Additional Evidence for the Exceptional Mechanism of the Acid-catalysed Hydrolysis of 4-Oxopyrimidine Nucleosides: Hydrolysis of 1-(1-Alkoxyalkyl)uracils, Seconucleosides, 3'-*C*-Alkyl Nucleosides and Nucleoside 3',5'-Cyclic Monophosphates

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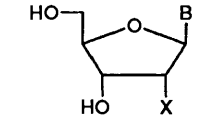
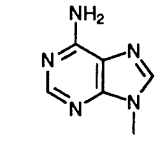
The rate constants for the acid-catalysed hydrolysis of 1-(1-alkoxyethyl)uracils and 1-alkoxymethyluracils have been determined. With both series of compounds, the hydrolysis rate is rather insensitive to the polar nature of the alkoxy group, in striking contrast with the hydrolysis of the corresponding analogues of adenine and cytosine nucleosides, which react *via* rate-limiting formation of an oxocarbenium ion intermediate. Furthermore, it has been shown that the 3',5'-cyclic monophosphates of thymidine and uridine undergo the hydrolysis of the *N*-glycosidic bond 760 and 260 times as fast as their parent nucleosides, while the cyclic monophosphates of 2'-deoxyadenosine and adenosine are depurinated much more slowly than the corresponding nucleosides. On this basis it is suggested that 4-oxopyrimidine nucleosides are hydrolysed by opening of the sugar ring. To obtain further evidence for this exceptional mechanism, comparative kinetic measurements with some *seco*- and 3'-*C*-alkyl nucleosides of uracil and adenine have been carried out.

The acid-catalysed hydrolysis of purine nucleosides (**1a**, **b**) has been shown[1,2] to proceed by a unimolecular rate-limiting departure of the protonated base moiety (Route A in Scheme 1).

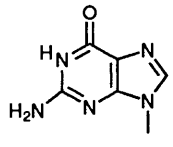


X = H; OH

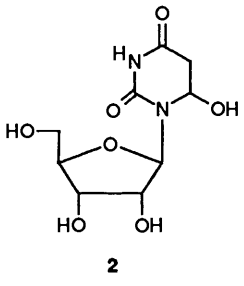
1a: B = Ade
b: B = Gua
c: B = Cyt
d: B = Thy
e: B = Ura



Adenine (Ade)



Guanine (Gua)



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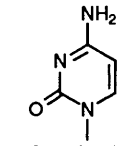
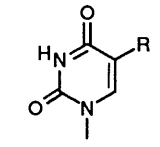


Cytosine (Cyt)

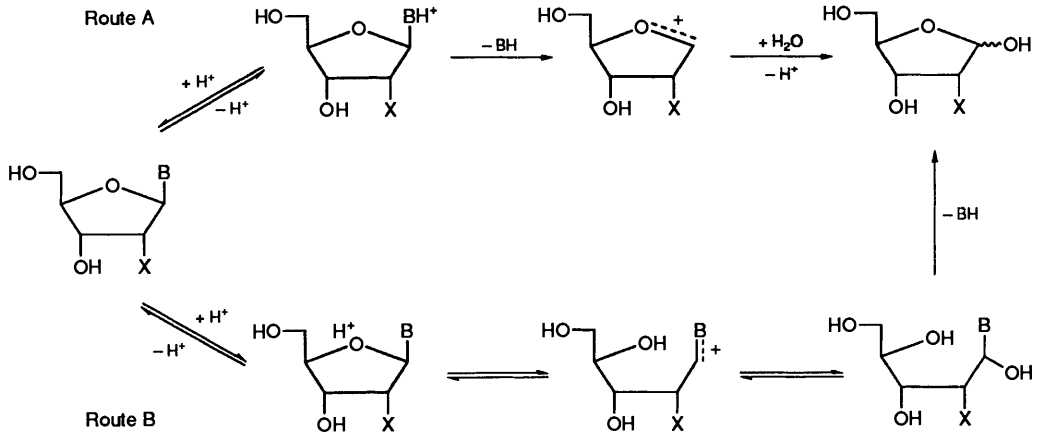


R = H: Uracil (Ura)
R = CH_3: Thymine (Thy)

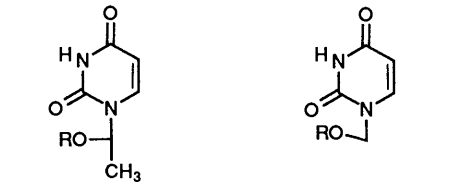
A cyclic oxocarbenium ion is formed from the sugar moiety as an intermediate. Hydrolysis of cytosine nucleosides (**1c**) has also been suggested to follow this mechanism.[3,4] In contrast, 4-oxopyrimidine nucleosides (**1d**, **e**) seem to behave differently. With thymidine (**1d**, X = H) and 2'-deoxyuridine (**1e**, X = H) anomerization to the corresponding α-anomer and isomerization to pyranoside derivatives have been shown to compete with the cleavage of the N-glycosidic bond under acidic conditions.[5] This has been suggested to refer to opening of the sugar ring and formation of a Schiff base intermediate (Scheme 1, Route B).[5] The hydrolysis of uridine (**1e**, X = OH), which is much slower than that of purine ribonucleosides, is not understood in detail. Prior and Santi have shown[6] that uridine equilibrates in aqueous acid with its covalent hydrate, 6-hydroxy-5,6-dihydrouridine (**2**), and that this hydrate may be hydrolyzed to uracil without intermediary formation of uridine. Accordingly, the hydrolysis of uridine to uracil may well proceed *via* **2**, or *via* an intermediate leading to formation of **2** from uridine. The kinetic isotope effect of the hydrolysis of [6-^{3}H]uridine, k_T/k_H = 1.12 at 363 K, has been taken as an indication of $sp^2 \rightarrow sp^3$ rehybridization at C6 during the hydrolysis.[6] To obtain further evidence for the exceptional mechanism of the acid-catalysed hydrolysis of 4-oxopyrimidine nucleosides, the effects of varying the sugar moiety structure on the hydrolysis rate have been studied. Firstly, the kinetics of the hydrolysis of 1-(1-alkoxyalkyl)uracils (**3** and **4**) have been studied and compared with the results obtained earlier with the corresponding purine[2] (**5**), cytosine[4] (**6**) and benzimidazole[7] (**7**) derivatives. Secondly, the hydrolysis rates of the *N*-glycosidic bond of 3',5'-cyclic monophosphates of adenine (**8**), cytosine (**9**), thymine (**10**) and uracil nucleosides (**11**) have been compared with those of their parent nucleosides. Thirdly, the effects that opening of the sugar-ring or alkylation of the C3' atom exert on the hydrolytic stability of uracil (or thymine) and adenine nucleosides have been determined.

Results and Discussion

Hydrolysis of 1-(1-*Alkoxyethyl*)*uracils* (**3a–e**) *and* 1-*Alkoxymethyluracils* (**4a–e**).—It has been shown that the effect of the alkoxy group, OR, on the hydrolysis of the 1-alkoxyethyl derivatives of purine[2] (**5**), cytosine[4] (**6**) and benzimidazole[7] (**7**) is similar to that of the non-departing alkoxy function, OR^x, on the hydrolysis of acyclic acetals (Route A in Scheme 2, Table 2).[8] Accordingly, all these acyclic nucleoside analogues appear to be hydrolysed by a mechanism which involves a rapid initial protonation of the base moiety and a unimolecular rate-limiting

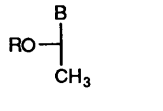


Scheme 1

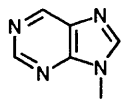


3a: R = CH $(CH_3)_2$
b: R = CH_2CH_3
c: R = CH_3
d: R = $CH_2CH_2OCH_3$
e: R = CH_2CH_2Cl

4a: R = CH $(CH_3)_2$
b: R = CH_2CH_3
c: R = CH_3
d: R = $CH_2CH_2OCH_3$
e: R = CH_2CH_2Cl

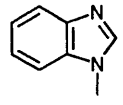


R = CH $(CH_3)_2$; CH_2CH_3; CH_3; CH_2CH_2Cl

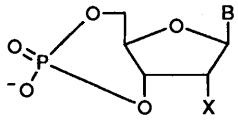


Purine

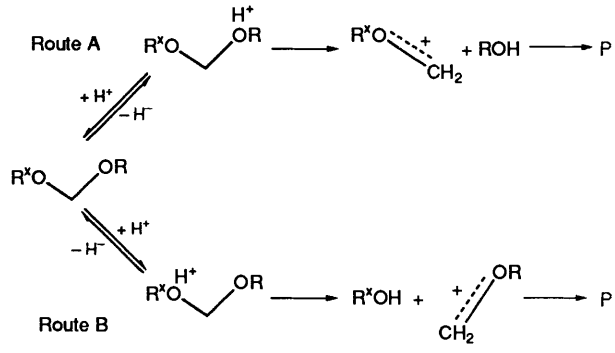
5: B = Purine
6: B = Cytosine
7: B = Benzimidazole



Benzimidazole



8a: B = Ade; X = H
b: B = Ade; X = OH
9a: B = Cyt; X = H
b: B = Cyt; X = OH
10: B = Thy; X = H
11: B = Ura; X = OH



Scheme 2

Table 1 First-order rate constants for the hydrolysis of 1-(1-alkoxyethyl)uracils (**3a–e**) and 1-alkoxymethyluracils (**4a–e**) in aqueous acid at 363.2 K

	$k_{obs}/10^{-6}$ s^{-1}	
Alkoxy group	**3a–e**[a]	**4a–e**[b]
Isopropoxy	26.9 ± 1.0	8.07 ± 0.18
Ethoxy	11.8 ± 0.4	6.03 ± 0.16[c]
Methoxy	5.13 ± 0.13	4.87 ± 0.10
2-Methoxyethoxy	3.48 ± 0.06	6.97 ± 0.19
2-Chloroethoxy	4.21 ± 0.10	9.00 ± 0.31[d]

[a] In 0.01 mol dm^{-3} aqueous hydrogen chloride, the ionic strength adjusted to 0.1 mol dm^{-3} with sodium chloride. [b] In 0.5 mol dm^{-3} aqueous hydrogen chloride. [c] k_{obs} = 16.5 × 10^{-6} s^{-1} in 1 mol dm^{-3} aqueous hydrogen chloride. [d] k_{obs} = 22.3 × 10^{-6} s^{-1} in 1 mol dm^{-3} aqueous hydrogen chloride.

departure of the protonated base.[2,4,7] As in Route A of acetal hydrolysis, a resonance-stabilized oxocarbenium ion is formed from the alkoxyalkyl group in the rate-limiting step, and the rate of the reaction is proportional to the stability of this intermediate: the more electronegative OR, the less stable the oxocarbenium ion. With the uracil derivatives (**3a–e**, **4a–e**), the dependence of the hydrolysis rate on the electronegativity of the alkoxy group clearly differs from that observed with the other acyclic nucleoside analogues (Tables 1 and 2). In fact, it more closely resembles the effect of the leaving alkoxy group on the rate of acetal hydrolysis (Route B in Scheme 2, Table 2), and may be rationalized as follows. Increasing the electronegativity of group R, for example, retards the preequilibrium protonation, but simultaneously ROH becomes a better leaving group, and hence the total influence on the hydrolysis rate remains small. The structural effects indicated above thus strongly suggest that **3a–e** and **4a–e** are not hydrolysed *via* an oxocarbenium ion derived from the alkoxyalkyl group, but rather by cleavage of the protonated alkoxy group with concomitant formation of a cationic Schiff base. In other words, the structure–reactivity correlations lend additional support to the previous suggestion, according to which the acid-catalysed hydrolysis of 4-oxopyrimidine nucleosides follows an open-chain mechanism (Route B in Scheme 1).[5] A possible explanation for this exceptional mechanism is that the π-electron delocalization of the uracil base is not as efficient as that of the other nucleobases, and hence the uracil nucleosides would be hydrolysed by the mechanism characteristic of aliphatic glycosylamines. The latter compounds are known to react by a

Table 2 Relative first-order rate constants for the acid-catalysed hydrolysis of the alkoxyalkyl analogues of nucleosides and the partial reactions A and B (Scheme 2) of the acetals of formaldehyde

	Compound					Acetal reaction	
RO or R^{x}O	3[a]	4[a]	5[b]	6[c]	7[d]	A[e]	B[e]
Isopropxy	5.2	1.7	12	9.3	9.4	22.1	2.27
Ethoxy	2.3	1.2	3.4	2.9	3.1	4.48	1.21
Methoxy	1	1	1	1	1	1	1
2-Methoxyethoxy		0.68	1.4				0.20 1.53
2-Chloroethoxy	0.82	1.8	0.077	0.10	0.087	0.048	1.96

[a] This work, see Table 1. [b] From Ref. 2. For the reaction of purine monocations at 313.2 K. [c] From Ref. 4. For the reaction of the substrate monocation at 363.2 K. [d] From Ref. 7. For the reaction of the substrate monocations at 353.2 K. [e] From Ref. 8. At 298.2 K.

Table 3 First-order rate constants for the acid-catalysed hydrolysis of the *N*-glycosidic bond of 2'-deoxynucleosides, and their 5'-monophosphates and 3',5'-cyclic monophosphates

			$k/10^{-3}$ s^{-1}		
Base moiety	T/K	[HCl]/mol dm^{-3}	2'-Deoxynucleoside	5'-dNMP	3',5'-cdNMP
Thy	363.2	0.1	0.005 3[a]	0.005 10	4.07
Cyt	363.2	4.0		0.158	0.465[b]
		1.0		0.032 1	0.149[b]
		0.01[c]	0.140[d]	0.029 1	0.119[e]
		3.9 × 10^{-5} [f]		0.009 08	0.017 7[e]
Ade	323.2	1.0	50.4[g,h]	24.3[g,i]	0.020 0[j]

[a] Ref. 17. [b] First-order rate constants for the hydrolysis of the 3',5'-cyclic phosphodiester: 1.08 × 10^{-3} s^{-1} and 1.83 × 10^{-4} s^{-1} in 4 and 1 molar acid, respectively. [c] The ionic strength adjusted at 0.1 mol dm^{-3} with sodium chloride. [d] Ref. 17. Refer to the pH-independent area of the rate profile.[3] [e] No phosphodiester hydrolysis detected. [f] An acetic acid–sodium acetate buffer at pH 4.4, the ionic strength adjusted at 0.1 mol dm^{-3} with sodium chloride. [g] Extrapolated from the values determined at lower concentrations of hydrogen chloride. [h] See Ref. 2. [i] See Ref. 13. [j] Extrapolated by Arrhenius eqn. from the values determined at higher temperatures. The activation entropy, $\Delta S^{\ddagger} = (-13 \pm 4)$ J K^{-1} mol^{-1}, and the activation enthalpy, $\Delta H^{\ddagger} = (104 \pm 2)$ kJ mol^{-1} at 323.2 K.

Table 4 First-order rate constants for the hydrolysis of the *N*-glycosidic bond of ribonucleosides, and their 5'-monophosphates and 3',5'-cyclic monophosphates at 363.2 K

		$k/10^{-3}$ s^{-1}		
Base moiety	[HCl]/mol dm^{-3}	Nucleoside	5'-NMP	3',3'-cNMP
Ade	1.0	7.1[a]	2.5[b]	*c*
Ura	1.0	0.001 00		0.251[d]
Cyt	1.0	0.001[f]		*e*
	0.01[g]	0.000 86[h]		0.007 96[i]

[a] Ref. 18. [b] Ref. 13. [c] For disappearance of the starting material. k = 0.184 × 10^{-3} s^{-1}; most likely refers to hydrolysis to 3'- and 5'-AMP. [d] For the competing hydrolysis of the 3',5'-cyclic phosphodiester group, k = 0.272 × 10^{-3} s^{-1}. [e] Only hydrolysis of the cyclic phosphodiester detected; k = 0.200 × 10^{-3} s^{-1}. [f] Approximated by the data in Refs. 15 and 19. [g] The ionic strength adjusted at 0.1 mol dm^{-3} with sodium chloride. [h] Ref. 19. For competing deamination of the cytosine moiety k = 3.05 × 10^{-5} s^{-1}. [i] By the method of initial rates applied to the accumulation of cytosine. No marked deamination of cytosine to uracil occurred on that timescale.

mechanism involving protonation of the glycosyl ring-oxygen, followed by ring-opening and formation of a cationic Schiff-base intermediate.[9] Anomerization and isomerization of the sugar moiety, observed to take place during the acidic hydrolysis of thymidine and deoxyuridine,[5] suggest that opening of the glycosyl ring takes place before the rate-limiting step of the reaction. It is impossible to distinguish, on the basis of the data available, whether hydration of the 5,6-double bond of the base moiety precedes the cleavage of the CO bond, as was suggested by Prior and Santi[6] in the case of uridine. This mechanism would most likely lead to a similar dependence of reactivity on structure as Route B (Scheme 1).

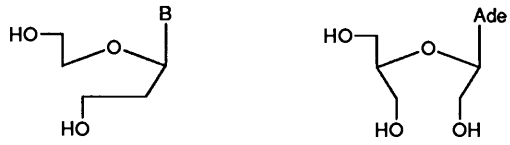
Hydrolysis of Nucleoside 3',5'-Cyclic Monophosphates.—It was observed[10,11] in early 1960s that thymidine (**10**) and uridine 3',5'-cyclic monophosphates (**11**) are hydrolysed in aqueous acid much more readily than the corresponding 5'-monophosphates, whereas the 3',5'-monophosphates of purine ribo- and 2'-deoxyribo-nucleosides (**8a**, **b**) are remarkably more stable than the parent nucleoside 5'-monophosphates. However, no mechanistic explanation for this difference could be given at that time. We have reinvestigated this subject in a more quantitative manner and our measurements fully corroborate these early observations. The hydrolyses of 3',5'-cyclic monophosphates of adenosine (**8b**), 2'-deoxyadenosine (**8a**), and thymidine (**10**) in 1.0 mol dm^{-3} aqueous hydrogen chloride were found to yield the nucleobase as the only chromophoric product. With 2'-deoxycytidine 3',5'-cyclic monophosphate (**9a**), the corresponding nucleoside 3'- and 5'-monophosphates were accumulated besides cytosine ([3'-dCMP]/[5'-dCMP] = 0.85/0.15), indicating that phosphodiester hydrolysis competes with the depyrimidination. With uridine (**11**) and cytidine 3',5'-cyclic monophosphates these reactions also compete, but because of the rapid phosphate migration[12] between 2'-and 3'-hydroxy groups an equilibrium mixture of nucleoside 2'- and 3'-monophosphates is obtained instead of the 3'-monophosphate. Depyrimidination of the cytosine derivatives becomes predominant under less acidic conditions (pH > 2), since the rate of this reaction is pH-independent at pH 1–3,[3,4] whereas the phosphodiester hydrolysis is approximately second-order in hydronium-ion concentration.[12] Table 3 summarizes the rate constants observed for the hydrolysis of the *N*-glycosidic bond of 2'-deoxyribonucleosides, their 5'-monophosphates and 3',5'-cyclic monophosphates. The corresponding data for ribonucleosides are presented in Table 4. As seen, the effect that the formation of the 3',5'-cyclic phosphodiester exerts on the

hydrolysis rate of the *N*-glycosidic bond is drastically different with various nucleosides, both in ribo and deoxyribo series. While thymidine (**10**) and uridine 3′,5′-cyclic monophosphates (**11**) are hydrolysed in aqueous acid 760 and 260 times as fast as their parent nucleosides, the cyclic monophosphate of 2′-deoxyadenosine (**8a**) is 2500 times more stable than 2′-deoxyadenosine and 1200 times more stable than the corresponding 5′-monophosphate. Adenosine 3′,5′-cyclic monophosphate (**8b**) yields adenine only 10 times less readily than the corresponding 5′-monophosphate, but in all likelihood **8b** is initially hydrolysed to a mixture of adenosine 3′- and 5′-monophosphates, and their much faster depurination then gives adenine. It is worth noting that the rate constant for the hydrolysis of **8b** is nearly equal to that of the phosphodiester hydrolysis of cytidine (**9b**) and uridine 3′,5′-cyclic monophosphates (**11**) [*ca.* 2×10^{-4} s^{-1} in 1 mol dm^{-3} aqueous hydrogen chloride at 363.2 K]. Depurination of adenosine 2′-, 3′ and 5′-monophosphates is, under these conditions, one order of magnitude faster,[12,13] *i.e.*, fast enough to prevent their marked accumulation.

The large opposing effects of the cyclic 3′,5′-phosphodiester group on the stability of the *N*-glycosidic bond of adenine and 4-oxopyrimidine nucleosides can hardly be of electronic origin. For comparison, the effect of the 5′-monophosphate group is only a minor one over all the nucleosides studied (Tables 3 and 4). The increased ring-strain of the sugar moiety that results from the formation of the fused six-membered phosphodiester ring offers a more attractive explanation. The phosphodiester ring adopts a fixed chair conformation, which forces the furanose ring into a strongly puckered high-energy conformation.[14] The hydrolysis of purine nucleosides involves rate-limiting formation of a cyclic oxocarbenium ion, where a partial double bond is formed between Cl′ and $O^{4'}$. The rigid and distorted furanose ring of a nucleoside 3′,5′-cyclophosphate can attain the required planarity only after considerable conformational changes, and the rate of hydrolysis is thus decelerated. With thymidine (**10**) and uridine derivatives (**11**), the increased ring-strain appears, in turn, to facilitate the depyrimidination. This is understandable, if it is assumed that the reaction proceeds *via* opening of the sugar ring; the ring-strain is now relieved on going from the initial state to the transition state.

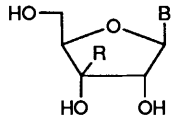
Cytosine nucleosides have been suggested to be hydrolysed by the same mechanism as the purine nucleosides,[3,4] although hydrolytic deamination to uridine competes with the hydrolysis of cytidine.[15] The structural effect of the alkoxy group, OR, on the hydrolysis of 1-(1-alkoxyethyl)cytosines (**6**) is, for example, similar to that observed with the corresponding 9-substituted purine derivatives.[4] Accordingly, one might expect that the 3′,3′-cyclic monophosphate of 2′-deoxycytidine would be more stable towards acid-catalysed depyrimidination than 2′-deoxycytidine. However, it is somewhat more labile (Table 3). The situation seems to be similar with the ribo analogues, although the competing phosphodiester hydrolysis and deamination hampered the kinetic measurements. One may speculate that while the depyrimidination *via* a cyclic oxocarbenium ion is retarded upon formation of the 3′,5′-cyclic monophosphate, the reaction *via* the acyclic Schiff base is accelerated. This would lead to a change in the hydrolysis mechanism, leaving the rate of depyrimidination practically unaffected.

Hydrolysis of Seconucleosides and 3′-C-*Alkylated Nucleosides.*—To learn more about the effect of varying the glycone moiety structure on the hydrolytic stability of adenine and 4-oxopyrimidine nucleosides, the rate constants for the hydrolysis of some seco analogues and 3′-*C*-alkylated derivates of these nucleosides were determined. The results obtained are listed in Table 5. As seen, the effects of these structural changes are rather small and do not lend marked additional support for the mechanistic conclusions drawn above. 2′,3′-Secoadenosine (**13**) is depurinated eight times as fast as adenosine, consistent with the four- to six-fold rate-enhancements reported previously for the seco analogues of benzimidazole ribonucleoside[16] and 7-methylguanosine 5′-monophosphate,[13] and also consistent with the argument that the increased flexibility of the sugar moiety would facilitate the formation of the oxocarbenium ion intermediate. However, the fact that 2′-deoxy-3′,4′-secoadenosine (**12a**) was found to be three times more stable than 2′-deoxyadenosine argues against the validity of this reasoning. With thymidine the corresponding structural change (**12b**) is moderately rate-accelerating. 3′-*C*-Methylation (**14a**) and ethylation (**14b**) has practically no effect on the hydrolytic stability of adenosine, while the 3′-*C*-ethyl substituent in uridine (**14c**) is moderately rate-retarding.



12a: B = Ade
b: B = Thy

13



14a: B = Ade; R = CH_3
b: B = Ade; R = CH_2CH_3
c: B = Ura; R = CH_2CH_3

Experimental

Materials.—The nucleobases, nucleosides, nucleoside 5′-monophosphates and nucleoside 3′,5′-cyclic monophosphates, except 2′-deoxycytidine 3′,5′-cyclic monophosphate, were products of Sigma, and they were used as received after the purity had been checked by HPLC. 2′-Deoxycytidine 3′,5′-cyclic monophosphate was prepared as described previously,[11] *i.e.*, by cyclization of the 4-morpholine-4-*N*,*N*′-dicyclohexylcarboxamidinium) salt of 2′-deoxycytidine 5′-monophosphate in anhydrous boiling pyridine in the presence of *N*,*N*′-dicyclohexylcarbodiimide.

1-(1-Alkoxyethyl)uracils (**3a–e**) were obtained by addition of trimethylsilylated uracil[20] on the appropriate alkyl vinyl ethers in dry 1,2-dichloroethane, using methanesulfonic acid as the catalyst. The product was isolated by the normal aqueous sodium hydrogen carbonate work-up, and purified by silica-gel chromatography (Merck silica gel 60). Elution with 0.5–3% methanol in dichloromethane gave **3a–e** in 10 to 20% yield. Ethyl, methyl and 2-chloroethyl vinyl ethers, used as starting materials, were commercial products, and isopropyl and 2-methoxyethyl vinyl ethers were synthesized from ethyl vinyl ether by mercury(II) acetate catalysed vinyl transetherification.[21] Tables 6 and 7 record the ^1H and ^{13}C NMR spectroscopic data and the EI MS molecular peaks of the compounds prepared. Consistent with the N1-alkoxyalkylation, the UV maxima and minima of all the compounds closely resembled those of uridine under both neutral and alkaline conditions. It should be noted that N3-alkylated uracils absorb in aqueous alkali at wavelengths 20 nm higher than do the N1-alkylated ones.[22]

Table 5 First-order rate constants for the acid-catalysed hydrolysis of some nucleosides, their C3′-alkylated derivatives, and acyclic secocounterparts

Compd.	T/K	$[H^+]$/mol dm^{-3}	$k/10^{-3}$ s^{-1}	
Adenosine (**1a**; X = OH)	363.2	0.10	0.450	±0.005[a]
C3′-Methyl adenosine (**14a**)			0.486	0.012
C3′-Ethyladenosine (**14b**)			0.528	0.030
2′,3′-Secoadenosine (**13**)			3.58	0.05
2′-Deoxyadenosine (**1a**, X = H)	323.2	0.10	3.19	0.03[b]
2′-Deoxy-3′,4′-secoadenosine (**12a**)			1.224	0.010
Thymidine (**1d**; X = H)	363.2	1.0	0.089 1	0.001 1
3′,4′-Secothymidine (**12b**)			0.357	0.02
Uridine (**1e**; X = OH)	363.2	1.0	0.001 00	0.000 02
C3′-Ethyluridine (**14c**)			0.000 169	0.000 011

[a] Ref. 18. [b] Ref. 2.

Table 6 ^{1}H NMR chemical shifts of 1-(1-alkoxyethyl)uracils in C^2HCl_3[a]

Compd.	N3-H	H5	H6	H1′	H2′	OR
3a	9.49 s	5.81 d	7.45 d	5.95 q	1.43 d	1.12 d; 1.21 d; 3.65 m
3b	9.23 s	5.81 d	7.40 d	5.85 q	1.45 d	1.21 t; 3.48 q
3c	9.38 s	5.83 d	7.37 d	5.76 q	1.46 d	3.31 s
3d	9.47 s	5.81 d	7.46 d	5.88 q	1.49 d	3.36 s; 3.64 t; 3.88 t
3e	9.59 s	5.84 d	7.45 d	5.94 q	1.51 d	3.6 m–3.8

[a] Given as ppm from internal Me_4Si.

Table 7 ^{13}C NMR chemical shifts and EI MS molecular peaks of 1-(1-alkoxyethyl)uracils.[a]

Compd.	C2	C4	C5	C6	C1′	C2′	OR	m/z
3a	150.9	163.6	103.2	139.2	79.7	22.9	21.4; 22.0; 70.6	198
3b	150.9	163.4	103.2	138.9	82.0	21.5	14.9; 64.7	184
3c	151.1	163.4	103.4	138.7	83.6	21.1	56.5	170
3d	151.3	163.6	103.1	139.1	82.5	21.4	59.1; 68.4; 71.2	214
3e	151.0	163.4	103.4	138.8	81.8	21.1	42.2; 68.9	218

[a] ^{13}C NMR shifts as ppm from internal Me_4Si in C^2HCl_3.

Table 8 ^{1}H NMR chemical shifts of 1-alkoxymethyluracils in C^2HCl_3[a]

Compd.	N3-H	H5	H6	H1′	OR
4a	9.12 s	5.78 d	7.36 d	5.17 s	1.18 d; 3.80 m
4b	9.48 s	5.78 d	7.34 d	5.16 s	1.22 t; 3.61 q
4c	9.20 s	5.79 d	7.31 d	5.12 s	3.41 s
4d	8.50 s	5.77 d	7.34 d	5.22 s	3.37 s; 3.54 m; 3.75 m
3e	9.99 s	5.82 d	7.39 d	5.24 s	3.65 t; 3.88 t

[a] Given as ppm from internal Me_4Si.

1-Alkoxymethyluracils (**4a–e**) were prepared by a one-pot reaction from trimethylsilylated uracil and appropriate alkoxymethyl methyl sulfides *via* transient displacement of the methylthio group with bromine. A modified Pummerer reaction[23] was used to obtain the alkoxymethyl sulfides used as starting materials. Accordingly, the appropriate alcohol (0.10 mol) was treated with a mixture of Me_2SO (0.47 mol), acetic anhydride (0.23 mol) and acetic acid (0.12 mol) for 2 days at room temperature. The reaction was stopped by addition of solid sodium carbonate, diluted with water, and the product was extracted with diethyl ether. Distillation of the crude product at normal pressure gave the desired alkoxymethyl methyl sulfide in about 20% yield. The product obtained was then treated with 1 equiv. of *N*-bromosuccinimide in dry 1,2-dichloroethane (30 min), the silylated uracil (1 equiv.) was added, and the product was isolated after half an hour by a normal aqueous sodium hydrogen carbonate work-up. The alkoxymethylated uracils (**4a–e**) were purified by silica-gel chromatography (Merck silica gel 60) eluting with 0.5–3% methanol in dichloromethane. The yield varied from 30 to 60%. Tables 8 and 9 record the ^{1}H and ^{13}C NMR spectroscopic data and the EI MS molecular peaks of the compounds prepared. As for the alkoxyethyl derivatives, they all were UV spectroscopically identical with uridine.

The preparation of 2′,3′-secoadenosine[24] (**13**), 3′,4′-secothymidine[25] (**12b**) and 2′-deoxy-3′,4′-secoadenosine[26] (**12a**) has been described previously. Compound **12a** was a generous gift from Prof. Florentiev. 3′-*C*-Ethyladenosine (**14b**) was obtained by glycosylation of N^6-bis(trimethylsilyl)adenine (5 mmol) with 1,2,3-tri-*O*-acetyl-5-*O*-benzoyl-3-*C*-ethyl-α,β-D-ribofuranose[27] in boiling 1,2-dichloroethane (40 cm^3; 2 h), using tin tetrachloride (1 equiv.) as the catalyst. The fully acylated **14b** was, after usual work-up and silica-gel chromatography, deprotected by methanolic ammonia. The overall yield was 43%. M.p. 142–143 °C, λ_{max} at pH = 1258 nm (ε = 14 300 dm^3 mol^{-1} cm^{-1}); λ_{max} at pH = 7–13 260 nm (ε 14 500 dm^3 mol cm^{-1}); δ 8.51 (s, H 8), 8.26 (s, H 2), 6.02 (d, H 1′), 4.61 (d, H 2′), 4.29 (t, H 4′), 3.77 (m, H 5′), 1.74 (q, CH_2) and 1.00 (t, CH_3). 3′-*C*-Methyladenosine[28] (**14a**) and 3′-*C*-ethyluridine[27] (**14c**) were synthesized as described earlier.

Table 9 ^{13}C NMR chemical shifts and EI MS molecular peaks of 1-alkoxymethyluracils[a]

Compd.	C2	C4	C5	C6	Cl′	OR	*m/z*
4a	151.0	163.4	103.1	143.1	74.4	22.2; 71.1	184
4b	151.2	163.6	103.2	143.2	76.5	15.0; 65.2	170
4c	151.2	163.4	103.3	143.1	78.0	57.1	156
4d	151.4	163.7	103.3	143.4	87.4	59.1; 69.1; 71.6	200
4e	151.4	163.9	103.5	143.3	76.7	42.7; 69.8	204

[a] ^{13}C NMR shifts as ppm from internal Me_4Si in C^2HCl_3.

Kinetic Measurements.—Reactions were followed by the HPLC method described previously.[29] Reaction mixtures were thermostatted in a stoppered flask in a water bath, the temperature of which was maintained to within ±0.1 K. The initial substrate concentration was 5×10^{-4} mol dm^{-3}. The compositions of the samples withdrawn at suitable intervals were analysed by HPLC on a Hypersil ODS5 column (4 × 250 mm, 5 μm). An acetic acid–sodium acetate buffer (pH 4.2), containing 0.1 mol dm^{-3} ammonium chloride and 0–8% (v/v) acetonitrile, was employed as the eluent. Within each kinetic run, the signal areas of the starting material and all the reaction products and intermediates were assumed to be proportional to their mole fractions, since the base moieties of the compounds remained unchanged. The reaction products were assigned by spiking with the authentic samples.

The hydrogen chloride solutions were prepared from commercial standards (J. T. Baker). The hydronium-ion concentrations of the acetate buffers under the experimental conditions were calculated with the aid of the literature data.[30]

The rate constants were calculated by applying the integrated first-order rate equation to the disappearance of the starting material. With nucleoside 3′,5′-cyclic monophosphates, the ratio of the rates of hydrolysis of the *N*-glycosidic bond and the phosphodiester group was determined on the basis of the product distribution at the early stages of the reaction.

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