rate constant for hydrolysis of **I** exceeds that for hydrolysis of **II** by 10^3-fold. Thus, the enhanced reactivity is dependent on the presence of the unprotonated neighboring quinoline nitrogen of **IH** as nucleophile.

A deuterium solvent kinetic isotope effect is not observed for the hydrolysis of **I** between pH(D) 2.8 and 6.8. Neither general-acid nor general-base catalysis of hydrolysis by added buffer species was found. Thus, there is no proton-transfer concerted with rate-determining P−O bond formation or P−O bond breakage. A mechanism in accord with the experimental data (Scheme 2)

Scheme 2

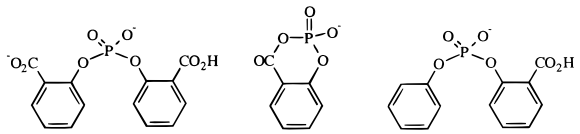


involves nucleophilic attack by the unprotonated neighboring quinoline nitrogen of **IH** on phosphorus with displacement of N-protonated 8-hydroxyquinolinate. There is no general-acid catalysis since the proton on the neighboring N-position is not transferred in the TS to the developing negative charge on the 8-oxygen. An intramolecular reaction, as in Scheme 2, is consistent with the low value of $\Delta S^{\ddagger} = -12.8$ eu.

Two Functional Group Catalysis

Originally, it had been reported that nucleophilic catalysis of hydrolysis of bis-2(carboxyphenyl) phosphate (**IV**) by

o-CO_2^- (to provide **V**) exhibited a surprisingly small rate enhancement over the hydrolysis of **VI** by the presence

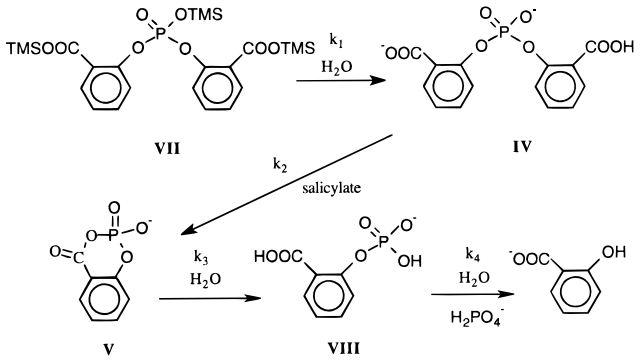


IV **V** **VI**

of the *o*-CO_2H.[21] Such a small rate enhancement by *o*-CO_2H general-acid participation appeared odd. Intramolecular hydrogen bonding would be expected to be of kinetic importance regardless of whether the rate-determining step was nucleophilic attack on P {with *o*-CO_2H hydrogen bonding to $-(PO_2^-)-$} or departure of the ^-O-Ar leaving group {with general-acid assistance from *o*-CO_2H}.[22] Semiempirical (AM1/SM2.1 solvation model) energies of rotamer conformations of the dianion of **IV,** with the *o*-CO_2^- oxygen at a 3.8 Å distance from P and free motion of the *o*-CO_2H substituent, reveal three minima conformations, **A**, **B,** and **C** (Figure 2).[23] In any instance where the *o*-CO_2^- oxygen is in position to attack in-line, the most stable conformation **B** has the *o*-CO_2H in a position as general-acid catalyst to hydrogen bond with a negative oxygen of $-(PO_2^-)-$. The next most favorable conformation (**A**) has the *o*-CO_2H adjacent to what will be the phenoxide leaving group. Thus, one would anticipate that intramolecular general-acid catalysis by the *o*-CO_2H would be important.

The first step (by ^{31}P NMR) in the sequence of events (Scheme 3) is the rapid deblocking of **VII** ($k_1 = 7.2 \times 10^{-2}$ s^{-1}) to provide the phosphodiester substrate (**IV**). In a slower ($k_2 = 1.4 \times 10^{-3}$ s^{-1}) but overlapping reaction, **IV** undergoes intramolecular attack by *o*-CO_2^- with elimination of salicylate and formation of the cyclic acyl phosphate **V,** which then undergoes a slower {$k_3 = 4.4 \times 10^{-4}$ s^{-1}} but overlapping ring opening to yield salicyl phosphate **VIII**. The hydrolysis of **VIII** is well studied ($k_4 = 10^{-5}$ s^{-1} [21]). The original assumption[21] of lack of catalysis of

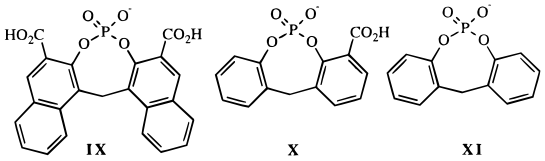
Scheme 3



VII **IV** **V** **VIII**

o-CO_2^- attack by neighboring *o*-CO_2H in hydrolysis of **IV** comes from the inability to distinguish the different processes by the use of the change in UV absorption.

In **IX**, the *o*-CO_2^- and *o*-CO_2H are more or less frozen in position such that the nucleophilic *o*-CO_2^- interacts with $-(PO_2^-)-$ and the *o*-CO_2H proton interacts with either $-(PO_2^-)-$ or departing RO^-, respectively. Thus, neighboring *o*-CO_2H general-acid catalysis is allowed regardless whether the critical transition state involves *o*-CO_2^- attack on phosphorus (Figure 3a) or departure of the leaving group (Figure 3b). A comparison of the rate constants for hydrolysis of diphenyl phosphate {6.3×10^{-8} s^{-1} at 50 °C} and **X**[24] shows that the *o*-CO_2^- group of the latter increases the rate constant by 10^4. In the phosphodiester **IX**, there is a second *o*-carboxy group and the associated rate enhancement is estimated to be ca. 10^8-10^9 over diphenyl phosphate and ca. 10^4 over **X**. Compound **XI** is equivalent to diphenyl phosphate in its



IX **X** **XI**

resistance to hydrolysis.[24] The cyclic acyl phosphodiester

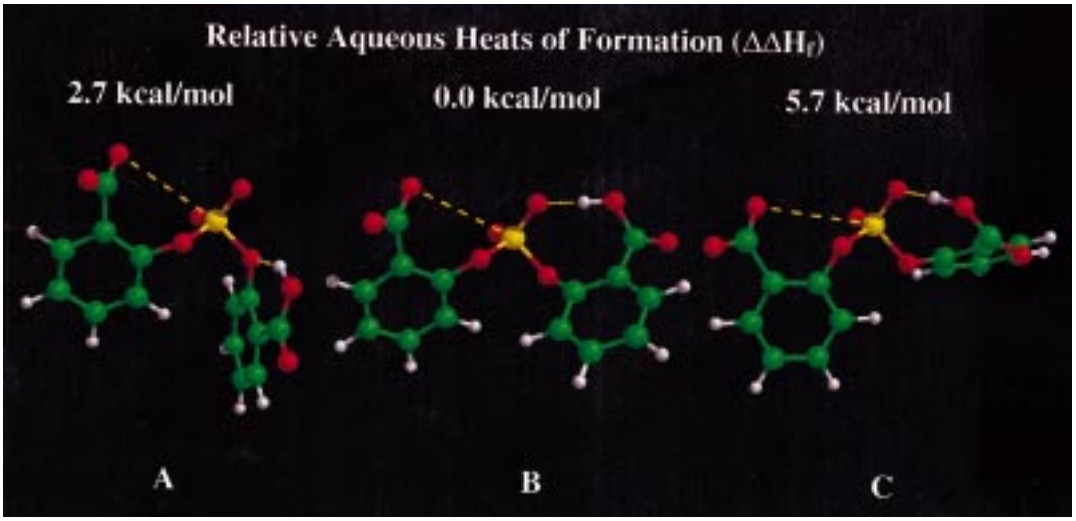


FIGURE 2. Low-energy conformations of bis-(2-carboxyphenyl) phosphate taken from a dihedral rotation study with a restraint upon the distance from *o*-CO_2^- oxygen to P of 3.8 Å.

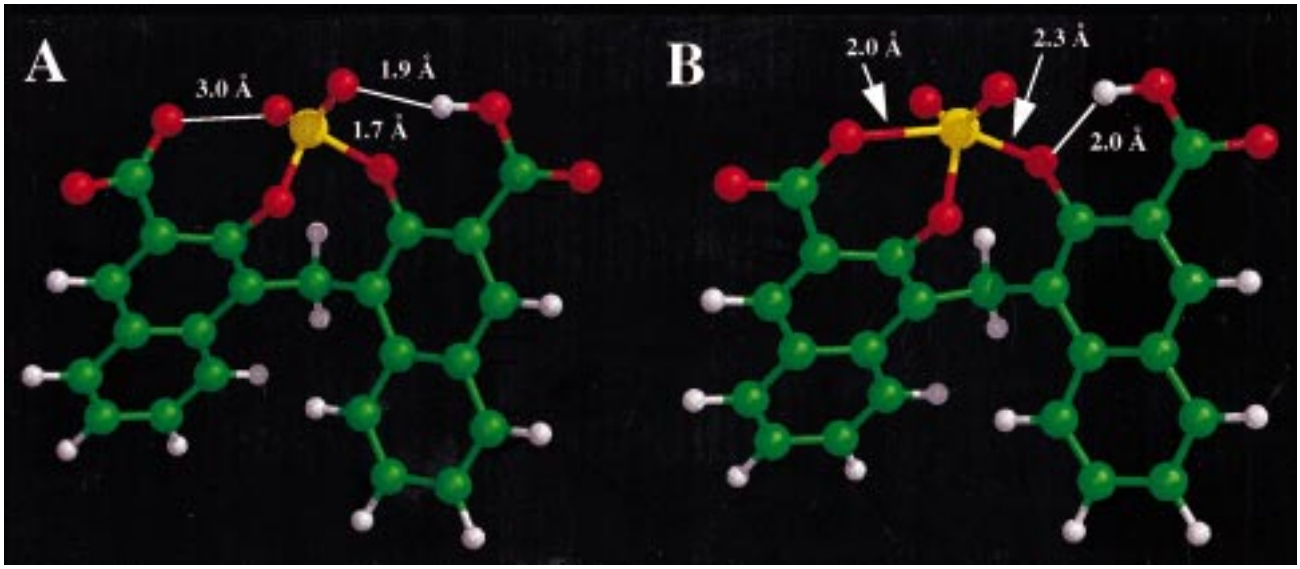
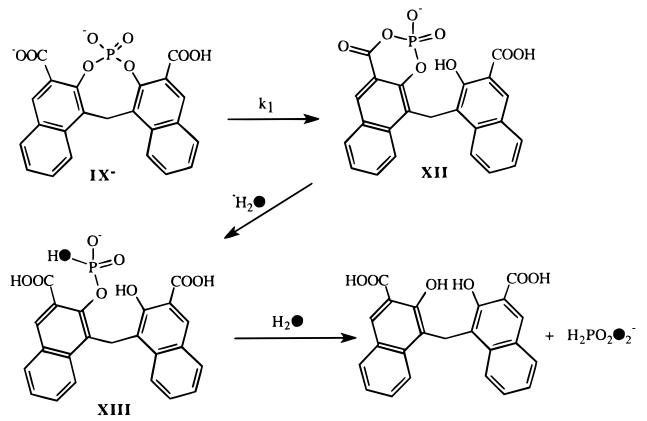


FIGURE 3. Structures taken from the reaction trajectory for hydrolysis of **IX** with neighboring group participation by *o*-CO_2^- and *o*-CO_2H substituents. (a) Rate determining *o*-CO_2^- attack for which case the *o*-CO_2H is hydrogen bonded to a -(PO_2^-)- oxygen. (b) The bond between *o*-CO_2^- oxygen and P is complete and phenoxide is departing with assistance from the *o*-CO_2H substituent.

Scheme 4



XII and monophosphate ester **XIII** are intermediates in the complete hydrolysis of phosphodiester anion **IX**$^-$ (Scheme 4). The rate of hydrolysis of **XII** to provide **XIII** is comparable to that of salicyloyl cyclic phosphate (**V**) {(39 °C), $k = 1.8 \times 10^{-5}$ s^{-1} (pH 5.50) vs $k = 4.7 \times 10^{-5}$ s^{-1} (pH 5.68),[22,24] respectively}.

In the hydrolysis of **IX**, the ^{18}O isotopic effect on the ^{31}P chemical shift reveals that three ^{18}O oxygens are incorporated into the H_3PO_4 product.[24] Considering that **XII** forms via intramolecular *o*-CO_2^- attack on phosphorus, the hydrolysis of **XII** to form **XIII** must also involve the attack of water on phosphorus, rather than the ester carbonyl carbon. Formation of the tetrahedral intermediate that would arise by HO^- attack on the acyl carbonyl of **XII** is sterically hindered by the bridged naphthalene ring (Figure 4). Also, as seen in Figure 4, hydrogen bonding of the *o*-COOH group to the phosphate oxygen partially quenches the charge on the $-(PO_2^-)-$ moiety of **XII** and this facilitates HO^- attack on phosphorus. Such hydrogen bonding, as well as metal ligation and increase in ionic strength, facilitates phosphate ester hydrolysis by diminishing the charges on the phosphate oxygens.[3a]

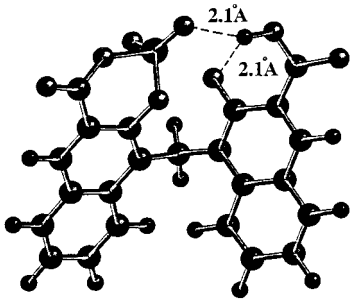


FIGURE 4. Ball and stick model of **XII** showing the bifurcated hydrogen bonding between the *o*-CO_2H and the free $-$OH group and $-(PO_2^-)-$ oxygen.

From the "bell-shaped" profile of the pH dependence of the log k_{obsd} for hydrolysis of **IX** in water (Figure 5), the reactive species must be **IX**$^-$. Since the hydrolysis of **IX**$^-$ occurs via a cyclic acyl phosphate intermediate (**XII**), there must occur a nucleophilic attack of the *o*-CO_2^- on phosphorus rather than a *o*-CO_2^--assisted general-base-catalyzed attack of water. In the transition state, the extent of formation of the carboxylate to phosphorus bond and rupture of the phosphorus to leaving oxygen bond remains unknown. The role of the *o*-CO_2H must be that of a general acid. The hydrogen bonding to the partially negative oxygens of $-(PO_2^-)-$ would explain the role of the *o*-CO_2H if departure of the leaving group had scarcely begun (Figure 3a). If, however, departure of the leaving group is rate controlling, the hydrogen bonding of the *o*-CO_2H would have transferred from the $-(PO_2^-)-$ oxygens to provide general-acid assistance to departure of the leaving group oxygen (Figure 3b).

The spontaneous hydrolysis of **X** is pH independent (Figure 5).[24] Such a flat profile might suggest that hydrolysis of **X** occurs by intramolecular participation of the *o*-carboxyl group in both un-ionized and ionized states (as an example, see ref 25).

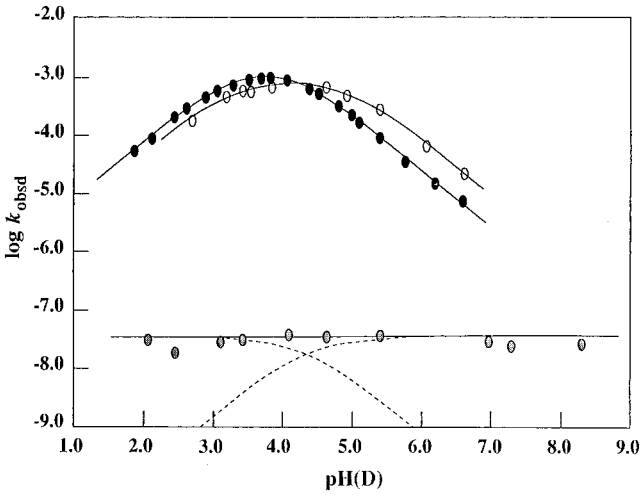


FIGURE 5. pH dependence of the pseudo-first-order rate constants (k_{obsd}) for the hydrolysis of **IX** and **X** {50 °C, μ 1.0}. Bell-shaped plots of log k_{obs} vs pH(D) for hydrolysis of **IX** in H_2O (closed circles) and **IX** in D_2O (open circles) and the pH independence of log k_{obs} vs pH for hydrolysis of **X** in H_2O (hatched circles). The pH independence of the reaction of **X** arises by addition of the contributions of proton ionized and un-ionized species as shown by the dashed lines.

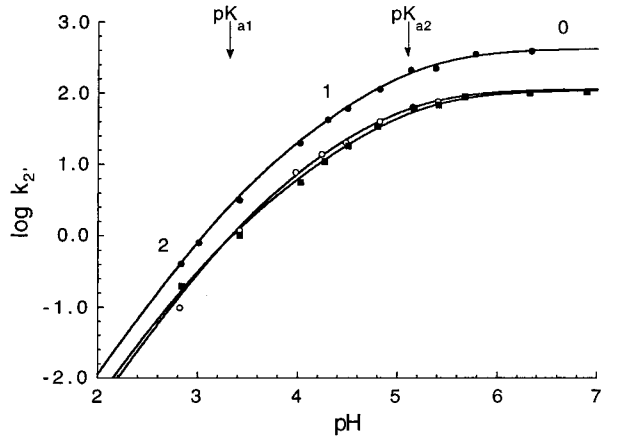
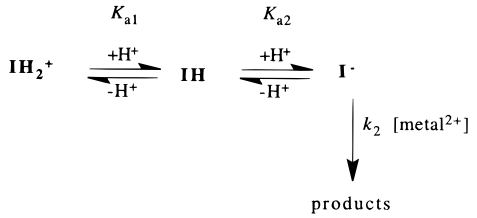


FIGURE 6. Plots of log k_2' vs pH for the hydrolysis of 2×10^{-4} M bis(8-hydroxyquinoline) phosphate at 30 °C (μ = 1.0, KCl) in the presence of Ni^{2+} (●), Co^{2+} (■), and Zn^{2+} (○).

One Metal Ion Catalysis

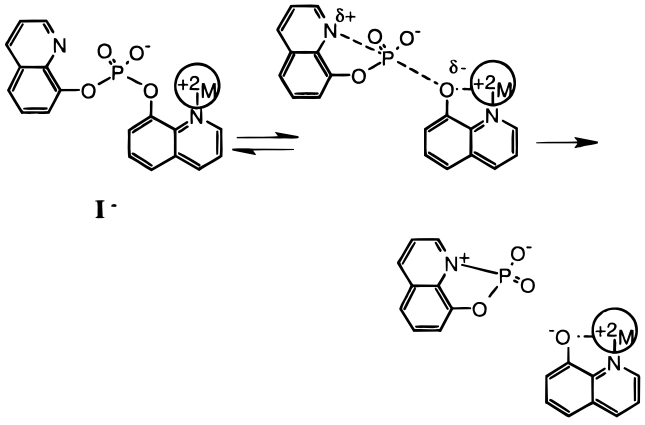
The rate of hydrolysis of bis(8-hydroxyquinoline) phosphate (**I**) between pHs 2 and 7 is first order in Ni^{2+}, Co^{2+}, and Zn^{2+}, but there is no catalysis by Mn^{2+}.[19] The pH−rate (log k_2') profile exhibits three distinct zones: (i) a region with a slope of 2; (ii) a region with a slope of 1; and (iii) a pH-independent region with a slope of zero (Figure 6). Thus, while the hydrolysis of bis(8-hydroxyquinoline) phosphate has **I**H as the reactive species in the absence of metal ions (Scheme 1), metal ion catalysis involves $\mathbf{I}^-$ (Scheme 5). The most likely mechanism for the metal ion catalyzed reaction involves one quinoline nitrogen as nucleophile while the other ligates a metal ion (Scheme 6). The rate of hydrolysis of the nonadjacent bis(6-hydroxyquinoline) phosphate is insensitive to the presence of Ni^{2+}, Co^{2+}, Zn^{2+}, or Mn^{2+}.

Scheme 5



Preassociation of a metal ion with the bis(8-hydroxyquinoline) phosphate ground state is weak because the only ligand groups available are a quinoline nitrogen and possibly a phosphate anionic oxygen. As the reaction progresses, a partial charge develops on the departing oxygen such that association of metal ion is enhanced in the transition state when compared to the ground state. The combined nucleophilic and metal ion catalysis provides, at 1 M [metal ion], a rate enhancement of 10^7 when compared to hydrolysis of the isomeric **II**. Thus, protonation of the quinoline nitrogen has a much smaller positive effect (inductive) upon the rate of hydrolysis of the bis(8-hydroxyquinoline) phosphate than does metal ion ligation (Lewis acid) of the quinoline nitrogen. This is because the proton does not directly interact[19] (Scheme 2) with the weakly basic leaving oxygen (pK_a of conjugate acid ~ 7.0), whereas the metal ion interacts directly with the departing oxygen of the leaving group (Scheme 6). It was established early on that there is a great kinetic advantage when Cu^{2+} ligates to the developing negative charge on the leaving oxygen in studies of the hydrolysis of phosphomonoesters.[20]

Scheme 6



A Phosphodiester Exhibiting Catalysis of Hydrolysis by One, Two, and Three Metal Ions. The hydrolysis of (8-hydroxy-2-quinolyl)methyl (8-hydroxyquinolyl)methylphosphonate (**XIV**) was chosen for study on the basis that two ligated metal ions can present themselves to the phosphonate moiety in such a manner as to cancel the negative charge of the $-(PO_2^-)-$ functionality and create an active site for nucleophilic attack and leaving group stabilization.[14] The alkyl phosphonate ester **XV** can only ligate one metal ion. Compound **XIV** is included in a discussion of phosphate diesters because, all other things

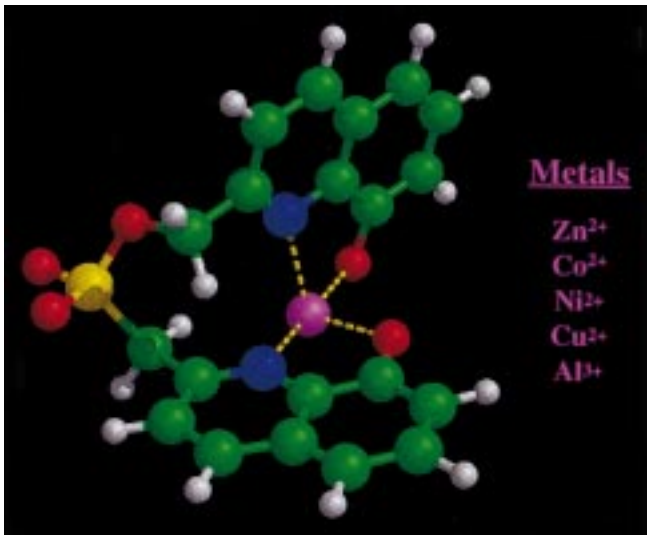
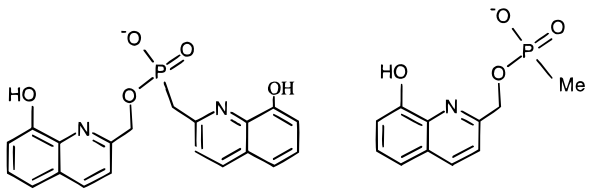


FIGURE 7. Sequestering of metal ions by **XIV** which prevents these metal ions from catalysis of the ester hydrolysis.

being equal, alkyl phosphonate[28] and dialkyl phosphate esters have comparable rates of hydrolysis.

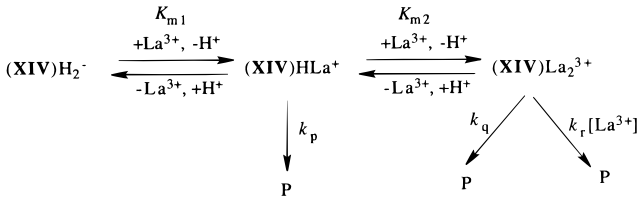


XIV **XV**

The divalent metal ions Zn^{2+}, Ni^{2+}, Co^{2+}, and Cu^{2+} as well as Al^{3+} form 1:1 complexes with **XIV** and do not catalyze its hydrolysis. Inspection of Figure 7 shows that the two quinoline nitrogens sequester the single metal ion such that it cannot interact with the $-(PO_2^-)O-$ moiety.[14]

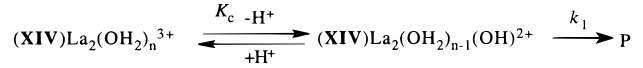
Kinetic studies of the catalysis of hydrolysis of **XIV** by La^{3+} show that the hydrolysis is first (k_p), second (k_q), and

Scheme 7



third (k_r) order in $[La^{3+}]$ (Scheme 7). The structure involving ligation of two metal ions, (**XIV**)$La_2(OH_2)_n^{3+}$, is provided in Figure 8a. It shows the lanthanum ions held in place such that one La^{3+} can ligate to a PO_2^- oxygen and a H_2O and the second La^{3+} is complexed to the second PO_2^- oxygen and the putative alkoxide leaving group. A plot of log k_q vs pH (not shown) is biphasic going from slope +1 to slope 0 with the break at pH $\approx$ 7. Since **XIV** has no functional group with pK_a near 7, the rate constant k_q must be associated with the structure (**XIV**)$La_2(OH_2)_{n-1}(HO)^{2+}$, in which an H_2O ligated to a La^{3+} becomes ionized (Scheme 8, $k_1 = 1.36 \times 10^{-3}$ s^{-1}, $t_{1/2}$ = 8.5 min). The pK_c = 7.19 (Scheme 8) is considerably smaller than that of H_2O ligated to a free lanthanum ion (pK_a = 9.06).[27] Inspection of Figure 8b shows how the ligated HO^- is in position for an in-line displacement reaction. Ligation bond lengths of ~2.5 Å, nonrigid stereochemistry of ligation, and a high coordination number of over 8 account for the ability of La^{3+} to enter into the structures of Figures 8a,b such that the nucleophilic HO^- is positioned properly for attack and the partially negative charges of both $-(PO_2^-)-$ oxygens and the leaving oxygen are ligated.

Scheme 8



The pH-dependent second-order rate constant, k_r, for hydrolysis of (**XIV**)La_2 by La^{3+} exhibits a first-order

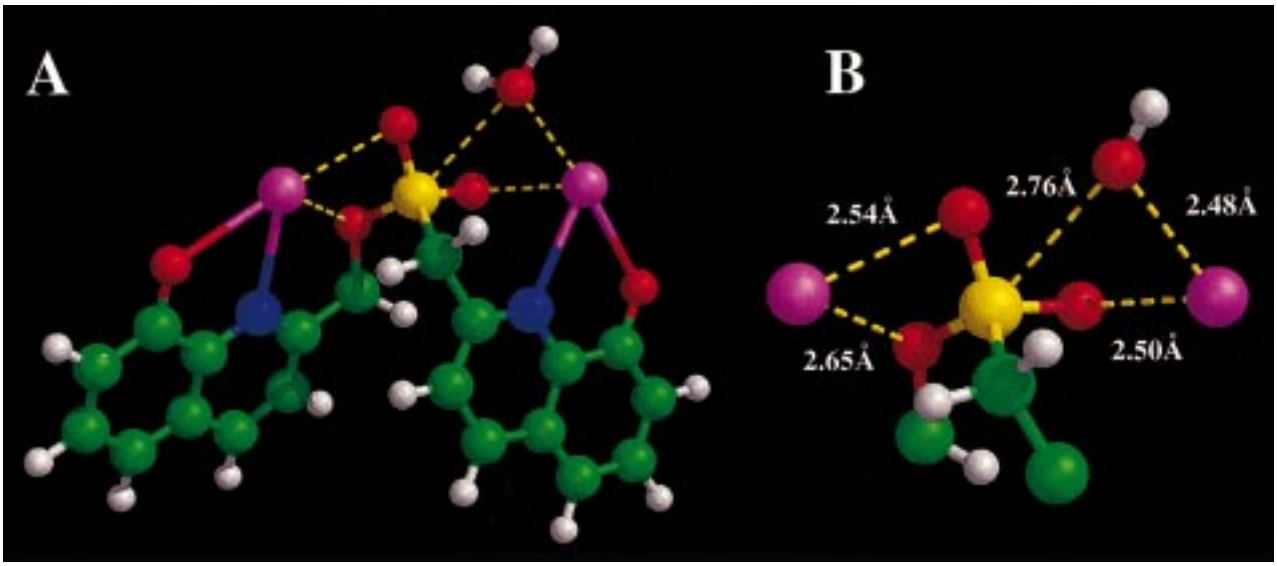
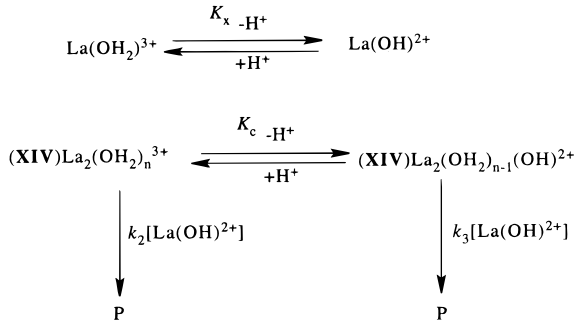


FIGURE 8. (A) Model of the complex of **XIV** with two La^{3+} one ligated to a water molecule. (B) Lanthanum bonding distances showing how one La^{3+} may hold the HO^- for in-line attack and also bond to a partially negative (PO_2^{2-}) oxygen, while the second La^{3+} may complex with the second (PO_2^{2-}) oxygen and the departing alkoxide oxygen.

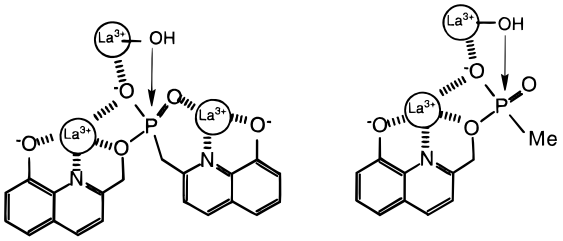
Scheme 9



dependence on hydroxide concentration (pH < 8.0) (not shown). This is in agreement with $La(OH)^{2+}$ being an effective nucleophile for the hydrolysis of (**XIV**)$La_2(H_2O)_n^{3+}$ (Scheme 9). The linearity of a plot of log k_r vs pH requires that the value of k_2 equals that of k_3. Thus, the ionization of a water molecule of (**XIV**)$La_2(OH_2)_n^{3+}$ to provide (**XIV**)$La_2(OH_2)_{n-1}(HO)^{2+}$ has no effect on the rate of the reaction with $La(OH)^{2+}$.

As is to be expected, **XV** forms only the 1:1 complex (**XV**)$La(X)_n$ {X = H_2O or HO^-}. This species also enters into a bimolecular reaction with La^{3+}, which leads to catalysis of hydrolysis. The first-order dependence of the rate constant k_s on [HO^-] establishes that (**XV**)$La(X)_n$, like (**XIV**)$La_2(X)_n$, is hydrolyzed by $La(OH)^{2+}$. A common mechanism is depicted in (Chart 2).

Chart 2



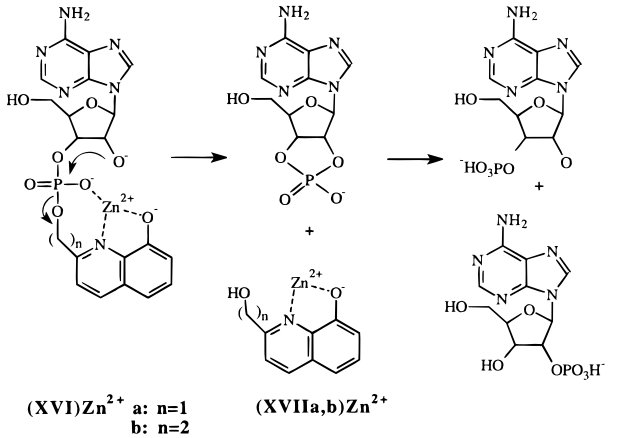
The calculated bimolecular rate constant for reaction of (**XV**)$La(X)_n$ with $La(OH)^{2+}$ is 7.61×10^{-2} M^{-1} s^{-1}, indicating that the $La(OH)^{2+}$ reacts with (**XIV**)$La_2(X)_n$ only four times faster than with (**XV**)$La(X)_n$. On the other hand, the complex (**XIV**)$La_2(X)_n$ is entirely active for spontaneous hydrolysis, but the complex (**XV**)$La(X)_n$ is not. The rate constant for reaction of $La(OH)^{2+}$ with (**XIV**)$La_2(X)_n$ ($k_2 = k_3 = 0.262$ M^{-1} s^{-1}) corresponds to one-fiftieth of the bimolecular rate constant for the reaction of $La(OH)^{2+}$ with 4-nitrophenyl methylphosphonate (14 M^{-1} s^{-1}).[28] Thus, the value of k_2 is exceedingly large when considering the difference of 7 pK_a units in the acidity of the leaving groups **XV** vs 4-nitrophenol. The notable reactivity of (**XIV**)$La_2(X)_n$ {X = H_2O or HO^-} with $La(OH)^{2+}$ must be provided by Lewis-acid catalysis by La^{3+} in the 1:2 complex. Comparing the rate constant (k_2) for this reaction with that for the intramolecular reaction of (**XIV**)$La_2(OH_2)_{n-1}(OH)^{2+}$ (k_1, Scheme 8) shows that only a 5×10^{-3} M concentration of $La(OH)^{2+}$ is required to give the same first-order rate as the intramolecular reaction. This small effective molarity suggests that $La(OH)^{2+}$, upon catalyzing the hydrolysis of (**XIV**)$La_2(X)_n$, weakly associates with phosphonate anion of the 1:2 complex and behaves as an intramolecular nucleophile (Chart 2). The binding constant of La^{3+} to the phosphonate moiety of (**XIV**)$La_2(X)_n$ is so small that the plot of k_{obsd} vs [La^{3+}] does not level-off until [La^{3+}] $\approx$ 0.08 M.[14] It is worthy to note that, to the best of our knowledge, the $La(OH)^{2+}$-catalyzed hydrolysis of the (**XIV**)$La_2(X)_n$ complexes is the first example of catalysis of hydrolysis involving three metal ions.

One must know the rate constant for hydrolysis of **XIV** in order to estimate the increase in rate constant for hydrolysis of **XIV** upon conversion into (**XIV**)$La_2(OH_2)_{n-1}(OH)^{2+}$. The bimolecular rate constant for HO^--catalyzed hydrolysis of **XIV** at 30 °C was estimated[14] to be $\sim 5.0 \times 10^{-11}$ M^{-1} s^{-1}. From this, at pH 8 and 30 °C, the pseudo-first-order rate constant of hydrolysis of **XIV** would be $\sim 5.0 \times 10^{-17}$ s^{-1}. Since at pH 8, k_q ($\approx k_1$) = 1.36×10^{-3} s^{-1}, a rate enhancement of 10^{13} has been observed.

The characteristics of high electronegativity, nonrigid stereochemistry of ligation, and the high coordination number over 8 for La^{3+} are caused by inner orbital shielding of outer electrons, filled d-orbitals, and a comparatively large ionic radius.[29] This behavior is in contrast to d-transition metal complexes where the involvement of the d-orbital in the bonding imparts strong directional characteristics and produces the well-defined geometry such as square planar or octahedral. The flexibility of the lanthanum complexes may satisfy the geometrical requirements of ligation of the ground and transition states in catalysis.

Models for One- and Two-Metal Ion Catalysis of RNA Hydrolysis. The phosphate diesters **XVIa** and **XVIb** (Scheme 10) have been used in modeling the metal ion catalysis of RNA cleavage. The purpose of their design was to place a metal ion at variable distance to the $-(PO_2^-)-$ function, the leaving oxygen, and nucleophilic 2′-hydroxyl group. Rate constants obtained with **XVIa**,**b** and any metal ion used were found to differ by no more than an order of magnitude ($\Delta\Delta G^{\ddagger}$ < 1 kcal/mol).

Scheme 10



All of the metal ions {Mg^{2+}, Zn^{2+}, Cu^{2+}, and La^{3+}} tested promoted intramolecular transesterification of **XVIa** and **XVIb,** yielding 2′,3′-cyclic adenosine monophosphate

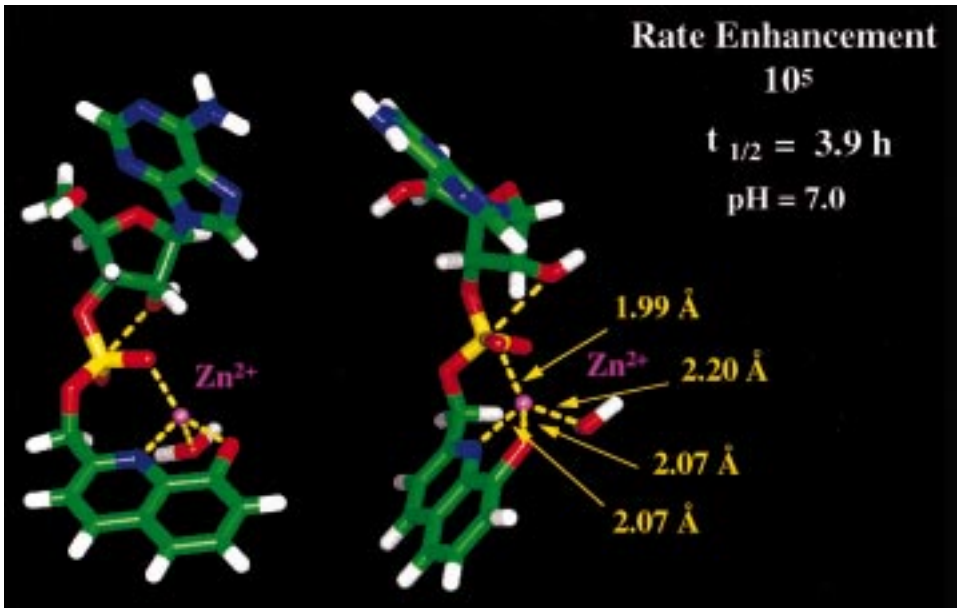


FIGURE 9. Modeling of the tetrahedral Zn^{2+} complex of **XVIa** showing how the metal can interact with an oxygen of the (PO_2^{2-}) moiety in a reaction which includes an in-line attack by the ribose 2′-oxygen.

and (8-hydroxyquinolyl)methanol (**XVIIa)** and -ethanol (**XVIIb)**, respectively.[30] The dependence of the pseudo-first-order rate constants for the transesterifications of the 1:1 phosphate ester:metal complexes on metal ion concentration at constant pH values established two reactions paths (eq 1). When the terms k_o and $k_M[M^{n+}]$ were determined at various pH values, it was found that both are dependent to the first order on HO^- concentration. This virtually establishes that ionization of the 2′-OH to provide the 2′-O^- nucleophile occurs by specific-base catalysis (i.e., HO^-) and by metal-ligated hydroxide ion [(M^{n+}(OH)]. These results parallel experimental work with the Hammerhead self-cleavage reaction which is first order in [HO^-] and interpreted to be both first and second order in [M^{n+}].[8c]

$$\mathbf{XIV(a/b)}^- + M^{n+} \underset{+H^+}{\overset{K_m}{\rightleftharpoons}} (\mathbf{XIVa/b}H_{-1})M^{(n-2)+} \xrightarrow{k_o + k_M[M^{n+}]} \text{product} \quad (1)$$

Molecular modeling of the 1:1 complexes of **XVIa**,**b** with divalent metal ions shows that interaction between the negative charge of the −(PO_2^-)− oxygens and a metal ion ligated to the 8-hydroxyquinoline is favored. This is so regardless of metal ions being tetrahedral Zn^{2+}, octahedral Mg^{2+}, or square planar Cu^{2+}. Modeling of the Zn^{+2} complex of **XVIa** is provided in Figure 9, which shows the tetrahedral Zn^{2+} to be correctly positioned to ligate with an oxygen of −(PO_2^-)− when the 2′-O^- is in position for an in-line displacement of the leaving group. Simultaneous ligation by Zn^{2+} of both leaving group and −(PO_2^-)− oxygens is not possible.[30] Interaction of metal ions with the leaving oxygen is possible for 1:1 complexes of Mg^{2+} and Cu^{2+}. However, simultaneous interaction in the ground state of Mg^{2+} or Cu^{2+} with both a −(PO_2^-)− oxygen and the leaving oxygen is impossible due to the rigid and well-oriented geometry of these metal ions.

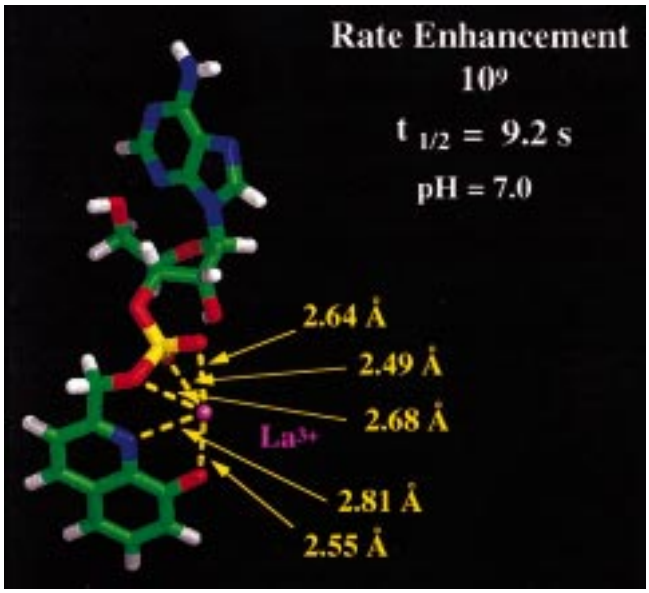


FIGURE 10. Modeling of the La^{3+} complex of **XVIa.** Due to the length of the bonds and the lack of geometrical constraints the metal ion is capable of complexing with both (PO_2^-) oxygens as well as to both ground state and incipient phenoxide in the transition state.

Molecular modeling of the 1:1 complex of La^{3+} with **XVIa** shows the ease in which this metal can simultaneously interact with both negative oxygens of −(PO_2^-)− and, also, the leaving oxygen moieties (Figure 10). This is due to the greater La^{3+}−O bond length (∼2.60 Å) and, unlike transition metal ions, a lack of directionality of bonding by La^{3+} due to filled d-orbitals.[30] The rate constants for the hydrolysis of the complexes of **XVIa,b** with La^{3+} are 10^3 times greater than those of the Zn^{2+} complexes, whereas the kinetically determined association constants of La^{3+} complexes are smaller than those of the Zn^{2+} complexes by a factor of 10^2. As supported by molecular modeling,[30] the remarkable activity provided

by La^{3+} must originate from a combination of Lewis-acid catalysis by coordination to the phosphate oxyanion and interaction with the leaving oxygen in the transition state associated with departure of the leaving group. The rate enhancement for hydrolysis of **XVIa,b** by complexation with metal ions is as follows: $\sim 10^5$ with Zn^{2+}, $\sim 10^3$ with Mg^{2+}, $\sim 10^5$ with Cu^{2+}, and $\sim 10^9$ with La^{3+}. For the La^{3+} complexes the rate enhancement exceeding 10^5 results, at least partially, from the additional interaction of La^{3+} with the leaving oxygen.

Conclusions

Recent studies in our laboratory on the catalysis of phosphate diester hydrolysis dealing with one and two functional group catalysis as well as with one, two, and three metal ion catalysis has been reviewed. The facile hydrolysis of bis(8-hydroxyquinoline) phosphate (**I**) between pHs 1 and 8 is dependent upon nucleophilic attack of one quinoline nitrogen upon phosphate and protonation of the second quinoline nitrogen to create a leaving group comparable to 4-nitrophenolate. The combination of nucleophilic and specific-acid catalysis provides a rate enhancement of 1.1×10^3 for **I** when compared to the hydrolysis of bis(6-hydroxyquinoline) phosphate. Proton transfer from N → O in the transition state is not important (no general-acid catalysis); however, there is assistance to departure of the leaving group if the proton is replaced by a metal ion.

Qualitative semiempirical evaluations of the transition states leading from conformations **A** and **B** of bis(2-carboxyphenyl) phosphate **(IV)** suggest participation of both functions in hydrolysis. Nucleophilic by *o*-CO_2^- and general acid by *o*-CO_2H catalysis of hydrolysis of **IV** is very rapid and proceeds via formation of salicyloyl cyclic phosphate, which ring opens to give salicyl monophosphate and finally salicylic acid. The ionic species of 4,4-methylenebis(2-carboxy-3-naphthyl) phosphate (**IX**) with one *o*-carboxylic acid ionized and the other not has been found to hydrolyze with a rate constant 10^9 greater than the estimated rate constant for hydrolysis of diphenyl phosphate. Both *o*-CO_2^- and *o*-CO_2H functions are involved in the catalysis of the rate-limiting step. The rate constant for the hydrolysis of the anionic species of **IX** exceeds that for the hydrolysis of a phosphodiester with only one *o*-carboxyl function (**X**) by 10^4. The hydrolysis occurs with intramolecular *o*-CO_2^- nucleophilic attack on phosphorus (assisted by *o*-CO_2H general-acid catalysis) to provide an acyl phosphate intermediate which undergoes hydrolytic cleavage by HO^- attack on phosphorus to provide phosphate monoester.

The **XVIa,b** "ribodinucleotides" undergo intramolecular transesterification by specific HO^- ionization of the ribose 2′-OH. The reaction is catalyzed by Zn^{2+} (10^5-fold), Mg^{2+} (10^3), Cu^{2+}(10^5), and La^{3+} (10^9-fold). Model building suggests that the rate enhancement by Zn^{2+} is due to the ligation of one of the negatively charged $-(PO_2^-)-$ oxygens (Figure 9), while the much larger catalytic effect of La^{3+} results from ligation of both $-(PO_2^-)-$ oxygens as well as the incipient alkoxide leaving oxygen (Figure 10) in the transition state. Reasons for the catalytic efficiency of La^{3+} are discussed.

Phosphodiesters and phosphonate esters have like rates of hydrolysis if the leaving groups are the same. The phosphonate ester **IX** forms a hydrolytically active 1:2 complex (**IX**)La_2 with La^{3+} but inert 1:1 complexes with Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, and Al^{3+}. The La^{3+} in the (**IX**)La_2 complex serve to (i) facilitate the formation of metal ligated hydroxide as an intramolecular nucleophile, (ii) stabilize the transition state of the hydrolysis by neutralization of the phosphonate negative charge, and (iii) interact with an incipient oxyanion of the leaving alcohol. The two La^{3+} functions operate in concert and provide $\sim 10^{13}$ rate enhancement. Consequently the 1:2 complex (**IX**)$La_2(OH_2)_{n-1}(OH)$ serves to show how two metal ions in concert may act as very effective catalysts in phosphodiester hydrolysis with alkoxide as the leaving group. First examples are described of three metal ion catalysis.

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References

(1) Wolfenden, R.; Ridgway, C.; Young, G., Spontaneous Hydrolysis of Ionized Phosphate Monoesters and Diesters and the Proficiencies of Phosphatases and Phosphodiesterases as Catalysts, *J. Am. Chem. Soc.* **1998**, *120*, 833−834 and references therein.

(2) (a) Bruice, T. C.; Benkovic, S. J. *Bioorganic Mechanisms*; Benjamin: New York, 1966; Vol. 2, Chapter 5. (b) Thatcher, G. R. J.; Kluger, R. *Adv. Phys. Org. Chem.* **1989**, *25*, 99−265.

(3) Dempcy, R. O.; Bruice, T. C. The Negative Charge of Alkyl Phosphate Diesters and the Slow-Gaited Hydrolysis of RNA and DNA-Catalysis of RNA Hydrolysis Through Metal Ion Ligation to the Ester-PO_2-Moiety, *J. Am. Chem. Soc.* **1994,** *116*, 4511−4512.

(4) Beese, L. S.; Steitz, T. A., Structural Basis for the 3′-5′ Exonuclease Activity of Escherichia-coli DNA Polymerase-I-A2 Metal Ion Mechanism. *EMBO J.* **1991**, *10*, 25−35.

(5) Kim, E. E.; Wyckoff, H. W. Reaction Mechanism of Alkaline Phosphatase Based on Crystal Structures-2-Metal Ion Catalysis. *J. Mol. Biol.* **1991**, *218*, 449−464.

(6) Hough, E.; Hansen, L. K.; Briknes, B.; Jynge, K.; Hansen, S.; Hordrik, A.; Little, C.; Dodson, E.; Derewenda, Z. High-Resolution (1.5Å) Crystal Structure of Phospholipase C from Bacillus Cereus. *Nature* **1989**, *338*, 357−360.

(7) Davies, J. F.; Hostomska, Z.; Hostomsky, Z.; Jordan, S. R.; Matthews, D. A. Crystal Structure of the Ribonuclease-H Domain of HIV-1 Reverse Transcriptase. *Science* **1991**, *252*, 88−95.

(8) (a) Steitz, T. A.; Steitz, J. A. A General 2-Metal-Ion Mechanism for Catalytic RNA. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6498−6502. (b) Piccirilli, J. A.; Vyle, J. S.; Caruthere, M. H.; Cech, T. R. Metal Ion Catalysis in the Tetrahymena Ribozyme Reaction. *Nature* (London) **1993**, *361*, 85−88. (c) Dahm, S. C.; Uhlenbeck, O. C. Role of Divalent Metal Ions in the Hammerhead RNA Cleavage Reaction. *Biochemistry* **1991**, *30*, 9464−9469.

(9) Cowan, J. A. Metal Activation of Enzymes in Nucleic Acid Biochemistry. *Chem. Rev.* **1998**, *3*, 1067−1087.
(10) Wilcox, D. E. Binuclear Metallohydrolases. *Chem. Rev.* **1996**, *96*, 2435−2458.
(11) Katayanagi, K.; Miyagawa, M.; Matsushima, M.; Ishikawa, M.; et al. Structural Details of Ribonuclease H from Eschericia coli as Refined to an Atomic Resolution. *J. Mol. Biol.* **1992**, *223*, 1029−1052.
(12) Bashkin, J. K.; Jenkins, L. A. The Role of Metals in the Hydrolytic Cleavage of DNA and RNA. *Comments Inorg. Chem.* **1994**, *16*, 77−93.
(13) (a) Young, M. J.; Chin, J. Dinuclear Copper(II) Complex That Hydrolyzes RNA. *J. Am. Chem. Soc.* **1995**, *117*, 10577−10578. (b) Chapman, W. H., Jr.; Breslow, R. Selective Hydrolysis of Phosphate Esters, Nitrophenyl Phosphates and UpU, By Dimeric Zinc Complexes Depends on the Spacer Length. *J. Am. Chem. Soc.* **1995**, *117*, 5462−5469.
(14) (a) Tsubouchi, A.; Bruice, T. C., Remarkable $\sim 10^{13}$ Rate Enhancement in Phosphonate Ester Hydrolysis Catalyzed by Two Metal Ions. *J. Am. Chem. Soc.* **1994**, *116*, 11614−11615. (b) Tsubouchi, A.; Bruice, T. C. Phosphonate Ester Hydrolysis Catalyzed by Two Lanthanium Ions—Intramolecular Nucleophilic Attack of Coordinated Hydroxide and Lewis Acid Activation. *J. Am. Chem. Soc.* **1995**, *117*, 7399−7411.
(15) (a) Morrow, J. R.; Buttrey, L. A.; Berback, K. A. Transesterification of a Phosphate Diester by Divalent and Trivalent Metal Ions. *Inorg. Chem.* **1992**, *31*, 16−20.
(16) Ihara, T.; Shimura, H.; Ohmori, K.; Tsuji, H.; Takeuchi, J.; Takagi, M. Hydrolysis of Nucleotides Using Actinoid Metal Ion. *Chem. Lett.* **1996**, 687−688.
(17) Kesicki, E. A.; DeRosch, M. A.; Freeman, L. H.; Walton, C. L.; Harvey, D. F.; Trogler, W. C. Interaction of Binuclear Transition Metal Complexes With DNA. *Inorg. Chem.* **1993**, *32*, 5851−5867.
(18) Mason, S. F. The Tautomerism of *N*-Heteroaromatic Hydroxy-Compounds, Part III, Ionization Constants. *J. Chem. Soc.* **1958**, 674−685 and references therein.
(19) Browne, K. A.; Bruice, T. C. Chemistry of Phosphodiesters, DNA and Models. 2. The Hydrolysis of Bis(8-Hydroxyquinoline) Phosphate in the Absence and Presence of Metal Ions. *J. Am. Chem. Soc.* **1992**, *114*, 4951−4958.
(20) Fife, T. H.; Pujari, M. P., Divalent Metal Ion Catalysis in the Hydrolysis of Phosphomonoesters. Hydrolysis of 2-(1,10-Phenanthrolyl) Phosphate. *J. Am. Chem. Soc.* **1988**, *110*, 7790−7797.
(21) Abell, K. W. Y.; Kirby, A. J. Intramolecular General Acid Catalysis of Intramolecular Nucleophilic Catalysis of the Hydrolysis of a Phosphate Diester. *J. Chem Soc., Perkin Trans. 2* **1983,** 1171−1174.
(22) Khan, S. A.; Kirby, A. J.; Wakselman, M. Intramolecular Catalysis of Phosphate Diester Hydrolysis. Nucleophilic Catalysis by the Neighbouring Carboxy-group of the Hydrolysis of Aryl 2-Carboxyphenyl Phosphates. *J. Chem. Soc. (B)* **1970**, 1182−1187.
(23) Bruice, T. C.; Blaskó, A.; Petyak, M. Participation of Two Carboxyl Groups in Phosphodiester Hydrolysis. 1. Hydrolysis of Bis(2-Carboxyphenyl) Phosphate. *J. Am. Chem. Soc.* **1995**, *117*, 12064−12069.
(24) Bruice, T. C.; Blaskó, A.; Arasasingham, R. D.; Kim, J.-S. Participation of Two Carboxyl Groups in Phosphodiester Hydrolysis. 2. A Kinetic, Isotopic, and ^{31}P NMR Study of the Hydrolysis of a Phosphodiester with Carboxyl Groups Fixed in an Attack Conformation. *J. Am. Chem. Soc.* **1995**, *117*, 12070−12077.
(25) Thanassi, J. W.; Bruice, T. C. Neighboring Carboxyl Group Participation in the Hydrolysis of Monoesters of Phthalic Acid. The Dependence of Mechanisms on Leaving Group Tendecies. *J. Am. Chem. Soc.* **1966**, *88*, 747−752.
(26) Withey, R. J. Lanthanum Ion Catalysis of Nucleophilic Displacement Reactions of Monoesters of Methyl Phosphonic Acid. *Can. J. Chem.* **1969**, *47*, 4383−4387.
(27) *Stability Constants of Metal-ion Complexes*; IUPAC Chemical Data Series No 22, Part B; Pergamon Press: New York, 1979.
(28) This value was calculated on the basis of the data in the literature (ref 26) using the pK_a value of 9.06 for H_2O ligated to La^{3+}.
(29) Sinha, S. P. *Structure and Bonding*; Springer-Verlag: New York, 1989; Vol. 25, p 69.
(30) Bruice, T. C.; Tsubouchi, A.; Dempcy, R. O.; Olson, L. One- and Two-Metal Ion Catalysis of the Hydrolysis of Adenosine 3′-Alkyl Phosphate Esters—Models for One- and Two-Metal Ion Catalysis of RNA Hydrolysis. *J. Am. Chem. Soc.* **1996**, *118*, 9867−9875.

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