

Efficient Intramolecular General Acid Catalysis of Nucleophilic Attack on a Phosphodiester

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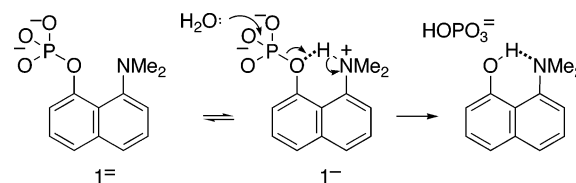
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Abstract: The hydrolysis of methyl 8-dimethylamino-1-naphthyl phosphate **4** and its reactions with a representative range of nucleophiles are catalyzed by the dimethylammonium group at acidic pH with rate accelerations of the order of 10^6 . The reaction persists up to pH 7 because the strong intramolecular hydrogen bond, which is the key to efficient general acid catalysis, is present also in the reactant. The sensitivity to the basicity of the nucleophile (Brønsted $\beta_{\text{nuc}} = 0.29$) lies between values measured previously for mono- and triesters. The comparisons suggest that general acid catalyzed reactions of phosphate mono- or diesters with strongly basic oxyanion nucleophiles (like those derived from a serine oxygen or a bound water molecule in an enzyme active site) will be fastest when their negative charges are neutralized by protonation. Reactions with NH_2OH and its N-methylated derivatives show an apparent α -effect, but $\text{NH}_2\text{-OMe}$ reacts no faster than a primary amine of similar basicity. It is suggested that the reaction involving NH_2OH as an oxygen nucleophile proceeds through the pre-equilibrium formation of the tautomer $\text{H}_3\text{N}^+\text{-O}^-$ as the active nucleophile: *ab initio* calculations support this idea.

General acid–base catalysis is evidently highly efficient in enzyme active sites, but in only a handful of model systems. The common feature of these efficient (and therefore necessarily intramolecular) model systems is the development of a strong intramolecular hydrogen bond in the product, and thus in the transition state leading to it (Scheme 1). Phosphate transfer from the 8-dimethylammonium-naphthyl-1-phosphate monoanion **1⁻** to water and to a range of nucleophiles shows general acid catalysis by the neighboring NH^+ group, through a strong intramolecular hydrogen bond which is in place even in the reactant.¹ The substantial acceleration (of the order of 10^6 -fold at 39 °C), achieved by the concerted action of an external nucleophile and an internal general acid, involves little or no prospect of synergy² (catalysis greater than the sum of the parts) between the two—as might reasonably be expected for a reaction with a low sensitivity to the incoming nucleophile.

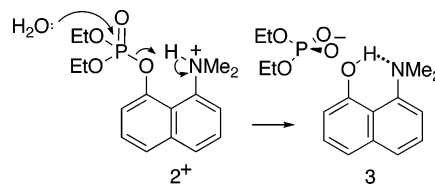
We concluded previously² that the transition state for the intramolecular general acid catalyzed hydrolysis of the diethyl triester **2⁺** differs significantly from that expected for a simple triester hydrolyzing at a similar rate, and the same is true for the reaction of **1⁻** compared with that of a simple phosphate monoester. The trend is the same in the two cases—more bond formation (later TS) from the nucleophile is needed to generate the negative charge on the leaving group needed to support a strong hydrogen bond and thus efficient proton transfer catalysis

Scheme 1. Concerted Dissociative Mechanism for the Hydrolysis of the Monoester **1⁻**.^{a,1}



^a Catalysis by the neighboring NH^+ group in this and related systems discussed in this paper is far too efficient for catalysis by external general acids to be detectable.

by the Me_2NH^+ group. In the case of the triester (**2⁺**) the intramolecular hydrogen bond is not present in the reactant.



Displacements at the phosphorus center of a triester may involve pentacovalent addition intermediates: those at the phosphorus center of a phosphate dianion (e.g., **1⁻**) are primarily dissociative, and will not,^{1,3} and the concerted mechanism expected for the latter system has for this reason more potential for effective synergy between the bond formation and bond breaking processes. In practice the accelerations occasioned by

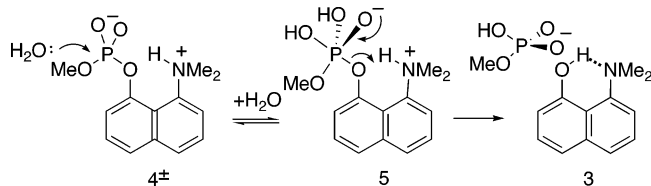
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Scheme 2. Possible Stepwise Mechanism for the Hydrolysis of Phosphodiester **4**[±]

the introduction of the dimethylammonium group are similar for triester and monoester, at least in part because of the significant extent of hydrogen bonding in the reactant monoester. This is substantial, as shown by the raised pK_a of the dimethylammonium group of **1**⁻, which is some 4.7 units higher than that of **2**⁺.¹ Our analysis¹ suggested that the expected combination of lower absolute reactivity, reduced reactant-state hydrogen bonding, and higher sensitivity to the nucleophile could support more effective synergy, and thus rate enhancements, in diesters derived from **1**—where efficient general acid catalysis of leaving group departure might tip the balance from a stepwise (Scheme 2) to a concerted mechanism. We report results for the methyl diester **4**, and compare them with those for the reactions of the mono- and triesters **1** and **2**.

Experimental Section

General. The hydroxylamines (as their hydrochlorides), other nucleophiles, and inorganic salts were of the highest purity available and were used as purchased.

¹H and ³¹P NMR spectra were recorded on a BRUKER Avance AM 250 spectrometer at 300 K, operating at 250 and 101 Mhz for ¹H and ³¹P, and using tetramethylsilane and 85% H₃PO₄ as internal and external standards, respectively. Chemical shifts are reported in δ (ppm). ESI-MS were recorded using a Fisons Instrument VG Platform II (note that this is a low resolution instrument). All organic solvents used were dried very carefully, and all reactions were carried out under strictly anhydrous conditions, under argon.

Dimethyl-(8-dimethylamino)-1-naphthyl Phosphate. To a stirred solution of 8-dimethylamino-1-naphthol (2 mmol) in 10 mL of dry dichloromethane (DCM) were added 1.3 equiv of pentane-washed sodium hydride in 2 mL of DCM. After 30 min 1.2 equiv of dimethyl phosphorochloridate in 7 mL of DCM was added dropwise to the resulted suspension, and the mixture was stirred for a further 20–30 min until reaction was complete (RP-HPLC, TLC). Then ca. 5 mL of dry silica gel, for direct preadsorption, was added to the reaction mixture, which was evaporated and chromatographed (silica; EtOAc/hexane, 7:3, $R_f = 0.25$) to yield the phosphate triester in 60–70% yield. ¹H NMR (DMSO-*d*₆, 250 MHz): 7.74 (d, 1H, $J = 8.1$ Hz), 7.54 (d, 1H, $J = 8$ Hz), 7.44 (q, 2H, $J = 3$ and 10.8 Hz), 7.3 (dt, 1H, $J = 7.6$ Hz), 7.12 (d, 1H, $J = 6.4$ Hz), 3.8 (d, 6H, $J = 11.3$ Hz, OCH₃), 2.74 (s, 6H, N(CH₃)₂). ESI-MS (ESI+): calcd. for C₁₄H₁₈NO₄P [M] 295.27; found 295.8 (100%), 317.8 (81.6%, +Na), and 613.1 (35.7%, dimer).

Methyl 8-Dimethylamino-1-naphthyl Phosphate 4. A saturated solution of NaI in acetone (10 mL) was added to the triester prepared above, and the resulting slightly yellow mixture refluxed for 30–40 min until the starting material had disappeared. After evaporation the solid residue was dry extracted (ultrasonic bath) with dry DCM (3 × 15 mL) and centrifuged. The combined supernatants were evaporated, and the solid obtained was redissolved in water (5 mL) and passed through a short RP(C18) column. Evaporation of the water gave the diester **4** as the sodium salt in almost quantitative yield. (Any remaining NaI could be extracted with acetonitrile, in which the sodium salt of the diester is insoluble.) ¹H NMR (DMSO-*d*₆, 250 MHz): 7.55 (dt, 1H, $J = 7.5$ and 1.2 Hz), 7.4–7.2 (m, 4H), 6.86 (dd, 1H, $J = 7.4$ and

Table 1. Rate Constants (s⁻¹) as a Function of pH for the Hydrolysis of Methyl 8-Dimethylamino-1-naphthyl Phosphate (**4**) in Water, at 60 °C and Ionic Strength 1.0 M (KCl)

in the pH region		in strong acid		
pH ^a	k_{obs} s ⁻¹	[HCl], M	$k_{obs}/$ HCl	$k_{obs}/$ DCI
0.30	1.00×10^{-5}	0.5	1.00×10^{-5}	5.11×10^{-6}
0.60	9.27×10^{-6}	1.0	2.83×10^{-5}	1.52×10^{-5}
1.00	8.83×10^{-6}	1.5		2.56×10^{-5}
1.60	8.69×10^{-6}	2.0	6.30×10^{-5}	4.13×10^{-5}
2.05	8.72×10^{-6}	2.5		5.33×10^{-5}
2.48	8.79×10^{-6}	3.0	1.07×10^{-4}	8.59×10^{-5}
2.97	8.68×10^{-6}	3.5		1.21×10^{-4}
3.50	8.66×10^{-6}	4	1.75×10^{-4}	
3.99	8.81×10^{-6}	6	3.18×10^{-4}	
4.50	8.86×10^{-6}	8	4.38×10^{-4}	
4.98	8.87×10^{-6}	10	4.20×10^{-4}	
5.48	8.73×10^{-6}	12	3.17×10^{-4}	
6.10	8.19×10^{-6}			
6.5	7.08×10^{-6}			
6.75	7.01×10^{-6}			
6.80	6.71×10^{-6}			
7.00	5.35×10^{-6}			
7.98	2.72×10^{-6}			

^a Buffers, etc., used: for pH (calculated) < 2, HCl; pH 3.0–4.0, formate; pH 4.0–5.5, acetate; pH 5.5–7.0, bis-Tris; pH 7.5–9.0, TRIS; pH 9.0–10, borate; pH > 10, NaOH.

1.1 Hz), 3.48 (d, 3H, $J = 10.8$ Hz, OCH₃), 2.76 (s, 6H, N(CH₃)₂). ³¹P NMR (DMSO) -1.69. ESI-MS (ESI+): calcd. for C₁₃H₁₅NNaO₄P [M⁻ + Na] 303.23; found 281.9 (17.6%, M), 303.9 (100%), 585.2 (8.7%, dimer -Na) and 607.1 (4.4%, dimer). ESI-MS (ESI-): calcd. for C₁₃H₁₆-NO₄P [M] 281.24; found 279.9 (100%, M⁻), 280.8 (16%), and 583.2 (3.6%, dimer).

Kinetics. Reactions were started by adding 100 μ L of a stock solution of the substrate (3.1×10^{-3} M) in water to 3 mL of reaction mixture containing a large excess (up to 1 M) of the nucleophile, assuring pseudo-first-order conditions. Strict first-order kinetics were observed for the reactions of the substrate, and reactions with nucleophiles were first order in both substrate and nucleophile. Reactions were followed at 60.0 °C for at least five half-lives, by monitoring the appearance of 8-dimethylamino-1-naphthol at 320 nm on diode-array spectrophotometers, equipped with thermostated cell holders. The pH (pD) of the reaction mixture was measured at 60.0 °C at the end of each run, using a digital pH-meter equipped with a combined electrode (with integrated reference electrode) and a Pt 1000 temperature sensor. (The pH-meter was calibrated at 60 °C with the appropriate buffers following the procedure of Bates.⁴)

Observed first-order rate constants (k_{obs}) were calculated by nonlinear least-squares fitting of the absorbance vs time curve, and correlation coefficients were always better than 0.999. Second-order rate constants were obtained by linear regression analysis from slopes of plots of the observed first-order rate constants against the concentration of the nucleophile. Solutions of hydroxylamine nucleophiles were self-buffered between pH 5.5 and 7.0 (standard aqueous NaOH (0.1 M; Merck) was added to aqueous hydroxylamine hydrochloride). Other buffers used are detailed in Table 1.

Products of the Reaction. The single detectable organic product was 8-dimethylamino-1-naphthol, identified by its UV–visible absorption and NMR spectrum. The initial phosphorus-containing product of attack by a nucleophile Nu will be Nu(MeO)PO₂⁻, and MeOPO₂-F was identified as the product of the reaction of **4** with KF by ESI(+)-MS and ESI(+)-MS/MS from the ion at $m/z = 115$. Hydroxylamine and its N-methylated derivatives are expected to act as oxygen

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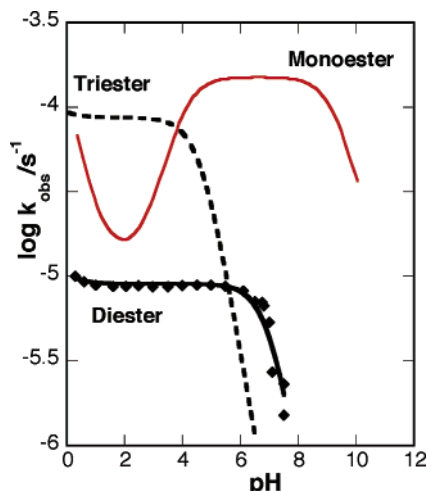
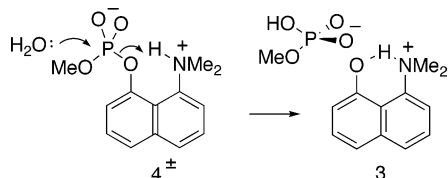


Figure 1. Rate profile for the hydrolysis of diester **4** in the pH-region, compared with profiles for the corresponding mono- and triesters **1** and **2** (data from Table 1, in water at 60 °C and ionic strength 1.0 M (KCl)). The points are experimental, the curves calculated.

Scheme 3. Mechanism Proposed for the pH-Independent Hydrolysis of **4[±]**



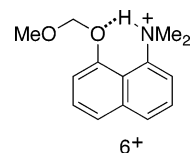
nucleophiles, since hydroxylamine is known to react in this way with (bis)-2,4-dinitrophenyl phosphate (an ester of similar reactivity to **4**).⁵

Results and Discussion

Hydrolysis of Methyl 8-Dimethylamino-1-naphthyl Phosphate **4.** The pH-rate profile for the hydrolysis of the diester **4** (Figure 1) shows a plateau between pH 1–7, defined by an apparent pK_a of 7.06 ± 0.06 , and the beginnings of an apparent acid catalyzed reaction at low pH. It is closely similar to that of the more reactive triester **2**, with the plateau defined in both cases by a single ionization in the pH-independent region. The corresponding plateau in the pH-rate profile for the hydrolysis of the monoester **1** is defined by two apparent pK_a s of 3.94 and 9.31. These were identified¹ as the (substantially shifted) ionizations of the phosphate group of **1[±]** and the dimethylammonium group of **1[±]**, respectively. Careful spectrophotometric titration of **4** shows a single pK_a of 6.96 ± 0.06 at 60 °C and ionic strength 1.0 M, which we can assign unambiguously to the dimethylammonium group of **4[±]**. (Measurements at 25.0 °C give pK_a values of 7.48 ± 0.01 (potentiometric) and 7.44 ± 0.02 (spectrophotometric).)

The pK_a of this group shifts remarkably (Figure 1) with the changing state of ionization of the neighboring phosphate group: from 4.63, a figure normal for a tertiary naphthylamine, for the triester, to 9.31 for the monoester **1[±]**. The figure for the diester **4** lies in between. We have discussed previously¹ why we consider that this variation depends primarily not on electrostatic interactions but on the strength of the intramolecular $O \cdots H-N^+$ hydrogen bond in **1[±]**, which increases with the

increasing basicity of the bridging ($P-O-Ar$) oxygen. In particular, the pK_a of the dimethylammonium group of **4[±]** is similar to that (7.40 at 65 °C) of the acetal **6**, with a neutral neighboring group, which is also hydrolyzed with relatively efficient intramolecular general acid catalysis (acceleration 10^{3-4} -fold).⁴



The hydrolysis of **4[±]** is only 18 times slower than that of the monoester **1[±]**. Phosphodiester esters are well known to be the least reactive esters of phosphoric acid; for example, the spontaneous hydrolysis of 2,4-dinitrophenyl phosphate dianion (hydrolyzed at a rate similar to that of monoester **1[±]**) is over 1000 times faster than that of the corresponding methyl 2,4-dinitrophenyl diester.^{6,7} Efficient intramolecular general acid catalysis reduces the difference in rate, because catalysis is more efficient for the diester. The acceleration due to general acid catalysis by the dimethylammonium group of **4[±]** is 2.56×10^6 at 60 °C, greater by an order of magnitude than that (2.4×10^5 at 60 °C) found for the monoester **1[±]**.¹ The effective pK_a of the leaving group is 3.6, similar to the value estimated for the triester **2[±]**.²

The simplest mechanism consistent with the pH-rate profile in the region pH 1–8 is outlined in Scheme 3. We have no evidence to implicate the involvement of a pentacoordinate addition intermediate, and any such species would be very short-lived. The solvent deuterium isotope effect k_{H_2O}/k_{D_2O} for the spontaneous hydrolysis of **4[±]** at pH 5.1 on the plateau (Figure 1) is 1.72 ± 0.14 . Values in this region are typical for general acid catalysis in these systems; relevant examples are the following: for the hydrolysis of the monoester **1[±]**, 1.46 ± 0.04 ;¹ for the hydrolysis of the triester **2[±]**, > 1.61 ;² and for that of the acetal **6[±]** 1.69.⁸ The simplest explanation is that neither bonding to nucleophilic water nor proton transfer from the general acid is far advanced in the transition state. (An alternative possibility is that the contribution from the in-flight proton (of the general acid) is actually inverse, if, as we believe, the intramolecular hydrogen bond is actually stronger in the transition state than in the reactant in these systems.)

Hydrolysis in Strong Acid. Hydrolysis under strongly acidic solutions, from 0.5 to 12 M HCl or DCl, shows a rate profile quantitatively similar, as might be expected, to that for the hydrolysis of the monoester **1[±]** and qualitatively similar to those observed for the acid hydrolyses of many esters and amides. Rate constants increase with acid concentration up to a maximum at about 8 M HCl or DCl (Table 1), before falling in very strong acid. This is expected behavior for a substrate which is reactive as the conjugate acid, but becomes fully protonated at high acid concentrations. The decrease in rate in very strong acid is accounted for by the decreasing activity of water, which acts as a nucleophile in the reaction.

Consistent with this picture, the data in the H_0 region fit eq i with $k_1 = 9.90 \times 10^{-4} \text{ s}^{-1}$ and a pK_a of -1.93 (corresponding

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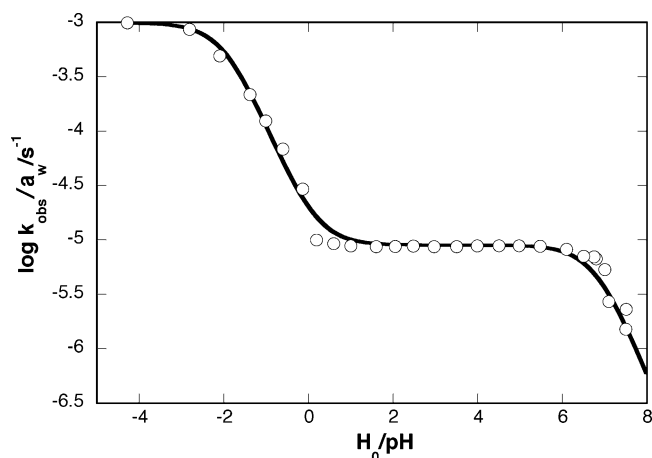
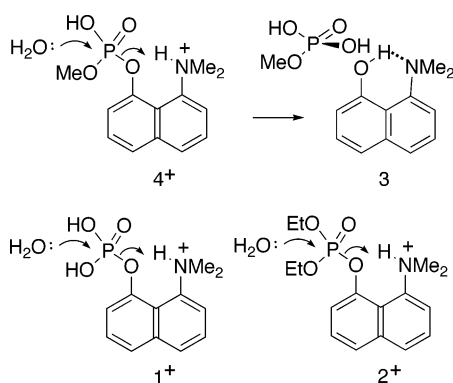


Figure 2. Full H_0 /pH-rate profile for the hydrolysis of diester **4**, at 60 °C. Data in the H_0 region are corrected for the activity of water (k_{obs}/a_w); see the text. The first pK_a corresponds to half-protonation at 5.6 M HCl.

Scheme 4. Suggested Common Mechanism for the Hydrolysis of the Cognate Cations 4^+ , 1^+ , and 2^+ under Strongly Acidic Conditions^a



^a Compound **3**, the non-phosphorus containing product in each case, will be protonated instantly under the conditions.

to half-protonation in 5.6 M HCl). Activities for water and HCl are based on the data (at 25 °C) of Randall and Young.⁹

$$k_{\text{obs}} = k_1 a_w \chi_{+} = k_1 a_w a_{\text{H}^+} / (a_{\text{H}^+} + K_a) \quad (\text{i})$$

Figure 2 shows the complete H_0 /pH-rate profile for the hydrolysis of **4**, corrected for the activity of water in strong acid. Plateaus below $H_0 = -3$ and from pH 1–7 correspond to the spontaneous hydrolyses of the two ionic species 4^+ and 4^\pm , with ionizations at $H_0 = -1.93$ and pH = 7.06, assigned unambiguously to the PO(OMe)OH and Me_2NH^+ groups, respectively. Both pK_a s are shifted by some 2.5 units, down and up, respectively, with respect to comparable free phosphoric acid ester and tertiary ammonium groups: a consequence, as discussed above, of the stabilization of the zwitterion 4^\pm by the rather strong hydrogen bond.

It seems certain that the apparent acid catalyzed hydrolysis of the zwitterionic diester 4^\pm actually represents the kinetically equivalent spontaneous hydrolysis of the cation 4^+ , by the mechanism shown in Scheme 4. Compound 4^+ is not expected to undergo further protonation on the neutral phosphoric acid group (next to the positively charged group in the *peri*-position) in any but very strong acid (acid catalysis of the hydrolysis of

Table 2. Second-Order Rate Constants for the Reactions of 4^\pm with Nucleophiles, at 60 °C and Ionic Strength 1.0 M

nucleophile	pK_a	pH	$k_{\text{Nu}} / \text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$
water	-1.74	5.5	1.60×10^{-7}
fluoride	3.17	6.3	1.16×10^{-4}
formate	3.75	6.3	1.31×10^{-5}
NH_2OMe	4.62	5.5	1.71×10^{-5}
acetate	4.81	6.3	1.20×10^{-5}
$\text{NH}_2\text{CH}_2\text{CF}_3$	4.86 ^a	5.5, 6.5	2.29×10^{-5}
$\text{Me}_3\text{N}^+\text{-O}^-$	4.99 ^a	5.9	3.52×10^{-5}
NMe_2OH	5.20	6.8	4.11×10^{-4}
NH_2OH	5.64 ^a	5.5	9.17×10^{-4}
NHMeOH	6.18	6.8	1.43×10^{-3}
phosphate	6.51	6.3	4.22×10^{-5}

^a pK_a s measured at 60 °C and ionic strength 1.0 M.

the triester 2^+ is not observed above pH 0). So the appropriate comparison is with the spontaneous rather than the acid catalyzed hydrolysis of a typical phosphodiester. For neutral diester acids this reaction is normally masked by the acid catalyzed reaction, so the more convenient comparison is with analogous phosphotriesters, where the spontaneous reaction can be observed well into the pH region (Figure 2).

In terms of reactivity 4^+ is effectively an activated triester, and it is interesting to compare the reactivities of the three different species 4^+ , 1^+ , and 2^+ , all of which we believe react by the same mechanism (Scheme 4). We noted previously¹ that the pH-independent hydrolysis of the diethyl triester 2^+ is 33.6 times slower than that of the phosphoric acid 1^+ , and this was attributed to “a combination of electronic and steric effects resulting from the replacement of the two OH protons of 1^+ by ethyl groups.” The data for 4^+ , which reacts 3.16 times more slowly than 1^+ , are consistent with this assignment and suggest that steric effects ($\text{Et} > \text{Me} > \text{H}$) are probably the more important. The pK_a s of the PO(OMe)OH and PO(OH)₂ groups of 4^+ and 1^+ are similar, with ionizations at $H_0 = -1.93$ and -1.48 , respectively. These values fall in the expected region for aryl phosphoric acid mono- and diesters, when the stabilization of the (zwitterionic) conjugate base by the hydrogen bond to the dimethylammonium group is taken into account. (For comparison, the first pK_a of phenyl phosphoric acid is 0.48.¹⁰)

Reactions with Nucleophiles. We have investigated the reactions of 4^\pm with a representative range of nucleophiles and report more detailed study of the reaction with the α -effect nucleophile hydroxylamine.

Data for the full set of nucleophiles studied (Table 2) are displayed in the form of a Brønsted plot in Figure 3. Fluoride shows its usual rate enhancement^{11–13} compared with oxygen nucleophiles, reacting as fast as expected for a hydroxylamine of similar pK_a . The point for formate also shows a (smaller) positive deviation from the line correlating reaction of oxygen nucleophiles. This is not unusual for attack on phosphate phosphorus;^{11,13} it is the result (we suggest¹⁴) of a combination of steric and electrostatic effects specific to nucleophilic attack (as opposed to general base catalysis).

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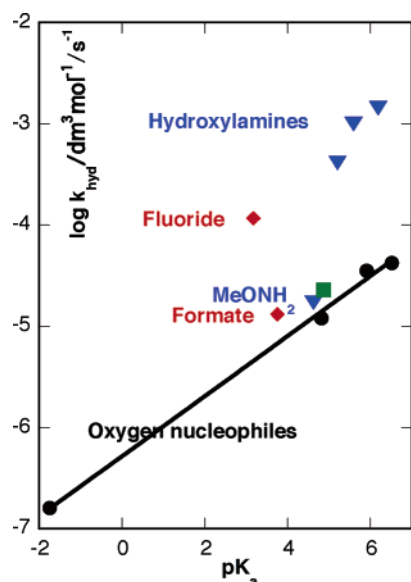


Figure 3. Brønsted plot for catalysis of the hydrolysis of 4^\pm by nucleophiles, in aqueous solution at 60° . Points generating the line are for water, acetate, trimethylamine *N*-oxide, and phosphate dianion (\bullet). Data for fluoride and formate (\blacklozenge), 2,2,2-trifluoroethylamine (\blacksquare), and hydroxylamine and its various methylated derivatives (\blacktriangledown) are discussed in the text. Data from Table 2.

Table 3. Brønsted Coefficients β_{nuc} for the Reactions of Nucleophiles with Phosphate Esters

	dimethylaminonaphthol derivatives		simple 2,4-dinitrophenyl esters	
	oxygen nucleophiles		oxygen nucleophiles	amine nucleophiles
ArOPO_3^{2-}	(1^-)	0.19 ± 0.01	— ^a	0
$\text{ArOP}(\text{OMe})\text{O}_2^-$	(4^\pm)	0.29 ± 0.01	0.31^b	0.31^b
$\text{ArOPO}(\text{OR})_2$	(2^+)	0.49 ± 0.03	$0.30^c, 0.32^d$	0.61^{12}

^a Reactions with oxyanions not observed.¹⁵ ^b Data from Kirby and Younas.¹³ ^{c,d} Data for reactions with oxygen nucleophiles of 2,4-dinitrophenyl phosphotriesters of 1,3-propane-diol and ethanol, taken from Khan¹² and Hay,¹⁶ respectively.

The slope of the line drawn in Figure 3 (i.e., the Brønsted coefficient β calculated for the reactions with oxygen nucleophiles) is 0.30 ± 0.01 . This is the same, within experimental error, as that for their reactions with methyl 2,4-dinitrophenyl phosphate (see Table 3).¹⁵

We now have Brønsted coefficients β_{nuc} for the reactions of oxygen nucleophiles with all three phosphate ester derivatives 4^\pm , 1^- , and 2^+ and can compare them with the same parameters for the reactions of corresponding 2,4-dinitrophenyl esters, which are activated to similar extents by virtue of the good leaving group and react at a similar rates to 4^\pm , 1^- , and 2^+ . β_{nuc} increases in the order monoester < diester < triester, as observed also for the reactions of simple esters of 2,4-dinitrophenol with neutral (amine) nucleophiles (Table 3). The reactions of 2,4-dinitrophenyl esters with oxygen nucleophiles are different: oxyanions do not react with the dianion of the 2,4-dinitrophenyl phosphate monoester at a measurable rate, so no data are available. β_{nuc} is the same for reactions of 2,4-dinitrophenyl di- and triesters, and it is the same also as that for the reaction with oxygen nucleophiles of the diester

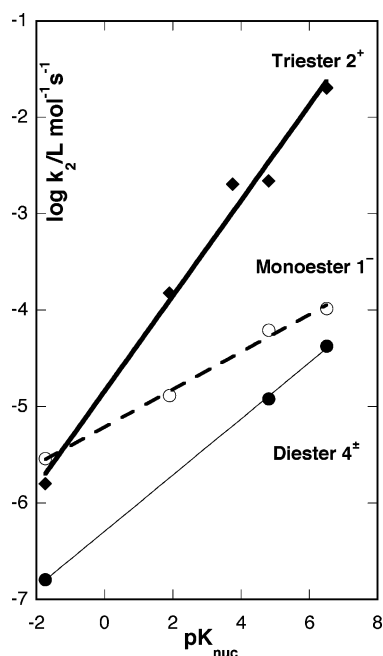


Figure 4. Brønsted plots compared for the reactions of oxygen nucleophiles with phosphate mono-, di-, and triesters 1^- , 4^\pm , and 2^+ , respectively, at 60°C and ionic strength 1.0 M. Data from Table 3 and refs 1 and 2.

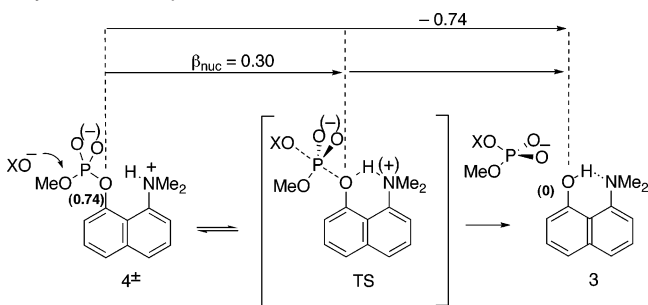
4^\pm . This has been explained¹⁵ in terms of the changing balance between the strength of the bond being formed and electrostatic repulsion for the anion–anion reactions.

Intramolecular general acid catalysis by the dimethylammonium group (Scheme 3, above) involves proton transfer to the oxygen atom of a leaving group which is initially relatively poor. Since Brønsted β_{nuc} values are greater for the reactions of phosphate triesters with poorer leaving groups,¹² it is reasonable to expect values to be greater for reactions with 4^\pm , 1^- , and 2^+ than for the reactions of the corresponding 2,4-dinitrophenyl esters, and this is what is observed for the mono- and triesters 1^- and 2^+ (Table 3). However, this is not the case for the reactions of the diester 4^\pm . It is important to understand this difference because the efficiency of intramolecular general acid catalysis, and its potential for synergy with nucleophilic catalysis, will depend on the increase in negative charge on the leaving group oxygen as bonding to the incoming nucleophile develops.

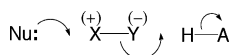
Figure 4 compares the reactivities of the mono-, di-, and triesters 1^- , 4^\pm , and 2^+ toward oxygen nucleophiles. The diester is the least reactive, as expected (though to an extent that depends on the leaving group). The higher sensitivity of the reactions of the monoester to leaving group basicity makes it the most reactive toward spontaneous hydrolysis, though it is several hundred times less reactive than the triester toward an oxygen nucleophile with a $\text{p}K_a$ of 7. The higher sensitivity to the basicity of the nucleophile predicts that the diester will react as fast as the monoester with a (hypothetical) oxygen nucleophile of $\text{p}K_a$ 11.15. A lesson reinforced by Figure 4 is that general acid catalyzed reactions of phosphate mono- or diesters with strongly basic oxyanion nucleophiles (like those derived from a serine oxygen or a bound water molecule in an enzyme active site) will be fastest when their negative charges are neutralized by protonation (or, presumably, by coordination to a metal cation).

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Scheme 5. Effective Charge Map for the Reaction of 4^\pm with an Oxyanion Nucleophile

Synergy between Nucleophilic Attack and General Acid Catalysis. We began this series of investigations with the proposition that “a logical way to break a very strong bond efficiently is to reinforce its natural polarity by the concerted application of complementary catalytic groups, to the point where cleavage becomes kinetically favorable”.²



Mechanisms of this sort, in which nucleophilic attack is concerted with proton transfer from a general acid, have often been written for enzyme catalyzed reactions, but it has proved difficult to model such mechanisms in simple systems. Fundamental problems are that the necessary three-body collision is entropically unfavorable outside an enzyme active site and that general acid catalysis is intrinsically inefficient even in most intramolecular situations.^{17,18} So realistic candidate models have to be based on one of the handful of systems supporting efficient intramolecular general acid catalysis. Of these only derivatives of 8-dimethylaminonaphthol have proved generally successful, with phosphodiester 4^\pm the most efficient. So the transition state for its reactions with nucleophiles is of special interest. Some relevant calculations are presented below, but a simple analysis of effective charge is also illuminating (Scheme 5).

The effective charge on the leaving group oxygen of a typical aryl methyl phosphate anion decreases from +0.74 to -1.0 on displacement by an oxyanion.^{19,20} The sum of β_{nuc} and $-\beta_{\text{LG}}$ (LG = leaving group), ca. 1.4 compared with $\beta_{\text{eq}} = 1.74$, suggests a significant build up of negative charge on the central PO_3^- group in the transition state. The leaving group in the reaction of 4^\pm is the neutral naphthol **3**, giving $\beta_{\text{eq}} = 0.74$. We do not know $-\beta_{\text{LG}}$, but since β_{nuc} is 0.3 it must be 0.44, less any build up of negative charge on the central PO_3^- group in the transition state. Any such charge build up is likely to be small: in the calculation described below for the reaction of 4^\pm with hydroxylamine the P–OAr bond is lengthened by 21% on going to the transition state, but the length of the P–O⁻ not involved in hydrogen bonding is unchanged. This is consistent with a progressive transfer of charge density from the breaking P–OAr bond to HN^+ , and coordinates $\alpha_{\text{Nu}} = 0.41$ and $\alpha_{\text{LG}} = 0.59$ in the More O’Ferrall–Jencks plot and thus a close-to-synchronous mechanism.

Table 4. Thermodynamic Parameters for the Reactions of 4^\pm with Nucleophiles, at 60 °C and Ionic Strength 1.0 M

nucleophile	ΔH		ΔS	
	kJ mol^{-1}	kcal mol^{-1}	$\text{J K}^{-1} \text{mol}^{-1}$	$\text{cal K}^{-1} \text{mol}^{-1}$
water	98.0 ± 5.2	23.4 ± 1.2	-50.3 ± 15.2^a	-12.0 ± 3.6^a
formate	79.4 ± 8.0	19.0 ± 1.9	-97.7 ± 23.2	-23.3 ± 5.5
fluoride ^b	83.4	19.3	-78.8	-18.8
NH_2OH	68.0 ± 7.4	16.2 ± 1.8	-104 ± 22	-24.8 ± 3.1

^a ΔS^\ddagger calculated on the basis of first-order rate constants. Using second-order rate constants figures are reduced by $33.4 \text{ J (8.0 cal) mol}^{-1}$. ^b Based on measurements at only two temperatures.

Synergy between nucleophilic and general acid catalysis^{2—}catalysis greater than the sum of the parts—will be apparent when the effective molarity (ref 17) for one or both of the intramolecular reactions is increased when they operate on the same group in the same molecule.^{21,22} The close-to-synchronous mechanism described in Scheme 5 offers close to optimum conditions for synergy, making a phosphodiester based on 4^\pm , with the nucleophile built in a target of interest. Work on candidate systems is in hand.

Thermodynamic Parameters. Thermodynamic parameters measured for the reactions of four representative nucleophiles with diester 4^\pm are summarized in Table 4. Entropies of activation fall in the range expected for bimolecular reactions.²³ Fluoride ion in particular behaves normally, so that the unusual values observed for its reaction with the monoester **1**⁻¹ seem to be specific to the anion–dianion interaction.

Reaction with Hydroxylamine. The α -effect nucleophiles are of special interest because of their high reactivity toward phosphorus, making them potential reagents of choice for the deactivation of organophosphorus poisons and chemical warfare agents.²⁴ Hydroxylamine is also of particular mechanistic interest because it is known to react with phosphate phosphorus through oxygen, rather than through its more basic nitrogen center.^{5,25}

Figure 5 shows the pH-rate profile for the reaction of 4^\pm with 0.5 M total hydroxylamine over a pH range that includes the pK_a s of both nucleophile and substrate, compared with the reaction in D_2O . The curve follows the expected bell-shape,¹ with a rate maximum in the region where the nucleophile is present as the free base and the substrate as the zwitterion 4^\pm , with the NMe_2 group protonated. The pH-rate constant profile was fitted to eq ii, where $k_{\text{obs,w}}$ is the rate constant for the water reaction, k_2 the second-order rate constant for the reaction of the zwitterion 4^\pm with neutral hydroxylamine, and $\chi_{\text{NH}_2\text{OH}}$ and χ_z the mole fractions of the two reactive species. ($[\text{NH}_2\text{OH}]_{\text{tot}}$ here represents the total concentration of hydroxylamine and its conjugate acid.) In practice catalysis by hydroxylamine is so strong that the contribution from the water reaction is not significant.

$$k_{\text{obs}} = k_{\text{obs,w}} + k_2 [\text{NH}_2\text{OH}]_{\text{tot}} \chi_{\text{NH}_2\text{OH}} \chi_z \quad (\text{ii})$$

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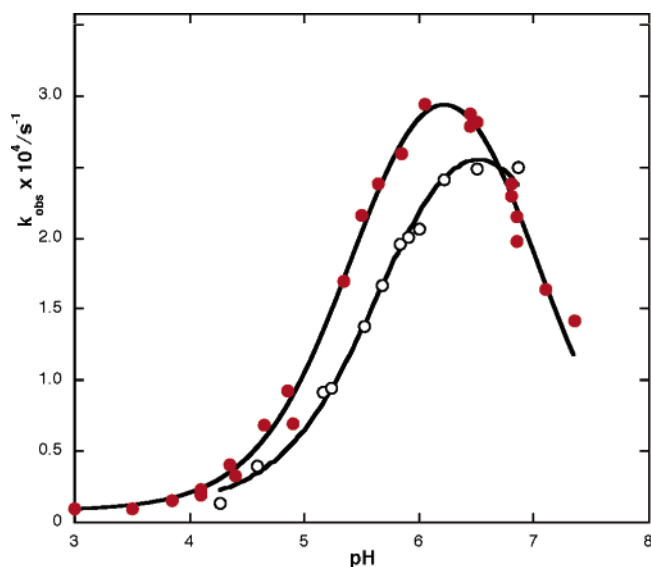
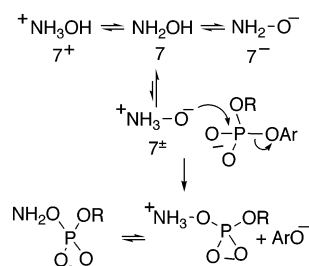


Figure 5. pH-rate profile for the reaction of diester 4^\pm with 0.5 M (total) hydroxylamine in H_2O (●) and D_2O (○). (Data in water at 60 °C and ionic strength 1.0 M (KCl) from Table 5). The points are experimental, the curves calculated (see the text).

Scheme 6



The least-squares fit gives second-order rate constants for the reaction of the free base $k_2(\text{NH}_2\text{OH}) = 8.30 \pm 0.12 \times 10^{-4}$ and $6.80 \pm 0.10 \times 10^{-4} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, for the reactions in H_2O and D_2O , respectively, and thus a solvent deuterium isotope effect of 1.22 ± 0.11 . (A second-order plot at pH 5.5, using 0.2–1.0 M hydroxylamine gave $k_2(\text{NH}_2\text{OH}) = 8.60 \pm 0.14 \times 10^{-4} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$.) The $\text{p}K_{\text{a}}$ s used for (the conjugate acid, NH_3^+OH) of hydroxylamine and the substrate 4^\pm , respectively, were 5.47 ± 0.05 and 6.96 ± 0.05 for the reaction in H_2O (consistent with our direct measurements of $\text{p}K_{\text{a}}$ s at 60 °C and ionic strength 1.0 M); and 5.67 ± 0.09 and 7.36 ± 0.16 for the reaction in D_2O . ($\text{p}K_{\text{a}}$ s in D_2O are higher by (0.2 ± 0.1) , as expected.^{26–28})

Reactions with other hydroxylamines show the usual pattern of reactivity: hydroxylamine and its *N*-methyl and *N,N*-dimethyl derivatives show an apparent α -effect, significantly greater than observed with monoester 1^- ; but the *O*-methyl derivative, which cannot react through oxygen, is 54 times less reactive than hydroxylamine itself (compared with 7 times when the two reactions with the monoester 1^- are compared). The reactivity of NH_2OMe is actually slightly lower than that of a primary amine of similar basicity (Table 2), consistent with hydroxylamine itself reacting through oxygen.

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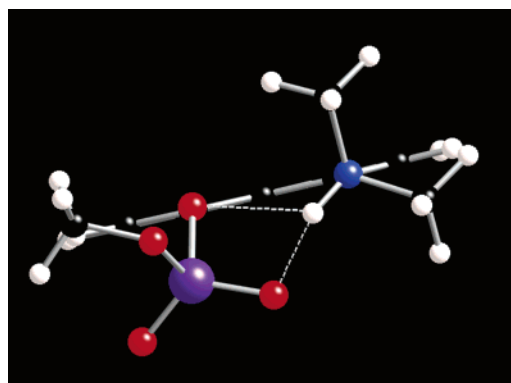


Figure 6. Calculated structure for 4^\pm , viewed perpendicular to the plane of the naphthalene ring. For details, see the text.

Calculations and the Mechanism of the Hydroxylamine

Reaction. The mechanism of the hydroxylamine reaction is a classical unresolved question in physical organic chemistry. NH_2OH is typically alkylated on nitrogen but often acylated²⁹ and generally phosphorylated⁵ on oxygen. *N*-Alkylated hydroxylamines also show enhanced reactivity in their reactions with such hard electrophiles, but NH_2OMe does not (as illustrated for example by the Brønsted plot of Figure 4).

The simplest explanation was proposed by Jencks,²³ who opined that “this extraordinary reactivity must almost certainly mean that ... the oxygen anion of the dipolar form ... is the reactive nucleophilic species.” (Scheme 6). However, there is no direct evidence for the presence of the zwitterionic tautomer 7^\pm in solution; and a small amount would in any case have to be highly reactive to support the observed enhanced rates of reaction.

We have shown recently³⁰ that the zwitterion 7^\pm is the preferred form of hydroxylamine in the crystal of its half hydrochloride. Ammonia oxide (7^\pm) is thus a stable species in the condensed phase when stabilized by a supporting network of hydrogen bonds, so potentially a stable species also in aqueous solution. In the absence of direct evidence for its existence in water we report calculations designed to test the mechanism of Scheme 6 in solution. Calculations were performed using the 6-31G(d) basis set in GAMESS-US (June 2005 version).³¹ Geometries of reactants, products, and transition state were optimized for solution in water using the conductor-like polarizable continuum solvation model (C-PCM).^{32,33}

The structure of the reactant phosphodiester 4^\pm is of interest because of the evidence, discussed above, for hydrogen bonding in solution between the phosphate group and the catalytic $\text{N}^+\text{—H}$ in the reactant. The calculated structure (Figure 6) shows the $\text{N}^+\text{—H}$ twisted out of the plane of the aromatic ring, with a torsion angle between the plane of the aromatic ring and the N—H bond of 33.2° . The MeOPO_2^- group is also out of plane, with the corresponding torsion angle $\text{C—C—O—P} = -105.2^\circ$.

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Table 5. Rate Constants for the Reaction of 4^\pm with Hydroxylamine as a Function of pH at Fixed Total ($[\text{NH}_2\text{OH}] + [\text{NH}_3^+\text{OH}] = 0.5 \text{ M}$) and as a Function of $[\text{NH}_2\text{OH}]$ at $\text{pH} = 5.5^a$

fixed total ($[\text{NH}_2\text{OH}] + [\text{NH}_3^+\text{OH}]$)				varying $[\text{NH}_2\text{OH}]$			
pH	k_{obs} , s^{-1}	pH	k_{obs} , s^{-1}	pD	k_{obs} , s^{-1}	$[\text{NH}_2\text{OH}]$, M	k_{obs} , s^{-1}
0.5	1.30×10^{-5}	5.35	1.70×10^{-4}	4.27	1.31×10^{-5}	0	8.77×10^{-6}
1.0	8.95×10^{-6}	5.50	2.16×10^{-4}	4.59	3.99×10^{-5}	0.2	8.25×10^{-5}
1.50	7.94×10^{-6}	5.65	2.39×10^{-4}	5.17	9.15×10^{-5}	0.4	1.49×10^{-4}
2.50	9.48×10^{-6}	5.85	2.59×10^{-4}	5.24	9.48×10^{-5}	0.6	2.30×10^{-4}
3.0	9.95×10^{-6}	6.05	2.94×10^{-4}	5.53	1.38×10^{-4}	0.8	3.13×10^{-4}
3.50	1.00×10^{-5}	6.45	2.87×10^{-4}	5.68	1.66×10^{-4}	1.0	3.88×10^{-4}
3.85	1.60×10^{-5}	6.45	2.79×10^{-4}	5.84	1.96×10^{-4}		
4.10	2.31×10^{-5}	6.50	2.81×10^{-4}	5.91	2.01×10^{-4}		
4.10	1.90×10^{-5}	6.80	2.38×10^{-4}	6.00	2.06×10^{-4}		
4.35	4.03×10^{-5}	6.80	2.30×10^{-4}	6.22	2.41×10^{-4}		
4.40	3.26×10^{-5}	6.85	1.98×10^{-4}	6.5	2.49×10^{-4}		
4.40	3.28×10^{-5}	6.85	2.15×10^{-4}	6.87	2.50×10^{-4}		
4.65	6.80×10^{-5}	7.10	1.64×10^{-4}				
4.85	9.23×10^{-5}	7.35	1.43×10^{-4}				
4.90	6.98×10^{-5}						

^a Data at 60 °C and ionic strength 1.0 M (KCl).

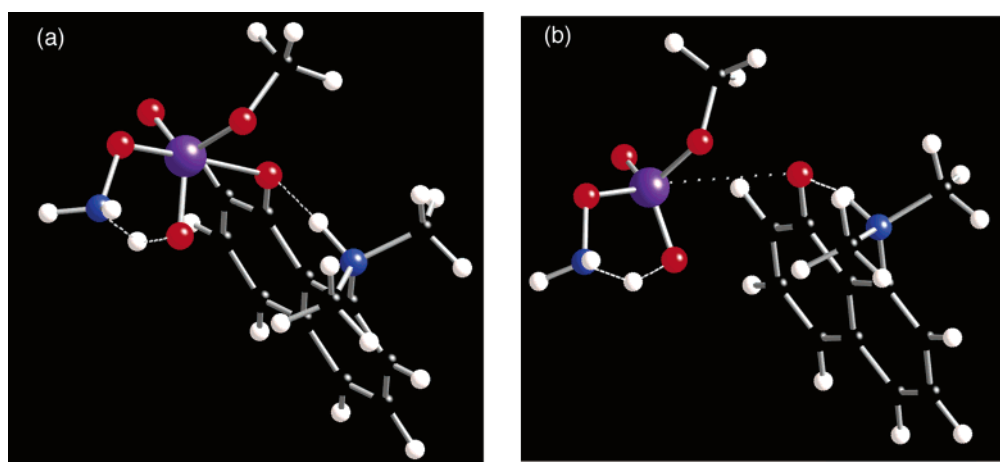
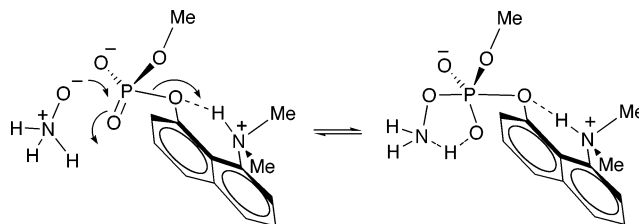


Figure 7. (a) Structure of the transition state for the reaction of 4^\pm with hydroxylamine (ammonia oxide tautomer 7^\pm). (b) The developing product of the reaction, with P–O (leaving group) distance = 3.5 Å.

The calculated P–O (leaving group) bond length is 1.646 Å, the $\text{N}^+\text{H}\cdots\text{O}(\text{P})$ distance 2.763 Å and the N–H–O angle 118.9°. A second, apparently stronger hydrogen bond to a P–O[−] center has an $\text{N}^+\text{H}\cdots\text{O}^-$ distance of 2.847 Å and an N–H–O angle of 164.4°. We found closely similar results (using a continuum model) for the monoester 1^- ;¹ but in a higher level calculation, including discrete water molecules, each P–O[−] center was strongly solvated by two or three waters, and the intramolecular H-bond to the leaving group oxygen the stronger (though still out of the plane of the aromatic system). The evidence, discussed above, that the $\text{p}K_a$ of the phosphoric acid group of 4^\pm is lowered by the interaction with the neighboring Me_2NH^+ group (rather than raised, as it should be by a strong H-bond to a P–O[−] center) is consistent with suggestions that the continuum model can underestimate H-bonding solvation compared with intramolecular hydrogen bonding.^{34–36}

The calculated transition state for the reaction using ammonia oxide ($^+\text{NH}_3\text{O}^-$) as the nucleophile shows a well-defined, trigonal-bipyramidal structure, pentacovalent at phosphorus

Scheme 7



(Figure 7a), consistent with a concerted $\text{S}_{\text{N}}2(\text{P})$ associative mechanism. (We note that the initial interaction between 4^\pm and the oxygen center of neutral NH_2OH under the same conditions is repulsive.) The length of the developing P–O bond to the incoming nucleophile is 1.807 Å, already shorter than the P–O bond to the leaving group: which has lengthened from 1.646 to 2.008 Å. At the same time the intramolecular hydrogen bond has strengthened substantially: the $\text{N}^+\text{H}\cdots\text{O}(\text{P})$ distance has shortened to 2.609 Å, the N–H–O angle is now 150.2°, and the in-flight proton is now almost in plane (C–C–N–H torsion angle 3.5°).

This picture for the reaction at phosphorus is consistent with what we know about transition states for the reactions of

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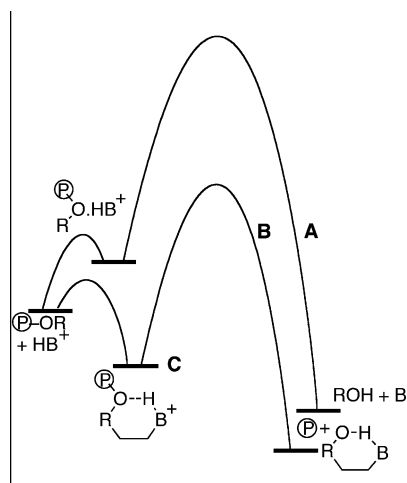


Figure 8. (Qualitative) energy profile diagram for the general acid catalyzed cleavage of a phosphate ester RO-P . Curve A: catalysis by an external general acid HA, or by a neighboring group HA not forming a significant hydrogen bond, in a conformationally flexible system. Curve B: catalysis by a neighboring general acid which makes a strong hydrogen bond to the leaving group oxygen even in the reactant.

nucleophiles with phosphate diesters.³⁷ Linear free energy relationships indicate transition states for reactions with bond breaking well advanced, but lesser amounts of bond formation to nucleophilic oxygen (β_{LG} and β_{nuc} are -0.97 and 0.31 , respectively, for the reactions of methyl 2,4-dinitrophenyl phosphate^{7,15}). Of particular interest is the incipient proton transfer from the H_3N^+ group of the nucleophile to a developing P-O^- center (Scheme 7), which is expected to be strongly basic in a phosphorane.³⁸ The two $\text{O}\cdots\text{H}$ distances are almost identical, at 1.233 and 1.221 Å, for $(\text{N})\text{O}\cdots\text{H}$ and $\text{H}\cdots\text{O}(\text{P})$, respectively (the $\text{N}\cdots\text{O}$ distance is 2.330 Å and the $\text{N}\cdots\text{H}\cdots\text{O}$ angle 143.49° for the proton being transferred in a five-membered ring).

We can follow this process along the reaction coordinate. Figure 7b shows the ensemble close to the end of the reaction, with the product structures readily recognizable. The P-O (leaving group) distance is 3.50 Å, the $\text{NH}_2\text{O-P}$ bond length 1.670 Å (compared with 1.571 Å for P-OMe) and the tetrahedral geometry around P distorted only by the need to accommodate the $\text{O}\cdots\text{H}\cdots\text{O}$ hydrogen bond (angle $\text{OPO} = 122.3^\circ$).

However, neither proton transfer is advanced much further: the proton from the H_3N^+ group of hydroxylamine is still about half-transferred (distances 1.259 and 1.225 Å for $(\text{N})\text{O}\cdots\text{H}$ and $\text{H}\cdots\text{O}(\text{P})$, respectively) and the naphthylammonium proton is still attached to nitrogen (the $\text{N}^+\text{H}\cdots\text{O}(\text{P})$ distance is a little shorter, at 2.548 Å, the N-H-O angle is now 154.5° , and the in-flight proton is a little closer to the plane of the aromatic system (C-C-N-H torsion angle 1.7°). This is consistent with our conclusion that the hydrogen bond is strongest in the transition state.

Conclusions

Hydroxylamine as an Oxygen Nucleophile. The reactions of phosphodiester with the α -effect nucleophile hydroxylamine typically give products phosphorylated on oxygen. Precedent, the kinetic evidence from this work, and the calculations described above are all consistent with a mechanism involving direct nucleophilic attack by the zwitterionic tautomer $^+\text{NH}_3\text{O}^-$ (7^\pm). It is to be expected that the same mechanism will apply to the reactions of hydroxylamine with other, similar electrophilic centers.

Efficiency of General Acid Catalysis. In the hydrolysis of the monoester 1^- the hydrogen bond is strong in the reactant, stronger in the product, and evidently strongest in the transition state.¹ We see the energetics of the process in the (simplified) terms illustrated in Figure 8, which distinguishes two typical cases. The rate determining step in both is P-O cleavage concerted with proton transfer to the leaving group oxygen; but in the typical case catalyzed by an external or a neighboring general acid (curve A), this must involve initial (rapid) formation of the encounter complex or the reactive conformation. However, in the special cases of 1^- , 4^\pm , and 6^+ , where the intramolecular hydrogen bond is so favorable that it is formed even in the reactant, this initial step is kinetically insignificant (curve B), because the reactant already exists in the reactive conformation (C). Furthermore, we have shown in a related system³⁹ that although this hydrogen bonding must be net stabilizing overall, its local effect on the bond to the leaving group is lengthening, and thus activating (thus reducing the overall stabilization of the reactant). But the largest effect must, by definition, be on the energy of the transition state. In the case of the phosphodiester 4^\pm under discussion the hydrogen bond is strong in the product, (naphthol $\text{O-H}\cdots\text{NMe}_2$) and significant in the reactant ($\text{P-O}\cdots\text{H-N}^+\text{Me}_2$). But once again it is strongest, and most stabilizing, in the transition state ($\text{P}\cdots\text{O}^{(-)}\cdots\text{H}\cdots\text{N}^{(+)}\text{Me}_2$): at the point where when the basicities of the leaving group oxygen and the general acid match, and the proton transfer becomes thermodynamically favorable.

This is reminiscent of Menger's split-site argument for enzyme efficiency.⁴⁰ The pre-equilibrium formation of a strong hydrogen bond is geometrically but not catalytically efficient, and enzymes have other and better means of bringing reacting groups into effective proximity. The strict geometrical requirements for strong intramolecular hydrogen bonding in water in (necessarily rigid) model systems mean that in such systems we will inevitably see them on occasion in the reactant.

Acknowledgment. We are grateful to PRONEX, CAPES, and CNPq-Brazil for financial support.

Supporting Information Available: Optimized geometries for 8-dimethylammonium-naphthyl-1-methyl phosphate 4^\pm , the transition state for its reaction with the ammonia oxide tautomer 7^\pm of hydroxylamine, and the developing product of the reaction.

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