Models for Nuclease Catalysis: Mechanisms for General Acid Catalysis of the Rapid Intramolecular Displacement of Methoxide from a Phosphate Diester

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The dianion of the dialkyl phosphate diester **3** is hydrolysed in water at 50 °C with a half-life of less than 2 min. The reaction involves highly efficient intramolecular nucleophilic catalysis (effective molarity $\geqslant 10^{10}$ mol dm^{-3}) by the phenol OH over the whole pH range. The initial products are the cyclic phosphate diester and methanol: no phosphate migration is observed. General acid catalysis is observed for the reactions of all ionic species, and has been characterised in detail for the mono- and di-anion. An important electrostatic effect, observed for general acids with a suitably positioned second NH^+ group, stabilises the transition state for the loss of methoxide from the dianion by up to 12.3 kJ mol^{-1}, even in water. The data allow an estimate of 10^4–10^5 for the factor by which protonation to give the triester activates a phosphate diester towards attack by a neighbouring OH group.

Mechanisms involving pentacovalent addition intermediates are proposed for the reactions of the neutral ester acid and the monoanion. The phosphorane dianion is considered to be an intermediate in the cyclisation of the substrate dianion, but with a lifetime too short for diffusional equilibration.

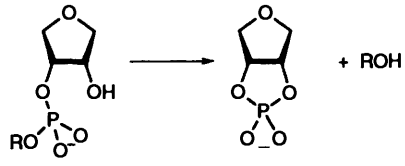
Of the phosphate esters which play a role in living systems, diesters are the most important, and the least reactive. The stability towards hydrolysis of DNA is crucial to the conservation of the genetic information, and even RNA, which is readily hydrolysed in alkali, is cleaved only very slowly at pH 7 in the absence of a relevant enzyme. This hydrolysis is in fact so slow that good quality data on catalysis of the reaction are difficult to obtain. This in turn makes it difficult to draw firm conclusions about mechanism, and there is conflicting evidence on the status—transition state or intermediate—of the pentacovalent species involved in such reactions.

Reactions involving DNA are slower still, but of growing interest as an increasing number of enzymes involved in DNA processing have been characterised in recent years. All these reactions,[1a-d] and related processes involving ribozymes,[1e] share a common chemistry, in which a phosphodiester undergoes one or more transesterifications, catalysed by one or more general bases, and one or more general acids or metal cations. The basic reaction is the displacement of one OH group by another, and it is a reasonable supposition that the number of mechanisms by which this can be achieved with high effficiency is small.

We are looking at a series of phosphate esters designed to show high reactivity in reactions catalysed by neighbouring OH groups, in order to define and understand these mechanisms, and the factors controlling the efficiency of their catalysis by general acids and bases. These factors hold the key to a proper understanding of the same reactions catalysed by enzymes.

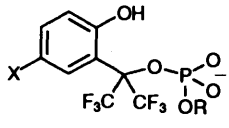
Most published work has focussed on the mechanism of action of the ribonucleases, particularly the familiar ribonuclease A,[1b] and the non-enzymic hydrolysis of systems based on the part-structure **1** has been studied by a number of authors.[2,3] Apart from the intrinsic interest of the reaction, catalysed by an enzyme whose three-dimensional structure was one of the earliest to be solved, this reflects also the relatively high reactivity of the system, in which the neighbouring 2'-OH group acts as a nucleophile to give a cyclic ester **2**. The reaction thus proceeds at a rate which is measurable, though by no means conveniently measurable, in the region of interest near pH 7. The simple dinucleotides UpU and ApA, for example, studied by Breslow and his co-workers, using HPLC to measure product formation,[3-6] have half-lives of days even at 80 °C.

These rates are controlled in large part by the effective molarity (EM)[7] of the participating hydroxy group. This has



1 **2**

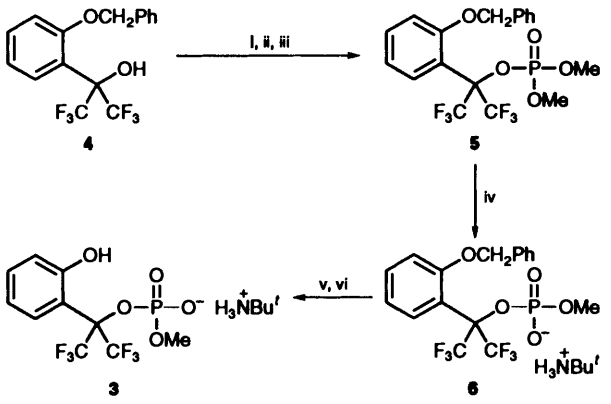
been estimated[7] at 3×10^7 mol dm^{-3} for the hydroxide-catalysed reaction of the phenyl ester (**1**, R = Ph).[8] A hydroxyalkyl phosphate diester with a higher effective molarity should undergo the same basic reaction at a proportionately faster rate. We report a detailed study of the hydrolysis of one such ester **3**, with supporting data for the reactions of the three derivatives **3a–c** shown. Compound **3** is designed to show an effective molarity large enough to allow us to observe the displacement of a simple alkoxy group from phosphorus in the absence of complications from the ionisation of the nucleophilic hydroxy-group; which in the case of a phenol can be fully ionised well within the pH range. The fully ionised phenolate is a simple surrogate for a partially deprotonated alcohol OH group, and models directly some less familiar enzyme-catalysed reactions. [At least two classes of nucleotide-modifying enzyme which form covalent enzyme–DNA intermediates (λ-integrases[1c] and topoisomerases[1d]) may use tyrosine as an active site nucleophile.] The phenol chromophore also makes it possible to follow the reaction continuously, while the two CF_3 groups are included to minimise competition from the S_N1 loss of phosphate (expected to be particularly rapid from the phenolate anion, to give a quinone methide).[9] The design was successful: we see no evidence of C–O cleavage products under any conditions. Ester **3** is hydrolysed over 50 000 times faster than UpU in imidazole-buffered solution, and its hydrolysis is strongly catalysed by general acids and bases.



3 X = H, R = CH_3
3a X = OMe, R = CH_3
3b X = H, R = CH_2CH_2F
3c X = H, R = CH_2CF_3

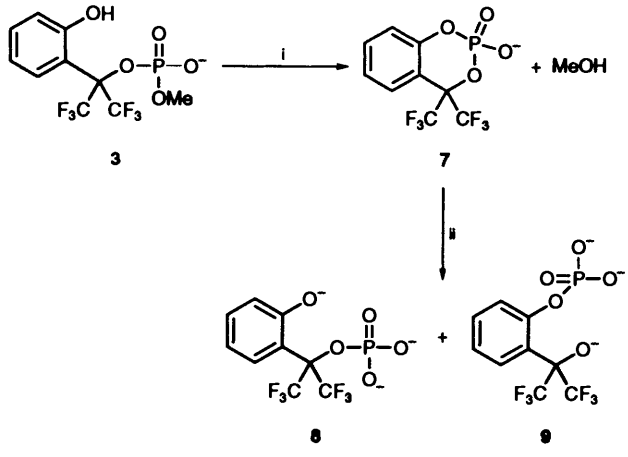
Results

Synthesis.—The synthesis of a molecule designed to cyclise rapidly requires a careful choice of protecting groups, to prevent premature cyclisation. The procedure we eventually developed for the preparation of the esters **3** and **3a–c** (described in Scheme 1 for **3**) uses the benzyl group to protect the phenol oxygen. This could be removed rapidly by hydrogenolysis; various other protecting groups proved unsatisfactory.



Scheme 1 *Reagents and conditions:* i, $MeOPCl_2$, DIEA; ii, MeOH, 45 min; iii, I_2, H_2O–THF–py; iv, Bu^tNH_2, 15 h; v, H_2, Pd–C, 10 min; vi, dil. HCl–MeCN pH 3

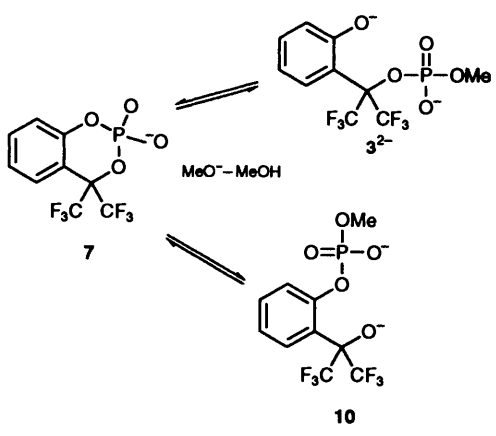
The phosphate diester **3** exhibits highly efficient intramolecular catalysis of hydrolysis over the entire pH range. The reaction is completely specific at all pH's, and involves cyclisation to **7**. This is the only product detectable by ^{31}P NMR spectroscopy after ten half-lives except in KOH solutions, where **7** is hydrolysed further to **8** and **9**.



Conditions: i, 50 °C, μ = 1.0 mol dm^{-3} (KCl), pH < 11; ii, 50 °C, μ = 1.0 mol dm^{-3} (KCl), pH > 11

There is no evidence for displacement of the better but endocyclic hexafluoroisopropoxy leaving group (the pK_a of hexafluoroisopropanol is 9.3, compared with 15.5 for methanol). We believe we can safely rule out a more complicated sequence of reactions involving phosphate transfer as the first step. The hydrolysis reaction shows a clean isosbestic point at all pHs. When the cyclic diester **7** is dissolved in methanol containing MeONa (1.75 mol dm^{-3}) rapid ring opening occurs to give the dianion of **3**, as shown by the appearance of the phenolate chromophore. This then disappears with a half-life of 11 min at room temperature, presumably with the formation of **10** as the thermodynamic product.

Kinetics.—Kinetic results were obtained in water at 50 °C and ionic strength 1.0 mol dm^{-3} (KCl). The reactions showed



strong buffer catalysis in almost every case. pH–Rate profiles for the uncatalysed hydrolysis of all four compounds (**3** and **3a–c**) were obtained by extrapolating to zero buffer concentration results from a range of concentrations at each pH. The resulting plots are brought together in Fig. 1. The curves are made up of contributions from four different reactions, simultaneous at some pH's, according to the rate law in eqn. (1): the acid catalysed hydrolysis of the neutral phenol acid HSH (k_1); the apparent spontaneous hydrolysis of the neutral species, which we presume represents the kinetically equivalent H_3O^+-catalysed reaction of the monoanion HS^- (k_2); the apparent spontaneous hydrolysis of the monoanion (k_3, which probably represents the kinetically equivalent H_3O^+-catalysed reaction of the substrate dianion, as discussed below); and the spontaneous hydrolysis of the dianion S^{2-} itself (k_4). The data were fitted by the method of weighted non-linear least squares* to eqn. (2) to give the values of up to four rate and two dissociation constants shown in Table 1. The data for compound **3** allow the dissection of the pH–rate profile into the separate contributions from the reactions of the various ionic forms, shown in Fig. 2.

$$k_{obs} = k_1[HSH][H] + k_2[HS^-][H] + k_3[HS^-] + k_4[S^{2-}] \quad (1)$$

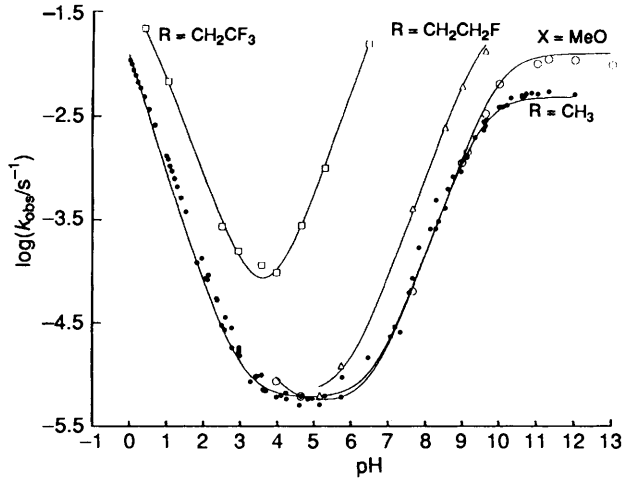


Fig. 1 pH–Rate profiles for the hydrolysis of **3**, **3a** (X = OMe), **3b** (R = CH_2CH_2F) and **3c** (R = CH_2CF_3), at 50 °C and ionic strength 1.0 mol dm^{-3} (KCl)

* Least squares fits of derived data were weighted according to the standard error of the slope or intercept concerned. For details see the Experimental section.

Table 1 Rate and kinetic dissociation constants for the hydrolysis of substrates **3** and **3a–c**, at 50 °C and ionic strength 1.0 mol dm^{-3}

	3[a]	**3a**[a]	**3b**	**3c**
k_1 (dm^3 mol^{-1} s^{-1})	1.26 ± 0.27 × 10^{-2}			3.72 × 10^{-2} (β_{LG}[c] −0.15)
k_2 (dm^3 mol^{-1} s^{-1})	7.60 ± 0.12 × 10^{-3}	4 × 10^{-2}[b]		9.14 × 10^{-2} (β_{LG}[c] −0.35)
k_3 (s^{-1})	5.97 ± 0.19 × 10^{-6}	5.26 × 10^{-6} (β_{nuc} −0.13)	6.47 × 10^{-6}	4.28 × 10^{-5} (β_{LG}[c] −0.28)
k_4 (s^{-1})	4.80 ± 0.32 × 10^{-3}	1.24 × 10^{-2} (β_{nuc} 0.95)	2.71 × 10^{-2}	17.7 (β_{LG}[c] −1.13)
K_1[P(O)OH]	0.13 ± 0.20			(Set at 0.2)
K_2(phenol)	2.85 ± 0.42 × 10^{-10}	1.05 × 10^{-10}		

[a] Rate constants for the hydroxide catalysed ring opening of the cyclic ester products from **3** and **3a** are 1.94 ± 0.06 × 10^{-3} and 1.0 × 10^{-3} dm^3 mol^{-1} s^{-1}, respectively. [b] Order of magnitude only: based on a single point. [c] The pK_a's (used for the calculation of β_{LG}) for the leaving group alcohols are: methanol, 15.5; 2-fluoroethanol, 14.2 and 2,2,2-trifluoroethanol, 12.43. (W. P. Jencks and J. Regenstein, *Handbook of Chemistry and Molecular Biology*, ed. G. D. Fasman, CRC Press, Inc., vol. 1, 1976).

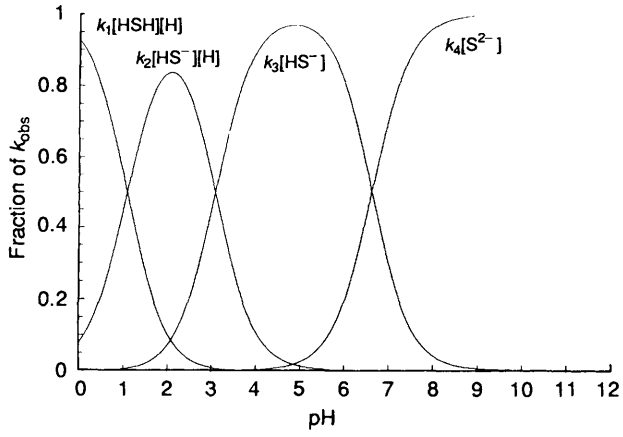


Fig. 2 Fraction of k_{obs} for the hydrolysis of **3** plotted as a function of pH, for the four kinetic terms of eqn. (2). The data are derived from the non-linear least squares fit of the pH–rate profiles to eqn. (2).

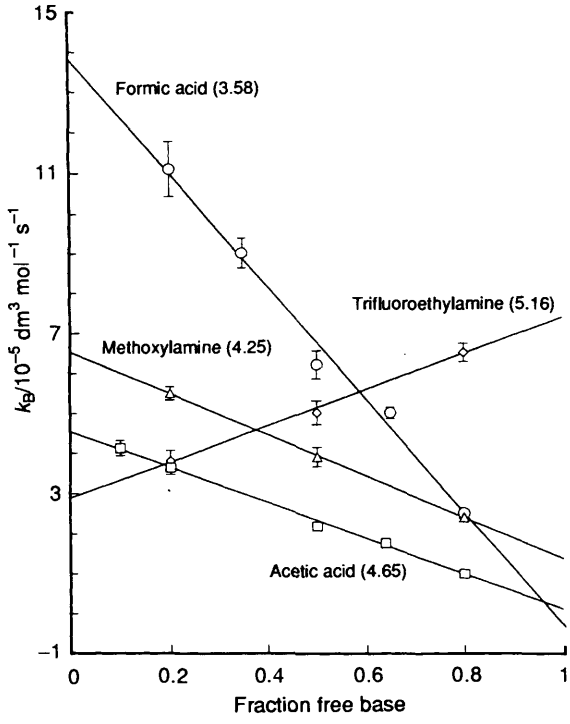


Fig. 3 Plot of buffer catalysis rate constants for the cyclisation of the monoanion of **3** at 50 °C and ionic strength 1.0 mol dm^{-3} (KCl), against the fraction of the free base component of the buffer

$$k_{obs} = \frac{k_1H^2 + k_2K_1H + k_3K_1}{H + K_1} + \frac{k_4K_2}{H + K_2} \quad (2)$$

Detailed pH–rate profiles for hydrolysis were not obtained for the derivatives of **3**, but several characteristic differences are evident (Fig. 1). For compound **3a** the pH–rate profile differs very little from that of **3** above pH 4, above which it follows the ionisation of its slightly more basic phenol. Compound **3b** is hydrolysed slightly faster than **3** between pH 5 and 9.6, the only region in which it was studied. Compound **3c** is significantly more reactive than **3** at all pH's. It has a very small pH independent region at pH 3.5.

Buffer catalysis. Second-order rate constants for catalysis by buffers (k_B) were calculated in terms of the major ionic species of the substrate present at the pH concerned. Plots of the rate constants thus obtained against the fraction of the buffer present in the free base form then gave second-order rate constants for catalysis by the acid and base forms of the buffer, as the intercepts of these linear plots at zero and 100% free base, respectively. Fig. 3 shows one such plot for a representative set of data for catalysis of the hydrolysis of the monoanion of **3**. Formic, the strongest acid represented, acts almost exclusively as a general acid, whereas 2,2,2-trifluoroethylamine, the most basic catalyst in this set, acts predominantly as a general base. We interpret this latter reaction, as discussed below, as the kinetically equivalent general acid catalysed reaction of the substrate dianion. Because of the high rate of the spontaneous reaction of the dianion, buffer catalysis was detectable for ester **3c** only for the most efficient general acid, the dication of TMEDA.

The large sets of rate constants obtained in this way for the reactions of the three different ionic forms of **3** are given in Tables 2–4. The best catalysts proved to be the dications derived from the protonation of 1,2-diamines, and a possible explanation in terms of electrostatic effects is discussed below. We explored possible electrostatic effects further by measuring catalysis by various other cationic general acids of interest, but none shows as large an effect as $Me_2NH^+(CH)_2NH^+Me_2$. As would be expected, the parent ethylenediammonium dication (pK_a 7.72) shows a comparable enhancement (1.8 times smaller than for the TMEDA dication before statistical correction). But acetamidinium and guanidinium cations are no more effective than expected for primary alkylammonium cations of the same high pK_a.

Deuterium isotope effects and thermodynamic parameters for the cyclisation reaction of **3** under representative conditions are given in Tables 5 and 6 respectively.

Discussion

The hydrolysis of **3** and its derivatives involves highly efficient intramolecular nucleophilic catalysis by the phenol OH across the whole pH range, with specific loss of the alkoxy group for all ionic forms of the substrate. The high effective molarity of the OH means that cyclisation is the only reaction observed. The same factor will stabilise pentacovalent addition intermediates on the reaction pathway. Alkylated pentaoxyphosphoranes are relatively stable compounds, and there is good evidence that such species can be intermediates in the hydrolysis of at least

Table 2 Statistically corrected pK_a's and rate constants for the cyclisation of the neutral form of **3** catalysed by general acids[a]

General acid	No. of runs	$pK_a + \frac{p}{q}$	$\left(10^5 \times \frac{k_{AH}}{p}\right)$/ dm^3 mol^{-1} s^{-1}	$\log \frac{k_{AH}}{p}$
Proton		−1.22	420 ± 90	−2.38
Betaine	15	1.65	209 ± 26	−2.68
Cyanoacetic acid	15	2.05	264 ± 47	−2.57
Chloroacetic acid	9	2.47	141 ± 33	−2.85
Methoxyacetic acid	9	3.15	138 ± 48	−2.86
Water		15.31	0.9 ± 1.4	−5.05

[a] The pK_a's used are the pH values of 50% free base buffer solutions under the conditions of the kinetic measurements. p corresponds to the number of protons that can be donated by the acid form of the buffer. q represents the number of sites on the conjugate base that can accept a proton.

Table 3 Rate constants for general acid catalysed cyclisation of the monoanion of **3** at 50 °C and ionic strength 1.0 mol dm^{-3} (KCl), catalysed by oxyacids and nitrogen acids with pK_a 1.95–6.68 (p and q are defined as for Table 2)

General acid	No. of runs	$pK_a + \frac{p}{q}$	$\left(10^5 \times \frac{k_{AH}}{p}\right)$/ dm^3 mol^{-1} s^{-1}	$\log \frac{k_{AH}}{p}$
Proton		−1.22	253 ± 4.0	−2.12
Betaine	15	1.65	24.1 ± 2.2	−3.62
Cyanoacetic acid	15	2.05	19.0 ± 1.6	−3.72
Chloroacetic	15	2.47	10.9 ± 0.43	−3.96
Methoxyacetic acid	15	3.15	7.27 ± 0.13	−4.14
Formic acid	15	3.28	13.92 ± 0.47	−3.86
DABCO	6	3.49	45.1 ± 0.04	−3.34
Acetic acid	15	4.35	4.521 ± 0.083	−4.34
Methoxylamine	9	4.73	2.189 ± 0.006	−4.66
Trifluoroethylamine	9	5.64	0.965 ± 0.032	−5.02
Hydroxylamine	15	5.96	7.16 ± 2.2	−4.15
Phosphate	15	6.29	0.82 ± 0.26	−5.09
Imidazole	9	6.98	12.6 ± 4.7	−3.90
Water		15.31	5.38 ± 0.17 × 10^{-3}	−7.27

cyclic phosphate esters.[2] It is therefore a reasonable presumption that such intermediates are involved in the reactions of the undissociated forms of our substrates, and probably also for the monoanions. The presence of two negative charges on the phosphorane will reduce its lifetime significantly: we conclude below that the phosphorane dianion is an intermediate in the cyclisation of the dianion of **3**, but that its lifetime is too short for diffusional equilibration.

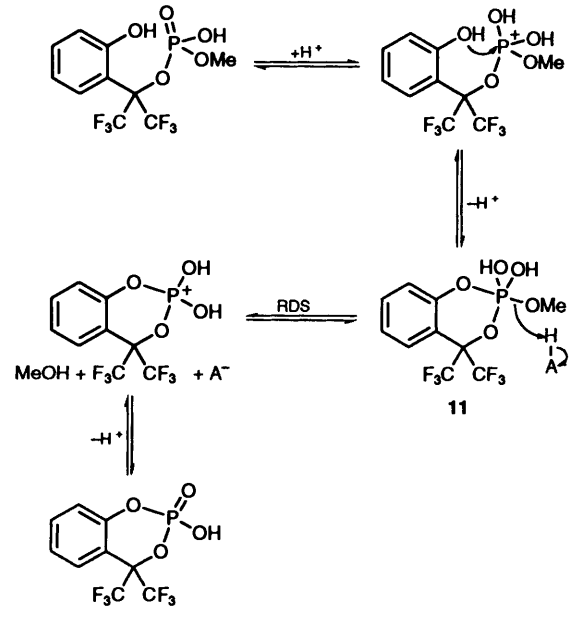
Effective Molarity.—We can estimate only an approximate value for the EM of the phenol oxygen of **3**, because the available intermolecular comparisons necessarily involve much better leaving groups than methoxide. Williams and his coworkers have measured the dependence on the leaving group of the displacement by phenolate of aryloxide anions from methyl aryl phosphate diesters, and from diethyl aryl triesters.[10] A long extrapolation (from 4-nitrophenoxy to methoxy) allows estimates of the rates for the displacement of methoxide in each case, and these can be compared with the observed rate constant for the cyclisation of the dianion of **3** (Table 1), and for the reaction of the reactive (phenolate-P–OH) form of the monoanion (Scheme 3), respectively. The two estimates are consistent in giving EM $\geqslant 10^{10}$ mol dm^{-3}.

Reactions of the Neutral Acid.—Cyclisation is only 2–3 times faster in 1 mol dm^{-3} acid than in the pH-independent region above pH 10, though the leaving group in acid will be methanol rather than methoxide. The likely mechanism is shown in Scheme 2.

Table 4 Rate constants for the general acid catalysed cyclisation of the dianion of **3**, at 50 °C and ionic strength 1.0 mol dm^{-3} (KCl), catalysed by oxyacids and by nitrogen acids with pK_a 4.25–10.67 p and q are defined as for Table 2)[a]

Buffer	$pK_a + \log \frac{p}{q}$	$10^{-3} \times k_{AH}$/ dm^3 mol^{-1} s^{-1}	$\log \frac{k_{AH}}{p}$
Proton	−1.22	2.09 ± 0.47 × 10^7	3.84
Acetic acid	4.35	94.0 ± 77	−1.03
Methoxylamine	4.73	2680 ± 39	−0.05
Trifluoroethylamine	5.64	1780 ± 27	−0.23
Cacodylic acid	5.89	*ca.* 80	*ca.* −1.1
Hydroxylamine	5.96	12 100 ± 790	0.61
PMEDA	6.01	1385	0.14
TMEDA diacid	6.27	34 200 ± 190	1.23
Phosphate	6.29	333 ± 11	−0.78
Imidazole	6.98	1371 ± 125	−0.16
EDA diacid	7.72	7600	0.10
2-Methylimidazole	7.88	356 ± 9	−0.75
TMPDA diacid	8.13	495 ± 17	−0.61
TRIS	8.14	273 ± 7	−1.04
Chloroquinuclidine	8.29	288 ± 58	−0.54
PMDETA diacid	8.33	80	−1.1
DABCO	8.45	250 ± 3	−0.60
PMDETA monoacid	8.90	13	−1.88
TMBDA diacid	8.91	100.9 ± 1.6	−1.30
TMEDA	8.81	28.6 ± 4.9	−1.54
Serine	9.02	108 ± 4	−1.44
Carbonate	9.10	3.65 ± 0.67	−2.44
TMPDA	9.34	19.6 ± 2.5	−1.71
DETA diacid	9.40	180	−1.52
Methoxyethylamine	9.47	83.7 ± 5.0	−1.55
TMHDA diacid	9.46	22.1 ± 3.5	−1.96
Glycine	9.60	80.0 ± 12	−1.57
TMBDA	9.77	15.1 ± 2.4	−1.82
DETA monoacid	9.97	56	−1.7
β-Alanine	10.10	43.7 ± 0.3	−1.84
Propylamine	10.47	42.4 ± 6.1	−1.85
Quinuclidine	10.67	28.5 ± 1.2	−1.54
Piperidine	10.90	22.8 ± 1.6	−1.94
Guanidinium	14	6	−3
Water	15.31	8.65 ± 0.58 × 10^{-2}	−4.36

[a] Abbreviations: TMXDA indicates *N,N,N',N'*-Tetramethyl-X-diamine: X = E,P,B,H = $(CH_2)_n$, when n = 2, 3, 4 and 6, respectively. PMEDA is pentamethylethylenediamine, PMDETA is pentamethyldiethylenetriamine, DABCO is diazabicyclooctane, and TRIS is tris-(hydroxymethyl)aminomethane.



Scheme 2

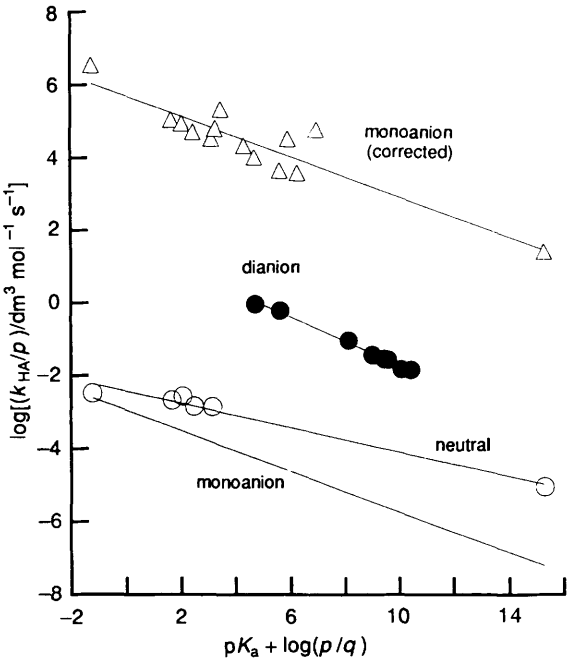


Fig. 4 Brønsted plots for general acid catalysis of the cyclisation reactions of the three ionic forms of **3**. The data are selected from Tables 2–4, and best least squares lines drawn through the points shown. The two lines for the monoanion represent the same set of data, shown (only) for the upper line, which is calculated for the minor ionic form [**3**$^-$] (Scheme 3).

We know from the work of McClelland and his group[11–13] that the first reaction of a phosphonium cation with a neighbouring hydroxy group is cyclisation to a phosphorane, in this case **11**. It is not obvious whether formation or breakdown of **11** will be rate determining, because appropriate timing of the proton transfer step can make an apparently poorer (more basic) leaving group leave faster. (As demonstrated for general acid catalysis of the hydrolysis of some acetals.[14]) However, the aryloxy group is lost selectively, up to at least pH 4.6, in the acid catalysed hydrolysis of the MePhP$^+$(OMe)OPh cation,[11] which involves a phosphorane with a similar choice of leaving groups, so we are on fairly safe ground in taking the loss of methanol to be rate determining. This is also consistent with the observation of general acid catalysis of the reaction: if the formation of the intermediate were rate determining this would involve rate determining general base catalysis of the addition of the phenol oxygen in the forward direction; not impossible in the circumstances, but not a known reaction.

The general acid catalysed reaction of the neutral form shows low sensitivity to the pK_a of the general acid. The data (not very good at low pH, and depending strongly on the value taken for the pK_a of the phosphoric acid group of the ester) are correlated by a Brønsted α of 0.1–0.2, for a small set of general acids with pK_a < 3.2, but both H_3O^+ and H_2O points fall close to the line (Fig. 4). This α-value is consistent with the early transition state expected for the breakdown of a high energy intermediate, but is very different from the α value of unity found by McGall and McClelland[12a] for the general acid catalysed expulsion of methanol from ethylenedioxy methoxydiphenylphosphorane (2,2-diphenyl-2-methoxy-1,3,2-dioxaphospholane). This α value of unity is not easy to explain,[12a] but we note that smaller values of α are observed for the general acid catalysed hydrolysis of similar systems with six- rather than five-membered rings,[12b] and particularly for acyclic phosphoranes;[13] and are in any case expected for a hydroxy- rather than an alkoxy-phosphorane. (The hydrolysis of hemiacetals routinely shows general acid–base catalysis whereas that of the corresponding acetals is specific acid catalysed.) However, our data are neither accurate nor extensive enough to warrant a detailed discussion.

The Monoanion Reaction.—As for the neutral compound, the absence of any observable phosphate migration means that the best evidence—direct observation apart—for the existence of a pentaoxyphosphorane intermediate (**11**, Scheme 2, or **12**, Scheme 3) is lacking. Such migration has been observed previously in Breslow's work on UpU;[3] and for reactions going by way of pentaoxyphosphorane monoanions formed by the addition of the carboxylate oxygen of a dialkyl or diaryl salicyl phosphate to the neighbouring phosphate triester phosphorus.[15] The breakdown of these latter phosphorane anions, which would be less stable than **12**, was shown to depend exclusively on the pK_a of the leaving group, and not at all on geometry. This is in sharp contrast to the behaviour of the corresponding phosphate diesters,[16] where displacement is exclusively exocyclic (either because of the high barriers to pseudorotation of phosphorane dianions, or because they do not exist as full intermediates, so that in-line displacement is enforced).

In neither case (**11** or **12**) under discussion here is pseudorotation precluded (see ref. 2 for a recent discussion of the Westheimer rules). The simplest explanation for the lack of phosphate migration is that the necessary pseudorotation is thermodynamically unfavourable for steric reasons: the hexafluoroisopropoxy group has about the same electronegativity (*i.e.* almost the same pK_a) as the phenolic oxygen, but is much bulkier. Because the two are in a ring they cannot both be apical, so the known high apicophilicity of the phenoxy group[2] is reinforced by the steric preference of the bulky group for the equatorial position.

Table 5 Solvent deuterium isotope effects for the cyclisation of **3**, at 50 °C and ionic strength 1.0 mol dm^{-3}

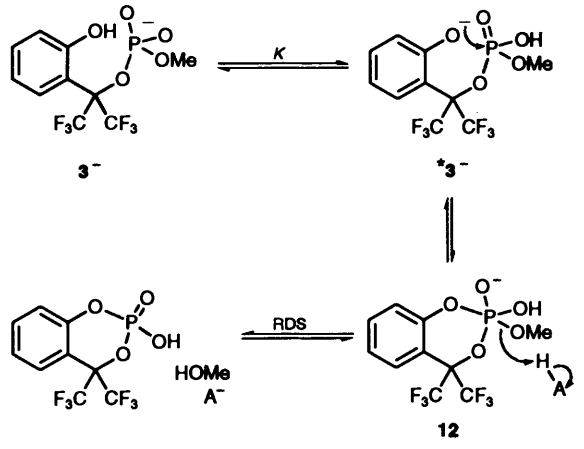
	k_H/k_D	
General acid	Monoanion	Dianion
H_2O	1.18 ± 0.07	1.188 ± 0.13
Acetic acid	1.63 ± 0.13	
TRIS		1.92 ± 0.44
β-Alanine		1.81 ± 0.17
$PrNH_2$		1.80 ± 0.29
Piperidine		1.76 ± 0.42

Table 6 Thermodynamic parameters for the cyclisation of **3**, at ionic strength 1.0 mol dm^{-3}

Reaction	E_a/ kJ mol^{-1}	$\Delta H^‡$/ kJ mol^{-1}	$\Delta S^‡$/ J K^{-1} mol^{-1}	$\Delta G^‡$/ kJ mol^{-1}
k_0, monoanion[a]	104.4 ± 1.2	101.7 ± 1.2	−65.5 ± 3.7	122.9 ± 1.2
Monoanion, k_{HA} (AcOH)[b]	88.9 ± 2.3	86.2 ± 2.3	−62.4 ± 6.9	106.4 ± 3.2
k_0, dianion[c]	72.5 ± 2.0	69.8 ± 2.0	−107.1 ± 6.4	104.4 ± 2.9
Dianion, k_{HB}·(piperidine)[c]	71.3 ± 4.9	68.6 ± 4.9	−65 ± 16	89.6 ± 7.0

[a] From measurements at five temperatures between 50 and 70 °C at pH 5.16 in trifluoroethylamine buffer, 50% free base. [b] From measurements at five temperatures between 50 and 70 °C in 0.4–1.0 mol dm^{-3} acetic acid–acetate buffers, 50% free base, pH 4.65 at 50 °C. [c] From measurements at three temperatures between 30 and 50 °C in 0.10–0.25 mol dm^{-3} piperidine buffers, 50% free base, pH 10.61 at 50 °C.

The reactions of the monoanion may be presumed to go by way of the less favourable ionic form $*3^-$ (Scheme 3). This conclusion is supported by the following order of magnitude calculation. The equilibrium constant K for the pre-equilibrium $3^- \rightleftarrows *3^-$ is 2.19×10^{-9}, the ratio of the dissociation constants (Table 1) for the phosphoric acid and the phenol OH. This figure allows a calculation of relative reactivity of the phosphate diester anion (Scheme 4, below) and the undissociated diester acid (the electrophile in Scheme 3) towards the phenolate nucleophile. The ratio depends on the general acid, since the Brønsted α differs for the two reactions, but lies in the range from 490 (for HA = H_3O^+) to 5.5×10^5 (for the spontaneous reaction: HA = H_2O). For general acids with pK_a near 7 the ratio is 10^4–10^5 (*e.g.* 8.1×10^4 for imidazolium). These figures for the rate acceleration available from protonation of the phosphate diester dianion are consistent with our recent results for related systems where both general acid and general base catalysis are observed,[17] and for derived systems with no kinetic ambiguity where diester anion reactivity is compared with the same reaction of a methyl triester:[18] and thus consistent with the pre-equilibrium proton transfer shown in Scheme 3.



Scheme 3

The mechanism of buffer catalysis of the hydrolysis of the monoanion, unusually, seems unambiguous. General acid catalysis, observed for buffer acids with $pK_a < 6.68$, is readily explained in terms of rate determining breakdown of the pentaoxyphosphorane monoanion **12** (Scheme 3). If this step is rate determining for the breakdown of the neutral phosphorane it certainly will be for the reaction of the anion, as more negative charge develops on the leaving group oxygen in the anionic transition state. (Measured values of Brønsted β_{LG} are significantly negative for the reactions of nucleophiles with diesters, and substituted phenolate anions are displaced selectively from aryl methyl phosphate ester dianions.[10,19,20]) This is consistent with the two-point Brønsted (leaving-group) plots of the data in Table 1 for **3** and **3c**, which show an increase in sensitivity from -0.15 to -0.35 in β_{LG} for the H_3O^+-catalysed reactions of the neutral compound and the monoanion, respectively.

The Brønsted α for general acid catalysis by substituted acetic acids is 0.27, and data points for most other general acids, including H_3O^+, lie close to the least squares line defined by them (Fig. 4). Apparent positive deviations are observed for the DABCO (diazabicyclooctane) dication, and for hydroxylammonium and imidazolium, but a hint of curvature in the plots corresponding to Fig. 3 suggests that the figures could be overestimated. In any case the kinetic data—the rate law, the entropy of activation and the sensitivity to structural variation—are all consistent with the mechanism of Scheme 3, with a fairly early transition state for P–O bond breaking. The cyclisation of the monoanion catalysed by acetic acid shows a small but significant solvent deuterium isotope effect (1.63), and a strongly negative entropy of activation (Table 6), also consistent with the mechanism of Scheme 3.

The apparent general base catalysis, observed for buffers with $pK_a > 4.5$, is unlikely to represent a general base catalysis *mechanism* for the monoanion reaction because it is still observed under conditions where substantial amounts of the starting material anion are, and significant amounts of the pentacovalent intermediate (**12**) might be expected to be, present in solution as the dianion;* so can be attributed with some confidence to the kinetically equivalent general acid catalysed reaction of the dianion (see Scheme 4, below). When the rate constants assigned in this way are included in the Brønsted plot for the latter reaction (Fig. 4), no discontinuity is apparent; consistent with the assignment of a common mechanism. This mechanism, including special cases where the general base catalysis mechanism might be operative, is discussed in detail below.

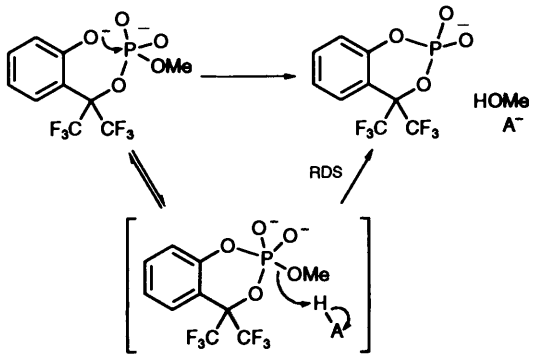
The Spontaneous Hydrolysis of the Monoanion.—This, the predominant reaction between pH 3 and 7, probably also represents the H_3O^+-catalysed hydrolysis of the dianion; even though the point calculated for water acting as a general acid falls on the Brønsted line for catalysis by substituted acetic acids (Fig. 4). This means reaction *via* transition state **13** (HA = H_3O^+) in Scheme 4. This would explain why this, unlike other reactions (Table 1) where the data are available, is slower for the methoxy compound, **3a**. β_{nuc} (-0.13, a crude figure based on two points only) is therefore negative, indicating that the phenol oxygen is more negative in the transition state than in the ground state. (The esters are present in solution overwhelmingly as the monoanion, with the phenolic OH undissociated. The reactive form is the phenolate anion, and this is not completely neutralised in the transition state.) This small negative value is consistent with more or less complete bond formation to P, as expected if the breakdown of a pentacovalent intermediate is rate determining. (Note that if this assignment of mechanism is correct, and the evidence is indicative rather than conclusive, an explanation is needed for the positive deviation of the point for H_3O^+ from the Brønsted plot [Figs. 4 and 5] for general acid catalysed hydrolysis of the dianion: this point is discussed below.) The small deuterium solvent isotope effect [$k(H_2O)/k(D_2O) = 1.18$] and the relatively high enthalpy of activation (102 kJ dm^3 mol^{-1}) must also reflect the proton-transfers involved as well as the effects of subsequent steps, and cannot be interpreted with confidence. The sensitivity to the leaving group (β_{LG} -0.28) indicates that little negative charge builds up on the leaving group oxygen in the transition state, as expected if bond breaking is concerted with protonation. There is a notable contrast in this parameter with the value for the spontaneous hydrolysis of the dianion (β_{LG} -1.13; see below), which involves no more proton transfer to the leaving group than is involved in hydrogen-bonding solvation.

Reactions of the Dianion.—The displacement from phosphorus of the methoxide anion, six powers of ten more basic, by the phenolate oxygen of the substrate dianion, is a remarkable reaction. Such a displacement by a concerted mechanism is unprecedented in carbon chemistry. Although even a neighbouring carboxylate will displace methoxide from a carboxylic methyl ester if the EM is high enough,[21] the reaction is viable only because it involves an addition–elimination mechanism.

* Available pK_a's of hydroxyphosphoranes[2] suggest a pK_a of 5–6 for the first dissociation of **11** (with two electron-withdrawing esterifying groups) and thus a second dissociation in the region pK_a 10–11.

We know of no case where alkoxide or hydroxide is displaced by neighbouring aryloxide from sp^3 carbon, and have in fact failed to observe such a reaction in more than one system of high EM. Displacement by oxyanion nucleophiles at phosphorus, even for the very unreactive phosphate diester monoanions, is faster than at sp^3 carbon because the transition state is stabilised by the availability of the fifth covalent bond at P, which specifically favours an associative transition state.

In the present case the pentaoxyphosphorane intermediate would be **13**, carrying two negatively charged oxygens and three leaving groups, two of them (the in-ring pair) relatively good leaving groups derived from conjugate acids with pK_a 9–10. No single piece of evidence is conclusive, but the sum of the evidence suggests that **13** is an intermediate in the hydrolysis of the dianion of **3**, but that its lifetime is too short for full diffusional equilibration.



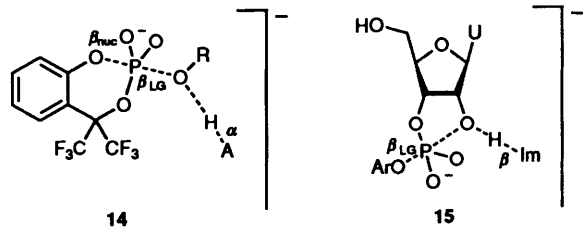
13

Scheme 4

Spontaneous Hydrolysis of the Dianion.—The high value of β_{LG} (−1.13) for the spontaneous reaction of the dianion* means that substantial negative charge has developed on the departing methoxide oxygen in the rate determining transition state. A crude estimate of its absolute magnitude is −0.4, made using Thea and Williams' figure[22] of −1.74 for β_{eq}, the Brønsted coefficient for complete transfer of the phosphate diester group between oxyanions of different basicities. This developing negative charge cannot be neutralised by proton transfer from the solvent beyond normal hydrogen-bonding solvation, because water is no more acidic than methanol, and proton transfer to incipient methoxide never becomes thermodynamically favourable. It is, however, largely neutralised by stronger general acids, which can transfer the developing negative charge to the conjugate base.

A measure of this last effect is the sharp fall in the magnitude of β_{LG}, from −1.13 to −0.28 when the general acid is H_3O^+ rather than solvent H_2O. (The comparison is with the spontaneous reaction of the monoanion, described above.) The degrees of bond formation to the nucleophilic oxygen are similar in the two cases: β_{nuc} *ca.* −0.13 for the spontaneous reaction of the monoanion (corresponding to a value of *ca.* 0.87 for the reaction of the reactive, phenolate form), and *ca.* 0.95 for the spontaneous reaction of the dianion. These figures tell us that bond formation is well-advanced in the transition state for the cyclisation of systems based on **3**. [Much further advanced, in particular, than for comparable intermolecular displacements of good leaving groups. Contrast, for example, the value of β_{nuc} *ca.* 0.95 for the present system with β_{nuc} = 0.31 for the intermolecular reactions of oxyanions (including phenoxide) with methyl 2,4-dinitrophenyl phosphate.[23] β_{LG} is similar in the two cases.]

The two reactions of **3** thus show bond formation to the nucleophile well-advanced and to similar degrees, consistent with rate determining breakdown of a common (phosphorane dianion) intermediate. However, the value of β_{LG} = −1.13 for the dianion reaction is numerically greater than β_{nuc} (0.95), so the increase in negative charge on the leaving group oxygen in the transition state **14** (HA = H_2O) is comparable to—if anything greater than—the decrease in charge at the nucleophilic centre. This allows little scope for significant build up of additional negative charge on the PO_2^- group of an intermediate, and is not consistent with a transition state structure very close to a phosphorane dianion, at least as represented by **13**, with full negative charges on the two equatorial oxygens.



14 **15**

This agrees with recent calculations, which find that the phosphorane dianion formed by the addition of hydroxide or methoxide to phosphate diester anions is unstable,[24] or barely stable[25,26] (at least in the gas phase) with respect to elimination of alkoxide, even when the diester forms a five-membered ring. The less stable the phosphorane, the closer it is in structure to transition states for its breakdown. The calculated structure of the trimethoxyphosphorane dianion[25] is in fact consistent with the transition state structure suggested by our results. It shows two very long bonds (P–O 1.803 and 1.895 Å) to the apical OMe groups (compared with 1.670 Å to the equatorial methoxy), corresponding to the considerable negative charges on the nucleophile and leaving group oxygens in the in-line displacement indicated by the measured β values.

Buffer Catalysis of the Cyclisation of the Dianion.—One of the most important results of this work is that it allows us to observe catalysis of the step in which a poor leaving group departs from the phosphorus centre of a phosphate diester, without complications from other reactions. A Brønsted plot (Fig. 5) displaying most of the data from Table 4 (not the two extreme points for water and the hydronium ion) shows extensive scatter. This is unusual for general species catalysis, and indicates that the effectiveness of a general acid depends not only on its pK_a, but on other factors also. The data for eight primary alkylammonium cations, spanning a range of almost 6 pK_a units, are well-correlated by a straight line of slope (Brønsted α) of −0.33 ± 0.01. [This is consistent with the Brønsted β of 0.67 obtained by Davis, Hall and Williams[20] for the hydrolysis of 4-nitrophenyl uridine-3′-phosphate catalysed by amine general bases, where the rate determining step (**15**) is mechanistically the microscopic reverse of that in Scheme 4.]

Deviations from this line appear to be associated primarily with the charge on the general acid. Neutral and especially anionic oxyacids show negative deviations, while the largest

* This is in fact about what would be expected from (a long extrapolation from) earlier data for the intramolecular displacement of aryloxide anions from diester phosphorus by carboxylate,[14] which also involves displacement of an oxyanion leaving group by an intramolecular nucleophile some six powers of ten less basic, and where β_{LG} was found to be −1.26. The calculation of β_{LG} assumes that the pK_a of the phenol is not affected by changing the leaving group OR of **3**. Any change is expected to be very small, and would have the effect of *increasing* β_{LG}.

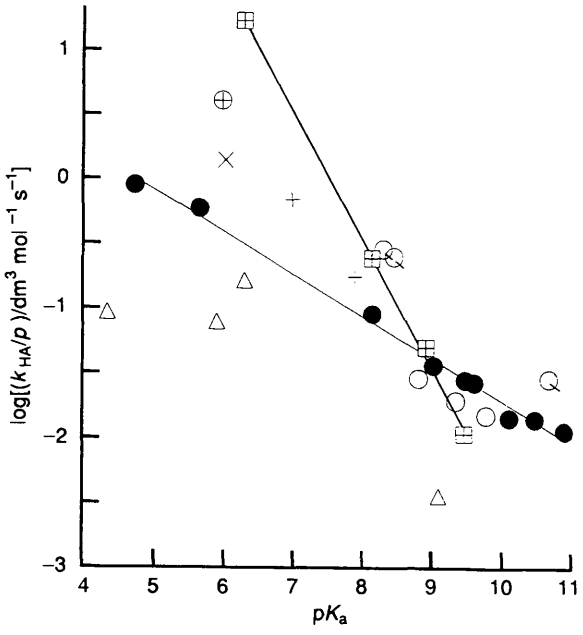
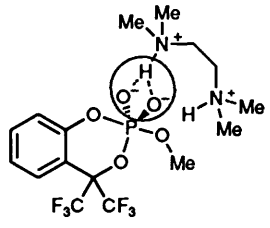


Fig. 5 Statistically corrected Brønsted plot for the general acid catalysed cyclisation of the dianion of **3** at 50 °C and ionic strength 1.0 mol dm^{-3} (KCl) in H_2O, by: primary and secondary ammonium ions (●); hydroxyammonium (⊕); protonated acyclic (○) and bicyclic (Q) tertiary amines; oxyacids (△); imidazolium cations (+) and the dications derived from tetramethylalkylene diamines (⊞) and pentamethylethylene diamine (×)

positive deviations are shown by diamine dications and by hydroxylammonium. This is most dramatically demonstrated by the direct correlation (Fig. 5) between the rate enhancement and the distance between the second positive charge and the general acid centre. For the series of dications Me_2NH^+-$(CH_2)_nNH^+Me_2$, with n = 2, 3, 4 and 6, the rate enhancement increases with decreasing n, to such an extent that the apparent Brønsted α for the series is 0.99. (This value, equal within experimental error to unity, is consistent with a rate determining diffusion-controlled proton transfer, but this possibility was ruled out by an experiment in which the viscosity of the solvent was increased without a proportionate decrease in the rate of the reaction catalysed by the TMEDA dication.) The effect on α of increasing the proximity of the positive charge is thus twice that of increasing the strength of the general acid.

The monocations derived from these bases define normal behaviour for tertiary ammonium cations: they are correlated by a line of slope similar to that drawn (Fig. 5) for alkylammonium cations, and the positive deviation from this line for the tetramethylethylenediammonium cation corresponds to a rate factor of over 100.[27] The effect in terms of free energy of activation is worth up to 12.3 kJ dm^3 mol^{-1} even in water. Assuming a simple point charge model for electrostatic stabilisation, an effective active site relative permittivity half that of bulk water would double this figure, equivalent to a factor of 10^4 in rate. (1,2-Diamine monocations should be the most efficient catalysts for the reverse reaction, the attack of neighbouring OH on a phosphate diester, and ethylenediamine has indeed been shown to be an efficient catalyst for the hydrolysis of polyadenylic acid at pH 8, where it will be present mainly as the monocation.[28])

The mechanism of this effect is of great interest. It may be presumed to stabilise the high concentration of negative charge on the pentacovalent transition state, and intermediate if one is involved, either electrostatically (through space) or by hydrogen bonding. The evidence suggests that both factors may be involved: when the second proton of the tetramethylethylenediammonium dication is replaced by a methyl group much but not all of the rate enhancement disappears (Fig. 5). Thus the rate constant for catalysis by the pentamethylethylenediammonium cation is 28 times smaller than that for the tetramethylethylenediammonium dication, but still some six-fold greater than expected for a tertiary ammonium cation of the same pK_a. The simplest explanation of these results is that the two effects—both normally weak in water—reinforce each other, possibly as in **16**: here (circled) intramolecular hydrogen bonding is strengthened by ion-pair association, which in turn is favoured by the close approach of the opposite charges made possible by the formation of the (possibly bifurcate) hydrogen bond (impossible for the $^+NMe_3$ group).* Related effects (as in **17**) could explain the similar positive deviation from the Brønsted plot of the point for H_3O^+, which is much more strongly polarising than an ammonium ion.



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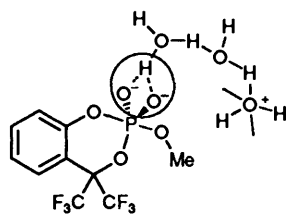


17

The Status of the Phosphorane Dianion Intermediate.—(i) We suggest that transition state **14** accounts for the spontaneous reactions of both monoanion (HA = H_3O^+) and dianion (HA = H_2O), for the general base catalysed reactions of the monoanion in most cases; and for the observed general acid catalysed reactions of the dianion.

(ii) Transition state **14** can be reached either directly, by the cyclisation of the phosphate ester dianion in the presence of a preassociated general acid; or in two or more steps, if the phosphorane dianion is an intermediate.

(iii) If the phosphorane *monoanion* is an intermediate in reactions at lower pH (Scheme 3), as both calculation and experiment suggest, its equilibrium concentration will be little changed up to pH 9 (*i.e.* in the region where the reactant is present predominantly as the monoanion). If the second pK_a of the phosphorane is indeed in the region 10–11,† then the transfer of the proton concerned to a general base with a pK_a greater than 10–11 (such bases show normal reactivity) will be diffusion controlled. Were the phosphorane dianion too unstable to exist, this proton transfer would necessarily be concerted with the elimination of a leaving group from the incipient dianion; *i.e.* general base catalysis of the breakdown of the monoanion would be enforced. However, these are clearly not conditions under which general base catalysis is to be expected, because the proton transfer concerned is already thermodynamically favourable.[29] Furthermore, the Brønsted β for this general base catalysis would have to be 0.67 (α = 0.33 calculated for general acid catalysis), clearly inconsistent with an early transition state for the proton transfer.

* Electrostatic effects are stronger in less polar media, and no doubt in enzyme active sites also. In the active site of ribonuclease, for example, up to three positively charged groups may be brought to bear on the dianionic transition state. It is not possible to introduce a third charged group to increase the effect observed in the present system simply by using, for example, pentamethyl diethylene triamine, $Me_2NCH_2CH_2$-$NMeCH_2CH_2NMe_2$, because the trication is formed only at very low pH, where the substrate dianion is present in vanishingly small amounts.

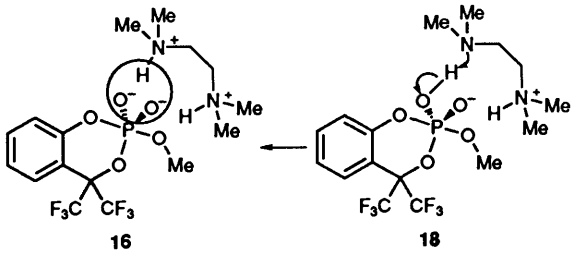
† This figure is a best estimate, as discussed above, but the argument presented here does not depend on an exact figure.

(iv) In the presence of a general acid with pK_a < 10–11 (the more usual case), proton transfer to the phosphorane dianion will be thermodynamically favourable, and thus faster than diffusional processes. The elimination of methanol *via* transition state **14** will be the slowest of three reactions available to the intermediate.

(v) We know that the phosphorane dianion, if formed, breaks down at a rate which is very sensitive to the pK_a of the leaving group ($\beta_{LG} = -1.13$, Table 1). In the absence of a general acid it will therefore revert almost exclusively to starting materials (up to 5×10^6 times faster than losing methoxide if β_{LG} is similar for reversion to starting material). In the presence of a general acid this figure becomes vastly more favourable to the forward reaction (for HA = H_3O^+, $\beta_{LG} = -0.28$, Table 1). This is the rate determining step of the reaction under most conditions, as discussed above; and both β_{LG}, and the low Brønsted coefficient ($\alpha = 0.33$) are consistent with the early transition state expected for the breakdown of a high energy intermediate.

(vi) If the phosphorane dianion is formed by a proton transfer from the monoanion to a simple general base, the preassociated general acid required to catalyse the breakdown step is automatically present. However, it is not instantaneously in a position to transfer a proton to the leaving methoxide, because this requires significant reorganisation of the solvation shell.

(vii) This reorganisation is not required when the general acid is a diammonium dication (transition state **16**) formed from the preassociated ion pair **18**.



16 **18**

Conclusions

The picture that emerges for the transition state **14** for the general acid catalysed cyclisation of the dianion of **3** is closely similar, though in this case approached experimentally from the opposite direction, to that (**15**, above) described by Davis *et al.*[20] for the cyclisation of *p*-nitrophenyl uridine-3′-phosphate. For **15** the Brønsted coefficient β for general base catalysis is 0.67, corresponding exactly to the value of $\alpha = 0.33$ observed for general acid catalysis in the present system. The picture for **14** is more detailed because we have values for both β_{nuc} and β_{LG} (for the spontaneous reaction, with HA = H_2O), and we can begin to identify the requirements for effective catalysis, particularly the advantage of two suitably positioned NH^+ centres.

The phosphorane dianion is a marginal intermediate in the system described in this paper. The corresponding pentacovalent species are probably not intermediates in intermolecular displacements at diester phosphorus, particularly where good leaving groups are involved. But bonding to the entering and leaving groups gets tighter as their basicity is increased,[19] and when ring formation or cleavage is involved. Kinetically significant phosphorane dianions are thus to be expected for reactions where only alkoxy groups are attached to phosphorus, and where they are stabilised by favourable ring formation and by complementary electrostatic interactions. All these factors are present in enzyme active sites; and all can also be identified in the transition state (**14**) for the most efficient catalytic process found in this work. We are currently looking to improve still further the efficiency of catalysis in these simple systems.

Experimental

Kinetic Measurements.—Inorganic buffers and potassium chloride were of AnalaR grade, and were dried *in vacuo* at 100 °C immediately before use without further purification. Potassium hydroxide and hydrochloric acid solutions were obtained by dilution of Convol® concentrates. Deuteriated solutions were prepared in the same manner. Chloroacetic acid, cacodylic acid, 2-methylimidazole, β-alanine, DL-serine, glycine, TRIZMA base, methoxylamine hydrochloride, and hydroxylamine hydrochloride were the best available commercial preparations (Aldrich or Sigma) and were used after drying *in vacuo* over calcium chloride without further purification. Glacial acetic and formic acid were dried by freeze–thaw cycles and used immediately. Methoxyacetic acid was fractionally distilled and the middle portion collected. Liquid amines were dried over calcium chloride for 24 h before purification by fractional distillation, DABCO was sublimed at 80 °C/0.5 mmHg. Imidazole was recrystallised from chloroform, betaine from water, cyanoacetic acid from acetone, and quinuclidine hydrochloride and chloroquinuclidine hydrochloride from ethanol before drying *in vacuo*. H_2O was distilled three times from all-glass apparatus and deoxygenated routinely with argon. D_2O was used directly from a freshly opened bottle.

Stock solutions were prepared by adding potassium hydroxide or hydrochloric acid solutions to preparations of the acid or base form of the buffer and then adjusting the ionic strength to 1.0 mol dm^{-3} with potassium chloride. The final volume was attained with water.

Data Collection and Analysis.—The cyclisation of the diesters were monitored by following the disappearance of the phenol chromophore at wavelengths where the largest change in absorbance was seen (which corresponds to λ_{max} when the phenol is ionised, and otherwise the shoulder of a complex peak) in the electrically thermostatted cell-holder of a Gilford 2600 single beam spectrophotometer. Wavelengths used for the substrate below the pK_a of the phenol, and those used for the phenolate (shown in brackets) were 285 (303), 312 (322), 285 (303) and 285 nm for **3**, **3a**, **3b** and **3c**, respectively.

Kinetic runs were started by injecting 2 mm^3 of a stock solution (usually *ca.* 0.077 mol dm^{-3} in H_2O, stored frozen) into buffer solution (280 mm^3) in the preheated cuvette. Buffer concentration was never less than 140 times substrate concentration, so that pseudo-first-order conditions were maintained. Standard conditions were 50 °C at ionic strength 1.0 mol dm^{-3}, maintained with potassium chloride. All pH measurements were made under the conditions of the kinetic experiments using a Radiometer PHM82 pH meter with a Russell CTWL electrode. The pD of solutions in D_2O was measured using the same electrode which had been standardised against (protium) standard buffers, and was taken to be 0.4 above the reading of the pH meter.[31] The absorbance was measured at set intervals, and recorded on a printer connected to the spectrophotometer. Data were analysed on an Apple Macintosh SE microcomputer using Kaleidagraph™ version 2.0.2 (Abelbeck Software). For faster reactions (half life < 100 min) the end-point was usually obtained after 10 half lives in the spectrophotometer. For slower reactions the cyclisation was followed for five half lives and the data analysed using the Guggenheim method,[32] using a time interval of more than 2.5 half lives. In both cases an excellent fit to the straight line was achieved (usually $r > 0.9999$) for data up to five half lives.

Fitting the pH–Rate Profiles for Substrate Hydrolysis.—The pH–rate profiles for the hydrolysis of the four phosphate diesters were obtained by determining the rate constants at several different buffer concentrations at each particular buffer ratio and extrapolating back to zero buffer concentration (Fig.

3). The pH–rate profile for the cyclisation of **3** requires four kinetic terms for an adequate description [eqn. (1), above]. Eqn. (2) was fitted to the pH–rate profile for the hydrolysis of **3** by a weighted non-linear least-squares method using SPSS version 4.1 for IBM computers. The uncertainties in the intercepts of the second-order buffer plots were used to weight the hydrolysis rate constants, and a 2% uncertainty was allowed for the rate constants in hydrochloric acid and potassium hydroxide. The fit gave reasonable values for all the parameters except the equilibrium constant for the phosphoric acid ionisation, which has a standard deviation greater than its magnitude.

Below pH 3.5 the cyclisation of **3** is dependent on the acid concentration (Fig. 1); the slope of the line changes, from 1.06 between pH 2.5–1.0, to 0.9 between pH 1.0–0. Since eqn. (2) used to describe the data indicates that the slope can be greater than 1.0 when $a_H < K_1$ but cannot be less than 1.0 in the region $a_H > K_1$, the equation clearly does not describe the observed behaviour in detail at low pH. In fact a good fit of the data down to pH 1.0 could be obtained simply by omitting the first term of eqn. (2): we consider that this is an artificial improvement, because there are good data in the region at low pH where the first term would be most important. The fit was not significantly improved by using standard activity coefficients for the proton, and no activity corrections have been made to the data shown. A common reason for such deviations involves specfic salt effects, but since they occur in a region of minor interest for this work, the question was not pursued. Between pH 3.5–6.0 the reaction is pH-independent; and above pH 6.5 the curve follows the second ionisation to the phenolate.

The pH rate profiles of the derivatives of **3** were fitted using eqn. (2). For **3c** the equilibrium constant for the ionisation of the phosphate was set at $K_1 = 0.2$. Only the rate constants k_3 and k_4 are therefore reliable values. For the cyclisation of **3a** in the limited pH region studied the fit required three terms, while for **3b**, for which the reaction was only studied down to pH 5.65, only two terms were necessary.

Buffer Catalysis.—The pK_a value of a buffer in this study was taken as the pH of the 50% free base solution measured at 50 °C. Increasing the concentration of added buffers at constant pH resulted in an increase in rate of cyclisation of **3** for all the buffers used, between pK_a 1.95 and 14. The catalysis can be expressed kinetically as general acid catalysis of the reaction of each of the three ions that are present in solution between these limits. For substituted acetic acids (pK_a 1.95–3.45), but not for formic acid (pK_a 3.58), or for acetic acid itself, a plot of the rate constant for buffer catalysis *vs.* fraction of free base showed upwards curvature towards low F_{base}. This curvature represents, formally, general acid catalysis of the reaction of the neutral substrate. The curvature disappears if the observed rate constants are converted to represent catalysis of the neutral substrate. The very poorly defined value for K_1 imparts a large error to the absolute magnitude of the derived rate constants, but it affects their relative values much less. There is also no doubt that catalysis is occurring: even where it is least efficient the rate is at least doubled in the presence of the general acid. The four point Brønsted plot derived from this treatment (see Fig. 4) should however be treated with a degree of caution.

The buffer catalysis apparent with buffers in the range pK_a 1.95–6.47 was, kinetically, general acid catalysis of the monoanion; while with buffers from pK_a 4.25 up to 14, more than four pK_a units above the pK_a of the phenol (kinetically) general acid catalysis of the dianion reaction was observed. This latter term becomes dominant for pK_a's above 5.16 (trifluoroethylamine). Above pH 7.58 the observed buffer catalysis rate constants were corrected for the ionisation of the phenol.

The derivatives of **3** also exhibit buffer catalysis, although for the more reactive substrate **3c** the background rate is too fast for catalysis of the dianion reaction to be detected with all but the most effective catalyst, TMEDA dication.

Effect of the Viscosity of the Medium.—To test the possibility that the reactions catalysed by diamine dications (for which the Brønsted α is close to unity) are diffusion controlled, the rate of cyclisation of **3** in water was compared with the rate in 60% (w/w) glycerol–water, which is nine times more viscous than water at 30 °C and expected to be 6–7 times more viscous at 50 °C.* In 0.2 mol dm^{-3} TMEDA, 50% dication, pH 6.2 at 50 °C, k_{obs} was depressed by only 30% in 60% glycerol solution (6.1×10^{-4} s^{-1} compared with 8.7×10^{-4} s^{-1} in the aqueous buffer).

Solvent Deuterium Isotope Effects.—Solvent deuterium isotope effects for the cyclisation of **3** were measured on the plateau region at high pH in 0.07 mol dm^{-3} potassium deuteroxide and in four amine buffers; piperidine, propylamine, β-alanine and TRIS, for the dianion reaction. As the extent of ionisation of both buffers and substrates differs in D_2O and H_2O the true values for k_H/k_D (buffer) were obtained by normalising the observed solvent isotope effect in the buffers to the value on the plateau where the substrate is fully ionised, and applying the correction to the observed values for buffer catalysis. For the pH-independent region the rates were measured in acetate buffer at two different buffer ratios and the results averaged.

Activation Parameters.—Activation parameters were measured for the uncatalysed cyclisation of the dianion and for catalysis by 50% free base piperidine buffer. Although the phenol is only 92% ionised in this buffer at 50 °C it was assumed that the variation in the equilibrium constant would be within the experimental error. For the pH-independent reaction trifluoroethylamine buffer (pH 5.16) was used to obtain the parameters for the water-catalysed reaction, and 50% free base acetate (pH 4.65) to obtain them for acetic acid catalysis. At these pH's more than 95% of the water catalysed reaction goes through the monoanion at 50 °C.

Product Analysis.—The hydrolysis products from **3** were investigated primarily by ^{31}P NMR spectroscopy, in solutions at ionic strength 1.0 mol dm^{-3} (KCl), at ambient temperature, using 5–10 mg of the substrate in 1 cm^3 of solution using trimethyl phosphite as an external standard and a drop of D_2O as an internal lock. Approximately 10 spectra were taken over the first half life; after which approximately 10 more were usually taken over the next 10 half lives. The solutions used (chosen so that reactions of all three ionic forms of the substrate are represented) and the spectral changes observed are recorded in Table 7. ^{1}H NMR spectra in D_2O established that loss of methanol coincided with the cyclisation.

The cyclic diester **7** is the only product after 10 half lives at all pH's below 11; above this pH ring opening of the diester occurs to give monoesters **8** and **9**. Ring opening is accompanied by an increase in absorbance at 285 nm, confirming the formation of **8**. No attempt was made to determine which isomer was the major product. In 1.75 mol dm^{-3} sodium methoxide in methanol at room temperature the initial ring closure of **3** is not observed because the ring is subsequently opened at a much faster rate. With the cyclic product **7** under the same conditions rapid ring opening reaction is observed; the chromophore due to the phenolate then disappears with a half life of approximately 11 min.

* Extrapolation from the data of M. L. Sheely, *Ind. Eng. Chem.*, 1932, **24**, 1063.

Table 7 Analysis of hydrolysis products for **3** by ^{31}P NMR spectroscopy at room temperature in selected kinetic solutions

Solution	Chemical shift relative to trimethyl phosphate		
	Starting material	Cyclic product	Ring opened product
1.00 mol dm^{-3} Hydrochloric acid	−146.0	−152.9	
0.10 mol dm^{-3} Hydrochloric acid	−145.9	−152.8	
1.00 mol dm^{-3} Acetic acid 50%	−145.9	−152.9	
0.25 mol dm^{-3} TMEDA dication 50% free base	−146.0	−153.0	
0.10 mol dm^{-3} KOH	−145.9	−152.9	Not observed after five half lives
1.00 mol dm^{-3} KOH	−145.9	−152.9	−141.7, −142.1[a]

[a] Peak ratio about 2:1. Both peaks start to appear within one half life for the cyclisation under these conditions.

Synthesis.—Benzyl 2-bromophenyl ether.[33] A mixture of benzyl bromide (1.18 g, 6.92 mmol), 2-bromophenol (0.6 g, 3.46 mmol), and anhydrous potassium carbonate (0.96 g, 6.92 mmol) was refluxed in dry acetone (15 cm^3) for 24 h. The mixture was filtered and the filtrate evaporated under reduced pressure to give an oily residue which was distilled on a Kugelrohr under reduced pressure. Benzyl 2-bromo-4-methoxyphenyl ether was prepared in the same way.

2-(2-*Benzyloxyphenyl*)-1,1,1,3,3,3-*hexafluoropropan*-2-*ol* **4**. The aryl bromide prepared above (0.25 cm^3 of a 3.8 mol dm^{-3} solution in ether, 0.95 mmol) was added to dry magnesium turnings (0.2 g, 8.37 mmol) and a small iodine crystal under argon. The mixture was warmed carefully and the remaining aryl bromide solution (1.75 cm^3, 6.65 mmol) added to keep the mixture refluxing gently. After stirring for 30 min dry THF (20 cm^3) was added, the mixture cooled to −30 °C and hexafluoroacetone added in excess from a gas cylinder *via* a Teflon tube. The mixture was allowed to warm to room temperature and stirred vigorously for 30 min to allow the excess hexafluoroacetone to escape *via* an argon line. Ether was added, the mixture poured onto ice and a solution of saturated ammonium chloride added. The mixture was extracted with ether, washed with saturated brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was distilled on a Kugelrohr to give the alcohol **4** (1.9 g, 75%) as needles, m.p. 61–63 °C (from 60–80 light petroleum); $R_f(CH_2Cl_2)$ 0.51; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3400 (OH), 1610, 1590, 1500 (aromatic C=C stretch), and 1200 (CF_3); δ_H(250 MHz; $CDCl_3$) 7.60 (1 H, d, *J* 8), 7.55 (1 H, s, exchangeable), 7.49–7.38 (6 H, m), 7.17–7.08 (2 H, m), and 5.18 (2 H, s); δ_C(400 MHz; $CDCl_3$) 157.7, 134.5, 131.5, 129.2, 129.1, 129.1, 127.9, 122.8 (q, *J* 1142.7), 122.3, 117.4, 114.4, 79.6 (septet, *J* 120), and 72.5; δ_F(250 MHz; $CDCl_3$) −75.7; *m*/*z* 350 (M^+, 3.5%), 91 (100%, C_7H_7), and 65 (14%, C_5H_5) (Found: M^+, 350.0714. $C_{16}H_{12}F_6O_2$ requires *M*, 350.0741).

2-(2-*Benzyloxy*-4-*methoxyphenyl*)-1,1,1,3,3,3-*hexafluoropropan*-2-*ol* was prepared from benzyl 2-bromo-4-methoxyphenyl ether in the same way. The alcohol was collected as a clear oil (5 g, 91%) which solidified in the refrigerator; R_f(chloroform) 0.62; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3440–3260 (OH stretch), 1630, 1600 (aromatic C=C stretch); δ_H(250 MHz; $CDCl_3$) 7.75 (1 H, s, exchangeable), 7.49–7.30 (5 H, m), 7.11 (1 H, s), 7.07 (1 H, d, *J* 9.1), 6.95 (1 H, dd, *J* 9.1 and 2.9), 5.13 (2 H, s), 3.78 (3 H, s); δ_C(400 MHz; $CDCl_3$) 154.2, 151.7, 134.7, 129.0, 129.0, 128.0, *ca.* 122 (*J ca.* 1000), 118.3, 116.1, 116.0, 115.1, 73.4, 55.7; δ_F(250 MHz; $CDCl_3$) −75.9; *m*/*z* 380 (58%, M^+), 151 (45, M − 2 × CF_3 and C_7H_7), and 91 (100, C_7H_7) (Found: M^+, 380.0818. $C_{17}H_{14}F_6O_3$ requires *M*, 380.0847).

2-(2-*Benzyloxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propyl dimethyl phosphate* **5**. Methyl dichlorophosphite (0.49 g, 3.7 mmol) was added dropwise to a solution of *N*,*N*-diisopropylethylamine (1.11 g, 8.6 mmol) in THF (20 cm^3) at −78 °C under argon and the solution stirred for 10 min, after which time the alcohol prepared above (5 cm^3 of a 0.58 mol dm^{-3} solution in THF, 2.9 mmol) was added dropwise.[34] After 2 h at 0 °C the mixture was cooled to −78 °C and anhydrous methanol (0.59 g, 2 mmol) was added dropwise. After 30 min, 85% MCPBA (10 cm^3 of a 0.86 mol dm^{-3} solution in THF, 8.6 mmol) was added, still at −78 °C, and then after 2 h at 0 °C 5% sodium bisulfite (20 cm^3) and water (150 cm^3) were added and the mixture extracted with chloroform (3 × 150 cm^3). The organic phase was washed with a solution of sodium hydrogen bicarbonate (pH 9) and brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was distilled (Kugelrohr, pressure < 0.03 mmHg) to give the triester (0.75 g, 56%), b.p. 160 °C/0.5 mmHg; $R_f(CHCl_3)$ 0.17; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1610, 1595 (C=C aromatic stretch); δ_H(250 MHz; $CDCl_3$) 7.64 (1 H, d, *J* 8.3), 7.43–7.29 (6 H, m), 7.05–6.99 (2 H, m), 5.14 (2 H, s) and 3.65 (6 H, d, *J* 11.6); δ_C(400 MHz; $CDCl_3$) 157.8, 136.5, 132.3, 129.4, 128.5, 127.9, 127.4, 120.5, 116.0, 113.6, 70.9 and 54.8 (d, *J* 26); two peaks not resolved; δ_F(250 MHz; $CDCl_3$) −71.5; δ_P(400 MHz; $CDCl_3$, relative to external trimethyl phosphate) −148.0; *m*/*z* 458 (M^+, 1%), 332 [44%, M − $HOPO(OMe)_2$], and 91 (100%, Bn) (Found: M^+, 458.0679. $C_{18}H_{17}F_6O_5P$ requires *M*, 458.0718). In later experiments oxidation by iodine was used as purification proved easier.

2-(2-*Benzyloxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propylmethyl* 2-*trifluoroethyl phosphate*. Prepared as for **5**, by adding trifluoroethanol to the intermediate chlorophosphite instead of methanol and oxidising with iodine to give the phosphate triester (120 mg, 45%) as a clear oil; R_f(chloroform) 0.32; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1610, 1600 and 1510 (C=C aromatic stretch); δ_H(250 MHz; $CDCl_3$) 7.59 (1 H, d, *J* 8.13), 7.45–7.30 (6 H, m), 7.06–7.00 (2 H, m), 5.14 (2 H, s), 4.33–3.88 (2 H, m) and 3.61 (3 H, d, *J* 11.8); δ_C(400 MHz; $CDCl_3$) 158.0, 136.3, 132.6, 129.1, 128.5, 128.0, 127.5, 121.7 (q, *J* 1153), 120.6, 113.7, 71.0, 63.8 (dd, *J* 160, 20), and 55.3 (d, *J* 28); δ_F(250 MHz; $CDCl_3$) −70.9 (q, *J* 11.5), −72.0 (q, *J* 11.5) and −76.0 (t, *J* 8.5); δ_P(400 MHz; $CDCl_3$) −150.1; *m*/*z* 526 (1.4%, M^+), 332, [29, M − $HOPO(OMe)(OCH_2CF_3)$], 91 (100, C_7H_7) (Found: M^+, 526.0558. $C_{19}H_{16}F_9O_5P$ requires *M*, 526.0817).

2-(2-*Benzyloxy*-4-*methoxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propyl dimethyl phosphate*. Prepared in the same manner using iodine oxidation to give the triester (0.5 g, 40%) as a clear oil that solidified in the refrigerator; R_F(chloroform) 0.07; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1590, and 1510 (C=C aromatic stretch); δ_H(250 MHz; $CDCl_3$) 7.42–7.30 (5 H, m), 7.24 (1 H, s), 6.93 (2 H, s); 5.08 (2 H, s), 3.77 (3 H, s) and 3.68 (6 H, d, *J* 11.6); δ_C(400 MHz; $CDCl_3$) 153.0, 151.8, 136.9, 128.4, 127.8, 127.3, 121.6 (d, *J* 1156), 117.0, 116.8, 115.7, 114.6, 71.3, 55.7, and 54.9 (d, *J* 25); δ_F(250 MHz; $CDCl_3$) −71.5; δ_P(400 MHz; $CDCl_3$) −149.7; *m*/*z* 488 (9%, M^+), 272 [59, M − C_7H_7 and $OPO(OMe)_2$], and 91 (100, C_7H_7); *m*/*z* 488 (27%, M^+), 362 [60, M − HOPO-

$(OMe)_2$], 91, (100, C_7H_7) (Found: M^+, 488.0793. $C_{19}H_{19}F_6O_6P$ requires M, 488.1094).

2-(2-*Benzyloxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propyl di*-2-*fluoroethyl phosphate*. Phosphorus trichloride (73 mm^3, 0.83 mmol) was added dropwise to a solution of *N*,*N*-diisopropylethylamine (0.56 cm^3, 3.18 mmol) in THF (4 cm^3) at −78 °C under argon and the solution stirred for 10 min after which time the alcohol (223 mg, 0.64 mmol) in THF (1 cm^3) was added dropwise. After 2 h at 0 °C the mixture was cooled to −78 °C and anhydrous fluoroethanol (292 mm^3, 5 mmol) was added dropwise. After 30 min at 0 °C, iodine (5 cm^3 of a 0.27 mol dm^{-3} solution in THF–water, 2:1, 1.35 mmol) was added with a few drops of pyridine. After 5 min the reaction mixture was evaporated under reduced pressure and then dissolved in chloroform. 5% sodium bisulfite (20 cm^3) and water (150 cm^3) were added and the mixture extracted with chloroform (3 × 150 cm^3). The organic phase was washed with a solution of sodium hydrogencarbonate (pH 9) and brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographed (SiO_2, chloroform) and distilled on a Kugelrohr to give the triester (185 mg, 55%); R_f(chloroform) 0.13; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1610, 1600 and 1510 (C=C aromatic stretch); δ_H(250 MHz; $CDCl_3$) 7.59 (1 H, d, *J* 8.3), 7.40–7.21 (6 H, m), 7.01–6.95 (2 H, m), 5.12 (2 H, s), 4.37 (4 H, dm, *J* 50) and 4.13 (4 H, dm, 30); δ_F(250 MHz; $CDCl_3$) −71.3 (6 F, s) and −225.5 (2 F, tt, *J* 50.3 and 29.8); δ_P(400 MHz; $CDCl_3$) −150.4; *m*/*z* 522 (0.9%, M^+), 332 [24, M − $HOPO(OCH_2CH_2F)_2$], 91 (100, C_7H_7) (Found: M^+, 522.0846. $C_{20}H_{19}O_5F_8P$ requires M, 522.1068).

tert-*Butyl ammonium* 2-(2-*benzyloxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propyl methyl phosphate* **6**. The phosphate triester **5** (200 mg, 0.44 mmol) was stirred in *tert*-butylamine (50 cm^3) overnight.[30] The amine was removed from the salt (that had precipitated out) under reduced pressure to give the diester in almost quantitative yield. The salt was purified further by twice precipitating it from a carbon tetrachloride–chloroform solution to give the diester **6** (90 mg, 40%) as a powder, m.p. 136–138 °C; ν_{max}(KBr)/cm^{-1} no diagnostic peaks; δ_H(250 MHz; D_2O) 7.83 (1 H, d, *J* 8), 7.52–7.38 (6 H, m), 7.18 (1 H, t, *J* 8.4), 7.11 (1 H, t, *J* 7.7), 5.20 (2 H, s), 3.63 (3 H, d, *J* 11.1), and 1.34 (9 H, s); δ_C(400 MHz; D_2O) 159.4, 139.3, 134.9, 133.0, 131.2, 130.8, 123.4, 120.6, 116.9, 73.5, 55.8 and 29.2 (four peaks not resolved); δ_F(250 MHz; D_2O) −71.4; δ_P(400 MHz; D_2O) −146.1 (Found: C, 48.2; H, 4.95. $C_{21}H_{26}F_6O_5PN$ requires: C, 48.75; H, 5.07%).

tert-*Butyl ammonium* 2-(2-*benzyloxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propyl methyl phosphate*. The 2-fluoroethyl phosphate triester (100 mg, 0.19 mmol) was refluxed in *tert*-butylamine (50 cm^3) for 48 h.[30] The amine was removed under reduced pressure to give an oily residue contaminated with less than 10% of the starting triester. This was removed by repeated washing with light petroleum to give the diester (70 mg, 67%) as a clear oil; δ_H(250 MHz; $CDCl_3$) 1.99 (1 H, d, *J* 5), 7.42–7.27 (6 H, m), 7.02 (1 H, d, *J* 8), 6.89 (1 H, d, *J* 8), 5.07 (2 H, s), 4.46 (2 H, dm, *J* 48), 4.09 (2 H, dm, *J* 26), and 0.76 (9 H, s); δ_P(400 MHz; $CDCl_3$) −148.3; δ_F(250 MHz; $CDCl_3$) −71.4, peak at *ca.* 226 not resolved.

tert-*Butyl ammonium* 2-(2-*benzyloxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propyl* 2,2,2-*trifluoroethyl phosphate*. The methyl trifluoroethyl phosphate triester (100 mg, 0.19 mmol) was stirred in *tert*-butylamine (50 cm^3) overnight.[30] The amine was removed from the salt (which had precipitated out) and the oily residue washed repeatedly with light petroleum to give the diester (60 mg, 60%); δ_H(250 MHz; $CDCl_3$) 7.98 (1 H, d, *J* 7.2), 7.39–7.27 (6 H, m), 7.01–6.92 (2 H, m), 5.09 (2 H, s), 4.45 (3 H, s), 4.27–4.15 (2 H, m), 1.09 (9 H, s); δ_C(400 MHz; $CDCl_3$) 157.2, 136.5, 131.5, 131.2, 128.5, 127.9, 127.3, 120.4, 119.3, 113.3, 70.8, 63.1 (q, *J ca.* 127), 51.3 and 27.3; δ_F(250 MHz; $CDCl_3$) −71.3 (s), −75.7 (t, *J* 9.1); δ_P(400 MHz; $CDCl_3$) −152.9 (Found: C, 45.2; H, 4.25. $C_{22}H_{25}F_9O_5PN$ requires C, 45.14; H, 4.31%).

tert-*Butyl ammonium* 2-(2-*benzyloxy*-4-*methoxyphenyl*)-1,1,-1,3,3,3-*hexafluoro*-2-*propyl methyl phosphate*. Prepared in the same manner as **6** to give the diester (200 mg, 60%) as a white powder, m.p. 155–156 °C; δ_H(250 MHz; $CDCl_3$) 7.75 (1 H, s), 7.36–7.30 (5 H, m), 5.01 (2 H, s), 3.76 (3 H, s), 3.55 (3 H, d, *J* 11.2) and 1.12 (9 H, s); δ_C(400 MHz; $CDCl_3$) 152.9, 151.1, 137.0, 128.4, 127.7, 127.2, 122.4 (q, 1170), 120.8, 117.3, 116.5, 114.4, 71.5, 55.7, 53.2 (d, *J* 26), 49.7 and 29.2; δ_F(250 MHz; $CDCl_3$) −71.2; δ_P(400 MHz; $CDCl_3$) −148.1 (Found: C, 47.1; H, 5.0. $C_{22}H_{28}F_6O_6PN$ requires C, 48.27; H, 5.16%).

tert-*Butyl ammonium* 2-(2-*hydroxyphenyl*)-1,1,1,3,3,3-*hexafluoro*-2-*propyl methyl phosphate* **3**. 10% Activated palladium on charcoal (100 mg) was suspended in 0.002 mol dm^{-3} HCl and stirred for a minimum of 30 min under hydrogen. A solution of the phosphate diester **6** (100 mg, 0.19 mmol) in 2:8 v/v acetonitrile–0.002 mol dm^{-3} HCl was added to the vigorously stirred catalyst. After about 10 min the mixture was filtered and the filtrate lyophilised to give the diester **3** as a fine white powder in almost quantitative yield; δ_H(400 MHz; D_2O) 7.7 (1 H, d, *J* 8.1), 7.39 (1 H, t, *J* 7.8), 7.03 (1 H, t, *J* 7.8), 6.98 (1 H, d, *J* 8.1), 3.66 (3 H, dd, *J* 11 and 1.7), and 1.33 (9 H, s); δ_C(400 MHz; D_2O) 157.3, 134.7, 132.5, 122.8, 120.8, 118.7, 55.9 (d, *J* 24.4), 54.5, and 29.2 (two signals not resolved); δ_F(250 MHz; D_2O) −71.6; δ_P(400 MHz; D_2O) −146.2.

All four substrates were deprotected in the same way. Hydrogenolysis times could be varied between 10 and 20 min, without affecting the result. The substrates thus prepared were not characterised, but used directly for the kinetic experiments. The preparation of the least reactive compound **3**, as described above, gave small amounts (*ca.* 5% by NMR) of cyclic product **7** when the deprotection was carried out on a larger scale.

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