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SOLUTION CONFORMATIONS AND HYDROLYTIC STABILITY OF 2′- AND 3′-SUBSTITUTED 2′,3′-DIDEOXYRIBONUCLEOSIDES, INCLUDING SOME POTENTIAL INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS

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Sugar ring conformations of a number of 2′- and 3′-substituted 2′,3′-dideoxyribonucleosides were determined in ^2H_2O by ^1H NMR spectroscopy. First-order rate constants for the cleavage of their *N*-glycosidic bond in aqueous acid were measured. The dependence of sugar ring conformation and hydrolytic stability on the polar nature of the 2′/3′-substitutent is discussed.

INTRODUCTION

In addition to 3′-azido-3′-deoxythymidine,[1] the generally known inhibitor of human immunodeficiency virus (HIV), several 2′,3′-dideoxyribonucleosides,[2–5] their 3′-azido[5–10] and 3′-fluoro derivatives,[3,7,10–17] and 2′,3′-didehydro analogues[3,10,11,18–21] show marked anti-HIV activity. 2′-Substituted 2′,3′-dideoxyribonucleosides are in this respect less promising, although 2′,3′-dideoxy-2′-fluororibonucleosides have been reported to exhibit some activity.[22,23] The relationship between structure and selective inhibition of HIV-1 replication *in vitro* has been reviewed.[24]

In view of the potential antiviral activity of 2′- and 3′-substituted 2′,3′-dideoxyribonucleosides, the conformations that these compounds adopt in aqueous solution are of considerable interest and may help in understanding their biological function, although the preferred conformation does not necessarily relate directly to the biological activity. X-ray structures have been reported for 2′,3′-dideoxycytidine,[25,26] 3′-deoxythymidine,[26] 3′-deoxy-2′-fluorothymidine,[27] 2′-azido-3′-deoxythymidine[28] and several 2′,3′-dideoxy-3′-fluoro-[11,29–34] and 3′-azido-2′,3′-dideoxyribonucleosides.[34–42] The data on conformations in solution are more scanty, being limited to NMR spectroscopic studies on 3′-azido-3′-deoxythymidine[43] and 2′- and 3′-halo-substituted pyrimidine nucleosides.[44] The results of these studies, together with comparative laser Raman spectroscopy,[45] suggest that the solid-state structures do not adequately represent the conformational behaviour in solution. 3′-Azido-3′-deoxythymidine, for example, exists in dimethyl sulphoxide as a mixture of C-2′-*exo*/C-3′-*endo* and C-2′-*endo*/C-3′-*exo* conformers, the former

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puckering being slightly favoured.[43] However, the compound crystallizes as a mixture of C-2′-*endo*/C-3′-*exo* and C-3′-*exo*/C-4′-*endo* forms.[35–39]

To obtain more extensive data on conformations of potentially antiviral nucleoside analogues in aqueous solution, in this work vicinal $^1H,^1H$ coupling constants of sugar protons of a great variety of 2′- and 3′-substituted 2′,3′-dideoxyribonucleosides were determined in ^2H_2O. The results are compared with the known solid-state conformations, and the effect of the polar nature of the 2′/3′-substituent on conformational parameters is discussed.

Hydrolytic stability is a factor that affects the applicability of nucleoside analogues as antivirals. To elucidate this property, first-order rate constants for cleavage of the *N*-glycosidic bond of a number of 2′- and 3′-substituted 2′,3′-dideoxyribonucleosides were determined in 0·1 mol dm^{-3} hydrochloric acid. The hydrolytic stability is correlated with the polar nature of the 2′/3′-substituent.

RESULTS AND DISCUSSION

Conformational analysis

Tables 1 and 2 record the ^1H NMR chemical shifts for the 2′,3′-dideoxyribonucleosides studied (structures **1** and **2**). The vicinal $^1H,^1H$ coupling constants of the sugar protons are listed in Tables 3 and 4. The pseudorotation parameters, which define the sugar ring puckering, were calculated from these values by applying the two-state (N and S) model of Haasnoot *et al.*[46] For the

Table 1. ^1H NMR chemical shifts of 3′-substituted 2′,3′-dideoxyribonucleosides in ^2H_2O[a]

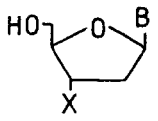
		δ(ppm)								
B	X	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-5″	X	B
A	OCH_2SCH_3	6·32	2·74	2·60	4·64	4·25	3·81	3·76	2·18, 4·75, 4·79	8·10, 8·20
A	$OCH_2SO_2CH_3$	6·44	2·86	2·76	4·78	4·39	3·81	3·79	3·12, 4·86	8·21, 8·27
A	N_3	6·39	2·93	2·67	4·55	4·17	3·84	3·78		8·20, 8·28
G	OCH_2SCH_3	6·24	2·79	2·62	4·65	4·23	3·80	3·76	2·19, 4·77, 4·80	7·97
C	OCH_3	6·18	2·29	2·57	4·12	4·18	3·81	3·74	3·39	6·18, 8·00
C[b]	OCH_2SCH_3	6·21	2·37	2·60	4·49	4·21	3·84	3·76	2·17, 4·73, 4·76	6·22, 8·06
C[b]	OCH_2OCH_3	6·21	2·28	2·52	4·36	4·14	3·81	3·75	3·40, 4·74, 4·76	6·02, 7·77
C	OCH_2N_3	6·21	2·31	2·56	4·42	4·17	3·80	3·75	4·80, 4·83	6·02, 7·77
T	OCH_2SCH_3	6·24	2·37	2·49	4·50	4·13	3·83	3·77	2·18, 4·73, 4·77	1·89, 7·61
T	$OCH_2OC(CH_3)_3$	6·22	2·33	2·48	4·41	4·11	3·81	3·74	1·25, 4·86, 4·88	1·87, 7·60
T	OCH_2OCH_3	6·24	2·38	2·49	4·40	4·13	3·83	3·76	3·41, 4·75, 4·77	1·88, 7·60
T	OCH_2N_3	6·24	2·41	2·52	4·46	4·16	3·83	3·78	4·82, 4·85	1·88, 7·60
T	OCH_2CN	6·25	2·38	2·50	4·47	4·15	3·82	3·77	4·90	1·88, 7·60
T	F	6·30	2·36	2·61	5·31	4·34	3·78	3·78		1·86, 7·63
[c]	N_3	6·15	2·49	2·53	4·31	4·02	3·86	3·77		8·12
[c]	F	6·29	2·35	2·69	5·32	4·38	3·79	3·79		8·12
[d]	F	6·24	2·32	2·79	5·31	4·44	3·80	3·78		8·63

[a] For B and X see structure **1**.
[b] Hydrochloride.
[c] 5-Chlorouracil.
[d] 5-Cyanouracil.

Table 2. ^1H NMR chemical shifts of 2′-substituted and 2′,3′-disubstituted 2′,3′-dideoxyribonucleosides in ^2H_2O[a]

			δ (ppm)							
B	X	Y	H-1′	H-2′	H-3′	H-3″	H-4′	H-5′	H-5″	B
T	H	N_3	5·86	4·4[b]	2·14	2·10	4·4[b]	3·93	3·74	1·85, 7·63
T	H	F	5·99	5·34	2·27	2·13	4·50	3·96	3·75	1·85, 7·67
T	F	F	6·09	5·28	5·42		4·42	3·88	3·83	1·86, 7·59

[a] For B, X and Y see structure **2**.
[b] Approximate values, due to overlapping.



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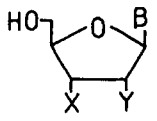

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phase angle, P_N, and amplitude, Φ_N, of pseudorotation of the C-2′-*exo*/C-3′-*endo* (N-type) conformer, constrained values[46] of 9·5° and 35·3° were usually employed. However, in cases where the N-type conformer predominates the data were recalculated with the S form constrained to typical values of $P_S = 164°$ and $\Phi_S = 38·8°$. With 3′-deoxy-2′,3′-difluorothymidine the amplitudes, Φ_N and Φ_S, were constrained to the values obtained with the N conformer of 3′-deoxy-2′-fluorothymidine and S conformer of 3′-deoxy-3′-fluorothymidine, leaving P_N, P_S and x_N adjustable. A value of 1·05 was used for the Huggins electronegativity of the azido group. Rotation of the hydroxymethyl group about the C-4′—C-5′ bond was described by populations of the conventional gg, gt and tg forms.[47] The conformational parameters obtained are given in Tables 5 and 6.

The data in Table 5 show that alkylation of the 3′-hydroxyl group of 2′-deoxyribonucleosides has only a moderate effect on the relative stability of C-2′-*exo*/C-3′-*endo* (N) and C-2′-*endo*/C-3′-*exo* (S) conformers, irrespective of the structure of the alkyl group. As with the parent nucleosides, the S type of puckering is favoured, the mole fraction, x_N, of the N form ranging from 0·2 to 0·3. Only the 3′-*O*-$CH_2SO_2CH_3$ derivative of 2′-deoxyadenosine exhibited a slightly

Table 3. Vicinal $^1H,^1H$ coupling constants of the sugar protons of 3′-substituted 2′,3′-dideoxyribonucleosides in ^2H_2O[a]

		J (Hz)						
B	X	1′,2′	1′,2″	2′,3′	2″,3′	3′,4′	4′,5′	4′,5″
A	OCH_2SCH_3	7·9(2)	6·2(1)	6·2(2)	2·8(1)	2·6(1)	3·5(1)	4·3(1)
A	$OCH_2SO_2CH_3$	8·3(2)	6·0(2)	5·9(3)	2·0(3)	2·1(2)	3·7(2)	3·9(2)
A	N_3	6·5(1)	6·5(1)	7·0(1)	4·8(1)	4·5(1)	3·5(1)	4·2(1)
G	OCH_2SCH_3	7·6(1)	6·3(1)	6·2(1)	3·0(1)	3·0(1)	4·0(1)	4·7(1)
C	OCH_3	7·2(1)	6·3(1)	6·4(1)	3·1(1)	3·2(1)	3·9(1)	5·2(1)
C	OCH_2SCH_3	6·6(1)	6·4(1)	6·8(1)	3·6(1)	3·3(1)	3·7(1)	5·1(1)
C	OCH_2OCH_3	7·1(1)	6·4(1)	6·8(1)	3·6(1)	3·6(1)	3·9(1)	5·1(1)
C	OCH_2N_3	7·1(2)	6·5(2)	6·6(2)	3·4(2)	3·6(2)	4·1(2)	4·9(2)
T	OCH_2SCH_3	7·2(1)	6·4(2)	6·9(2)	3·3(2)	3·4(2)	3·9(1)	5·0(1)
T	$OCH_2OC(CH_3)_3$	7·1(2)	6·5(2)	6·9(2)	3·4(2)	3·4(2)	3·7(2)	5·0(2)
T	OCH_2OCH_3	7·1(1)	6·5(1)	6·9(1)	3·7(1)	3·7(1)	3·8(1)	4·9(1)
T	OCH_2N_3	7·3(1)	6·5(1)	6·8(1)	3·4(1)	3·5(1)	3·9(1)	4·9(1)
T	OCH_2CN	7·6(1)	6·3(1)	6·6(1)	3·2(1)	3·4(1)	4·1(1)	5·0(1)
T	F	9·1(4)	5·7(3)	5·4(4)	0·1(3)	0·0(3)	3·9(3)	3·9(2)
[b]	N_3	5·9(3)	6·3(3)	7·7(4)	5·7(4)	5·6(4)	3·5(4)	4·4(4)
[b]	F	8·8(2)	5·7(2)	5·3(2)	1·2(1)	1·3(1)	4·3(2)	4·3(2)
[c]	F	8·4(2)	5·9(2)	5·2(2)	0·0(2)	0·0(2)	3·7(2)	4·4(2)

[a] For B and X see structure **1**. Uncertainty of the first decimal (standard deviation of the optimized value) is given in parentheses.
[b] 5-Chlorouracil.
[c] 5-Cyanouracil.

Table 4. Vicinal $^1H,^1H$ coupling constants of the sugar protons of 2′-substituted and 2′,3′-disubstituted 2′,3′-dideoxyribonucleosides in ^2H_2O[a]

			J (Hz)						
B	X	Y	1′,2′	2′,3′	2′,3″	3′,4′	3″,4′	4′,5′	4′,5″
T	H	N_3	2·3(1)	6·3(1)	3·0(1)	9·4(2)	6·1(1)	2·9(1)	4·6(1)
T	H	F	0·0(1)	4·7(1)	0·2(1)	11·2(2)	5·1(1)	2·9(1)	4·7(1)
T	F	F	4·5(2)	4·7(2)		4·6(2)		2·9(2)	4·0(2)

[a] For B, X and Y see structure **2**.

Table 5. Conformational parameters for 3′-substituted 2′,3′-dideoxyribonucleosides in ^2H_2O[a]

B	X	Pseudo-rotational parameters[b]			Populations[c]		
		x_N	$P_S(^\circ)$	$\Phi_S(^\circ)$	gg	gt	tg
A	OCH_2SCH_3	0·20	167	33·8	0·58	0·29	0·13
A	$OCH_2SO_2CH_3$	0·15	170	35·5	0·61	0·24	0·15
A	N_3	0·40[f]	154	33·4	0·59	0·28	0·13
G	OCH_2SCH_3	0·24	166	33·7	0·50	0·32	0·18
C	OCH_3	0·26	165	32·6	0·46	0·37	0·17
C	OCH_2SCH_3	0·31	168	30·5	0·48	0·37	0·15
C	OCH_2OCH_3	0·30	157	31·8	0·46	0·37	0·17
C	OCH_2N_3	0·29	160	31·7	0·46	0·34	0·20
T	OCH_2SCH_3	0·27	158	30·6	0·48	0·35	0·17
T	$OCH_2OC(CH_3)_3$	0·28	160	30·2	0·49	0·36	0·15
T	OCH_2OCH_3	0·31	156	31·2	0·49	0·35	0·16
T	OCH_2N_3	0·28	158	31·1	0·48	0·35	0·17
T	OCH_2CN	0·26	156	32·9	0·46	0·35	0·19
T	F	0·00	174	31·7	0·59	0·23	0·18
[d]	N_3	0·49[g]	134	35·6	0·57	0·30	0·13
[d]	F	0·07	164	34·3	0·51	0·26	0·23
[e]	F	0·00	186	32·1	0·55	0·30	0·15

[a] For B and X see structure **1**.
[b] Pseudo-rotational parameters according to Haasnoot *et al.*[47] Constrained values $P_N = 9·5^\circ$ and Φ_N were employed for the conformer.
[c] Populations of the C-4′—CH_2OH rotamers calculated on the basis of the coupling constants of conventional gg, gt and tg forms.[48]
[d] 5-Chlorouracil.
[e] 5-Cyanouracil.
[f] $x_N = 0·59$, $P_N = 15·5^\circ$, $\Phi_N = 27·8^\circ$ when constrained values of $P_S = 164^\circ$, $\Phi_S = 38·8^\circ$ were employed.
[g] $x_N = 0·49$, $P_N = 6·4^\circ$, $\Phi_N = 26·5^\circ$ when constrained values of $P_S = 164^\circ$, $\Phi_S = 38·8^\circ$ were employed.

Table 6. Conformational parameters for 2′-substituted and 2′,3′-disubstituted 2′,3′-dideoxyribonucleosides in ^2H_2O[a]

B	X	Y	Pseudo-rotational parameters[b]			Populations[c]		
			x_N	$P_N(^\circ)$	$\Phi_N(^\circ)$	gg	gt	tg
T	H	N_3	0·82[d]	17·0	30·1	0·60	0·35	0·05
T	H	F	1·00[d]	7·8	31·5	0·59	0·35	0·06
T	F	F	0·46[e]	22·2		0·67	0·28	0·05

[a] For B, X and Y see structure **2**.
[b] Pseudo-rotational parameters according to Haasnoot *et al.*[46]
[c] See footnote c of Table 5.
[d] Constrained values of $P_S = 164^\circ$ and $\Phi_S = 38·8^\circ$ were employed for the S conformer.
[e] Constrained values of $\Phi_N = 31·5^\circ$ and $\Phi_S = 31·7^\circ$ were employed.

smaller value of x_N (0·15). The spread in the optimized pseudo-rotation parameters of the S form is also relatively small: $154^\circ < P_S < 170^\circ$, $31^\circ < \Phi_S < 35^\circ$.

Replacing the 3′-hydroxyl group with an azido group shifts the N–S equilibrium towards the N form, as reported previously for 3′-azido-3′-deoxythymidine.[43] The values observed for x_N of 3′-azido-2′,3′-dideoxyadenosine and 3′-azido-5-chloro-2′,3′-dideoxyuridine were 0·40 and 0·49, respectively. In contrast, 2′,3′-dideoxy-3′-fluororibonucleosides adopt almost entirely an S-type conformation. Both findings are expected on the basis of the polar nature of the 3′-substituent. The 3′-fluoro group, as a highly electronegative substituent, tends to take a pseudo-axial orientation, and hence S-type conformations are favoured. The 3′-azido group is, in turn, slightly less electronegative than the 3′-hydroxy group, and the equilibrium is thus shifted towards the N-type conformations. At the 2′-position the effect of these substituents on the sugar ring puckering is naturally opposite (Table 6). The tendency of the 2′-fluoro group to take a pseudo-axial orientation compels the sugar ring to be entirely in the N conformation. The situation is analogous with 2′-azido nucleosides, but the predominance of N-type conformations is less complete than with the 2′-fluoro derivative. When both C-2′ and C-3′ bear a fluorine atom, an approximately equimolar mixture of N and S conformers is obtained.

The conformation about the C-4′—C-5′ bond is not markedly sensitive to the 3′-substituent. The gg form predominates with all the 3′-substituted nucleosides studied, the mole fraction of this rotamer falling in the range 0·45–0·67. The gt form is more stable than the tg form ($0·24 < x_{gt} < 0·37$, $0·13 < x_{tg} < 0·23$).

The gg form is slightly more favoured with 2′- than with 3′-substituted derivatives.

The *syn–anti* equilibrium about the *N*-glycosidic bond was studied with only two compounds by applying the method of Davies *et al.*[48] The ^3J(C2,H1′) and ^3J(C6,H1′) values obtained with the 3′-*O*-CH_2SCH_3 derivatives of thymidine (2·2 and 3·8 Hz) and 2′-deoxycytidine (1·6 and 3·2 Hz) were almost identical with those reported earlier[48] for the unsubstituted nucleosides, indicating that the conformation about the *N*-glycosidic bond is not significantly influenced by 3′-*O*-alkylation.

Table 7 summarizes the solid-state structures reported in the literature for 2′- and 3′-substituted 2′,3′-dideoxyribonucleosides. As can be seen, most of the 3′-substituted derivatives are crystallized in two forms. One of them shows conformational features which are fairly common in nucleosides, viz. a C-2′-*endo*/C-3′-*exo* of the furanose ring, gg conformation of the C-4′—CH_2OH group and a glycosidic torsion angle (C-2—N-1—C-1′—$O^{4'}$) of about −120°. The other crystal structure may closely resemble this, exhibiting a more negative glycosidic torsion angle (−160° to −170°), or the sugar ring pucker may be C-3′-*exo*/C-4′-*endo*, the C-4′—CH_2OH group in the gt form and the glycosidic torsion angle −180°. It is worth noting that although 3′-substituted 2′,3′-dideoxyribonucleosides have been crystallized only as S conformers, or in forms resembling the typical S conformation, the proportion of N-type conformers in solution is not negligible. The pseudo-rotation parameters obtained for the S form in solution, keeping those of the N form constrained to the typical values ($P_N = 9·5°$, $\Phi_N = 35·3°$), do not deviate markedly from the values observed in the solid state. Accordingly, the conventional two-state model appears to describe adequately the conformational behaviour of these compounds in solution. 2′-Substituted 2′,3′-dideoxyribonucleosides are crystallized as N conformers, consistent with the predominance of this form in solution.

Table 7. Conformational parameters of the solid-state structures reported for 2′- and 3′-substituted 2′,3′-dideoxyribonucleosides[a]

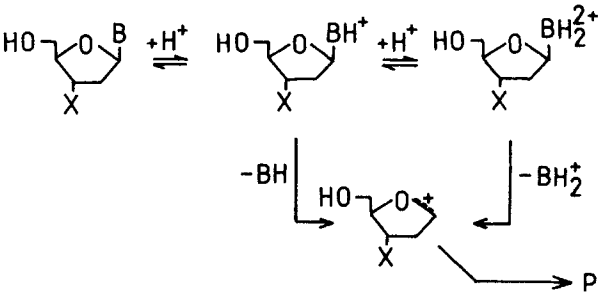
B	X	Y	P(°)	Φ(°)	χ(°)	τ(°)	Ref.
C[b]	N_3	H	135	38	−130	48	42
C	F	H	154	40	−143·5	63·4	31
			164	36	−153·0	−71·4	31
T[c]	N_3	H	175·2	32·3	−126·6	gg	35
			214·5	36·6	−177·7	gt	35
			174	33	−127·1	50·7	36
			213	37	−176·5	173·6	36
			173·6	33·0	−127·2	50·6	37
			212·3	36·7	−177·0	173·4	37
T	H	N_3	12	34	−165·7	52·8	28
T	F	H	164	36	−138·4	50·2	29
			169	32	−159·6	52·8	29
T	H	F	12	35	−152·2	55·1	27
			8	35	−168·0	49·7	27
U	N_3	H	174	35	−160	56	41
U	F	H	165	35	−121·1	51·3	32
			176	26	−150·5	47·4	32
[d]	N_3	H	173	33	−128·2	50·4	32
			208	36	−168·9	170·8	32
[d]	F	H	179	32	−168·8	55·8	11
			163	32	−131·3	51·1	11
[e]	N_3	H	173	33	−129·8	50·5	32
			203	37	−168·4	172·1	32
[e]	F	H	179	31	−168·8	53·9	32
			161	35	−131·8	51·2	32
[f]	F	H	184	29	−107	43·8	33
g	F	H	174	34	−134·7	54·3	30

[a] For B, X and Y see structure **2**. χ is the torsion angle C-2—N-1—C-1′—$O^{4'}$ and τ is the torsion angle $O^{5'}$—C-5′—C-4′—C-3′
[b] Hydrochloride.
[c] See also Refs 38–40.
[d] 5-Chlorouracil.
[e] 5-Bromouracil.
[f] 5-Iodouracil.
[g] 5-Cyanouracil.

Hydrolytic stability

Table 8 summarizes the effects that various substituents at C-3′ have on the rate of acid-catalysed hydrolysis of 2′,3′-dideoxyribonucleosides. Part of these data have been published previously in a congress report.[49]

It has been well established[50–52] that 2′-deoxyribonucleosides derived from adenine, guanine and cytosine are all hydrolysed by rate-limiting unimolecular cleavage of the protonated base moiety with concomitant formation of a cyclic oxocarbenium ion (Scheme 1). As seen from Table 1, replacing the 3′-hydroxyl group of these nucleosides with an azido or fluoro group reduces the hydrolysis rate to one fifth of its original value. Alkylation of the 3′-hydroxyl function also moderately retards the hydrolysis, the rate retardation being increased with increasing electronegativity of the alkyl group. Replacing the 3′-



Scheme 1

Table 8. Effect of 3′-substituent on the hydrolytic stability of 2′-3′-dideoxyribonucleosides in 0·1 mol dm^{-3} hydrochloric acid at 333·2 K (363·2 K for thymine derivatives)[a]

	k_X/k_{OH}			
X	A	G	C	T
H	25[b]		18·6[b]	11[b]
OH	1[c]	1[d]	1[e]	1[i]
OCH_3		0·79[b]	0·70[b]	
OCH_2SCH_3	0·63[b]	0·65[b]	[f]	[f]
OCH_2OCH_3	0·64	0·51	[f]	[f]
$OCH_2OCH_2CH_2OCH_3$	0·62			
$OCH_2OC_6H_5$		0·40		
OCH_2SOCH_3	0·32[b]		0·40[b,g]	[f]
$OCH_2SO_2CH_3$	0·22	0·28[b]	0·30[b]	0·47[b]
OCH_2N_3	0·42	0·46	0·23[b,h]	[f]
OCH_2CN				[f]
N_3	0·19[b]			0·34[b]
F	0·16[b]		0·18[b]	0·54[b]
1,2,4-Triazol-1-yl				0·05

[a] See structure **1**. k_X and k_{OH} are first-order rate constants obtained with 3′-substituted 2′,3′-dideoxyribonucleosides and their 2′-deoxyribonucleoside counterparts, respectively.
[b] From Ref. 50.
[c] First-order rate constant = $9·64 \times 10^{-3}$ s^{-1}.[50]
[d] First-order rate constant = $7·68 \times 10^{-3}$ s^{-1}.[50]
[e] First-order rate constant = $3·0 \times 10^{-6}$ s^{-1}.[50]
[f] Cleavage of X.
[g] First-order rate constant for cleavage of X = 6×10^{-7} s^{-1}.
[h] First-order rate constant for cleavage of X = $1·2 \times 10^{-6}$ s^{-1}.
[i] First-order rate constant = $5·3 \times 10^{-6}$ s^{-1}.[50]

hydroxyl group with a less electronegative hydrogen atom, in turn, accelerates the cleavage of the *N*-glycosidic bond by more than one order of magnitude. All these effects may be attributed to changes in the stability of the oxocarbenium intermediate. Electron-withdrawing groups at C-3′, for example, diminish the electron density at the C-1′—O$^{4'}$ region, and hence destabilize the positively charged carbonium ion. This destabilization is partially reflected to the transition state, resulting in deceleration.

Hydrolysis of thymine nucleosides appears to be slightly less susceptible to the polar nature of the 3′-substituent than hydrolyses of adenine, guanine and cytosine nucleosides. A possible explanation for this is that thymine nucleosides are not hydrolysed via a cyclic oxocarbenium ion. Thymidine and 2′-deoxyuridine have been shown to undergo anomerization concurrent with hydrolysis, and hence a route via an acyclic Schiff base intermediate has been preferred.[53] With the exception of 3′-deoxythymidine, all the thymine derivatives studied in this work seemed to be anomerized concurrent with hydrolysis, as indicated by the intermediary appearance of three minor signals in high-performance liquid chromatograms (HPLC). These signals were UV spectroscopically identical with the starting material.

EXPERIMENTAL

Materials. Of the compounds studied, 2′-deoxy- and 2′,3′-dideoxyribonucleosides were commercial products from Sigma. 2′,3′-Dideoxy-3′-fluoroadenosine was obtained by treating N^6-benzoyl-9-(5-*O*-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)adenine[54] with diethylaminosulphur trifluoride (DAST) in dichloroethane (0·5 h, room temperature), followed by removal of the benzoyl groups with methanolic ammonia (overnight). The overall yield was 60%; m.p. 191–192 °C. Preparation of the other nucleosides employed has been described previously.[3,7,11,16,28,30,55–57]

NMR spectroscopy. NMR spectrometric data were obtained on a Jeol GX-400 spectrometer at 298·2 K. The chemical shifts were measured relative to external TMS. Computational simulation of the ^{1}H resonance spectra gave the vicinal ^{1}H,^{1}H coupling constants at an accuracy of ±0·1 Hz. The PSEUROT program[58] was applied to determine the pseudo-rotational parameters.

Kinetic measurements. First-order rate constants of hydrolysis were obtained by the HPLC technique described previously.[51]

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