

## Nucleophilic Attack by Oxyanions on a Phosphate Monoester Dianion: The Positive Effect of a Cationic General Acid

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It has been known for many years that the dianions of phosphate monoesters are not readily attacked by anions. Kirby and co-workers showed that amines displace the aryloxy anion from the dianions of phosphate esters with good leaving groups (Scheme 1), but could find no reaction of fluoride or various oxyanions at the phosphorus center of *p*-nitrophenyl<sup>1</sup> or 2,4-dinitrophenyl phosphate<sup>2</sup> dianions, and concluded "as might be expected, the two negative charges provide a considerable electrostatic barrier toward reactions with nucleophilic reagents which have a negative charge on the attacking atom. However, this barrier is not absolute, as shown by the reactions of fluoride with phosphoramidate and acetyl phosphate."

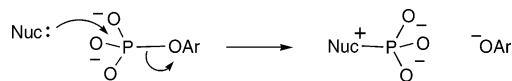
This broad generalization remains sound. The single negative charge on a phosphate diester anion is clearly not a serious barrier, as evidenced by the relatively rapid alkaline hydrolysis of such compounds, and both the exceptions cited involve what are formally monoanions. Thus, only the monoanion of acetyl phosphate reacts with fluoride,<sup>3</sup> and phosphoramidates have the structure R<sub>3</sub>N<sup>+</sup>–PO<sub>3</sub><sup>2-</sup>. Since these original observations, there has appeared abundant evidence of S<sub>N</sub>(P) reactions of fluoride and oxyanions with phosphoramidates,<sup>4–7</sup> with no apparent discrimination relative to neutral amine nucleophiles of similar basicity.<sup>5</sup>

For true dianions, the work of Herschlag has defined the "bottom line". The fluoride anion reacts at about the same rate as a molecule of water with the (terminal PO<sub>3</sub><sup>2-</sup> group of the) tetraanion of ATP, and similarly with the *p*-nitrophenyl phosphate dianion, which gives a few percent of fluorophosphate on hydrolysis in the presence of 1–3 M KF at 95 °C.<sup>8</sup> This result is consistent with the involvement of either an indiscriminately reactive electrophilic species (the metaphosphate monoanion) or an S<sub>N</sub>(P) reaction with fluoride (pK<sub>a</sub> 3.1, more phosphorophilic) reacting by coincidence at a rate similar to that of water (which is considerably less basic, but neutral). Because simple phosphate transfers in water have been shown to involve consistent inversion at P, the latter explanation is more likely<sup>5</sup> – presumably with a small electrostatic effect disfavoring attack by fluoride. (Herschlag estimates a maximum 50-fold electrostatic effect on rate at high ionic strength in water.<sup>5</sup>)

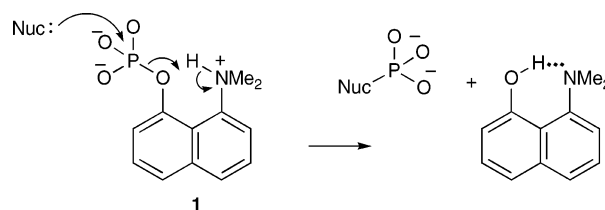
We report that electrostatic repulsion disappears when the hydrolysis of an aryl phosphate monoester is catalyzed by a neighboring cationic general acid. We are studying the hydrolysis of the phosphate monoester **1** of 8-(dimethylamino)-1-naphthol, a system known to support efficient intramolecular general acid catalysis (Scheme 2).<sup>9,10</sup>

The pH–rate profile for the hydrolysis of **1** shows a pH-independent plateau stretching almost from pH 4 to 9,<sup>11</sup> with hydrolysis in this region almost 10<sup>6</sup> times faster than expected for the spontaneous hydrolysis of a simple aryl phosphate dianion.<sup>12</sup> We propose the mechanism shown in Scheme 2 (**1**, Nuc = H<sub>2</sub>O), with nucleophilic participation by water enforced by the high energy

### Scheme 1

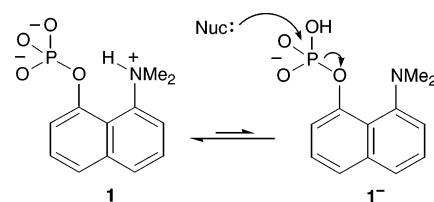


### Scheme 2



of the metaphosphate intermediate that would otherwise be formed.<sup>4</sup> Replacement of water by a better nucleophile provides nucleophilic catalysis, concerted with the general acid catalysis already in place (**1**, Nuc = H<sub>2</sub>O, fluoride, acetate, etc.). This is equally true for neutral amine, oxyanion, and fluoride anion nucleophiles, and the rate constants define a "normal" Brønsted plot (Figure 1), showing the expected low sensitivity to the pK<sub>a</sub> of the nucleophile (β<sub>nuc</sub> = 0.19 for reactions with oxyanions) and evidence for the (modest) α-effect expected for hydroxylamines. The point for fluoride (○) falls almost exactly on the line defined by the points (●) for oxyanion nucleophiles.

This result was unexpected, so it is important to rule out alternative explanations. As pointed out by referees, the (less favored) tautomer **1**<sup>-</sup> of the reactive ionic form of **1** can in principle react as a phosphate diester, and would thus not discriminate against anionic nucleophiles.

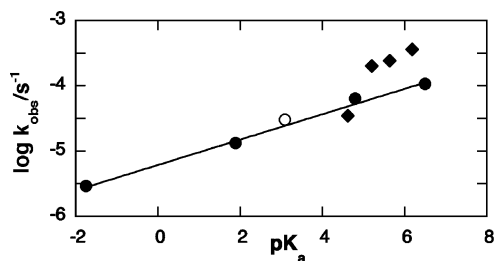


This alternative mechanism (**1**<sup>-</sup>, arrows) may be considered unlikely simply on the grounds that a phosphate diester is hydrolyzed more slowly than a corresponding monoester dianion under almost all conditions.<sup>13</sup> More specifically, we can estimate a rate constant for the hydrolysis of tautomer **1**<sup>-</sup> under the conditions of our reaction. Good data (at 100 °C) are available for the hydrolysis of diaryl phosphates.<sup>14</sup> Symmetrical diaryl phosphate monoanions are less reactive than alkyl aryl phosphates, and the (hypothetical) direct displacement reaction of an aryl hydroxy phosphate such as **1**<sup>-</sup> can confidently be expected to be slower still.

The rate constant for hydrolysis of the diphenyl phosphate monoanion at 100 °C can be estimated from the linear free energy

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**Figure 1.** Brønsted plot for phosphate transfer to the nucleophile (Scheme 1). Points are for oxyanions and water (●), fluoride (○), and hydroxylamines (◆: the point with a negative deviation is for MeONH<sub>2</sub>).

**Table 1.** Relative Reactivity of Nucleophiles toward the PO<sub>3</sub><sup>2-</sup> Group<sup>a</sup>

phosphate derivative	T (°C)	$k_{\text{rel}} = k_2(\text{nuc})/k_2(\text{H}_2\text{O})$			
		hydroxide	acetate	fluoride	pyridine
triester/2,4D <sup>13</sup>	39	$7.7 \times 10^6$	179	$1.1 \times 10^6$	907
diester Me/2,4D <sup>14</sup>	39	$1.45 \times 10^5$	21	5500	$1.76 \times 10^4$
$\gamma$ -picoline-PO <sub>3</sub> <sup>2-</sup> , <b>2</b> <sup>6</sup>	25	157	4.5	56	$1.75 \times 10^4$
2,4-DNPP <sup>2-</sup> , <b>3</b> <sup>2</sup>	39	<i>b</i>	<i>b</i>	<i>b</i>	206
pNPP <sup>2-</sup> , <b>4</b> <sup>1</sup>	39	<i>b</i>	<i>b</i>	<i>b,c</i>	896
salicyl P <sup>2-</sup> , <b>5</b> <sup>16</sup>	39	<i>b,d</i>	<i>b</i>	<i>b</i>	88
<b>1</b>	60	<i>b,d</i>	22	10.5	$\sim 22^e$

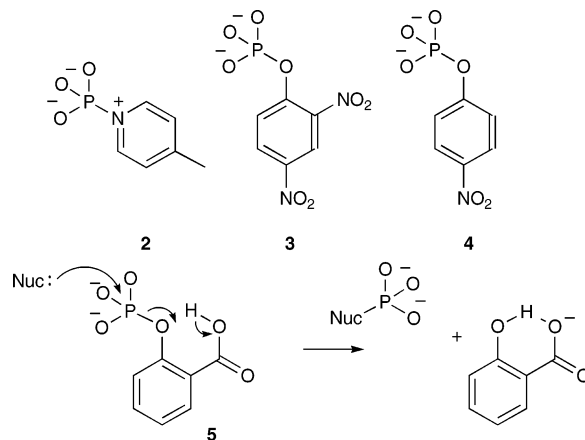
<sup>a</sup> The triester is 2-(2,4-dinitrophenyl)-2-oxo-1,3,2-dioxaphosphorinan, and the diester methyl is 2,4-dinitrophenyl phosphate. <sup>b</sup> No detectable reaction. <sup>c</sup> Estimate: <2. <sup>d</sup> Reactive form absent at high pH. <sup>e</sup> Estimated from the Brønsted plot (Figure 1).

relationship of Kirby and Younas<sup>14</sup> as  $4.72 \times 10^{-10} \text{ s}^{-1}$ ; at 60 °C, the reaction would be expected to be some 80 times slower (using  $E_a = 27 \text{ kcal/mol}$ ), viz.  $6 \times 10^{-12} \text{ s}^{-1}$ . This is  $2.7 \times 10^7$  times slower than that observed for **1**. Yet **1**<sup>-</sup> is present in only one part in  $2.34 \times 10^5$ ,<sup>11</sup> so the observed rate of hydrolysis is over  $6 \times 10^{12}$  times faster than that expected for reaction via **1**<sup>-</sup>. Furthermore, **1**<sup>-</sup> has no viable catalytic group; nucleophilic catalysis by NMe<sub>2</sub> would require in-line displacement, but the only viable leaving group is the naphthol, which cannot get in-line. We conclude that the hydrolysis of **1** involves direct nucleophilic attack on the PO<sub>3</sub><sup>2-</sup> group, assisted by intramolecular general acid catalysis by the neighboring dimethylammonium group, well known to be efficient in this role in this situation.

Thus, the negative, repulsive electrostatic effect on the attack of nucleophilic anions on the PO<sub>3</sub><sup>2-</sup> group can be “neutralized” not only by a positive charge directly attached to the electrophilic phosphorus center, but even by one on a neighboring group in the reactant and transition states. Relevant data are summarized in Table 1, which compares rates for attack of representative nucleophiles on various phosphate derivatives, taking water as the (nominally neutral) standard. Figures for reactions with activated di- and triesters are included for a broader comparison.<sup>15</sup>

There is no detectable catalysis by any anion of the hydrolysis of activated esters <sup>2</sup>-O<sub>3</sub>P-OAr (**3** and **4**), although the absolute reactivity of **1** is comparable to that of **3**. The result with **1** also stands in significant contrast to previous experience with general acid catalysis of phosphate transfer by the carboxyl group, in salicyl phosphate **5**. There too the neighboring general acid is a highly effective catalyst for the hydrolysis of the phosphate monoester (**5**, Nuc = H<sub>2</sub>O),<sup>16</sup> and there too attack by better nucleophiles than

water is observed. Yet catalysis is limited to reactions with neutral nucleophiles (**5**, Nuc = substituted pyridines).



This result suggests a further potential role for the abundance of cationic amino acid side chains (lysines and histidines, possibly also arginines) in the active sites of enzymes such as phosphatases and kinases catalyzing phosphate transfer reactions from monoesters (presumably also from ATP) to oxyanion nucleophiles such as carboxylate and phosphate. It seems that cationic groups may do more than stabilize the developing negative charge on the leaving group and equatorial oxygens of the pentacovalent transition state. The mechanism of the effect will be discussed in our full paper; a clue is that the second pK<sub>a</sub> of the phosphate group of the reactant **1** is reduced to  $3.94 \pm 0.06$ , unmistakable evidence for the presence of a strong intramolecular hydrogen bond.<sup>11</sup>

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**Supporting Information Available:** Synthesis and characterization of 8-(dimethylamino)-1-naphthyl phosphate **1**. Tables of kinetic data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216.
- (2) Kirby, A. J.; Varvoglis, A. G. *J. Chem. Soc. B* **1968**, 135–141.
- (3) di Sabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4393–4400.
- (4) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7579–7586.
- (5) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7587–7596.
- (6) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1990**, *112*, 1951–1956.
- (7) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1990**, *112*, 1942–1950.
- (8) Admiraal, S. J.; Herschlag, D. *J. Am. Chem. Soc.* **1999**, *121*, 5837–5845.
- (9) Kirby, A. J.; Percy, J. M. *J. Chem. Soc., Perkin Trans. 2* **1989**, 907–912.
- (10) Asaad, N.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1708–1712.
- (11) Ionizations at pK<sub>app</sub> 3.94 and 9.31 were identified (by NMR titration) with the phosphate and dimethylammonium groups, respectively. These pK<sub>a</sub>'s are shifted substantially as compared to those for similar unperturbed groups, consistent with a strong intramolecular hydrogen bond between them in the ground state. This hydrogen bond plays a significant role in enabling anion attack, even though stabilization of the reactant will in general reduce the extent of general acid catalysis.
- (12) Kirby, A. J.; Dutta-Roy, N.; da Silva, D.; Lima, M. F.; Nome, F., to be published.
- (13) Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1970**, 1172–1182.
- (14) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165–1172.
- (15) Note that reactions of <sup>2</sup>-O<sub>3</sub>P-X compounds are typically insensitive to the pK<sub>a</sub> of the nucleophile. This is the main reason for the higher values of  $k_{\text{rel}}$  for the di- and triesters.
- (16) Bromilow, R. H.; Kirby, A. J. *J. Chem. Soc. B* **1972**, 149–155.

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