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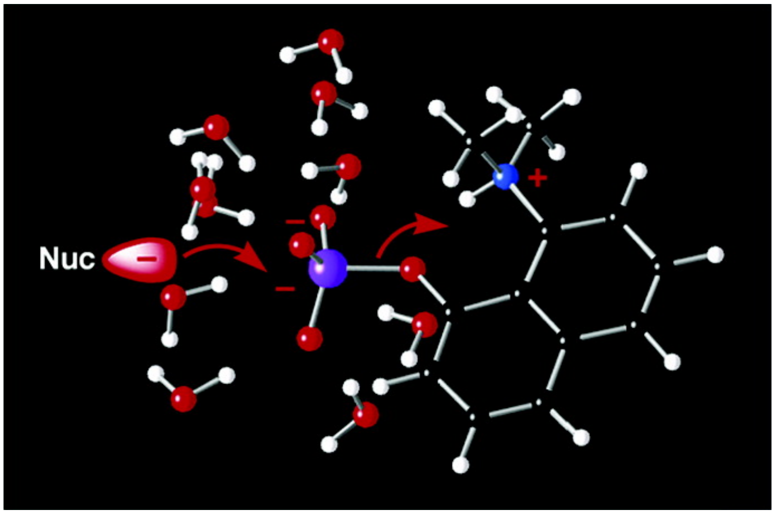

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Intramolecular General Acid Catalysis of Phosphate Transfer. Nucleophilic Attack by Oxyanions on the PO_3^{2-} Group

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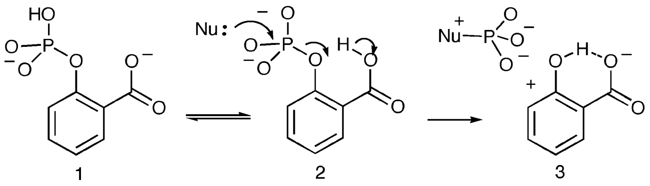
Abstract: Phosphate transfer from the 8-dimethylammonium-naphthyl-1-phosphate monoanion **4m** to water and to a range of nucleophiles shows general acid catalysis by the neighboring NH^+ group, through the strong intramolecular hydrogen bond. Reactivity is insensitive to the charge on the nucleophile, so that fluoride and oxyanions displace dimethylaminonaphthol from the PO_3^{2-} group as effectively as do amines of the same basicity. Reactivity is (predictably) relatively insensitive to the basicity of the nucleophile and to the α-effect. A strong intramolecular hydrogen bond is present in the product, but also in the reactant, as evidenced by major perturbations in the pK_a's of the phosphate and dimethylamino groups, to 3.94 and 9.31, respectively, and by ab initio calculations. Rate accelerations are of the order of 10^6-fold despite this stabilization: the strength of the hydrogen bond is evidently significantly enhanced in the transition state. The evidence suggests that it also depends remarkably strongly on the degree of ionization of the reacting phosphate group and will be significantly reduced for the neutral $PO(OH)_2$ group. Thus, the hydrolysis of the substrate cation $\mathbf{4}^+$ shows a correspondingly greater, $>10^8$-fold acceleration.

Introduction

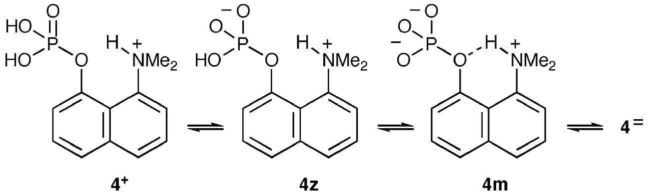
Enzyme-catalyzed transfer of the phosphoryl (PO_3^-) group appears always to involve participation by the acceptor nucleophile, as shown by the observation of inversion of configuration at phosphorus.[1,2] The mechanism is thus formally associative in character. However, reactions involving the P−O cleavage of phosphate monoester dianions are highly sensitive to the basicity of the leaving group, indicating a major dissociative element in the process. Thus, for the displacement of the typical poor leaving groups of biology, such as simple alcohol and sugar hydroxyls, assistance from a properly placed electrophile is essential. In many cases, this is a metal cation, but for enzymes which do not use an active site metal, a general acid is involved.

The only simple model for general acid catalysis in phosphate-transfer reactions is the hydrolysis of salicyl phosphate **1**, which involves efficient intramolecular general acid catalysis of the attack of both water and (neutral) amine nucleophiles at phosphorus (**2**, Scheme 1).[3,4] However, the choice of amine nucleophile is severely limited to those present in the free base form below pH 4 (where the COOH group (pK_a 3.76) is protonated), and reactions with oxyanions such as acetate are not detectable.

Scheme 1



Scheme 2



To extend our understanding of catalysis of such reactions, and especially the efficiency of general acid catalysis, we need model systems active near pH 7. We report our results with the phosphate ester **4** of 8-(dimethylamino)-l-naphthol (Scheme 2), which not only (perhaps unexpectedly) meets this requirement but also (definitely unexpectedly!) transfers the PO_3^- group to oxyanions as well as to neutral nucleophiles.[5] The work includes a detailed investigation of the reactions of **4** with the α-effect nucleophile hydroxylamine and its various methylated derivatives.

Experimental Section

Materials. The triester diethyl 8-(dimethylamino)naphthyl phosphate was prepared as described previously.[6] The hydroxylamines, as their

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† University Chemical Laboratory.
‡ Universidade Federal de Santa Catarina.

(1) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877−919.
(2) Thatcher, G. R. J.; Kluger, R. *Adv. Phys. Org. Chem.* **1996**, *25*, 99−265.
(3) Bender, M. L.; Lawlor, J. M. *J. Am. Chem. Soc.* **1963**, *85*, 3010−3017.
(4) Bromilow, R. H.; Kirby, A. J. *J. Chem. Soc. B* **1972**, 149−155.
(5) Kirby, A. J.; Lima, M. F.; da Silva, D.; Nome, F. *J. Am. Chem. Soc.* **2004**, *126*, 1350−1351.
(6) Asaad, N.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1708−1712.

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hydrochlorides, other nucleophiles, and inorganic salts were of the highest purity available and were used as purchased.

8-(Dimethylamino)-1-naphthylphosphate. Iodotrimethylsilane (0.28 g, 1.38 mmol) was added to a stirred solution of diethyl 8-dimethylaminonaphthyl-1-phosphate[6] (0.2 g, 0.6 mmol) in dichloromethane (15 cm^3) at room temperature. After 12 h, the solvent was removed under reduced pressure to give a yellow solid. MeOH (10 mL) was added to the solid, and after the mixture was stirred for 10 min, the solvent was removed under vacuum to give a brown solid: mp 106−107 °C; ν_{max} (KBr)/cm^{-1} 3690−3448 (br, OH), 1654 and 1610 (ArH), 1459 (CH_3), 1255 (P=O); NMR δ_H (200 MHz; D_2O) 7.97 (1H, dd, *J* 0.8 and 8.3), 7.65 (1H, dd, *J* 0.9 and 7.8), 7.72 (1H, dd, *J* 2.0 and 7.32) 7.55 (3H, dt, *J* 1.5 and 7.4), 3.41 (6H, s); δ_C (200 MHz; D_2O) 145.71, 138.41, 135.96, 130.07, 127.16, 125.92, 124.92, 123.99, 120.40, 119.14, 46.90; ^{31}P NMR (MeOD) $\delta = 1.81$; *m/z* (+ve FAB) 268.0760 (99.2, MH^+) $C_{12}H_{15}NO_4P$ requires M, 268.0739).

Kinetics. Reactions were started by adding 50 μL of a stock solution of the substrate (6.66×10^{-3}M) in water to 3 mL of reaction mixture containing a large excess (up to 1 M) of the nucleophile, ensuring strictly first-order kinetics for the reaction with the substrate. 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 either a Varian Cary 50 or a Hewlett-Packard 8453 diode-array spectrophotometer, both equipped with thermostated cell holders. The pH of the reaction mixture was measured at the end of each run at 60.0 °C, using a Hanna Instruments model pH 200 pH-meter. Observed first-order rate constants (k_{obs}) were calculated by nonlinear least-squares fitting of the absorbance vs time curve (Hewlett-Packard UV−Visible ChemStation software or the Scanning Kinetics program of the Cary 50): 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 and (first-order) rate constants for hydrolysis from the intercepts of these plots. Solutions of hydroxylamine nucleophiles were self-buffered between pH 5.5 and 7.0 (aqueous standard NaOH (0.1 M; Merck) was added to aqueous amine hydrochloride). Other buffers used are detailed in Table 1.

NMR spectra were measured in D_2O at 25 °C using a BRUKER Avance DPX 400 spectrometer at 300 K. ^1H and ^{13}C chemical shifts are referred to internal sodium 3-(trimethylsilyl) propionate (TSP) and $\delta^{31}P$ to external 85% H_3PO_4. Values of pD of solutions in D_2O were obtained by adding 0.4 to the observed pH-meter reading at 60 °C.

Products of the Reaction. The single detectable organic product was 8-(dimethylamino)-1-naphthol, identified by its UV−vis absorption and NMR spectrum. The initial phosphorus-containing product of attack by a nucleophile Nu will be Nu-PO_3^{2-}; hydroxylamine and its *N*-methylated derivatives are expected to act as oxygen nucleophiles, since hydroxylamine reacts with the dianion of 2,4-dinitrophenyl phosphate (an ester of similar reactivity to **4m**) to give $NH_2OPO_3^{2-}$, which is stable under the conditions of the reaction.[7]

Results and Discussion

Hydrolysis of 8-(Dimethylamino)-1-naphthyl Phosphate. The pH−rate profile for the hydrolysis of the phosphate monoester **4** shows that it is hydrolyzed rapidly (half-life 72 min at 60 °C) between pH 4 and 9 (Table 1 and Figure 1). The points in Figure 1 are experimental, the solid curve calculated by fitting to eq i, which describes the different equilibria and reactions in solution (Scheme 3).

$$k_{obs} = k_H\chi_z[H^+] + k_w\chi_z + k_m\chi_m \qquad \text{(i)}$$

Table 1. Rate Constants (s^{-1}) as a Function of pH for the Hydrolysis of 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}/DCl
0.3	6.16×10^{-5}	0.5	4.5×10^{-5}	6.167e^{-5}
0.6	3.5×10^{-5}	1	1.317×10^{-4}	1.867×10^{-4}
0.7	3.23×10^{-5}	1.5	2.583×10^{-4}	3.35×10^{-4}
1.1	1.53×10^{-5}	2	4.55×10^{-4}	5.267×10^{-4}
1.7	1.18×10^{-5}	2.5	5.517×10^{-4}	7.217×10^{-4}
3.0	3.00×10^{-5}	3	7.033×10^{-4}	9.183×10^{-4}
3.1	2.86×10^{-5}	3.5	8.683×10^{-4}	0.00116
3.2	3.31×10^{-5}	4	0.00106	0.00137
3.6	5.12×10^{-5}	4.5	0.00122	0.00156
4.0	8.83×10^{-5}	5	0.00135	0.00167
4.2	8.85×10^{-5}	5	0.0013	
5.0	1.50×10^{-4}	6	0.0015	
5.1	1.45×10^{-4}	7	0.0016	0.00199
6.0	1.55×10^{-4}	8	0.0015	0.00179
6.1	1.57×10^{-4}	9	0.00138	
7.0	1.57×10^{-4}	10	0.00121	0.00133
7.0	1.60×10^{-4}	12	7.7×10^{-4}	7.92×10^{-4}
7.8	1.58×10^{-4}			
8.0	1.57×10^{-4}			
8.1	1.55×10^{-4}			
9.0	1.33×10^{-4}			
9.2	9.16×10^{-5}			
9.5	7.50×10^{-5}			
9.6	4.00×10^{-5}			
9.9	2.77×10^{-5}			
10.0	3.17×10^{-5}			

[a] Buffers, etc. used: pH < 2, HCl; pH 3.0−4.0 formate; 4.0−5.5 acetate; 5.5−7.0 bis-Tris; 7.5−9.0 TRIS; 9.0−10 borate; pH > 10, NaOH.

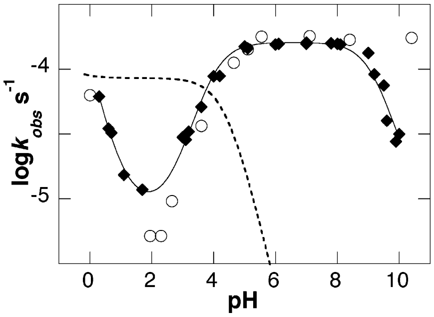

Figure 1. pH−rate profile for the hydrolysis of phosphate ester **1** at 60 °C and ionic strength 1.0 M (KCl). The main curve shows the fit for **1** (◆, 60 °C): the dashed curve is the pH−rate profile for the hydrolysis of the corresponding diethyl triester (diethyl 8-(dimethylamino)-1-naphthyl phosphate (also at 60 °C).[6] The open circles (○) show data for 2,4-dinitrophenyl phosphate at 39 °C, for comparison.

Here, χ_z and χ_m are the mole fractions of the zwitterionic and monoanionic forms **4z** and **4m**, respectively.

The rate and equilibrium constants derived from the least-squares fit give $pK_a(1) = 3.94 \pm 0.06$, $pK_a(2) = 9.31 \pm 0.05$, and k_m for the hydrolysis of the monoanion **4m** $= 1.60 \pm 0.06 \times 10^{-4}\ s^{-1}$. The data at low pH give $k_z = 1.0 \pm 0.33 \times 10^{-5}\ s^{-1}$ for the spontaneous hydrolysis of the zwitterion **4z** and $k_H = 9.0 \pm 2.0 \times 10^{-5}$ L mol^{-1} s^{-1} for its apparent acid-catalyzed reaction (see below). The dianion $\mathbf{4}^{2-}$ does not react at a measurable rate under the conditions.

The plateau in the pH−rate profile is defined by the two ionizations, identified (by UV−vis spectroscopy and the variation of the ^1H NMR chemical shifts of adjacent C−H protons) as the second dissociation of the phosphate (apparent pK_a 3.94) and that of dimethylammonium NH^+ group (pK_{app} 9.31). These pK_a's are shifted substantially compared with those for similar

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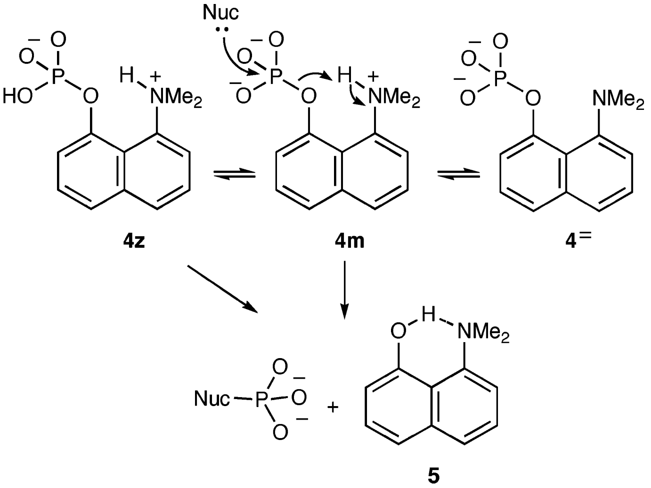
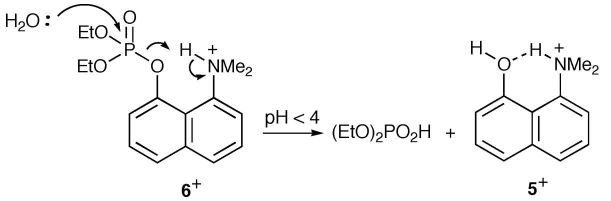
(7) Domingos, J. B.; Longhinotti, E.; Bunton, C. A.; Nome, F. *J. Org. Chem.* **2003**, *68*, 7051−7058.

Scheme 3



Scheme 4



unperturbed groups. The pK_a of the dimethylammonium NH^+ is higher by some 4.7 units than the apparent pK_a of 4.63 reported for the triester diethyl 8-dimethylaminonaphthyl-1-phosphate,[6] which falls in the general region expected for a naphthylamine. We conclude (see below) that the basicity of the POAr oxygen is significantly greater in the phosphate dianion $ArOPO_3^{2-}$. This results in rather strong hydrogen bonding between the N^+H and this bridging oxygen in **4m**, in contrast to the little or none observed to the (less basic) oxygen of the P−OAr group of the corresponding phosphate triester **6** (Scheme 4). The triester is hydrolyzed by a mechanism formally similar to that shown (Scheme 3) for **4m**, with slightly more efficient general acid catalysis by the neighboring Me_2NH^+ group.[6] The geometry of course is essentially identical in the two cases and patterned on that of Proton Sponge,[8,9] which specifically—and strongly—favors such intramolecular hydrogen bonding. (The alternative intramolecular hydrogen bonding, $N^+H\cdots{}^-O{-}P$, through a $7^1/_2$-membered[11] ring is not expected to be significant in water, a conclusion supported by the results of calculations discussed below.)

The strong hydrogen bond to the bridging OAr oxygen in **4m** is expected to channel negative charge from the phosphate dianion oxygens to the dimethylammonium center, lengthening the P−OAr bond (we have observed the consequent bond length changes in the case of a related acetal[10]) and thus making the PO_3^{2-} group less basic. The pK_a of an aryl phosphate monoanion $ArOP(OH)O_2^-$ is typically close to 6 but is reduced by electron withdrawal in the Ar group: the sensitivity to the pK_a of the parent phenol ArOH is 0.23 ± 0.03. (Correlation based on data for four phosphate monoesters derived from phenols with pK_a's between 4.11 and 9.95. Interpolation using this plot gives an initial estimate of 2.5 ± 1.5 for the effective pK_a of the naphthol leaving group of **4m**.)

It is not possible to estimate an effective molarity (EM[11]) for catalysis by the Me_2NH^+ group of **4m** because the corresponding intermolecular general acid catalyzed reaction is too slow to measure: we know only that it must be high. Alternative, though less specific, measures of efficiency are (a) the rate acceleration for the particular reaction under given conditions and (b) the (related) estimated effective pK_a of the leaving group (as compared with the rate of hydrolysis of an ester $ArOPO_3^{2-}$ lacking the intramolecular catalytic group). (a) The estimated rate enhancement for the hydrolysis of the monoanion **4m**, compared with an aryl phosphate dianion derived from a naphthol of pK_a 9.4 (based on the recent results of Lad et al., which add a key point to the earlier linear free energy relationship), is 2.4×10^5 at 60 °C and 8.8×10^5 at 39 °C.[12,13] (b) This translates into an effective pK_a of the leaving group of **4m** of 5.2 at 39 °C (in the case of the triester **6**$^+$ the figure is 3.4.[6])

This acceleration clearly results from the intramolecular interaction of the adjacent ionising groups, which may involve some through-space electrostatic contribution but is based primarily on the stronger intramolecular hydrogen-bond which develops between NH^+ and the leaving group oxygen in the transition state for P−OAr cleavage.[14] The strong intramolecular hydrogen bond in the product dimethylaminonaphthol has been well-characterized[15] and is the basic design feature of this model system.[16] We have shown previously that the strong intramolecular hydrogen bond in the product **5**, like that in the salicylate monoanion **3**, is the key to efficient general acid catalysis.[4,16,17] The product **5** is stabilized by up to 25−30 kJ mol^{-1} (based on the elevation of its pK_a[5]) and the reactant similarly by 20−25 kJ mol^{-1}. Stabilization of the transition state (late for the P−O cleavage of monoester dianions) must be greater than that of the reactant by a further 30−35 kJ (7.2−7.8 kcal) mol^{-1}.

Ab Initio Calculations. Two closely related questions of interest for the mechanism suggested in Scheme 3 are the strongly perturbed pK_a's of the two groups which ionize in the pH region and the mechanism by which the dimethylammonium group catalyzes phosphoryl transfer, especially to anionic nucleophiles. We suggest above that the key in both cases is the intramolecular hydrogen bond, and have performed ab initio calculations to define the structure of **4m** in more detail.

Gas-phase structures are unlikely to be revealing for a zwitterionic structure in aqueous solution, so we carried out calculations using two different approximations for modeling solvent water. In Cambridge, we applied a continuum water model,[18] as implemented in the Jaguar program,[19] at the B3LYP/6-31G** level,[20−22] specifically for **4m**. In Florianópolis, we

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[8] Alder, R. W.; Bowman, P. S.; Steele, W. R. S.; Winterman, D. R. *J. Chem. Soc., Chem. Commun.* **1968**, 723−724.

[9] Staab, H. A.; Saupe, T. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 865−879.

[10] Bond, A. D.; Kirby, A. J.; Rodriguez, E. *J. Chem. Soc., Chem. Commun.* **2001**, 2266−2267.

[11] Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183−278.

[12] Lad, C.; Williams, N. H.; Wolfenden, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5607−5610.

[13] Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415−423.

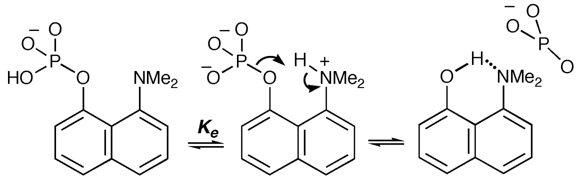
[14] We note that the pK_a of the acetal 8-dimethylammonium-1-methoxymethoxynaphthalene (7.40 at 65 °C, ref 17) is also raised, by about 2 pK units. The observation of a substantial effect with the neutral acetal group suggests strongly that it is due primarily to the intramolecular hydrogen-bond, rather than to an electrostatic interaction.

[15] Grech, E.; Nowicka-Scheibe, J.; Olejnik, Z.; Lis, T.; Pawelka, Z.; Malarski, Z.; Sobczyk, L. *J. Chem. Soc., Perkin Trans. 2* **1996**, 343−348.

[16] Kirby, A. J. *Acc. Chem. Res.* **1997**, *30*, 290−296.

[17] Kirby, A. J.; Percy, J. M. *J. Chem. Soc., Perkin Trans. 2* **1989**, 907−912.

Scheme 5



4- **4m** **P**

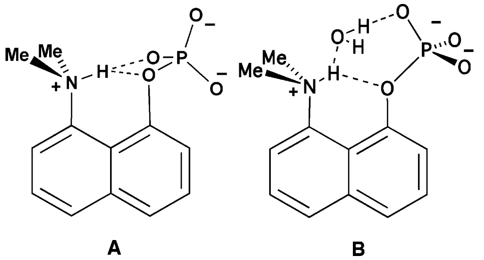
added 15 discrete water molecules, designed to represent the inner solvation shell of the PO_3^{2-} group (GAMESS program,[23] RHF/6-31G(d) level*), and compared **4m** with methyl phosphate and with 1-naphthyl phosphate, the cognate system lacking the dimethylammonium group. Minimizations gave the zwitterion **4m** in both environments, without the need for geometrical restriction when solvent was present. Detailed calculational results are included in the Supporting Information to this paper.

The lowest energy minimum found using the continuum model has the zwitterionic stucture **4m**, consistent with the experimental evidence that the proton is attached to naphthylamine nitrogen rather than the normally more basic phosphate dianion group. The next lowest minimum is the tautomer **4-** (Scheme 5), higher in energy by 21 kJ mol^{-1} (compared with an experimental[24] ΔpK_a with difference of 3.3, corresponding to 19 kJ mol^{-1}). A third minimum, corresponding to the P−O cleavage products, metaphosphate anion,[25] and the naphthol P, is 72 kJ mol^{-1} higher in energy. (This figure can be compared with the experimental value for $\Delta H^{\ddagger}$ for hydrolysis of 126 kJ (30.06 ± 0.80 kcal) mol^{-1}). The geometry calculated for the hydrogen-bonding geometry in the product **P** is closely similar to that found in the crystal structure.[15]

In both environments, the scissile P−O(C) bond is significantly lengthened (1.702 Å in the continuum model, 1.693 Å with discrete water molecules present: compared with 1.667 and 1.648 Å for the 1-naphthyl and methyl phosphate dianions). The ammonium proton is involved in bifurcate hydrogen bonding to the adjacent P−O(C) and to a second more basic center. In the absence of a discrete water molecule the strongest H-bond (N−H···O 2.479 Å, bond angle 169.7°, torsion angle CCNH −43.4°) in the continuum model is from N^+H to a $(P)O^-$ (the $P-O^-$ bond concerned is correspondingly lengthened, 1.558 compared with 1.515 ± 0.001 Å for the other two $P-O^-$). The charge on the N^+H group is further delocalized through a weaker hydrogen bond (N−H···O 2.805 Å, bond angle 105.8°) to the leaving group oxygen.

With discrete water molecules present each of the three $P-O^-$ centers is strongly solvated by H-bonding to two or three waters and the intramolecular H-bond is stronger (N−H···O 2.607 Å, bond angle 123.3°). It is still out of the plane of the aromatic system (torsion angle CCNH 38.3°) to allow a second, weak H-bond (N−H···O 3.03 Å, bond angle 136.3°) from the N^+H to a water molecule solvating one of the $P-O^-$ group. These arrangements are summarized in Scheme 6.

Scheme 6. Bifurcate Hydrogen Bonding Involving the N^+H Group of 4m[a]



[a] Based on structures calculated using the continuum model (**A**) and discrete water molecules (**B**). See the text.

Detailed Mechanism. We know that—other things being equal—longer bonds (including the P−O bonds of phosphate esters) break more easily.[11] The increasing electron withdrawal which makes RO^- a better leaving group (and ROH a stronger acid) polarizes a P−OR bond in the sense P^+OR^- and so lengthens it. Evidently, sufficiently strong hydrogen bonding to the leaving group oxygen can have the same effect, as shown by the significant lengthening of the P−O(C) bond of **4m** revealed by both calculations. This lengthening of the P−O(C) bond from compound to compound, together with the associated reduction in the mean PO^- bond length, can be seen as progress along the reaction coordinate[11] for P−O cleavage, although the overall result for reactant **4m** is a net stabilization. Further lengthening of this bond during the course of thermal vibration leads to reaction.

The final stage of the reaction involves rotation of the catalytic N^+-H bond into the plane of the aromatic system to set up the strong intramolecular hydrogen bond in the product **P**, as a result of the increasing electron density on the leaving group oxygen driven by the cleavage of the P−O bond and the transfer of the PO_3^- group to a molecule of water. (There is no water molecule "in position"—along the extended O−P bond—in the calculated structure **B**.)

The mechanism of hydrolysis of **4m** is simply represented as **4m** → **5** (Scheme 3, Nuc = H_2O). The involvement of water as a nucleophile is typically weak but essential in such phosphate transfer reactions, enforced by the high energy of the metaphosphate intermediate PO_3^- which would be produced by an unassisted $S_N1(P)$ process. This conclusion is based on the general experience that PO_3^- transfer goes with inversion of configuration:[1] though the reaction shows little (sometimes no[26]) sensitivity to the reactivity of the nucleophile, and hydrolysis shows an entropy of activation near zero, generally considered typical of a unimolecular process.[13] The hydrolysis of **4m** follows the usual pattern, being controlled by the enthalpy term, $\Delta H^{\ddagger} = 125.8 \pm 3.3$ kJ (30.06 ± 0.80 kcal) mol^{-1}, with a significantly positive entropy of activation, $\Delta S^{\ddagger} = 59.0 \pm 9.9$ J (14.1 ± 2.3 cal) K^{-1} mol^{-1}. The involvement of the general acid is expected to have no significant effect on the entropy term, since the proton transfer takes place within a strong hydrogen bond which remains in place throughout the reaction. For comparison, observed enthalpies of activation are 38 ± 2

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(18) Marten, B.; Kim, K. C., C.; Friesner, R. A.; Murphy, R. B.; Ringnalda, M. N.; Sitkoff, D.; Honig, B. *J. Phys. Chem.* **1996**, *100*, 11775-.

(19) Jaguar 4.2, S., Inc., 2000.

(20) Hehre, W. J.; Radom, L. S., P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory;* Wiley: New York, 1986.

(21) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(22) Lee, C. T.; Yang, W. T.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789.

(23) Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A. B.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S. J.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. *J. Comput. Chem.* **1993**, *14*, 1347−1363.

(24) The pK_a's used are the observed value of 9.31 for the dimethylammonium group of **4m** and for **4-** the typical observed value of 6.0 for the second dissociation of an aryl phosphate monoester.

(25) Metaphosphate is not expected to exist for a significant length of time in real water.[2]

(26) Kirby, A. J.; Varvoglis, A. G. *J. Chem. Soc. B* **1968**, 135−141.

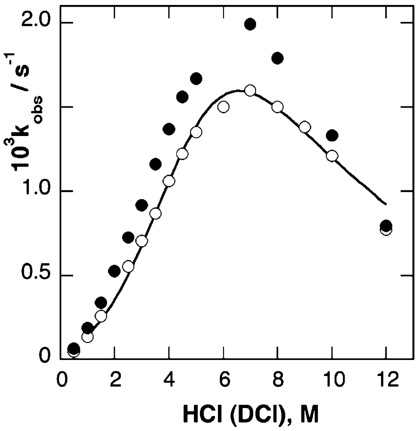


Figure 2. Rate constants for hydrolysis of phosphate ester **1** in concd HCl (○) and DCl (●) at 60 °C. The curve is based on points calculated from eq ii, using $k_1 = 2.92 \times 10^{-3}$ s^{-1} and p$K_a = -1.48$.

and 26 kcal/mol for the hydrolysis of the dianions of phenyl[12] and 2,4-dinitrophenyl[13] phosphate, respectively, which show similar, positive entropies of activation of ~7 cal K^{-1} mol^{-1}. (For the hydrolysis of salicyl phosphate **2** $\Delta H^{\ddagger} = 23.5$ kcal/mol, with $\Delta S^{\ddagger} = -1.2$ cal.[4]) Reactivity is controlled in each case primarily by the enthalpy term: the entropy of activation indicates that the involvement of water as a nucleophile in these reactions is comparable to its involvement in the solvation shell of the phosphate dianion reactant. Presumably the weak involvement of the nucleophilic water is balanced by the release of water from the strongly solvated PO_3^{2-} group as its charge decreases.

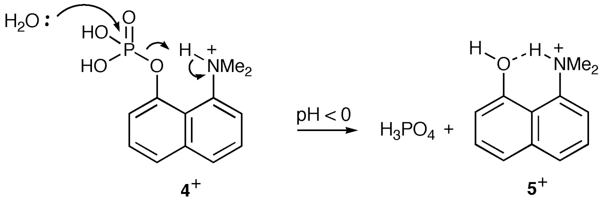
Hydrolysis in Strong Acid. Hydrolysis under strongly acidic solutions, from 0.5 to 12 M HCl or DCl, shows a rate profile qualitatively similar to those observed for the acid hydrolyses of many esters and amides, with rate constants increasing up to a maximum at about 7 M HCl or DCl (Figure 2 and Table 1) and then falling at higher acid concentrations. This is behavior expected for a substrate which is reactive as the conjugate acid and becomes fully protonated at high acid concentrations: the fall in rate in this latter region is accounted for by the decreasing activity of water, which acts as a nucleophile. Consistent with this picture, the data can be fit reasonably well to eq ii

$$k_{obs} = k_1 a_w \chi_+ = k_1 a_w a_H/(a_H + K_a) \qquad \text{(ii)}$$

The curve in Figure 2 is derived from eq ii using $k_1 = 2.92 \times 10^{-3}$ s^{-1}; the mole fraction χ_+ of the substrate cation $\mathbf{4}^+$ (calculated using a pK_a of -1.47, corresponding to half-protonation in 4.3 M HCl) and activities for water and HCl (at 25 °C) based on the data of Randall and Young.[27]

It seems clear that this reaction, the apparent acid-catalyzed hydrolysis of the zwitterion **4z**, actually represents the kinetically equivalent spontaneous hydrolysis of the cation $\mathbf{4}^+$ by the mechanism shown in Scheme 7. (Adding a $[a_H][\mathbf{4}^+]$ term does not improve the fit to eq ii). $\mathbf{4}^+$ is unlikely to undergo further protonation on the phosphate group (next to the positively charged group in the *peri*-position) in any but very strong acid, and its estimated pK_a is a normal 5−6 units lower than that of the conjugate base **4z**. In terms of reactivity, $\mathbf{4}^+$ is effectively an activated triester:

Scheme 7



The estimated rate enhancement for the hydrolysis of $\mathbf{4}^+$, compared with a dialkyl aryl phosphate[28] derived from a naphthol of pK_a 9.4, is over 10^8 at 60 °C, substantially greater than the rate enhancement estimated above for the hydrolysis of the monanion **4m**. This is not unexpected because there is little or no H-bonding stabilization of the reactant when the phosphate group is neutral, as indicated by the observed pK_a = 4.63 of the Me_2NH^+ group of the diethyl ester $\mathbf{6}^+$.[6] The rate constant for the reaction of $\mathbf{4}^+$ (as suggested in Scheme 7) is 33.6 times faster than the corresponding reaction of the diethyl ester (Scheme 4). We attribute this to a combination of electronic and steric effects resulting from the replacement of the two OH protons of $\mathbf{4}^+$ by ethyl groups.

Deuterium Isotope Effects. A small inverse solvent deuterium isotope effect is observed over the whole range of acid concentration used. In the linear region of the rate vs [HCl] or [DCl] plots (below 5 M acid), the apparent second-order rate constants are 3.8×10^{-4} and 3.0×10^{-4} $M^{-1}s^{-1}$, respectively, giving $k_H/k_D = 0.78$. This is consistent with the mechanism of Scheme 7, with the inverse isotope effect for the protonation equilibrium partially offset by (small) normal kinetic isotope effects for the attack of water as a nucleophile on $\mathbf{4}^+$ and the accompanying intramolecular proton transfer.

The reaction was also followed in both H_2O and D_2O at pH (pD) 7.0, as a function of hydroxylamine concentration. The observed intercepts and slopes of the second-order plots give kinetic isotope effects for the reactions of **4m** with water ($k_{H_2O}/k_{D_2O} = 1.46 \pm 0.04$) and with NH_2OH ($k_{H_2O}/k_{D_2O} = 1.67 \pm 0.05$). The results are consistent with nucleophilic attack on **4m** in both cases: kinetic solvent deuterium isotope effects are typically small for the hydrolysis of phosphate monoester dianions,[13] no doubt because of the weak involvement of the nucleophile in the rate determining transition state, and the same is evidently true for the reaction with hydroxylamine. (The reaction with hydroxylamine is discussed in more detail below.)

Further evidence consistent with this mechanistic interpretation (Scheme 3) comes from a proton inventory study, conducted for the pH-independent reaction of **4m** at 60.0 °C, pH 8.0 (phosphate buffer 0.01 mol L^{-1}, 1 mol L^{-1} KCl). The solvent isotope effect in mixtures of HOH ($n = 1-0$) and DOD ($n = 0-1$) shows a linear dependence of k_n/k_0 on the atom fraction n of deuterium (Figure 3) This result is consistent with the Gross−Butler equation in its simplified form ($k_n/k_0 = 1 + n(\Phi^T - 1)$), indicating that only one transition-state proton transfer is involved in the reaction. The isotope effect k_{H_2O}/k_{D_2O} observed for the water reaction of 2,4-dinitrophenyl phosphate is close to unity;[13] thus, the overall small normal isotope effect (k_H/k_D) observed for the reaction of **4m** is consistent with a small degree of proton transfer from the NH^+ group in the transition state. The small isotope effect is also consistent with the Brønsted

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(27) Randall, M. R.; Young, L. E. *J, Am. Chem. Soc.* **1928**, *50*, 989−1004.

(28) Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1970**, 1172−1182.

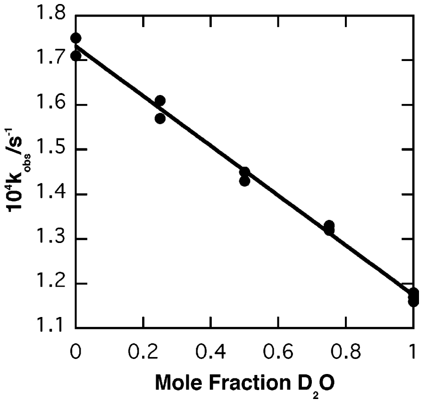

Figure 3. Proton inventory plot for the hydrolysis of **4m** at 60 °C and ionic strength 1.0M (KCl).

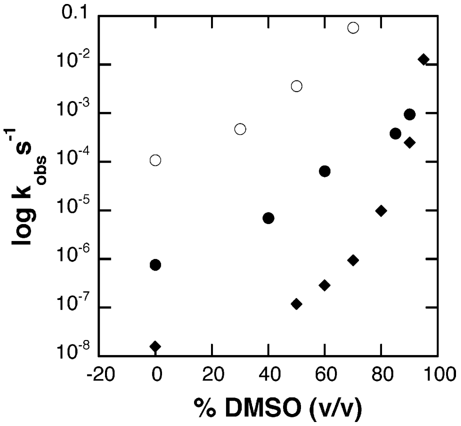

Figure 4. Rates of hydrolysis of phosphate monoester dianions as a function of dimethyl sulfoxide concentration. Data are for 4-nitrophenyl phosphate at 39 °C (◆), 2,4-dinitrophenyl phosphate at 39 °C (○),[13] and **4m** at 25 °C (●). Solutions of **4m** in water at pH 7.0 (0.01 M phosphate buffer, ionic strength 1.0 M (KCl)) were diluted with the organic solvent.

β_{nuc} of 0.19 (see below) and a transition state that is early on the reaction coordinate for bond formation to, and thus any consequent proton transfer from, the nucleophile.

Solvent Effects. The hydrolytic cleavage of the dianions of phosphate monoesters with good leaving groups is strongly accelerated by the addition of dipolar aprotic solvents to the aqueous reaction medium.[13,29] In principle, the hydrolysis of **4m**, which involves neutralization of charge in the transition state, might be expected to show an enhanced effect. We have examined the hydrolysis of **4m** in a range of mixed solvents and find effects in the expected direction, but relatively small rate enhancements. Typical results are illustrated in Figure 4 for the reactions of **4m**, which also includes data for the hydrolysis of the dianions of 2,4-dinitrophenyl and 4-nitrophenyl phosphate, in the presence of increasing amounts of dimethyl sulfoxide. Qualitatively similar effects are observed with other water-miscible solvents. As in other ways, the hydrolysis of **4m** is typical of the hydrolysis of the dianion of a phosphate monoester with a good leaving group, no doubt because the key intramolecular hydrogen bond is present throughout the reaction and the general acid is not separately solvated.

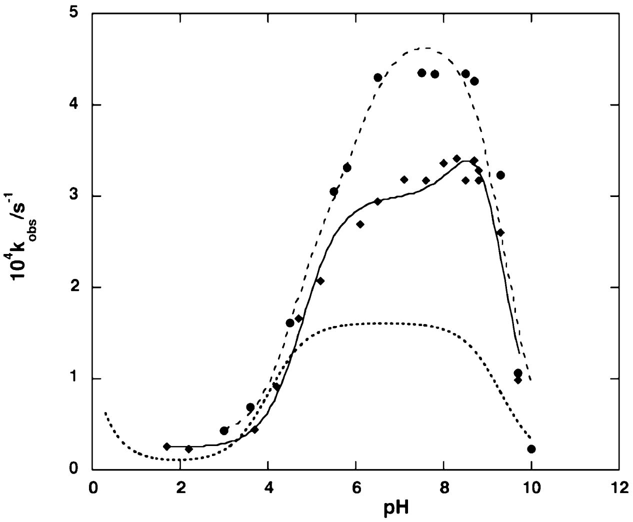

Figure 5. Reaction of **4m** with hydroxylamine. Rate constants at 60 °C as a function of pH for the reaction of **4m** in the absence (dotted curve) and presence of 0.6 M (◆) and 1.0 M (●) NH_2OH. The curves are calculated using equations i and iii and the rate constants listed in Tables 1 and 2.

Table 2. Rate Constants for the Reaction of 4m with Hydroxylamine

(a) At Fixed Total ($[NH_2OH] + [NH_3^+OH]$), as a Function of pH at 60 °C and Ionic Strength 1.0 M (KCl)

$[NH_2OH]$ 0.6 M		$[NH_2OH]$ 1.0 M	
pH	k_{obs} (s^{-1})	pH	$k_{obs'}$ (s^{-1})
1.7	2.56×10^{-5}	3.0	4.30×10^{-5}
2.2	2.27×10^{-5}	3.6	6.88×10^{-5}
3.7	4.40×10^{-5}	4.5	1.61×10^{-4}
4.2	9.13×10^{-5}	5.5	3.05×10^{-4}
4.7	1.66×10^{-4}	5.8	3.31×10^{-4}
5.2	2.07×10^{-4}	6.5	4.30×10^{-4}
6.1	2.69×10^{-4}	7.5	4.35×10^{-4}
6.5	2.94×10^{-4}	7.8	4.336×10^{-4}
7.1	3.18×10^{-4}	8.5	4.34×10^{-4}
7.6	3.17×10^{-4}	8.7	4.26×10^{-4}
8.0	3.36×10^{-4}	9.3	3.23×10^{-4}
8.3	3.41×10^{-4}	9.7	1.06×10^{-5}
8.5	3.17×10^{-4}	10.0	2.29×10^{-5}
8.7	3.39×10^{-4}		
8.8	3.17×10^{-4}		
8.8	3.28×10^{-4}		
9.3	2.60×10^{-4}		
9.7	9.85×10^{-5}		

(b) As a Function of $[NH_2OH]$ at pH = 7.0 and 60 °C and Ionic Strength 1.0 M (KCl).

$[NH_2OH]$, M	k_{obs} (s^{-1}) in H_2O	k_{obs} (s^{-1}) in D_2O
0		1.28×10^{-4}
0.1	2.10×10^{-4}	
0.2	2.42×10^{-4}	1.58×10^{-4}
0.4	2.85×10^{-4}	1.92×10^{-4}
0.6	3.35×10^{-4}	2.17×10^{-4}
0.8	3.83×10^{-4}	2.48×10^{-4}
1.0	4.30×10^{-4}	2.72×10^{-4}

Reactions of 8-(Dimethylamino)-1-naphthyl Phosphate with Nucleophiles. The reaction of **4** with 0.6 and 1.0 M (total) NH_2OH was measured over the pH range 2−10 to categorize the efficiency of catalysis by this α-effect nucleophile as a function of pH. Reactions with a small number of other nucleophiles, measured for comparison, produced the unexpected results described below.

Results for the reaction with hydroxylamine are shown in Figure 5 and Table 2. (Hydroxylamine is known to react through oxygen with phosphate mono-, di-, and triesters,[7] as discussed above). The reaction shows the expected bell-shape, with a rate maximum in the region where the nucleophile is present as the

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(29) Abell, K. W. Y.; Kirby, A. J. *Tetrahedron Lett.* **1986**, 1085−1088.

Table 3. Second-Order Rate Constants for the Reactions of 4m with Nucleophiles at 60 °C and Ionic Strength 1.0 M

nucleophile (concd)	pK_a	k_{Nu} (L mol^{-1}s^{-1})
H_2O	−1.74	2.88×10^{-6}
$H_2PO_4^-$	1.9	1.3×10^{-5}
Fluoride	3.1	2.97×10^{-5}
$MeONH_2$	4.62	3.5×10^{-5}
Formate	3.81	7.8×10^{-5}
Acetate	4.81	6.2×10^{-5}
Me_2NOH	5.20	2.02×10^{-4}
NH_2OH	5.64	2.43×10^{-4}
NH_2OH in D_2O		$1.45 \pm 0.03 \times 10^{-4}$
MeNHOH	6.18	3.6×10^{-4}
HPO_4^{2-}	6.51	1.04×10^{-4}

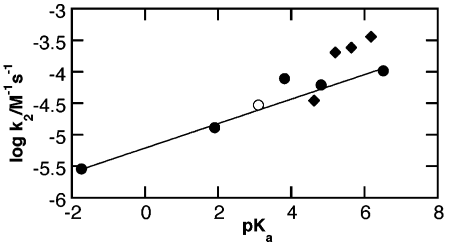

Figure 6. Brønsted plot for catalysis of the hydrolysis of **4m** by nucleophiles, in aqueous solution at 60 °C. Points are for water, phosphate mono- and dianion, formate and acetate (●), fluoride (○), and hydroxylamine and its various methylated derivatives (◆). Data from Table 3.

free base and the substrate as the monoanion **4m**, with the NMe_2 group protonated. The pH−rate constant profile for the reaction between **4** and hydroxylamine was fitted to eq iii, where k_m(Nu) is the second-order rate constant for the reaction of the monoanion **4m** with neutral hydroxylamine and the terms χ_{NH_2OH} and χ_m are the mole fractions of the two reactive species.

$$k_{obs} = k_{obs,w} + k_m(Nu)\,\chi_{NH_2OH}\,\chi_m \qquad \text{(iii)}$$

The second-order rate constant and the pK_a of (the conjugate acid, NH_3^+OH, of) the nucleophile derived from the fit are k_m(Nu) $2.5 \pm 0.3 \times 10^{-4}$ L mol^{-1} s^{-1} and pK_a = 5.6 ± 0.2. The same reaction was also followed at constant pH (7.0, self-buffered) as a function of hydroxylamine concentration (in both H_2O and D_2O, see above). The observed slope of the second-order plot gives a more precise second-order rate constant of $2.41 \pm 0.04 \times 10^{-4}$ L mol^{-1} s^{-1}.

The rate of hydrolysis in the plateau region was examined as a function of concentration (in the range 0.1 to 1 M) for several other, representative, nucleophiles. Second-order rate constants were calculated from the expression $k_{obs} = k_0 + k_{Nu}$[nucleophile], and the results are summarized in Table 3 and Figure 6. Remarkably, oxyanions react with **4m** just as fast as do neutral amine nucleophiles of similar pK_a. The Brønsted plot of Figure 6 shows a least squares line drawn through the four points for water, acetate, and phosphate mono- and dianions. The points for the α-effect nucleophiles hydroxylamine and its *N*-methyl and *N*,*N*-dimethyl derivatives show only modest enhancements, as expected for reactions with the dianion of a phosphate monoester.[26] Only NH_2OMe, which can only react through N, shows no rate enhancement, consistent with the oxygen attack mechanism for the other hydroxylamines.

The attack of oxyanions on the phosphorus center of a monoester dianion is unique, as discussed in our preliminary communication.[5] Generally, there is no detectable reaction between oxyanion nucleophiles and true[5] dianions even of activated phosphate monoesters. We presume that electrostatic repulsion normally disfavors the anion−dianion reaction. The effect need not be very large to make such slow reactions undetectable: Herschlag[30] estimates a maximum 50-fold electrostatic effect on the rate of attack by fluoride on the *p*-nitrophenyl phosphate dianion, at high ionic strength in water at 95 °C.

To investigate the anion−dianion reaction in more detail, we measured thermodynamic parameters for the reaction with fluoride anion, which will show minimal steric effects. A disadvantage is its low basicity, which means that the second-order rate constants were measured (at 60, 70, and 80 °C) against the background of the faster hydrolysis reaction. Within this caveat, the results are strongly suggestive. The enthalpy of activation $\Delta H^\ddagger$ is a massive 169.4 ± 2.9 kJ (41.1 ± 0.7 kcal) mol^{-1}, substantially offset by a very large positive entropy of activation, $\Delta S^\ddagger = 176 \pm 9$ J (42.0 ± 2.9 cal) K^{-1} mol^{-1}. This increase of 10 kcal/mol in $\Delta H^\ddagger$ compared with attack by water is consistent with significant electrostatic repulsion disfavoring the anion/dianion reaction: it reflects also the cost of desolvating a lone pair of the tightly solvated fluoride anion to allow it to act as a nucleophile. By way of compensation the release of water involved will contribute to the large positive entropy of activation. In practical terms, the overall effect of this unusual combination of thermodynamic parameters is to make the fluoride reaction substantially more sensitive to temperature than competing reactions, so that it rapidly becomes relatively more significant at higher temperature.

The Brønsted coefficient (slope of the least squares line drawn in Figure 6) is $\beta_{nuc} = 0.19 \pm 0.01$) for the reactions with oxygen nucleophiles. This is smaller, as would be expected, than that (0.50) measured for the triester diethyl-8-dimethylamine-naphthyl phosphate **6**$^+$ but significantly higher than the value of zero observed for the reactions of (necessarily neutral) nucleophiles with the dianion of 2,4-dinitrophenyl phosphate,[13] a monoester dianion hydrolyzed at a similar rate to **4m** (Figure 1). This, together with the identical Brønsted coefficient of $\beta_{nuc} = 0.19 \pm 0.01$ for the attack of (substituted pyridine) nucleophiles on salicyl phosphate **2**,[4] suggests that intramolecular general acid catalysis of PO_3^- transfer involves somewhat tighter transition states than the corresponding intermolecular reactions. This behavior differs from that for reactions at the acetal center of 8-dimethylammonium-1-methoxymethoxynaphthalene, which are closely similar for catalyzed and intermolecular reactions with nucleophiles.[17]

Finally we comment, at the suggestion of a reviewer, on why we are confident that the reactions of **4m** with nucleophiles do involve nucleophilic, rather than general base catalysis of hydrolysis. Apart from its reactions with anions the PO_3^{2-} group of this system behaves *in detail* as expected for a monoester $ROPO_3^{2-}$ with a good leaving group: and the reactions with anions (Figure 6) closely parallel those of neutral nucleophiles with phosphorylated pyridines.[31]

General base catalysis has never been reported for the reactions of nucleophiles with these simple PO_3^{2-} systems. The reactions with amines show steric effects and entropies of activation typical of nucleophilic, bimolecular processes, and

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(30) Admiraal, S. J.; Herschlag, D. *J. Am. Chem. Soc.* **1999**, *121*, 5837−5845.
(31) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7587−7596.

no solvent deuterium isotope effects, and the reactive intermediates formed on reaction with tertiary amines can be trapped with fluoride anion to give phosphofluoridate, stable under the conditions.[26,30,31] The reaction of fluoride with **4m** itself actually shows a positive entropy of activation, hard to reconcile with a termolecular process; formate reacts faster than the more basic acetate, as found for other, similar reactions of the PO_3^{2-} group;[31] and the low solvent deuterium isotope effect is not consistent with the involvement of a committed second molecule of water in the transition state for hydrolysis. All the evidence is consistent with nucleophilic catalysis, albeit with the weak involvement of the committed nucleophile in the transition state typical of PO_3^- group transfers.

Conclusions

The plateau rate for the hydrolysis of **4m** represents a substantial acceleration, achieved by the concerted action of an external nucleophile and an internal general acid. The synergy involved is not high, as is to be expected for a reaction with a low sensitivity to the incoming nucleophile. (We know little about the sensitivity to the pK_a of the general acid, but in the related reaction of salicyl phosphate **2** this too is known to be low.[4])

We concluded previously that the transition state for the intramolecular general acid catalyzed hydrolysis of the diethyl triester **6**$^+$ is significantly different from that expected either for a triester hydrolyzing at a similar rate or for one with a comparable leaving group. The same appears to be true for phosphate monoesters. The trend is the same in the two cases—more bond formation is necessary from the nucleophile (later TS) to generate the negative charge on the leaving group needed to support a strong hydrogen bond and thus efficient proton-transfer catalysis. Displacements at the phosphorus center of a triester (**6**$^+$) may involve pentacovalent addition intermediates; those at the phosphorus center of **4m** will not, and the concerted mechanism expected for the latter system should for this reason have more potential for effective synergy between the bond-formation and bond-breaking processes. In practice the accelerations observed are similar for triester and monoester, at least in part because of the low sensitivity of the reactions of monoester dianions to the nucleophile, and the significant extent of hydrogen bonding in the reactant. Absolute reactivity can be increased by using α-effect nucleophiles, but the α-effect is also minimized by this low sensitivity. This analysis suggests that the combination of lower absolute reactivity, reduced reactant-state hydrogen bonding, and higher sensitivity to the nucleophile expected for phosphate diesters could support optimally effective synergy and, thus, rate enhancements in diesters derived from **4**, where efficient general acid catalysis of leaving group departure could tip the balance from a stepwise to a concerted mechanism. This work is underway.

Acknowledgment. We are grateful to EPSRC for a studentship (to N.D.-R.) and to PRONEX, CAPES, and CNPq-Brazil for financial support.

Supporting Information Available: Optimized geometries for 8-dimethylammonium-naphthyl-1-phosphate **4** (**4m**, **4-**, and cleavage products **P** (Scheme 5)) and for methyl and naphthyl-1-phosphate. This material is available free of charge via the Internet at http://pubs.acs.org.

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