Models for Enzyme-catalysed Phosphate Transfer: Comparisons of Reactivity towards a Neighbouring Hydroxy Group for Phosphodiester Anions and Acids. General Base Catalysis of the Cyclisation of a Hydroxyalkyl Phosphate Triester

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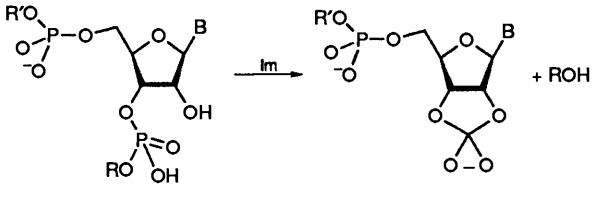
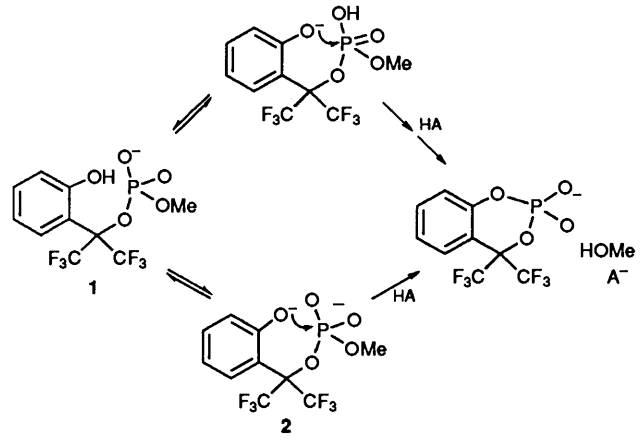
Methylation of phenyl 1,2-isopropylidene-β-D-xylofuranose 3'-phosphate increases the rate of intramolecular cyclisation by a factor of over 10^5. This confirms previous estimates of the effect of protonation on the reactivity of phosphate diesters towards a neighbouring hydroxy group, which depend on the correct assignment of kinetically equivalent mechanisms, and makes available reliable data on the magnitude of the effect for reactions catalysed by a range of general acids and bases. General base catalysis is characterised for the intramolecular cyclisation of one diastereoisomer (**7b**) of methyl phenyl 1,2-isopropylidene-β-D-xylofuranose 3'-phosphate triester: the Brønsted β is 0.65 and catalysis is enhanced by the proximity of the positive centres of suitable diamine monocations.

Breslow[1,2] has suggested that the initial step of the ribonuclease-catalysed hydrolysis of RNA may involve attack by the 2'-hydroxy group on the protonated phosphate diester group, with assistance by the imidazole group of an active-site histidine acting as a general base.

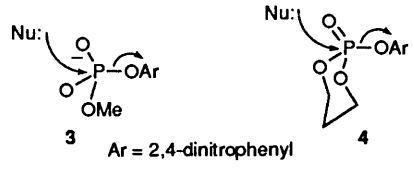


There is no doubt that protonation of the exceedingly unreactive phosphate diester anion will increase its reactivity towards nucleophilic attack, but it is not a simple matter to quantify the rate factor involved. In recent work[3] on the hydrolysis of a methyl phosphate diester **1** undergoing efficient intramolecular nucleophilic attack by phenolate oxygen, we identified separate reactions of the monoanion **1** and the dianion **2** to which we assigned similar mechanisms, involving nucleophilic attack by the phenolate anion on the neutral phosphoric acid and the phosphate anion. The data thus allowed very precise estimates of the rate enhancement due to protonation of the diester anion, of 5.5×10^5 for the uncatalysed reaction (HA = H_2O), and 8.1×10^4 for the reaction catalysed by the imidazolium cation. These figures compare well with a similar estimate available from results for a system more closely related to the nucleotide structure,[4] but are much larger than those suggested by earlier work,[5] discussed below, on the relative reactivity of similar di- and triesters. Furthermore, as is always the case when kinetically equivalent mechanisms differ only in the position of a proton in the transition state, the new estimates depend absolutely on the correctness of the assignment of mechanism. The kinetic ambiguity disappears if the proton is replaced by an alkyl group, so we have compared the rate of the intramolecular cyclisation of a model hydroxyalkyl phosphate diester anion with that for the corresponding methyl phosphate triester, where the proton of the diester acid is replaced by a methyl group. The study also allows us to characterise general base



catalysis of the reaction of the triester, and thus, in principle, of the diester acid.

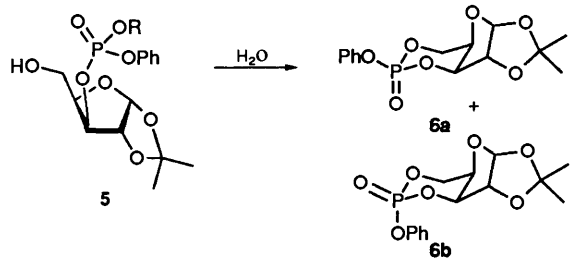
The earlier work[5] showed that the relative rates of reaction with external nucleophiles of triesters and the corresponding diesters depend strongly on the charge on the nucleophile. For the displacement of 2,4-dinitrophenolate from the phosphate phosphorus of the esters **3** and **4** the ratio is less than 100 for neutral nucleophiles (26 for water, 2–40 for pyridines), but several thousand for anions (4000 for phosphate, nearly 5000 for fluoride).[5] This suggested that the electrostatic repulsion



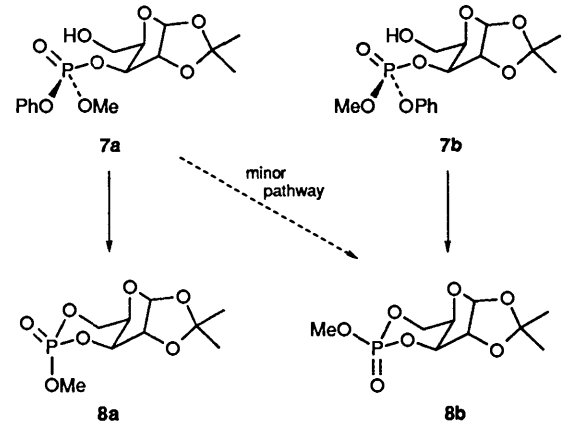
involved in the anion–anion reaction is at least as important as the difference in the intrinsic electrophilicity of the two phosphorus centres. However, phosphate-transfer reactions which do not depend on metal ions generally involve general base catalysed attack by hydroxy oxygen. This makes the degree of charge development at the attacking oxygen, and thus the potential electrostatic repulsion for attack on the

phosphorus centre of a phosphate diester anion, difficult to assess.

The reactions of triesters derived from nucleotides are rapid, and have to be followed by methods such as HPLC, which do not allow continuous monitoring. We have used triesters **5** derived from 1,2-isopropylidene-β-D-xylofuranose, which cyclise at convenient rates in reactions involving nucleophilic attack by the free 5'-hydroxy group, and can be monitored continuously in the UV. The leaving group RO^- is phenolate, allowing direct comparison with our results for the reverse reaction of **1** where a neighbouring phenolate is the nucleophile.* Phenolate is also a convenient surrogate for the partially protonated alkoxide leaving group involved in reactions where its departure is general acid catalysed.



We have measured rate constants for the hydrolysis of three derivatives of **5**, the diphenyl ester (**5**, R = Ph) and the methyl phenyl esters (**5**, R = Me). The cyclisation of **5** (R = Ph) gives two diastereoisomeric products **6a** and **6b**, and their relative rates of formation and hydrolysis (to the cyclic phosphate diester) are such that the kinetic analysis becomes inconveniently complicated.[7] So for detailed investigation we prepared and separated the diastereoisomeric esters **7a** and **7b**, which cyclise to **8a** and **8b**, respectively. We report a partial pH–rate profile for the cyclisation of **7a**, and evidence that the reaction is general base catalysed, together with a more extensive study of catalysis by general bases of the hydrolysis of **7b**.



Results

Diphenyl Ester **5** (R = Ph).—Detailed results with this compound are not reported, but the main features of its reactions are of interest. ^{31}P NMR experiments show that it is hydrolysed rapidly in 0.005 mol dm^{-3} NaOH in 1:1 1,4-dioxane–water at room temperature to an 85:15 mixture of

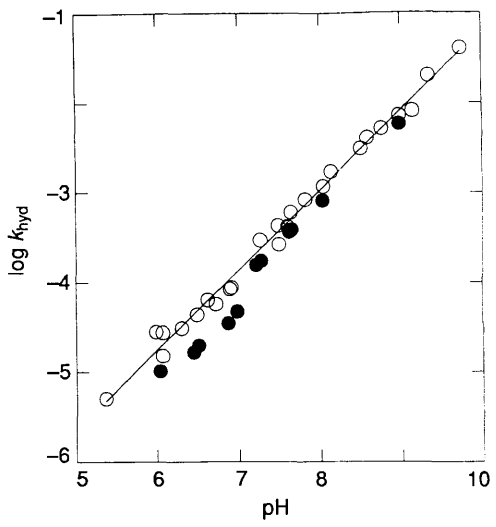
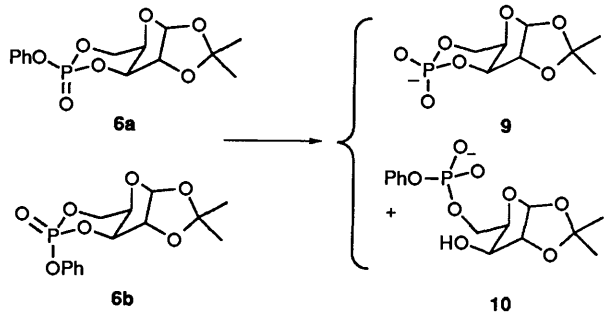


Fig. 1 pH–rate profiles compared for the cyclisation of **7b** (○) and **7a** (●) at 50 °C and ionic strength 1.0 mol dm^{-3}. The equation of the (least-squares) line drawn for the reaction of **7b** is $\log k_{hyd} = 0.89 \pm 0.02$ pH − 10.09 ± 0.12. The slope of the corresponding line (not drawn) through the points for the slower reacting isomer **7a** is 1.01 ± 0.05 pH − 11.21 ± 0.34. The rate constants k_{OH} given in Table 1 are calculated from the data at pH 7.

cyclic triesters **6a** and **6b**. The subsequent hydrolysis of these cyclic triesters is much slower, and observed under these conditions only after the addition of more base. The same products are observed near pH 7 (at 50 °C in 0.1 mol dm^{-3} imidazole buffer, 50% free base, and in 1:1 1,4-dioxane–water) and also in anhydrous 1,4-dioxane in the presence of 5 equivalents of triethylamine. **6a** is hydrolysed nearly six times faster than **6b**, as expected if relative reactivity is determined by the ground-state energies. (**6b**, with the PhO group axial, is stabilised by the generalised anomeric effect.[7]) The major product is the cyclic diester **9** for both isomers, but significant amounts of the ring-opened diester **10** are also produced (13% from **6a**, 22% from **6b**).



Methyl Phenyl Triesters **7a** *and* **7b**.—Diastereoisomers **7a** and **7b** are hydrolysed a few times more slowly than the diphenyl ester, at rates which differ by only a small factor, which depends on the conditions (relevant rate constants are given in Table 1, and pH–rate profiles shown in Fig. 1). The major product from the hydrolysis at 50 °C in 0.5 mol dm^{-3} TRIS buffer, 50% free base (in 1:1 1,4-dioxane–water) of the faster hydrolysing diastereoisomer can be identified by its NMR spectrum as the cyclic triester **8b**, with the methoxy group equatorial. This identifies the faster-hydrolysing isomer as **7b** (S_P). The slower isomer is therefore **7a** (R_P): this gives as the major product the cyclic triester **8a**, with the methoxy group axial. Small amounts (*ca.* 6%) of the diastereoisomeric triester

* In both cases six-membered rings are formed. We have discussed[3,6] the advantages of using systems where the effective molarity can be varied, rather than those based on ribose, where it is fixed.

Table 1 Rate constants for the hydrolysis of triesters and diesters at 50 °C and ionic strength 1.0 mol dm^{-3}

Compound	k_{OH}	$k_B/10^{-4}$ dm^3 mol^{-1} s^{-1} B = phosphate	$k_B/10^{-4}$ dm^3 mol^{-1} s^{-1} B = TRIS
5, R = Ph	580[a]	32	180
7a	190[a]	4.6	8.3
7b	365[a]	8.9	17.6
11	$(3.75 \pm 0.15) \times 10^{-4}$ [b]		

[a] Based on hydrogen ion activities, calculated from the pH and $K_w = 5.476 \times 10^{-14}$ at 50 °C. [b] Based on hydroxide ion concentrations.

Table 2 Second-order rate constants for catalysis by general bases of the cyclisation of **7b**, at 50 °C and ionic strength 1.0 mol dm^{-3}

General base	Concn. range/mol dm^{-3} (No. of runs)	pK_a	$k_B/10^{-4}$ dm^3 mol^{-1} s^{-1}	C–O cleavage (%)[a]
TMEDA monocation[b]	0.08–0.20 (9)	5.99	17.0 ± 3.2	13.2
MES	0.08–0.20 (3)	6.07	0.95	6.5
Phosphate	0.12–0.40 (9)	6.49	8.87 ± 2.40	—
Imidazole	0.08–0.20 (3)	6.72	13.1	1.6
EDA monocation	0.08–0.20 (9)	6.89	23.4 ± 5.5	0.6
4-Methylmorpholine	0.08–0.20 (3)	7.49	25.7	3.2
Triethanolamine	0.08–0.20 (3)	7.61	10.1	1.7
2-Methylimidazole	0.08–0.20 (3)	7.60	20.9	0.3
TRIS	0.08–0.20 (3)	7.64	17.6 ± 3.2	—
TMPDA monocation	0.08–0.20 (9)	7.82	77	1.9
Carbonate	0.12–0.40 (3)	8.98	*b*	—
TMBDA monocation	0.08–0.20 (3)	8.59	187.0	—
DABCO	0.08–0.20 (9)	8.76	1019 ± 5	3.5
TMEDA free base	0.08–0.20 (9)	9.14	158	2.0

[a] Measured by HPLC analysis of reactions performed with 0.1 mol^{-1} dm^{-3} buffer, 50% free base, under the conditions of the kinetic experiments (see the Experimental section). [b] TMEDA (*etc.*) are tetramethylEthylene(Propane, Butane)diamine: MES is morpholine-*N*-ethanesulfonate: DABCO is diazabicyclooctane.

8b are also formed from **7a**, by a pathway which involves retention of configuration at phosphorus.

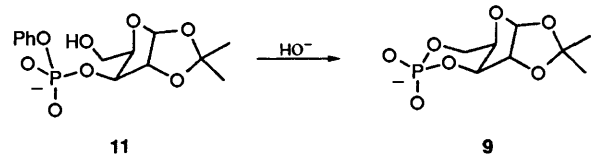
Slower subsequent reactions lead in both cases to the formation of the cyclic diester **9**, also produced from the diphenyl ester **5** (R = Ph), as described above. It is likely that these reactions involve C–O cleavage (S_N2 attack on the CH_3 groups of **8a** and **8b**, with displacement of a phosphate diester monoanion), but they do not interfere with the much faster reactions of interest for this work.

Both diastereoisomers **7a** and **7b** also give small amounts of demethylation product (7% for **7b**, 15% for the slower-hydrolysing isomer **7a** under these conditions (TRIS buffer, 1:1 1,4-dioxane–water). This reaction is UV-transparent, and does not interfere directly with the kinetic measurements. Under the conditions of the kinetic experiments (solvent water) this pathway generally accounts for no more than a few percent of the observed reaction (see below).

The cyclisation reactions show strong buffer catalysis at pHs below about 9. Above this pH the hydroxide-catalysed reaction is dominant. For example, for the most basic amine used, TMEDA (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine), buffer catalysis can be observed for the 20% and 50% free-base buffers, but is no longer detectable for 80% free base, even though the free base is the catalytic species. Buffer catalysis data have been measured for the cyclisation of **7b** catalysed by a range of amines and oxyanions, in six cases at three different buffer ratios (Table 2). We observe exclusively general base catalysis, with significant differences in efficiency depending on the structure of the general base, as discussed below. A small set of data measured for the reaction of the slower reacting isomer **7a** appear in Table 1.

Demethylation was a major pathway in the product-determination experiments in 50% 1,4-dioxane–water, particularly for tertiary amines known to be good carbon nucleophiles. Using 0.1 mol dm^{-3} buffer, 50% free base, demethylation accounted for 54, 62 and 75% of the initial products from the reactions of TMEDA, *N*-methylmorpholine and DABCO, respectively. Products were therefore carefully monitored, by HPLC, for almost all buffer-catalysed reactions, under the conditions of the kinetic experiments. It was found that reactions were considerably slower in the less polar mixed solvent, which specifically favours the S_N2 mechanism: evidently this is less sensitve to the changing polarity of the medium. In water demethylation is a minor side-reaction, generally accounting for less than 2% of the observed rate. The exceptions were the TMEDA cation (13.2%), MES (6.5%) and DABCO (3.5%, of which 2% is accounted for by the amine reaction). Up to about 1% of the hydroxide reaction involves C–O cleavage. These results are indicated in Table 2, where the rate constants shown are uncorrected. The data used for the Brønsted plot (Fig. 2) have been corrected for C–O cleavage where this amounts to more than 3.5% of total reaction, and refer specifically to P–O cleavage.

Cyclisation of the Monophenyl Diester **11**.—The cyclisation of the diester (half-life 50.4 h in 0.01 mol dm^{-3} KOH at 50 °C) is much slower than that of the triesters. Buffer catalysis is not observed in the high pH region, so data are available only for the hydroxide reaction. k_{OH} was measured in a series of KOH solutions as $(3.75 \pm 0.15) \times 10^{-4}$ dm^3 mol^{-1} s^{-1}. The comparison (Table 1) with the triesters, which are hydrolysed too fast to be measured under these conditions, involves a correction for the activity of the hydroxide ion. Using an activity coefficient $\gamma = 0.69$ we estimate the difference in reactivity $k_{triester}/k_{diester}$ to be 4.6×10^5 for **7b** at pH 7.



11 **9**

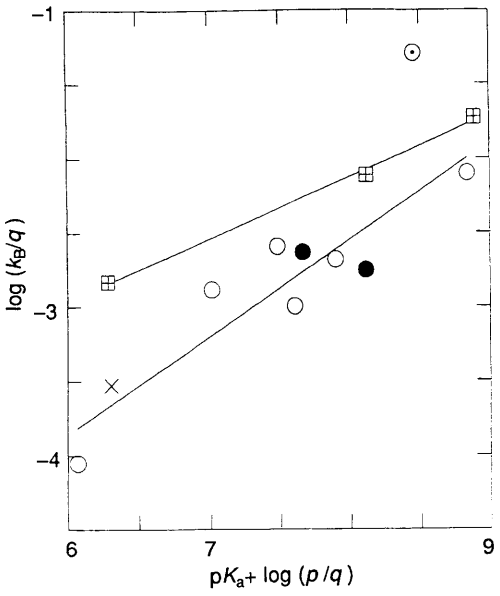


Fig. 2 Statistically corrected Brønsted plot comparing rate constants for catalysis of the cyclisation of **7b** by uncharged tertiary amines (○) with data for catalysis by the monocations of tertiary amines (⊞), at 50 °C and ionic strength 1.0 mol dm^{-3}. The filled circles represent points for primary amines, × the point for phosphate dianion, and the dotted circle that for the reaction with DABCO (see the text). The data are taken from Table 2, and are corrected for C–O cleavage where this amounts to more than 3.5% of the total reaction.

Discussion

Because of the large difference in reactivity between the phosphate di- and tri-esters we were able to measure the ratio $k_{triester}/k_{diester}$ only for the hydroxide-catalysed reactions, which under strongly basic conditions may be presumed to involve the cyclisations of the substrate conjugate bases (**12**, OR = OMe and O$^-$, respectively).

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The ratio ($k_{triester}/k_{diester}$ 4.6 × 10^5) is similar to ratios estimated indirectly for buffer-catalysed reactions from our work with diesters.[3,4] The ratio observed in this work for the intramolecular displacement of phenoxide is also similar to the ratio of k_{OH} for the hydrolysis of trimethyl and dimethyl phosphates which can be estimated by extrapolation from literature data at various temperatures[8a] as 2.7 × 10^5, and a similar ratio can be estimated by comparing the rate of base-catalysed hydrolysis of UpU and the dimethyl ester of uridine 3′-phosphate.[8b] These figures confirm the accuracy of our estimates based on the kinetically equivalent reactions of **1** and **2**, of the activating effect for attack on phosphorus of protonation of a phosphate diester, and thus make available reliable data for the magnitude of this effect for reactions catalysed by a range of general acids and bases.

Effective molarities (EM)[9] of the nucleophilic OH group in the triesters **7** and the diester **11** can be estimated as 10^4 and 10^3 mol dm^{-3}, respectively. These figures are approximate, and not necessarily significantly different: the best intermolecular reactions available for comparison are for hydroxide ion reaction with a dialkyl phenyl phosphate[10] and with diphenyl phosphate,[11] respectively; they involve various minor corrections. In particular it is assumed that the pK_a of the CH_2OH group of the xylose derivative **7b** has the same pK_a as water. Since the EM for the diester reaction is at least no higher than that for the triester reaction, electrostatic repulsion between the oxyanion nucleophile and the diester anion centre, thought to be absent for intramolecular reactions in more rigid systems,[12] must still be operative for **11**.

General Base Catalysis.—The substantial set of buffer catalysis data (Table 2) for the cyclisation of **7b** allows us to characterise the transition state for this reaction in some detail. The mechanism is undoubtedly classical general base catalysis of the rate determining attack of the neighbouring 5′-OH group on the phosphate triester phosphorus (**13**): the nucleophile and leaving group are identified by the structure of the product, the nucleophile would be the poorer leaving group from the pentacovalent intermediate which is likely to be involved (see the discussion in ref. 3); and the rate depends on the pK_a of the general base with a Brønsted coefficient β which lies between zero and unity.

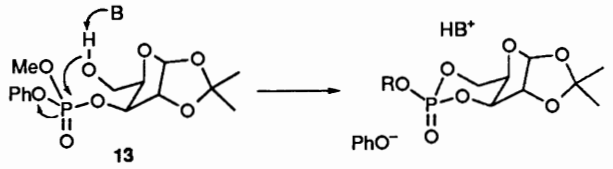
Demethylation is a minor side-reaction under the conditions of the kinetic experiments, though it becomes the dominant pathway in 1 : 1 1,4-dioxane–water, as discussed above. (The dealkylation of methyl phosphate triesters by amines is a useful synthetic procedure,[13] and the compact structure of bicyclic amines like DABCO minimises their steric demands, making them particularly good nucleophiles towards sp^3 carbon.[14] DABCO, for example, reacts up to 40 times faster than triethylamine with methyl bromide.)

The Brønsted plot of all the data (Fig. 2, corrected for the small amounts of C–O cleavage) shows considerable scatter, indicating that reactivity depends on factors other than basicity. We observed similar, even more pronounced, scatter in the corresponding plot for general acid catalysis of the displacement of methoxide from the diester anion **2**, and can analyse the data of Fig. 2 in the same way. Rate constants for catalysis by uncharged tertiary amines show a normal dependence on their pK_a, with a Brønsted β (the slope of the lower line in Fig. 2) of 0.65 ± 0.13. (Catalysis by primary or secondary amines might be expected to be correlated by a different line of similar slope.) This is consistent with substantial proton transfer to the general base in the transition state, and β is the same as found by Davis *et al.*[15] for general base catalysis of the cyclisation of 4-nitrophenyl uridine 3′-phosphate. It is also complementary to the Brønsted α of 0.33 we found for general acid catalysis of the departure of methoxide from **2**. It appears that at least the proton-transfer part of the transition state for the P–O bond-breaking process in these reactions is closely similar for the triester **7b** and for these two (fairly reactive) phosphodiesters.

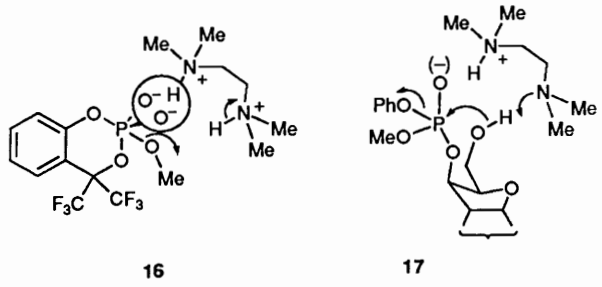
Two types of tertiary amines show significant positive deviations from this line (lower line, Fig. 2). The rate constants for catalysis by the monocation conjugate acids of diamines $Me_2N(CH_2)_nNMe_2$ are consistently higher than those for comparable neutral amines of the same basicity, and the positive deviation increases with decreasing length of the methylene chain. The effect is smaller, but similar to the effect we observed for the reverse reaction of the phosphate diester dianion **2**.[3] In that case the effect on the Brønsted exponent of increasing proximity of the positive charge was twice that of increasing the strength of the general acid. In the present case the two effects are comparable [the upper line correlating the points for the three monocations (Fig. 2) has a slope, β = 0.39 ± 0.03]. The fastest reacting amine of all is diazabicyclo-octane (DABCO). The conjugate acid of this base is also a particularly effective general acid catalyst for the cyclisation of **2**,[3] but the effect is unique in being greater for the reaction of **7b**. We have no satisfactory explanation for this result: general base catalysed reactions are not usually sensitive to steric effects, and a specific effect on solvent structure seems unlikely.

(Various rate comparisons rule out direct nucleophilic attack on phosphorus as an alternative explanation.) It is only as a result of this exceptional reactivity that catalysis is observable at the highest pH used with DABCO (80% free base, pH 9.34). General base catalysis is not detected for 80% free-base TMEDA, because the specific base-catalysed reaction is dominant at pH 9.74. The kinetic isotope effect for both the TMEDA and the DABCO-catalysed reactions is small (1.2), suggesting that proton transfer and P–O bond formation are not strongly coupled in these extreme cases. There is known to be an important cross-interaction between nucleophile and leaving group in the reactions of phosphate triesters,[16] and this particular reaction represents an extreme case, on the borderline of detectable general base catalysis.

It will be interesting to compare the data of Table 2 with similar results involving the reaction of a phosphate diester anion. This requires a more reactive diester than any currently available, and we are preparing suitable candidates.[17] The relevant comparison that is possible is with our data for general acid catalysis of the cyclisation of the dianion **2** of **1**,[3] the microscopic reverse of which (**14**) would be the (inaccessible) general base catalysed attack of methanol on the cyclic diester produced from **15**.



We ascribed the larger effects of positively charged general acids on the cyclisation of the dianion of **1** to an electrostatic effect stabilising the transition state for the reaction, as in **16**.[3] The same mechanism extended to the reaction of **7b** is shown as **17**. Though these effects are relatively small in water, multiple



interactions between ammonium cation centres and phosphate anions can lead to significant binding, as shown by the work of Lehn on model phosphate-transfer reactions of ATP.[18] Where the interactions concerned involve electrostatic attraction and hydrogen-bond formation substantially larger effects are to be expected in less polar environments, including the active sites of enzymes catalysing phosphate-transfer reactions of phosphodiesters. The general conclusion, that an enzyme catalysing a phosphate-transfer reaction of a phosphodiester needs a general base, a general acid and positive charge situated where it can best stabilise the pentacovalent transition state, is not surprising. However, the detailed information about structure and reactivity emerging from this work allows the informed comparison of detailed mechanisms for the enzyme reactions, and provides reliable guidelines for the design of more efficient artificial catalysts for these important reactions.

Experimental

Kinetic Measurements.—Conditions and methods were generally as recently described.[3] Standard conditions were 50 °C at ionic strength 1.0 mol dm^{-3}, maintained with potassium chloride. pH measurements were made under the conditions of the kinetic experiments using a Radiometer PHM82 pH meter with a Russell CTWL electrode. Buffer pK_as were 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 the rate of cyclisation of all three triesters (**5**, **7a** and **7b**) for all the buffers used, except for carbonate and DABCO at the highest pH used.

Product Analysis.—The hydrolysis products of the phosphate esters used in this work were investigated primarily by ^{31}P NMR spectroscopy, in most cases by comparison with authentic samples, using 20–30 mg of the substrate in 2 cm^3 of solution with 2% phosphoric acid in D_2O as an external standard. ^1H NMR spectra were also recorded. Conditions are described for individual cases.

The faster-hydrolysing diastereoisomer of methyl phenyl 1,2-isopropylidene-β-D-xylofuranose 3-phosphate **5** (R = Me, δ_P − 5.52) gives as the main product a compound with a resonance at δ − 6.74 (m). This must be the cyclic diester with an axially oriented phosphoryl oxygen **8b**. (The assignment is based on the ^{31}P chemical shifts of the known cyclic esters **8a** and **8b**, where the stereochemistry was identified by NOE experiments,[19] and similar and consistent results with related methyl phosphonate esters.[20]) This identifies the faster-hydrolysing diastereoisomer as the S_P isomer **7b**.

The slower-hydrolysing diastereoisomer of **5** (R = Me, δ_P − 5.81) gives as the main product a compound with a resonance at δ − 7.96 (m). This must be the cyclic diester with an equatorially oriented phosphoryl oxygen (**8a**).[19,20] This identifies the slower-hydrolysing diastereoisomer as the R_P isomer **7a**. In addition small amounts (6% of total, 8% of cyclic product) of the compound (**8b**) resonating at −6.74 ppm can be detected.

Both reactions give small amounts (7% from **7b**, 15% from **7a**) of a compound with a resonance at −5.16 ppm (d, $^3J_{PH}$ = 8–9 Hz). This was isolated by preparative TLC, and identified by its (^1H and coupled ^{31}P) NMR spectra as the demethylation product phenyl 1,2-isopropylidenexylose 3′-phosphate (**11**), which had already been prepared by demethylation with sodium iodide (see below).

The final product from both diastereoisomers is the 3′,5′-cyclic ester **9** (δ_P − 5.9, $^3J_{PH}$ 20.5 Hz), also produced as the final product from the diphenyl ester **5** (R = Ph).

Diphenyl 1,2-*O*-isopropylidene-β-D-xylofuranose 3-phosphate **5** (R = Ph, δ_P − 12.2) in 0.005 mol dm^{-3} NaOH in 1:1 1,4-dioxane–water at room temperature gave 85% of **6a** (δ_P −14.0, d, $^3J_{PH}$ = 20.6 Hz) and 15% of **6b** (δ_P − 15.5, d, $^3J_{PH}$ = 20.7 Hz). This process was complete before any subsequent events could be detected. After addition of more NaOH and longer time the mixture of the cyclic triesters was hydrolysed to form 84% of the cyclic diester **9** (δ_P − 5.9, d, $^3J_{PH}$ = 20.5 Hz) and 16% of the ring-opened compound **10** (δ_P − 4.3, t, $^3J_{PH}$ = 8.1 Hz). These two compounds were also isolated by preparative TLC and their structures confirmed by ^1H NMR. The cyclization of **7** takes a similar course in 0.1 mol dm^{-3} imidazole buffer (50% free base, in 1,4-dioxane–water 1:1) as well as with 5 equivalents of triethylamine in anhydrous 1,4-dioxane.

Sodium phenyl 1,2-isopropylidene-β-D-xylofuranose 3-phosphate (**11**, δ_P − 4.3, t, $^3J_{PH}$ 8.1 Hz) in 0.01 mol dm^{-3} NaOH in

1 : 1 aqueous 1,4-dioxane at 25 °C gave as the single product the 3′,5′-cyclic diester **9** (δ_P − 5.9, $^3J_{PH}$ 20.5 Hz). These three compounds (**9**, **10** and **11**) were also isolated by preparative TLC and their structures confirmed by ^{1}H NMR spectroscopy.

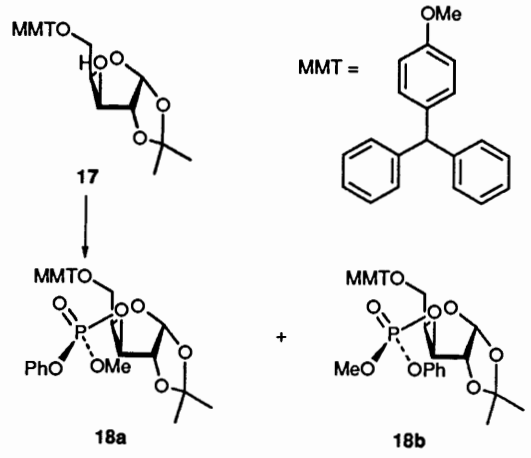
The main product from the buffer-catalysed reaction of **7b** in 1 : 1 1,4-dioxane was the diester **11**, so quantitative product analyses were run for these reactions, using reversed-phase HPLC. Reactions were run to completion in 1 cm^3 of buffer, under the conditions of the kinetic experiments; then 110 μl aliquots were diluted with 160 μl of triethylammonium acetate (2 mol dm^{-3}), and 200 μl injected. (Waters Resolve RP-18 column, 4.6 mm × 125 mm; eluted using a linear gradient from 0.1 mol dm^{-3} triethylammonium acetate, pH 5.2 to 0.1 mol dm^{-3} triethylammonium acetate in 25% acetonitrile–water.) Retention times were 4.8 and 9.9 min for phenol and the diester **11**, respectively. Integration used the Dynamax software package from Rainin, and areas were weighted for the different absorption coefficients.

Materials and Methods.—Pyridine (Merck *pa*) was distilled from calcium hydride and stored over molecular sieves (4 Å). Acetonitrile (Merck *pa*) was distilled from calcium hydride and stored over molecular sieves (3 Å). Triethylamine was distilled from calcium hydride and stored over a few large lumps of the same reagent. 1,4-Dioxane was distilled from lithium aluminium hydride and kept over sodium wire. 1,2-*O*-Isopropylidene-β-D-xylofuranose was prepared from xylose *via* the diisopropylidene derivative according to Svanberg and Sjöberg.[21] All other chemicals and solvents were of standard commerical grade. ^{31}P NMR was performed at 109.4 MHz (resolution 1.0 Hz) and chemical shifts are measured relative to 2% H_3PO_4 in D_2O. ^{1}H NMR was performed at 269.7 MHz (resolution 0.4 Hz) and chemical shifts are relative to $CDCl_3$ (7.27 ppm). All resonances were assigned using homonuclear decoupling experiments.

Synthesis

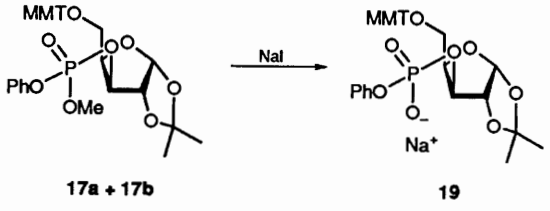
5-O-(4-*Methoxytriphenylmethyl*)-1,2-*O*-*isopropylidene*-β-D-*xylofuranose* (**17**).—1,2-*O*-Isopropylidene-β-D-xylofuranose (10 mmol) was rendered anhydrous by evaporation of added pyridine and then dissolved in the same solvent (50 cm^3). The solution was kept on ice and 4-methoxytriphenylmethyl chloride (12 mmol) added. The reaction was left stirring overnight (the ice melted after a few hours). The reaction was quenched with 1 cm^3 of methanol, and after 30 min the solvent was evaporated under reduced pressure. The mixture was then dissolved in dichloromethane and washed twice with saturated aqueous sodium hydrogencarbonate then once with water. The organic phase was dried with sodium sulfate and subsequently evaporated to yield a yellow syrup. The product was purified by chromatography on silica gel (eluent toluene–ethyl acetate 3 : 1) evaporation of the appropriate fractions gave 8.5 mmol of **1**.

5-O-(4-*Methoxytriphenyl*)*methyl*-1,2-*isopropylidene*-β-D-*xylofuranose* 3-(*Methyl Phenyl Phosphate*) **18**.—Tetrazole (0.84 g, 12 mmol) was rendered anhydrous by evaporation of added acetonitrile and then dissolved in the same solvent (5 cm^3). The solution was kept on ice and phenyl dichlorophosphate (0.9 cm^3, 6 mmol) was added followed by triethylamine (1.8 cm^3, 14 mmol). A solution of **1** (0.69 g, 1.5 mmol, rendered anhydrous by evaporation of added acetonitrile and then dissolved in the same solvent (5 cm^3) was then added to the mixture over a period of 5 min. The reaction was then taken off the ice–water bath and stirred at room temperature for 1 h. The mixture was cooled on the ice–water bath again and 0.5 cm^3 of methanol was added and the solution was kept another 30 min at room temperature. The solvent was evaporated off under reduced pressure and the residue dissolved in dichloromethane. The organic phase was washed twice with saturated sodium hydrogencarbonate (aq.) and once with water and subsequently dried with sodium sulfate and evaporated. The mixture was subjected to silica gel chromatography (toluene–ethyl acetate 5 : 1) whereby the two diastereoisomers **18a** (slower moving, 335 mg, 35%) and **18b** (faster moving, 270 mg, 28%) could be separated. A mixed fraction (210 mg, 22%) was also obtained but not further purified since this could be used in synthesis of the diester **11** (see below).



(R_P)-1,2-O-*Isopropylidenexylose* 3-(*Methyl Phenyl Phosphate*) **7a**.—The slower moving isomer of **18** (*i.e.* **18a**, 200 mg, 0.32 mmol) was dissolved in 5 cm^3 80% aqueous acetic acid. After 1 h dichloromethane was added followed by enough water to create a two-phase system. The dichloromethane phase was washed repeatedly with water, dried with sodium sulfate and evaporated. The product was purified by silica-gel chromatography (eluent: toluene–ethyl acetate 1 : 2) to give 80 mg of **7a** (70%). δ_P [25 °C; dioxane–Tris buffer (0.5 mol dm^{-3}: pH = 8.21) 1 : 1] −5.81 (quintet, $^3J_{PH}$ = 10.3 Hz); δ_H (25 °C; $CDCl_3$) 5.95 (d, 1 H, $^3J_{HH}$ = 3.7 Hz, H-1), 4.69 (d, 1 H, $^3J_{HH}$ = 4.0 Hz, H-2), 4.91 (dd, 1 H, $^3J_{HH}$ = 2.2 Hz, $^3J_{PH}$ = 8.8 Hz, H-3), 4.37 (m, 1 H, H-4), 3.72–3.83 (m, 1 H, H-5a), 3.58–3.68 (m, 1 H, H-5b), 7.38 (t, 2 H, $^3J_{HH}$ = 7.7 Hz, *m*-H), 7.22 (d t, 3 H, $^3J_{HH}$ = 7.7 Hz, *o*,*m*-H), 3.94 (d, 3 H, $^3J_{PH}$ = 11.7 Hz, MeOP), 1.55 (s, 3 H, *Me*CMe) and 1.52 (s, 3 H, MeC*Me*).

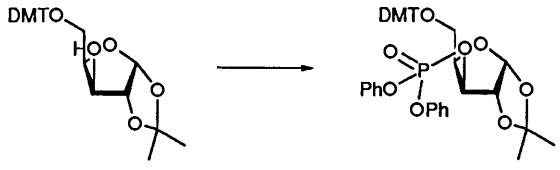
(S_P)-1,2-O-*Isopropylidenexylose* 3-(*Methyl Phenyl Phosphate*) **7b**.—Prepared using the same procedure as for **7a**, starting from the faster moving isomer of **18** (*i.e.* **18b**, 250 mg, 0.4 mmol) to give 110 mg of **7b** (76%). δ_P [25 °C; 1 : 1 dioxane–Tris buffer (0.5 mol dm^{-3}; pH = 8.21)] −5.52 (quintet, $^3J_{PH}$ = 10.6 Hz); δ_H (25 °C; $CDCl_3$) 5.91 (d, 1 H, $^3J_{HH}$ = 3.7 Hz, H-1), 4.55 (d, 1 H, $^3J_{HH}$ = 3.7 Hz, H-2), 4.94 (dd, 1 H, $^3J_{HH}$ = 2.4 Hz, $^3J_{PH}$ = 9.0 Hz, H-3), 4.39 (m, 1 H, H-4), 3.76–3.93 (m, 2 H, H-5), 7.39 (t, 2 H, $^3J_{HH}$ = 8.1 Hz, *m*-H), 7.24 (dt, 3 H, $^3J_{HH}$ = 8.4 Hz, *o*, *m*-H), 3.93 (d, 3 H, $^3J_{PH}$ = 11.7 Hz, MeOP), 1.55 (s, 3 H, *Me*CMe) and 1.52 (s, 3 H, MeC*Me*).



5-O-(4-*Methoxytriphenylmethyl*)-1,2-O-*isopropylidene*-β-D-*xylofuranose* 3-(*Phenyl Sodium Phosphate* **19**.—**17a** and **17b**

(210 mg, 0.33 mmol, mixture of diastereoisomers) was dissolved in acetonitrile (1 cm^3) together with 2 equiv. of sodium iodide (0.66 mmol). The mixture was kept for 40 h at room temperature then all liquids were evaporated off under reduced pressure and the residue dissolved in dichloromethane. The organic phase was washed twice with water and evaporated to dryness. Purification by chromatography on a short silica-gel column (chloroform–methanol 9:1) gave 180 mg of **19** (85%).

1,2-O-*Isopropylidene*-β-D-*xylofuranose* 3-(*Phenyl Sodium Phosphate*) **11**.—**19** (180 mg, 0.28 mmol) was dissolved in 5 cm^3 of 80% acetic acid (aq.). After 1 h one volume of water was added followed by diethyl ether. The aqueous phase was washed several times with ether and subsequently evaporated. The residue was redissolved in water and then lyophilized to give 85 mg (82%) of the authentic sample of **11** used for the identification of the minor product formed from **7a** and **7b** under hydrolytic conditions (δ_P − 5.16, $^3J_{PH}$ = 8–9 Hz); δ_H (25 °C; D_2O) 1.26 (s, 3 H, *Me*CHe), 1.46 (s, 3 H, MeC*Me*), 3.6–3.8 (m, 2 H, H-5), 4.31 (m, 1 H, H-3), 4.6–4.7 (m, 2 H, H-2,4), 5.94 (d, 1 H, J = 3.6 Hz, H-1), 7.15 (d, 2 H, J = 8.4 Hz, *o*-H), 7.16 (t, 1 H, J = 8.4 Hz, *p*-H) and 7.36 (t, 2 H, J = 7.9 Hz, *m*-H).



20

5-O-(4,4′-*Dimethoxytriphenyl*)-1,2-O-*isopropylidene*-β-D-*xylofuranose* 3-*Diphenyl Phosphate*) **20**.—A mixture of 5-*O*-(4,4′-dimethoxytriphenylmethyl)-1,2-*O*-isopropylidene-β-D-xylofuranose (prepared using the same procedure as for **1** but using 4,4′-dimethoxytriphenylmethyl chloride instead of 4-methoxytriphenylmethyl chloride, 0.49 mg, 1 mmol) and sodium iodide (1.1 mmol) was rendered anhydrous by evaporation of added pyridine and then dissolved in the same solvent (5 cm^3). Diphenyl phosphorochloridate (1.5 mmol) was added with stirring and the reaction was kept for 1 h at room temperature. Most of the pyridine was evaporated off and the residue dissolved in dichloromethane. The solution was washed twice with saturated sodium hydrogen carbonate solution and once with water. The organic phase was dried with sodium sulfate and subsequently filtered and evaporated under reduced pressure. The crude product was then purified by silica-gel flash chromatography (toluene–ethyl acetate 5:1) to yield 687 mg (95%) of **20**.

1,2-O-*Isopropylidene*-β-D-*xylofuranose* 3-(*Diphenyl Phosphate* (**5**; R = Ph).—**20** (0.36g, 0.5 mmol) was treated with 10 cm^3 of 80% aqueous acetic acid for 30 min. Water (10 cm^3) and diethyl ether (20 cm^3) were added to the mixture. The ether phase was washed twice with water and then dried with sodium sulfate, filtered and evaporated under reduced pressure. The crude product was chromatographed (short-column flash chromatography on silica gel, eluent 1:1 toluene–ethyl acetate) to give 156 mg (74%) of **5** (R = Ph). δ_P (25 °C; dioxane–water 1:1) −12.2 (d, $^3J_{PH}$ = 7.3 Hz); δ_H (25 °C; [$^2H_6$]acetone) 6.05 (d, 1 H, $^3J_{HH}$ = 3.7 Hz, H-1), 4.84 (d, 1 H, $^3J_{HH}$ = 3.8 Hz, H-2), 4.94 (dd, 1 H, $^3J_{HH}$ = 2.6 Hz, $^3J_{HH}$ = 7.5 Hz, H-3), 4.46 (m, 1 H, H-4), 4.08 (t, 1 H, $^3J_{HH}$ = 6.1 Hz, H-5a), 3.84 (t, 1 H, $^3J_{HH}$ = 6.1 Hz, H-5b), 7.42–7.63 (m, 10 H, Ar-H); 1.59 (s, 3 H, *Me*CMe) and 1.41 (s, 3 H, MeC*Me*).

Phenyl 1,2-O-*Isopropylidene*-β-D-*xylofuranose* 3,5-*Cyclic Phosphates* **6a** *and* **6b**.—1,2-*O*-Isopropylidene-β-D-xylofuranose (190 mg, 1 mmol) was dissolved in dry tetrahydrofuran (10 cm^3) with triethylamine (0.32 cm^3, 2.5 mmol). The solution was cooled on ice and phenyl phosphorodichloridate (0.18 ml, 1.2 mmol) added. The reaction was left to stir for 2 h at room temperature. Triethylammonium hydrogen carbonate (pH = 7.5; 1 cm^3; 2 mol dm^{-3}) was added and the tetrahydrofuran evaporated off. The residue was partitioned between dichloromethane and 1 mol dm^{-3} triethylammonium hydrogen carbonate. The organic phase was washed with water, dried over sodium sulfate, filtered and evaporated. **6a** and **6b** were separated using short-column flash chromatography on silica gel (eluent 2:1 toluene–ethyl acetate) to give 140–150 mg (*ca.* 45%) of each isomer. The compounds are identical with those made and identified by Neeser *et al.*[19]

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