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THE SYNTHESIS AND HYDROLYSIS OF A SERIES OF DEOXYFLUORO-D-GLUCOPYRANOSYL PHOSPHATES

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ABSTRACT

The synthesis of all four deoxyfluoro-α-D-glucopyranosyl phosphates is described. Rate constants for their acid-catalyzed hydrolysis were determined, and fluorine substitution was shown to have a significant effect in lowering the rate, particularly when the substitution is adjacent to the anomeric center. Relative rate-constants measured in M $HClO_4$ at 25° are 60.30:1.00:7.05:3.97:16.5 for α-D-glucopyranosyl phosphate and the 2-, 3-, 4- and 6-deoxyfluoro derivatives, respectively. The hydrolysis of 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate was studied in more detail, and an activation entropy and enthalpy of 4.1 e.u. (M reactant) and 113.5 kJ.mol^{-1}, respectively, were determined for hydrolysis in M $HClO_4$ at 60°. The pH dependence of its hydrolysis was investigated, and rate constants for hydrolysis of the monoanion ($k_M = 1.88 \times 10^{-6}$ s^{-1}) and neutral ($k_N = 6.23 \times 10^{-5}$ s^{-1}) species were thus extracted. Hydrolysis of the monoanion is not significantly affected by fluorine substitution, as expected. The ability or inability of several mechanistically distinct enzymes to utilize these fluorinated substrates is rationalized in the light of these findings.

INTRODUCTION

Considerable effort has been expended in recent years in the synthesis of deoxyfluoro sugars[1], primarily as a result of interest in such analogs for use as probes of enzyme active-sites[2,3], as possible anticancer agents[4], as tools for probing the biosynthesis of glycoproteins[5], and, more recently, as carriers of ^{18}F for positron emission tomographic (PET) studies[6–8]. Substitution of a fluorine atom for a hydroxyl group is of interest, as it is sterically conservative; thus, binding of fluoro sugars to proteins should not be inhibited sterically. In addition, because fluorine is more electronegative, it should be capable of accepting, albeit weakly, a hydrogen bond, and such electronegativity may also have important mechanistic consequences when the substitution is adjacent to a reaction center.

However, despite these extensive synthetic and biochemical endeavors, very little has been reported on the mechanistic consequences of such substitutions as

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applied to simple glycosyl-transfer reactions, be they enzyme-catalyzed or spontaneous. This should, nevertheless, be of considerable interest as the very usefulness of 2-deoxy-2-[^{18}F]fluoro-D-glucose in PET studies resides in the resistance of the 2-deoxy-2-fluoroglucosyl moiety to enzyme-catalyzed glycosyl-transfer reactions. Thus 2-deoxy-2-fluoroglucose is able to enter brain cells and become processed into phosphorylated derivatives, but it cannot be processed beyond that stage, and so it remains trapped in the cell at sites of high metabolic activity, because the sugar phosphate is incapable of efflux. The exact stage at which its processing is blocked is not yet clear, but, as hexokinase[9] and phosphoglucomutase[9a] (from nonbrain sources) can successfully process 2-deoxy-2-fluoroglucose and its 6-phosphate, whereas glycogen phosphorylase[9a], and, probably, other glycosyltransferases are incapable of action, or at least can only act slowly, it is likely that it is trapped as a mixture of the 1- and 6-phosphates, or as nucleoside diphosphate derivatives. Experiments with rat brain *in vivo*[10] would tend to support this concept.

The mechanisms of action of such glycosyltransferases, including lysozyme[11], are generally considered to be analogous to that established for acid-catalyzed glycoside hydrolysis[12], and to proceed *via* a glycosyl oxocarbonium-ion intermediate, or, at least, *via* a transition state having substantial oxocarbonium-ion character. Substitution of the (electronegative) fluorine atom adjacent to this site should result in significant destabilization of the oxocarbonium ion, and thus, to inhibition of the reaction. However, no studies have been published on the simple, acid-catalyzed hydrolysis of deoxyfluoroglucosides or deoxyfluoroglucosyl phosphates to test this, despite a wealth of information[12–14] on the hydrolysis of derivatives of the native sugar and of other analogs.

The mechanism of acid-catalyzed hydrolysis of α-D-glucopyranosyl phosphate was elucidated in some elegant work by Bunton *et al.*[15,16], in addition to determination of the mechanism of bond cleavage at a variety of pH values. More-recent work[17] addressed the effects of structural changes in the sugar on the rate of acid-catalyzed hydrolysis of its 1-phosphate. Results were found to be quite consistent with those obtained for the acid-catalyzed hydrolysis of alkyl and aryl glucopyranosides[12–14].

We now describe the synthesis of a full series of monodeoxyfluoro-α-D-glucopyranosyl phosphates, a systematic study of their acid-catalyzed hydrolysis, and a more detailed study of the hydrolysis behavior of the 2-deoxy-2-fluoro derivative.

RESULTS

Fluorination reactions were generally performed by using the reagent diethylaminosulfur trifluoride (DAST) (Aldrich Chemical Co.) with the appropriate, partially protected sugar. These deoxyfluoro sugars were deprotected, and the products acetylated by fairly standard procedures, but overall yields in these syntheses were generally improved over the previously published routes, and full ^{1}H- and ^{19}F-n.m.r. assignments are provided. All of the phosphorylations were per-

TABLE I

^{1}H- AND ^{19}F-N.M.R. CHEMICAL SHIFTS FOR MONODEOXYFLUORO-D-GLUCOPYRANOSYL PHOSPHATES

Position of fluorination	*Anomeric configuration*	*Chemical shift*[a]							
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*F*
6	α	5.44	3.46	3.78	3.51	4.00	4.74	—[b]	237.48
4	α	5.52	3.45	3.97	4.26	4.04	3.80	3.69	199.07
3	α	5.44	3.72	4.67	3.68	—	3.91	3.84	201.25
2	α	5.53	4.29	3.96	3.36	3.86	3.81	3.66	200.20
2	β	5.09	4.07	3.79	3.36	3.50	3.90	3.64	197.54

[a]^{1}H-Chemical shifts are referenced to external sodium 4,4-dimethyl-4-silapentane-1-sulfonate. ^{19}F-Chemical shifts are reported in parts per million upfield from $CFCl_3$. [b]Indicates that the resonance position could not be assigned, due to coincidence of signals.

formed by the MacDonald phosphorylation procedure[18] on the β-peracetates, and products crystallized, and were recrystallized, as their bis(cyclohexylammonium) salts. Successful synthesis of these largely novel deoxyfluoroglucopyranosyl phosphates was evidenced by their satisfactory microanalyses and by the ^{1}H- and ^{19}F-n.m.r. data presented in Tables I and II. In all cases, the most downfield signal is from the anomeric proton, which is coupled with both H-2 and the phosphorus atom, the magnitude of $J_{1,2}$ indicating an equatorial proton at C-1, and thus, the α-anomeric configuration for the sugar phosphate in all cases except that of 2-deoxy-2-fluoro-β-D-glucopyranosyl phosphate. The magnitude of the ^{1}H–^{1}H coupling-constants observed suggests a *trans*-diaxial orientation of H-2 and H-3; thus, the sugar adopts the $^4C_1$(D) conformation, and is therefore not distorted upon fluorine substitution. Several interesting long-range couplings involving the fluorine atoms are observed; for example, when fluorine is present at C-3 and C-4, H-1 is found to be coupled to the fluorine. Such couplings had been observed in the spectra of the α-tetraacetates of 3-deoxy-3-fluoro-[19] and 4-deoxy-4-fluoro-D-glucose[20]. Similarly, for the 2-deoxy-2-fluoro- and 6-deoxy-6-fluoro-α-D-glucopyranosyl phosphates, H-2 is coupled over four bonds to phosphorus, as seen for α-D-glucopyranosyl phosphate[21], suggesting that the phosphate group favors a trans relationship to H-2.

The second ionization constant (pKa_2) for 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate was determined both potentiometrically and by ^{19}F-n.m.r. measurements of chemical shift *and* $^2J_{F,H}$ value, and was found to be 5.90 (±0.03). An estimate of 1.00 (±0.05) for the first ionization constant (pKa_1) was obtained by ^{19}F-n.m.r. spectroscopy. The second ionization constant (pKa_2) for 2-deoxy-2-fluoro-β-D-glucopyranosyl phosphate was determined by ^{19}F-n.m.r. spectroscopy to be 5.60 (±0.05). All pKa values were determined for a 0.1M solution of the sugar. The first and second ionization constants of both α- and β-D-glucopyranosyl phosphate had been determined[17] under identical conditions, and were reported to be 1.4 and 6.08 (α) and 1.3 and 5.88 (β), respectively. Thus, substitution of fluorine

TABLE II

^{1}H-N.M.R. COUPLING CONSTANTS FOR MONODEOXYFLUORO-D-GLUCOPYRANOSYL PHOSPHATES

Position of fluorination	*Anomeric configuration*	*Coupling constants (Hz)*											
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	$^2J_{F,H}$	$^3J_{F,H}$	$J_{F,H}$	$^3J_{P,H\text{-}1}$	$^4J_{P,H\text{-}2}$
6	α	3.4	9.7	10.0	9.4	2.7	—[a]	10.7	47.2	31.0	—	7.2	1.0
4	α	3.5	9.0	9.0	9.0	—	4.0	12.4	50.8	15.4	3.5[b]	7.3	—
3	α	3.7	8.0	8.8	8.9	—	—	12.0	54.6	13.8	3.7[c]	7.4	—
2	α	3.6	9.0	9.6	9.6	—	4.8	12.0	47.8	12.4	—	8.0	1.0
2	β	8.0	8.1	9.5	9.5	—	5.1	12.0	50.5	15.2	—	8.0	—

[a]Indeterminate, non-zero coupling. [b]$^5J_{F\text{-}4,H\text{-}1}$. [c]$^4J_{F\text{-}3,H\text{-}1}$.

TABLE III

OBSERVED FIRST-ORDER RATE-CONSTANTS FOR ACID-CATALYZED HYDROLYSIS[a] OF MONODEOXYFLUORO-D-GLUCOPYRANOSYL PHOSPHATES

D-*Glucopyranosyl phosphate*	*Hydrolysis temperature (°C)*	*Rate constant* $[k_{obs} \times 10^5 (s^{-1})]$
α-	25	4.10
6-deoxy-6-fluoro-α-	25	1.12
4-deoxy-4-fluoro-α-	25	0.270
3-deoxy-3-fluoro-α-	25	0.480
2-deoxy-2-fluoro-β-	25	0.175
2-deoxy-2-fluoro-α-	25	0.068
	45	1.17
	65	16.3
	82	120

[a]Hydrolysis was performed in M $HClO_4$. Aliquots were removed at time intervals and assayed for release of phosphate.

at C-2 depresses pKa_2 by 0.18 and pKa_1 by 0.4 for the α anomer, and depresses pKa_2 by 0.28 for the β anomer.

First-order rate-constants for the acid-catalyzed hydrolyses (M $HClO_4$) of the series of deoxyfluoroglucopyranosyl phosphates at 25° are presented in Table III, in addition to values for 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate at several different temperatures. Because the sugar phosphates under investigation had been crystallized as their bis(cyclohexylammonium) salts, control experiments were performed with the parent compound α-D-glucopyranosyl (dipotassium phosphate) in order to test for any effect of this amine on hydrolysis rates. Rate constants measured for the acid catalyzed hydrolyses (M $HClO_4$, at 25°) of both the dipotassium (4.16×10^{-5} s^{-1}) and bis(cyclohexylammonium) (4.06×10^{-5} s^{-1}) salts were essentially identical to that recorded[22] (4.0×10^{-5} s^{-1}) for the dipotassium salt.

The pH dependence of the hydrolysis rate-constant was determined at 82° for that deoxyfluoro sugar most severely affected by fluorine substitution, namely 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate, and it is presented in Fig. 1, together with the data (solid lines) for α-D-glucopyranosyl phosphate and methyl phosphate, determined previously[15,22]. Data points presented are those determined in this study for hydrolysis in potassium hydrogenphthalate buffer at the pH values

TABLE IV

OBSERVED RATE CONSTANTS FOR HYDROLYSIS OF 2-DEOXY-2-FLUORO-α-D-GLUCOPYRANOSYL PHOSPHATE AT 82° AS A FUNCTION OF pH

pH	1.00	2.01	3.01	4.01	5.01	6.20
Rate constant $\times 10^5$ (s^{-1})	6.250	0.726	0.213	0.199	0.169	0.068

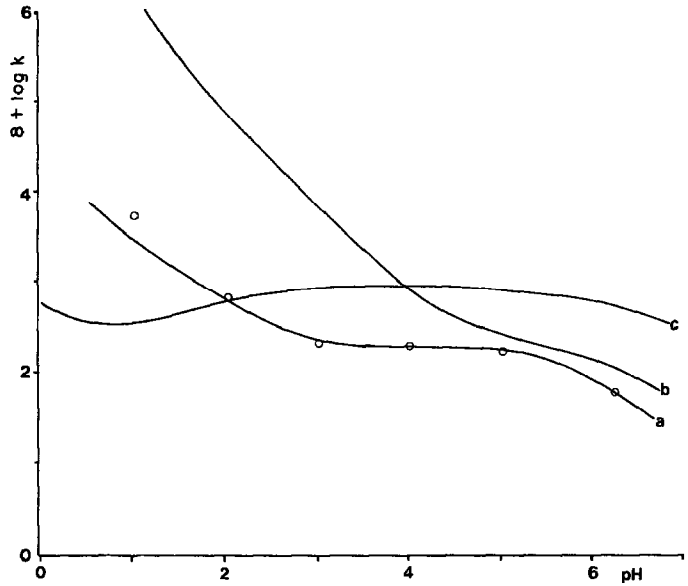


Fig. 1. A plot of observed first-order rate-constant for hydrolysis *versus* pH for (a) 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate, (b) α-D-glucopyranosyl phosphate[16], and (c) methyl phosphate[15]. [Data points are those determined for hydrolysis of 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate in 50mM potassium hydrogenphthalate buffer at the pH values indicated, at 82°. The solid line through the points is that calculated for its hydrolysis by using pKa values of 1.16 and 6.08 and assuming rate constants of 6.23×10^{-5} and 1.88×10^{-6} s^{-1} for the neutral species and monoanion, respectively. Deviation of the line at low pH is due to hydrolysis of the conjugate acid species, which was not included in the calculation.]

indicated. The solid line through these points is that calculated for hydrolysis of 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate by using the approach of Bunton *et al.*[22], wherein it is assumed that the observed rate (k_{obs}) in this range will be the sum of the rates for each contributing species

$$k_{obs} = k_n(C_n/C_p) + k_m(C_m/C_p) + k_d(C_d/C_p),$$

where k_n, k_m, and k_d are the specific rate-constants of the neutral, monoanion, and dianion species, respectively, and the ratios C_n/C_p, C_m/C_p, and C_d/C_p are the mole fractions of each species. The line shown is calculated on the basis of pKa_1 and pKa_2 values of 1.00 and 5.90 at 25°, appropriately corrected to 1.16 and 6.08 at 82° by using the equation of Harned and Embree[23], which describes changes in the dissociation constants of weak acids with temperature, and assuming for the 2-deoxy-2-fluoro sugar a temperature dependence of the pKa similar to that observed[15] for the parent α-D-glucopyranosyl phosphate. Values for the hydrolysis rate-constants for the neutral and monoanion species were obtained by application of the iterative method described previously[22]; this yielded values of 6.23×10^{-5} and 1.88×10^{-6} s^{-1}, for the neutral species and the monoanion, respectively. These

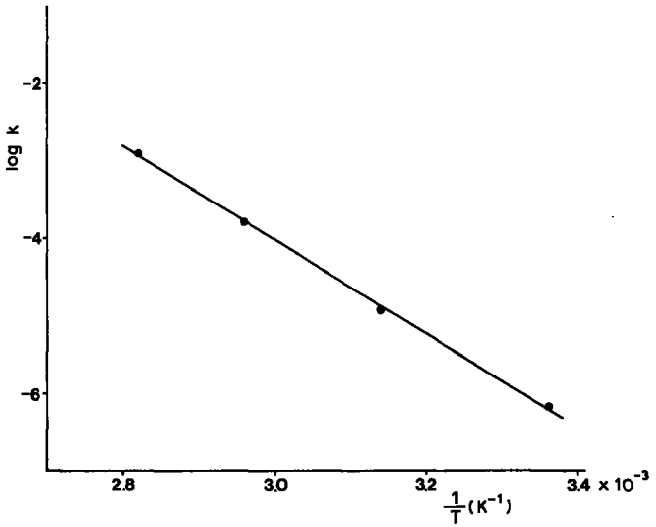


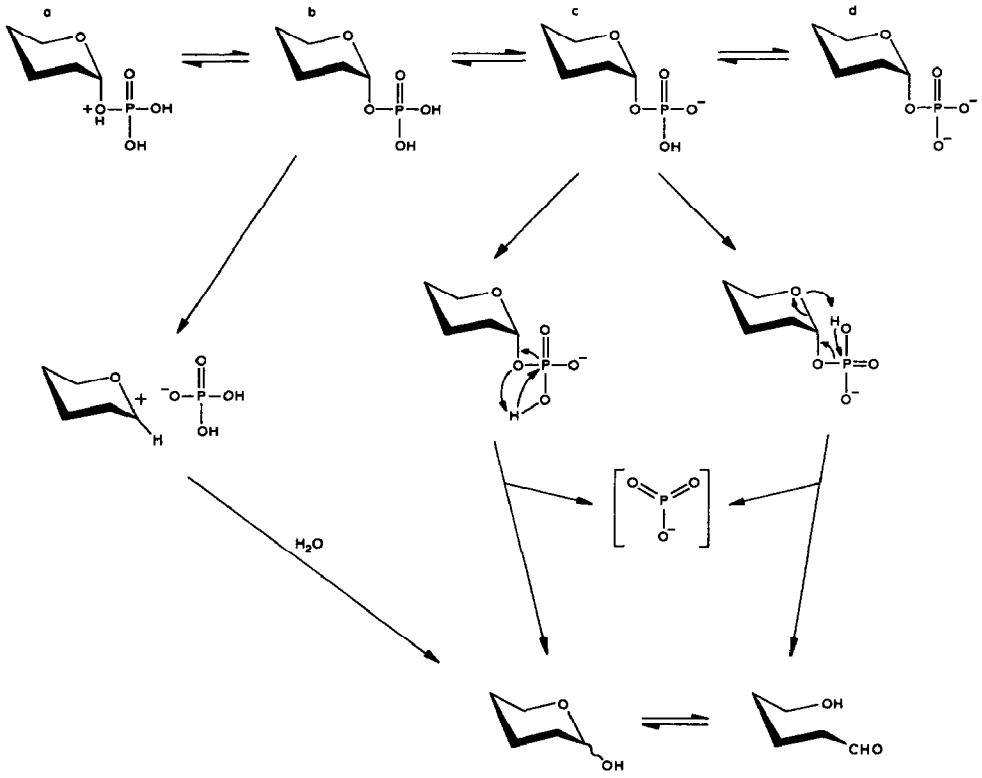
Fig. 2. Arrhenius plot of observed first-order rate-constants for the hydrolysis of 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate in M $HClO_4$. [Observed rate-constants represent a combination of rate constants for the hydrolysis of the neutral and conjugate acid species.]

may be compared to values of 3.97×10^{-3} and 1.45×10^{-6} s^{-1}, respectively, determined[15] for α-D-glucopyranosyl phosphate under similar conditions. The departure of the experimental points from the calculated line at low pH values is due to the increasing contributions from hydrolysis of the conjugate acid form **a**, which has not been included in the calculations.

Fig. 2 shows the Arrhenius plot relating to the temperature dependence of hydrolysis of 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate in M $HClO_4$. It is a linear plot yielding $\Delta H^{\ddagger} = 113.5$ kJ.mol^{-1} and $\Delta S^{\ddagger}_{25} = 4$ e.u.

DISCUSSION

The consensus mechanism of hydrolysis[15–17] of α-D-glucopyranosyl phosphate at a series of different pH values is presented in Scheme 1. As may be seen, hydrolysis may, depending upon the pH, be occurring from any of the ionic forms present, although hydrolysis of the dianion **d** is very slow, and will not therefore be discussed. Reaction through the monoanion **c** (at pH values >5) occurs, with P–O bond-cleavage, probably *via* the metaphosphate mechanism or some preassociative mechanism that has a transition state having considerable metaphosphate character. This mechanism does not involve an oxocarbonium ion intermediate, and as there is no significant build-up of charge on the anomeric carbon atom in the transition state, it is relatively insensitive to the nature of the sugar group. Indeed, alkyl, aryl, and pyranosyl phosphates are hydrolyzed at very similar rates under these conditions[22]. At pH values lying between 2 and 5, hydrolysis occurs primarily through



Scheme 1. The mechanisms of glycopyranosyl phosphate hydrolysis at different pH values. [Species **a** is the conjugate acid; species **b**, the neutral species; **c**, the monoanion; and **d** the dianion.]

the neutral species **b**, and involves C–O bond-cleavage. The linear dependence on hydrogen-ion concentration of the rate observed[15] eliminates the possibility of the conjugate acid species **a** being involved because a steeper dependence on concentration of hydrogen ion could then be expected. Hydrolysis of the neutral species therefore proceeds *via* an oxocarbonium-ion intermediate, or at least *via* a transition state having substantial, oxocarbonium-ion character. Such a species is required to adopt a conformation in which C-5, O-5, C-1, and C-2 are coplanar, in order to allow for overlap between the empty p orbital on C-1 and the p-type, lone pair on C-5. Likely conformations are a half chair or a boat conformation. A true oxocarbonium-ion intermediate may not be involved, as it has been suggested recently[14,24] that solvolysis reactions at the anomeric carbon atom of sugars (with C–O bond-cleavage) all proceed through a preassociation mechanism that may be concerted in some cases. Thus, there may well be significant participation by, and delocalization of positive charge onto, the nucleophile in such reactions.

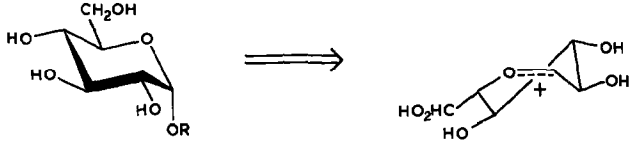
Hydrolysis of the conjugate acid is the principal route below pH 1, and it is more complex, apparently involving C–O bond-cleavage primarily, and, therefore, a carbonium-ion-like mechanism, but with increasing amounts of P–O bond-cleavage as the acidity is increased[15].

Interpretation of the data on hydrolysis of the deoxyfluoro α-D-glucopyranosyl phosphates is presented on the assumption of a similar, general mechanism. This is not unreasonable, as essentially identical mechanisms are obtained for hydrolysis of both α-D-glucopyranosyl phosphate and methyl phosphate at all pH values, despite a very large difference (by a factor of 10^5) in the magnitudes of acid hydrolysis rate-constants. It is therefore unlikely that the overall mechanism for the two sugars will be different when only a 58-fold rate difference is found in this case; thus, it is assumed that hydrolysis of the monoanion occurs by P–O bond-fission and of the neutral and conjugate-acid species by C–O bond-fission. This point has not, however, been proved.

The data in Table III refer to hydrolysis in M perchloric acid; therefore, reactions will be proceeding *via* an oxocarbonium-ion-like transition-state and, as such, should be sensitive to substitution on the glucopyranose ring. It is clear that fluorine substitution decreases the hydrolysis rate-constant in all cases, but most severely if it is on C-2, and also that the rate constant does not simply reflect the distance of the fluorine atom from the anomeric center, as the observed order is 6-fluoro > 3-fluoro > 4-fluoro > 2-fluoro. Interestingly, the inverse order was observed in studies of the acid hydrolysis rate-constants of a series of alkyl[25] and aryl[26] deoxy-D-glucosides; that is, 2-deoxy > 4-deoxy > 3-deoxy > 6-deoxy. These two results must be closely related, and they suggest the presence of at last two competing factors giving rise to this particular order of reactivities. Indeed, for the deoxyglucosides, it had been suggested that it results from a combination of steric and electronic factors[12,26].

Steric considerations. — It has been demonstrated[12,17,27] that steric factors that favor the transformation of a chair conformation of the ground-state glycoside into the partially planar conformation of the transition-state oxocarbonium ion, as shown in Scheme 2, will cause an increase in reaction rate. This explain, in part, the increased acid-catalysed hydrolysis-rates for methyl deoxy-α-D-glucopyranosides[12,25] and phenyl deoxy-β-D-glucopyranosides[26] as the increase in non-bonded interactions in progressing from the ground state to the transition state for deoxyglucosides will be less than that for the parent sugar. However, because the fluorine substituent is similar in size to, and in fact slightly smaller than, a hydroxyl group, the rate decrease observed in each case cannot be due to steric factors.

Electronic considerations. — Two different electronic factors must be considered, inductive and dipolar. Inductive destabilization of positive charge at the anomeric center in the transition state by nearby electronegative substituents had



Scheme 2. The chair to half-chair transition in hydrolysis of an α-D-glucoside *via* an oxocarbonium ion intermediate.

been proposed[12,13,25,28,29] as the cause of significant rate-decreases. Quite large effects are observed, as the substituents will affect, in a similar manner, the equilibrium constant for protonation (in the conjugate acid pathway) and the rate constant for the decomposition of the conjugate acid. Thus, "2-deoxyglucosides" are hydrolyzed some 2000 times faster than glucosides[25,26], whereas 2-amino-2-deoxyglucosides react at some 140th the rate[12]. Thus, inductive effects are important, but, because substituents on C-2 will affect both the equilibrium constant for formation of the conjugate acid and the rate constant describing its decomposition, it is not a simple matter to deconvolute these two factors and ascribe numerical importance to each. Although inductive effects would seem to account for most of the rate decrease occasioned by fluorine substitution on C-2, such inductive effects would be expected to be smaller for the other deoxyfluoroglucopyranosyl phosphates, and they might not account for the full rate diminution observed. Furthermore, inductive effects cannot account for the presence of a larger hydrolysis rate-constant for the 3-deoxy-3-fluoro than for the 4-deoxy-4-fluoro derivative.

An additional explanation, electronic in nature, relates to the relative orientations of dipoles associated with C–OH and C–F bonds in the ground state and in the half-chair transition-state. The importance of such dipolar interactions has been amply illustrated by the anomeric effect. Inspection of models and of "Newman" projections of α-D-glucopyranosyl phosphate, and assuming that a greater dipole is associated with C–F than with C–OH, leads to the following conclusions (see Fig. 3). 2-Deoxy-2-fluoro-α-D-glucopyranosyl phosphate should be hydrolyzed considerably more slowly, because there is a large increase in dipole alignment in going from the ground state to the transition state; 3-deoxy-3-fluoro-α-D-glucopyranosyl phosphate should be hydrolyzed very slightly faster *on this criterion*, due to a decrease in overall dipolar alignment; 4-deoxy-4-fluoro-α-D-glucopyranosyl phosphate should undergo no net change in fluorine dipole orientations, and therefore this effect should not affect the rate; and effects associated with C-6 could not be predicted easily. Thus, the predicted hydrolysis rate order would be $3 > 4 > 2$, which is that observed.

The converse would, of course, be true for deoxyglucosides, as there is no significant dipole associated with the C–H bond. Therefore, superimposition of such dipolar effects onto the inductive effects discussed previously could well account for the observed extent and order of reactivities. Similar inhibition of reactions proceeding *via* carbocationic intermediates as a result of adjacent fluorine substitution had been observed[30,31].

Detailed interpretation of the activation entropy and enthalpy ($\Delta H^{\ddagger} = 113.5$ kJ.mol^{-1}; $\Delta S^{\ddagger}_{25} = 4$ e.u.) is unwarranted, as they represent a mixture of kinetic terms relating to hydrolysis of the neutral species **b** and its conjugate acid **a**. However, it is interesting to compare these values with those determined[16] for acid-catalyzed hydrolysis (1.47M $HClO_4$) of α-D-glucopyranosyl phosphate ($\Delta H^{\ddagger} = 116.6$ kJ.mol^{-1}, $\Delta S^{\ddagger}_{25} = 14.9$ e.u.) and note that the major difference between these two sets of data is in the activation entropy. A similar lowering of this value upon

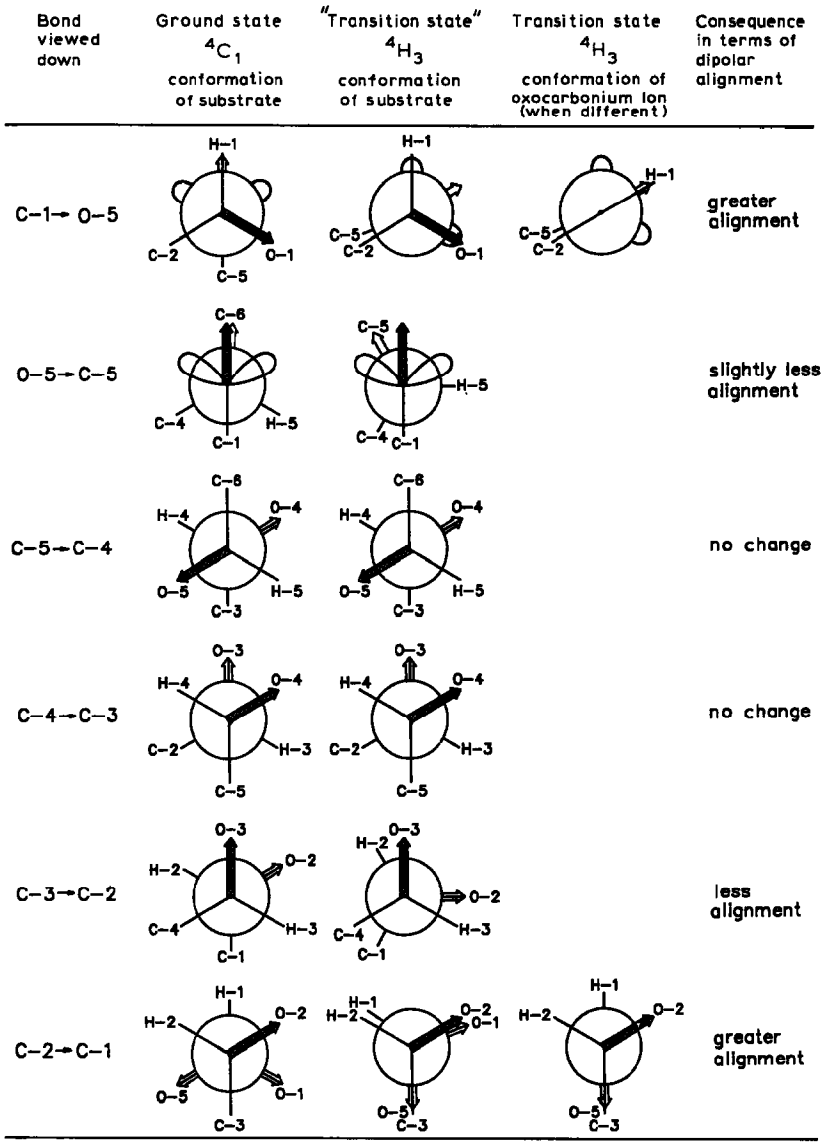


Fig. 3. "Newman" projections viewed down each of the sugar-ring bonds for the $^4C_1$(D) ground state and the putative, half-chair transition-state for D-glucoside hydrolysis *via* an oxocarbonium ion-like transition state. [Bold arrow represent dipoles at each carbon atom.]

halogen substitution at C-2 was noted[29] in comparing activation entropies for acid-catalyzed (2M HCl) hydrolysis of methyl β-D-glucopyranoside ($\Delta S^{\ddagger}_{60}$ = 16.5 e.u.) and its 2-chloro-2-deoxy derivative ($\Delta S^{\ddagger}_{60}$ = 7.6 e.u.). Such effects have been ascribed[29] to partial bonding of a water molecule to the glycosidic carbon atom in the transition state upon inductive destabilization of developing positive charge.

This is a reasonable extension of current suggestions[14,24] that all solvolytic reactions at the anomeric carbon atom of sugars proceed through a preassociation mechanism, and it is entirely consistent with more recent observations[32] of inductive enhancement of preassociative participation in other displacement reactions.

The ratio of acid-catalyzed hydrolysis rates for the two anomers of 2-deoxy-2-fluoro-D-glucopyranosyl phosphate ($\beta:\alpha$ = 2.6:1) is very similar to that measured previously for α- and β-D-glucopyranosyl phosphate and a variety of other hexopyranosyl phosphates. Thus, substitution of fluorine at C-2 does not affect this value, suggesting relatively similar ground-state energies for the two phosphates.

The pH dependence of the hydrolysis rate, given in Fig. 1, provides a striking demonstration of the inhibitory effect of fluorine substitution upon mechanisms involving an oxocarbonium ion-like intermediate, and highlights the fact that this has little or no effect on the metaphosphate mechanism. Observed rate-constants are essentially identical at pH >5, but considerably (58 fold) lower at very acid pH values. The line drawn through the data points is that calculated in the manner described previously[21], assuming pKa values of 1.00 and 5.90 at 25°, appropriately corrected for 82°, and rate constants for the neutral species and monoanion of 6.23×10^{-5} and 1.88×10^{-6} s^{-1}, respectively. These may be compared to values of 3.97×10^{-3} and 1.45×10^{-6} s^{-1} for α-D-glucopyranosyl phosphate[22] under essentially identical conditions.

Thus, fluorine substitution at C-2 results in greatly decreased reaction-rates through oxocarbonium ion-like intermediates (63-fold slower for the neutral species in this case), but has very little effect on the metaphosphate mechanism. This is completely consistent with the observed abilities of a variety of enzymes to utilize 2-deoxy-2-fluoroglycosides as substrates, as follows. Two enzymes whose mechanism presumably proceeds *via* an oxocarbonium-ion intermediate, namely, glycogen phosphorylase and yeast α-glucosidase, were either completely unable to catalyze conversion of 2-deoxy-2-fluoro-substituted substrates, or did so only slowly[9a]. One enzyme that is believed to operate *via* a mechanism involving P–O bond-cleavage, with no oxocarbonium-ion intermediate, namely, phosphoglucomutase, utilizes 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate very efficiently[9a]. The current data therefore essentially confirm the currently proposed mechanisms for these enzymes, and provide a rigorous explanation for the observed retention of 2-deoxy-2-fluoro-D-glucose in brain cells as its phosphoric esters.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Thin-layer chromatography was performed on precoated plates of silica gel (60-F_{254}, E. Merck, Darmstadt), with detection by quenching of fluorescence, or by charring after spraying with 10% H_2SO_4 in ethanol. Column chromatography was performed on Merck silica gel 60 (180–230 mesh). Solutions were evaporated *in vacuo* at <50°.

Nuclear magnetic resonance spectra were recorded at either 400 MHz (^1H

data) or 254 MHz (^{19}F data), unless noted otherwise. ^1H-Chemical shifts are given relative to external sodium 4,4-dimethyl-4-silapentane-1-sulfonate for samples in D_2O, and relative to Me_4Si for all other solvents. ^{19}F-Chemical shifts were measured against external trifluoroacetic acid and are given in p.p.m. upfield from $CFCl_3$. Trifluoroacetic acid resonates 76.53 p.p.m. upfield from $CFCl_3$. Reaction times required in the phosphorylations for optimal production of the α anomer in each case are presented. Shorter reaction-times often led to significant production of the β anomer.

1,2,3,4-Tetra-O-acetyl-6-deoxy-6-fluoro-β-D-glucopyranose (**1**). — 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranose[33] (1.0 g, 3 mmol) and 2,4,6-trimethylpyridine (0.7 mL, 6 mmol) were dissolved in anhydrous dichloromethane (20 mL) cooled to −20°, and DAST (0.8 mL, 6 mmol) was slowly added during 15 min to the stirred solution under a nitrogen atmosphere. The mixture was slowly warmed to room temperature, and, after 24 h, cooled to 0°; and methanol (5.0 mL) was added to decompose the excess of reagents. The solution was evaporated *in vacuo*, and the resulting yellow oil purified by column chromatography on silica gel, using 9:1 dichromethane–ethyl acetate as the eluant. The pure product (0.71 g, 68%) crystallized from methanol; m.p. 125–126° (lit.[34] m.p. 125–126°); ^1H-n.m.r. data (400 MHz, $CDCl_3$): δ 5.73 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.1–5.3 (m, 3 H, H-2,3,4), 4.49 (ddt, 1 H, $J_{6,F}$ 47.2, $J_{6,6'}$ 10.6, $J_{5,6}$ 2.4 Hz, H-6), 4.45 (ddt, 1 H, $J_{6',F}$ 47.0, $J_{6,6'}$ 10.6, $J_{5,6'}$ 4.1 Hz, H-6'), 4.10 (m, 1 H, H-5), 2.11, 2.06, 2.04, and 2.01 (4 s, 12 H, OAc); ^{19}F-n.m.r. (254 MHz, $CDCl_3$): 233.93 (dt, $J_{F,6}$ 47, $J_{F,5}$ 25 Hz) p.p.m.

6-Deoxy-6-fluoro-α-D-glucopyranosyl [bis(cyclohexylammonium) phosphate] (**2**). — Anhydrous, crystalline phosphoric acid (1.0 g, 10 mmol) was heated at 55° under vacuum until molten, compound **1** (0.5 g, 1.4 mmol) was added, and the melt was heated under vacuum for 2.5 h at 55°. Aqueous 2M lithium hydroxide (20 mL) was then added, and the mixture kept overnight at room temperature. Lithium phosphate was removed by filtration through Celite and the cooled (4°) filtrate was passed through a precooled (4°) column of Dowex 50W-X8 (H^+) ion-exchange resin into an excess of cyclohexylamine in deionized water. The solution was evaporated *in vacuo* at 30°, and the residue crystallized by addition of acetone. The crystals were recrystallized from water–acetone, to give **2** (0.45 g, 70%); m.p. 179–185°, $[\alpha]_D^{25}$ +48.2° (*c* 0.1, water).

Anal. Calc. for $C_{18}H_{38}FN_2O_8P$: C, 46.95; H, 8.32; N, 6.08. Found: C, 46.96; H, 8.29. N, 6.06.

Methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-fluoro-α-D-glucopyranoside (**3**). — Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside[35] (15 g, 30 mmol) and 4-(dimethylamino)pyridine (7.35 g, 60 mmol) were dissolved in anhydrous dichloromethane, and treated with DAST (9.6 mL, 45 mmol) as described for **1**. The reaction was quenched after 24 h, the solution concentrated, and the concentrate eluted from a column of silica gel with 19:1 dichloromethane–ethyl acetate. Recrystallization from ethanol yielded **3** (8.58 g, 56%); m.p. 138–139° (lit.[36] m.p. 139–141°); ^1H-n.m.r. data (400 MHz, $CDCl_3$): δ 8.2–7.9 (m, 6 H, Ph), 7.7–7.3 (m,

9 H, Ph), 6.11 (dt, 1 H, $J_{3,F}$ 15, $J_{3,2}$ 9, $J_{3,4}$ 9 Hz, H-3), 5.22 (dd, 1 H, $J_{2,3}$ 9, $J_{2,1}$ 4 Hz, H-2), 5.18 (t, 1 H, $J_{1,2}$ 4, $J_{1,F}$ 4 Hz, H-1), 4.78 (dt, 1 H, $J_{4,F}$ 52, $J_{4,3}$ 9, $J_{4,5}$ 9 Hz, H-4), 4.33 (m, 2 H, H-6,6'), 4.33 (m, 1 H, H-5), and 3.48 (s, 3 H, OCH_3); ^{19}F-n.m.r. (254 MHz, $CDCl_3$): 197.8 (dd, $J_{F,4}$ 52, $J_{F,3}$ 15 Hz) p.p.m.

*Methyl 4-deoxy-4-fluoro-α-*D*-glucopyranoside* (**4**). — To a suspension of **3** (5.6 g) in absolute methanol (24 mL) was added M NaOMe in methanol (2.5 mL), and the mixture was stirred for 18 h at room temperature. The base was neutralized with Dowex 50W-X8 (H^+) resin, the suspension filtered, the filtrate concentrated *in vacuo* and the concentrate eluted from a column of silica gel by using 9:1 ethyl acetate–methanol, yielding **4**, which was recrystallized from 1:1 ethyl acetate–acetone; 1.71 g, 78%; m.p. 129–130° (lit.[36] m.p. 129–130°); ^{1}H-n.m.r. data (80 MHz, D_2O): δ 4.76 (t, 1 H, $J_{1,2}$ 3.3, $J_{1,F}$ 3.3 Hz, H-1), 4.1–3.2 (m, 5 H, H-2,3,4,6,6'), and 3.48 (s, 3 H, OCH_3); ^{19}F-n.m.r. (254 MHz, D_2O): 199.2 (dd, $J_{F,3}$ 16, $J_{F,4}$ 51 Hz) p.p.m.

*4-Deoxy-4-fluoro-*D*-glucose* (**5**). — A solution of **4** (0.45 g) in water (40 mL) containing Dowex 50W-X8 (H^+) ion-exchange resin (20 mL) was heated at reflux, with stirring, for 25 h. After cooling, filtering, and evaporating, the resultant gum crystallized, to yield **5** (0.36 g, 85%); m.p. 184–186° (lit.[36] m.p. 189–190°); ^{19}F-n.m.r. data (254 MHz, D_2O): 199.2 (dd, $J_{F,3}$ 15, $J_{F,4}$ 51 Hz, α anomer) and 201.2 (dd, $J_{F,3}$ 16, $J_{F,4}$ 51 Hz, β anomer) p.p.m.

*1,2,3,6-Tetra-*O*-acetyl-4-deoxy-4-fluoro-β-*D*-glucopyranose* (**6**). — A solution of **5** (1.8 g) in dry pyridine (16 mL) was cooled to 0°, acetic anhydride (9.5 mL) was slowly added, and the mixture kept for 48 h at room temperature. It was then cooled to 0°, methanol (5 mL) was added, and, after 1 h, the solution was evaporated *in vacuo* and the residue dried *in vacuo* for 18 h. To the resultant gum was added 45% HBr–HOAc (16 mL) and acetic anhydride (1 mL), and the solution was stirred for 1 h at room temperature, dissolved in dichloromethane, washed with 5 portions of water at 0°, dried (Na_2SO_4), and evaporated *in vacuo*. The resulting gum was dissolved in glacial acetic acid (60 mL), mercuric acetate (5 g) was added, and the mixture stirred for 1 h at room temperature in the dark. Dichloromethane was added, and the solution washed with 5 portions of water at 0°, dried (Na_2SO_4), and evaporated *in vacuo*; the residue crystallized from methanol, yielding **6** (1.9 g, 69%); m.p. 126–128° (lit.[37] m.p. 127–129°); ^{1}H-n.m.r. data (270 MHz, $CDCl_3$): δ 5.78 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.44 (dt, 1 H, $J_{3,F}$ 16, $J_{3,4}$ 9, $J_{3,2}$ 9 Hz, H-3), 5.13 (t, 1 H, $J_{2,3}$ 8, $J_{2,1}$ 8 Hz, H-2), 4.57 (dt, 1 H, $J_{4,F}$ 52, $J_{4,3}$ 9, $J_{4,5}$ 9 Hz, H-4), 4.45–4.30 (m, 2 H, H-6,6'), 3.93 (m, 1 H, H-5), 2.06 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), and 2.14 (s, 6 H, OAc); ^{19}F-n.m.r. (254 MHz, $CDCl_3$): 200.9 (dd, $J_{F,4}$ 51, $J_{F,3}$ 15 Hz) p.p.m.

*4-Deoxy-4-fluoro-α-*D*-glucopyranosyl [bis(cyclohexylammonium) phosphate]* (**7**). — Reaction of **6** (1 g) with anhydrous phosphoric acid (2 g) was performed exactly as for **2**, yielding **7** (0.62 g, 46%); m.p. 148–151°, $[\alpha]_D^{25}$ +51.3° (*c* 0.19, water).

Anal. Calc. for $C_{18}H_{38}FN_2O_8P$: C, 46.95; H, 8.32; N, 6.08. Found: C, 46.69; H, 8.30; N, 6.15.

3-Deoxy-3-fluoro-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**8**). — 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose[38] (0.5 g) and 4-(dimethylamino)-pyridine (0.5 g) were dissolved in dry dichloromethane (20 mL), and treated with DAST (0.5 mL) as described for **1**. The reaction was quenched after 20 h, the solution concentrated, and the concentrate eluted from a column of silica gel with 24:1 dichloromethane–ethyl acetate, to yield **8** (ref. 39; 0.32 g, 64%); ^{1}H-n.m.r. data (400 MHz, $CDCl_3$): δ 5.96 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.01 (dd, 1 H, $J_{3,F}$ 50, $J_{3,4}$ 2 Hz, H-3), 4.71 (dd, 1 H, $J_{2,F}$ 11, $J_{2,1}$ 4 Hz, H-2), 4.29 (m, 1 H, H-5), 4.2–4.0 (m, 3 H, H-4,6,6'), 1.41, 1.36, 1.27, and 1.23 (4 s, 12 H, CH_3); ^{19}F-n.m.r. (254 MHz, $CDCl_3$): 200.5 (ddd, $J_{F,3}$ 50, $J_{F,4}$ 29, $J_{F,2}$ 11 Hz) p.p.m.

3-Deoxy-3-fluoro-D-glucose[39] (**9**). — A solution of **8** (1.2 g) in water (60 mL) containing ethanol (12 mL) and Dowex 50W-X8 (H^+) resin (20 mL) was stirred for 4.5 d at room temperature. After filtration and concentration, the product was dissolved in 7:2:1 ethyl acetate–ethanol–water, and eluted from a short column of silica with the same solvent. Concentration, and drying of the residue for two days *in vacuo*, yielded **9** (0.8 g, 93%) as a colorless gum; ^{19}F-n.m.r. data (254 MHz, D_2O): 197.8 (ddt, $J_{F,3}$ 54, $J_{F,4}$ 13, $J_{F,2}$ 5 Hz, α anomer) and 202.7 (dt, $J_{F,3}$ 53, $J_{F,4}$ 14 Hz) p.p.m.

Anal. Calc. for $C_6H_{11}FO_5$: C, 39.56; H, 6.09. Found: C, 39.90; H, 6.23.

1,2,4,6-tetra-O-acetyl-3-deoxy-3-fluoro-β-D-glucopyranose (**10**). — A solution of **9** (0.59 g) in dry pyridine (5 mL) was treated exactly as in the synthesis of **6**, appropriately scaled, and the product crystallized from dichloromethane–pentane, to give **10** (0.51 g, 45%); m.p. 116–118° (lit.[39] m.p. 116–120°); ^{1}H-n.m.r. data (400 MHz, $CDCl_3$): δ 5.65 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.25 (m, 2 H, H-2,4), 4.60 (dt, 1 H, $J_{3,F}$ 52, $J_{3,4}$ 9, $J_{3,2}$ 9 Hz, H-3), 4.26–4.12 (m, 2 H, H-6,6'), 3.75 (m, 1 H, H-5), 2.11, 2.10, 2.09, and 2.08 (4 s, 12 H, 3 OAc); ^{19}F-n.m.r. (254 MHz, $CDCl_3$): 196.7 (dt, $J_{F,3}$ 52, $J_{F,4}$ 13, $J_{F,2}$ 13 Hz) p.p.m.

3-Deoxy-3-fluoro-α-D-glucopyranosyl [bis(cyclohexylammonium phosphate] (**11**). — Reaction of **10** (0.3 g) with anhydrous phosphoric acid (0.6 g) was performed exactly as for **2**, yielding **11** (0.16 g, 37%); m.p. 140–143°, $[\alpha]_D$ +52.3° (*c* 0.2, water).

Anal. Calc. for $C_{18}H_{38}FN_2O_8P \cdot 2\ H_2O$: C, 43.54; H, 8.53; N, 5.64. Found: C, 43.05; H, 8.12; N, 5.77.

3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (**12**). — Trifluoromethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranoside[40] (3 g) was dissolved in 45% HBr–HOAc (30 mL) containing acetic anhydride (3 mL), and the solution kept for 6 d at room temperature; dichloromethane (50 mL) was added, and the mixture was washed with water (0°), dried ($MgSO_4$), concentrated *in vacuo*, and the product crystallized from diethyl ether–pentane. Recrystallization from this solvent gave **12** (2.4 g, 81%); m.p. 83° (lit.[40] m.p. 79–80°); ^{1}H-n.m.r. data (270 MHz, $CDCl_3$): δ 6.30 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.64 (dt, 1 H, $J_{3,F}$ 12, $J_{3,2}$ 10, $J_{3,4}$ 10 Hz, H-3), 5.14 (t, 1 H, $J_{4,3}$ 10, $J_{4,5}$ 10 Hz, H-4), 4.56 (ddd, 1 H, $J_{2,F}$ 50, $J_{2,3}$ 10, $J_{2,1}$ 4 Hz, H-2), 4.38–4.10 (m, 3 H, H-5,6,6'), 2.05, 2.07, and 2.09 (3 s, 9 H, 3 OAc).

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranose (**13**). — To a solution of **12** (3 g) in glacial acetic acid (60 mL) was added mercuric acetate (5.1 g), and the mixture was stirred for 20 h at room temperature. After evaporation *in vacuo*, the white solid was suspended in diethyl ether, the suspension filtered, and crystallization induced by addition of pentane. Recrystallization yielded **13** (2.1 g, 73%); m.p. 91–92° (lit.[20] m.p. 91–92°); ^{1}H-n.m.r. data (400 MHz, $CDCl_3$): δ 5.79 (dd, 1 H, $J_{1,2}$ 8, $J_{1,F}$ 3 Hz, H-1), 5.38 (dt, 1 H, $J_{3,F}$ 14, $J_{3,4}$ 9, $J_{3,2}$ 9 Hz, H-3), 5.07 (t, 1 H, $J_{4,3}$ 9, $J_{4,5}$ 9 Hz, H-4), 4.45 (dt, 1 H, $J_{2,F}$ 51, $J_{2,3}$ 8.5, $J_{2,1}$ 8.5 Hz, H-2), 4.30–4.10 (m, 2 H, H-6,6′), 3.86 (m, 1 H, H-5), 2.19, 2.10, 2.09, and 2.05 (4 s, 12 H, 4 OAc); ^{19}F-n.m.r. (270 MHz, $CDCl_3$): 201.7 (ddd, $J_{F,2}$ 51, $J_{F,3}$ 14, $J_{F,1}$ 3 Hz) p.p.m.

2-Deoxy-2-fluoro-α-D-glucopyranosyl [bis(cyclohexylammonium) phosphate] (**14**). — Reaction of **13** (2 g) with anhydrous phosphoric acid (4 g) was performed as for **2**, except that 48 h at 55° was required for complete reaction. Crystallization from water–acetone yielded **14** (0.93 g, 35%); m.p. 139–144°, $[\alpha]_D^{25}$ +48.6° (*c* 0.52, water).

Anal. Calc. for $C_{18}H_{38}FN_2O_8P$: C, 46.95; H, 8.32; N, 6.08. Found: C, 46.91; H, 8.50; N, 5.96.

2-Deoxy-2-fluoro-β-D-glucopyranosyl [bis(cyclohexylammonium) phosphate] (**15**). — Compound **12** (0.2 g) was dissolved in dry acetonitrile (4 mL), and silver tosylate (0.165 g) was added; the mixture was protected from light, and kept for 30 min at room temperature. The solvent was removed *in vacuo*, and the mixture suspended in 4 mL of anhydrous tetrahydrofuran. After filtration through Celite, anhydrous phosphoric acid (0.264 g) was added to the filtrate, and the mixture was protected from moisture and boiled under reflux for 90 min. The reaction was quenched by cooling (0°) followed by addition of 2M lithium hydroxide (3 mL), and the mixture was kept for 18 h at room temperature. Lithium phosphate was removed by filtration through Celite, and the cooled filtrate was passed down a precooled column of Dowex 50W-X8 (H^+) resin into an excess of cyclohexylamine in de-ionized water. After lyophilization, ^{1}H-n.m.r. spectroscopy of the crude product (0.156 g, 63%) indicated that both anomers of the 1-phosphate were present, and in the ratio of 1:1.

The β anomer was purified by dissolving the product mixture in the minimal volume of water, followed by adding acetone, and cooling the mixture in an ice bath for 1 h. The crystalline product was removed by filtration, and dried *in vacuo*. Recrystallization in the same manner gave pure **15** (0.026 g, 25%); m.p. 146–149°, $[\alpha]_D$ +2.3° (*c* 0.26, water). Reliable microanalyses for this compound could not be obtained, but the n.m.r.-spectral data were completely consistent with the structure assigned (see Tables I and II), and fast-atom-bombardment mass spectrometry showed a parent ion (M + 1 = 461), as would be required.

Hydrolysis studies. — Hydrolysis conditions employed were essentially identical to those described previously[15,17]. Acid-catalyzed hydrolysis was monitored by incubating a solution of the sugar phosphate (~1.5mM) in 1.0M perchloric acid at the required temperature (±0.02), removing 1.0-mL aliquots at appropriate inter-

vals, and analyzing for phosphate released by using the assay of Fiske and Subbarow[41]. Total-hydrolysis data were obtained by heating samples (in triplicate) for 30 min in a boiling-water bath, and data obtained in this way agreed closely with calculated values in all cases. Due to the limited quantities of 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate available, studies on the pH-dependence of the hydrolysis rate-constant were performed by using a more sensitive phosphate assay[42], allowing smaller (0.1 mL) aliquots to be taken. All such hydrolyses were performed in a buffered system, using 50mM potassium hydrogenphthalate as the buffer throughout the pH range studied, and adjusting to the desired pH with standard M hydrochloric acid or M sodium hydroxide as described previously[17].

Rate constants were determined from plots of $\log[(A_T - A_t)/A_T]$ *versus* time, where A_t is the absorbance value at time t, and A_T the absorbance value for the totally hydrolyzed sample. All plots were clearly linear to at least two half-lives for each compound studied, with correlation coefficients ranging from 0.981 to 0.998 and comprising from 6 to 20 data points in each case.

Ionization constants. — The ionization constants for 2-deoxy-2-fluoro-α-D-glucopyranosyl phosphate at 25° were determined by potentiometric titration of the compound (0.1M) with 1.00M hydrochloric acid stirred in a thermostatted vessel by using a Radiometer pHM 82 pH meter. Ionization constants for both anomers of 2-deoxy-2-fluoro-D-glucopyranosyl phosphate were also determined by ^{19}F-n.m.r. measurement of chemical shifts and coupling constants for a 0.1M solution of the salts at a series of pH values. Results obtained were identical to those determined potentiometrically.

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