Reaction of Acetyl Hypofluorite with Pyrimidines. Part 3.[1] Synthesis, Stereochemistry, and Properties of 5-Fluoro-5,6-dihydropyrimidine Nucleosides

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The reaction of acetyl hypofluorite (AcOF) with unprotected uracil and cytosine nucleosides in acetic acid or water has been studied using ^{18}F as a tracer. For the nucleosides in general two *cis*-diastereoisomers of both the 6-acetoxy-5-fluoro and 5-fluoro-6-hydroxy adducts were obtained, ^1H n.m.r. analysis of which showed that they all possessed the *anti*-conformation. The 6-acetoxy-5-fluoro adducts of the uracil nucleosides showed a remarkable stability and appeared to be interesting versatile compounds. They could be converted into their hitherto unknown corresponding 5-fluoro-6-hydroxy-O^6,5′-anhydrocyclouracil nucleosides. For the cytosine nucleosides the 6-acetoxy-5-fluoro adducts were not observed, while the other cytosine adducts were found to rapidly deaminate at C-4 in water yielding the corresponding uracil analogues. Interestingly, even within a pair of diastereoisomers different deamination rates were observed.

Until 1976 the synthesis of 5-fluorinated pyrimidine nucleosides involved *de novo* construction of the base from small aliphatic precursors. In 1976 Robins *et al*.[2] reported the direct fluorination of cytosine and uracil nucleosides using CF_3OF. However, fully protected starting material is necessary for this process while a second drawback is the decreasing availability of CF_3OF in Europe. Recently we reported on the reaction of acetyl hypofluorite (AcOF) with uracil and cytosine[3] and some of their *N*-1 alkyl substituted derivatives.[1] It appeared to be unnecessary to protect the NH function at C-4, while the presence of hydroxy groups does not necessarily affect the fluorination when AcOF is used,[4,5,6] possibly eliminating the requirement of the use of protected nucleosides.

On reaction of AcOF with enamides it was found that to a certain degree[1] a cationic intermediary is formed. Therefore, using unprotected uracil nucleosides, in acetic acid the 5′-OH group of the sugar moiety might compete with the incoming nucleophile leading to 5-fluoro-6-hydroxy-O^6,5′-anhydrocyclouracil derivatives. A similar reaction has been observed on fluorination of O^6-cyclouracil arabinoside and xyloside with F_2 in acetic acid[7] and during iodination of deoxycytidine and thymidine.[8] On the other hand, the 6-acetoxy-5-fluoro adducts of *N*-1-alkyl substituted uracils proved to be rather stable compounds. They could be isolated by column chromatography and be converted into the corresponding 5-fluorouracils by treatment with triethylamine (TEA).[1] The unprotected 6-acetoxy-5-fluorouracil nucleosides could possibly be converted into O^6,5′-cyclo derivatives with the aid of a Lewis acid. From a biological point of view the 5-fluoro-6-hydroxy-O^6,5′-anhydrocyclouracil nucleosides might be an interesting new class of prodrugs for the toxic 5-fluorouracil, especially in view of the promising results obtained with 5′-deoxy-5-fluorouridine.[9]

In this paper the stereochemistry of the reaction of AcOF with several unprotected uracil and cytosine nucleosides [(**1**), (**15**)] and the conversions of the corresponding intermediaries is described. In order to simplify the detection of the several intermediaries most of the experiments were carried out using ^{18}F as a tracer.

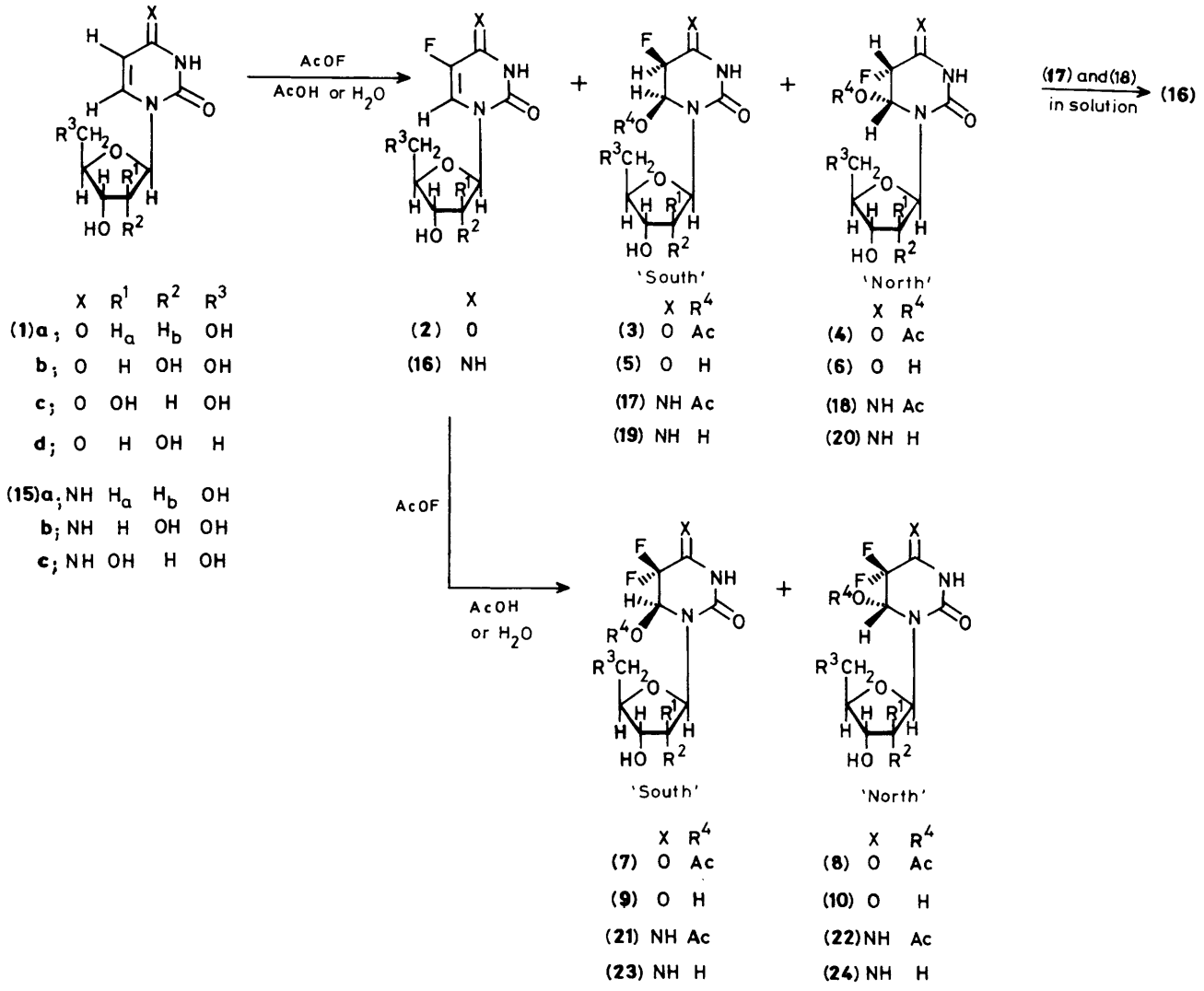
Results and Discussion

The results of the reaction of gaseous AcOF with the pyrimidine nucleosides (**1**) and (**15**) are given in Scheme 1 and Tables 1, 2, and 3. In general, the overall yield of the reaction was 70—85%. In the fluorination of the uracil nucleosides (**1**) using acetic acid as solvent the two 6-acetoxy-5-fluoro adducts (**3**) and (**4**) were obtained as the main products in a ratio of about 2:1. In the fluorination of ARA-U (**1c**) in acetic acid a by-product identified as a 5-fluoro-6-hydroxy-O^6,2′-anhydrocyclonucleoside (**13c**) was found, but no O^6,5′-cyclonucleosides were observed for any of the substrates (**1**). This means that in acetic acid the 5′-OH of (**1**) does not compete with the acetoxy anion, whilst the 2′-OH of the arabinofuranosyl moiety only partly competes. In water, the expected 5-fluoro-6-hydroxy adducts (**5**) and (**6**) were formed in a ratio of about 1.4:1; now for (**1c**) no 2′-cyclonucleoside was observed. However, as with the *N*-1 alkyl substituted uracils,[1] the formation of the hydroxy adducts (**5**) and (**6**) was accompanied by the formation of a relatively large amount (ratio OH:OAc adduct 1.5, Table 2) of the corresponding acetoxy isomers (**3**) and (**4**). Similar results [(**7**)—(**10**)] were obtained when the 5-fluorouracil nucleosides (**2**) were used as starting material (Table 2); the mechanistic aspects of this phenomenon have been discussed recently.[1]

The small $J_{5F,6H}$ coupling constants observed for compounds (**3**) and (**4**) and compounds (**5**) and (**6**) [for a comparison, those of the difluoro analogues (**7**)—(**10**) are included in Table 1] establish the *cis* stereochemistry of each adduct. However, it is of note that on h.p.l.c. analysis using ^{18}F as a tracer the radioactive peaks belonging to the *cis*-diastereoisomers were followed by small peaks (<2%) that could not be isolated. In line with the chemistry found for uracil[3] and the *N*-1-alkyl substituted uracils[1] these small peaks most probably belong to the 'missing' *trans*-diastereoisomers.

As with the *N*-1-alkyl uracils, the adducts (**3**) and (**4**) were rather stable compounds and could be isolated by column chromatography. They appeared to be interesting versatile compounds (Scheme 2). With TEA they could easily be converted into their analogous 5-fluoro compounds (**2**). In water at room temperature each acetoxy diastereoisomer underwent a slow solvolytic elimination reaction (a few % a day) leading to a mixture of the 5-fluoro compound and its corresponding 5-fluoro-6-hydroxy diastereoisomer; heating at 60 °C enhanced this solvolytic elimination reaction, different rates being observed for the individual diastereoisomers (*vide infra*).

When the Lewis acid $FeCl_3$ in wet MeCN was used, the



	X	R^1	R^2	R^3
(1)a;	O	H_a	H_b	OH
b;	O	H	OH	OH
c;	O	OH	H	OH
d;	O	H	OH	H
(15)a;	NH	H_a	H_b	OH
b;	NH	H	OH	OH
c;	NH	OH	H	OH

	X
(2)	O
(16)	NH

	X	R^4
(3)	O	Ac
(5)	O	H
(17)	NH	Ac
(19)	NH	H

	X	R^4
(4)	O	Ac
(6)	O	H
(18)	NH	Ac
(20)	NH	H

	X	R^4
(7)	O	Ac
(9)	O	H
(21)	NH	Ac
(23)	NH	H

	X	R^4
(8)	O	Ac
(10)	O	H
(22)	NH	Ac
(24)	NH	H

Scheme 1.

conversion of (**3**) and (**4**) into the diastereoisomers (**5**) and (**6**) occurred—with retention of conformation—almost instantaneously. Interestingly, treatment with $FeCl_3$ in dry MeCN resulted in the formation of the corresponding diastereoisomeric O^6-cyclonucleosides (**11**)—(**14**) (Scheme 2). This hitherto unknown and possibly biologically interesting new class of fluorinated pyrimidines appeared once formed to be fairly stable and could be isolated by column chromatography. In water at room temperature they were only slowly converted into their 5-fluoro analogues (**2**) with a chemical half life of about 15 days.

In the fluorination of the cytosine nucleosides (**15**) using acetic acid as solvent the 5-fluorocytosine nucleoside (**16**) was the main product; the adducts (**17**) and (**18**) were not observed. As by-products two 6-acetoxy-5,5-difluoro adducts (**21**) and (**22**) in a ratio of about 2:1 were obtained, formed by a consecutive reaction of preformed (**16**) with AcOF. These latter products were stable in acetic acid solution, which illustrates the high acidity of 5-H: the adducts (**21**) and (**22**) are stable with respect to the substituent at C-6 because 5-H is absent and the substituent at N-1 blocks the acylimine formation.[1]

On reaction of AcOF with (**15**) in water the two 5-fluoro-6-hydroxy adducts (**19**) and (**20**) were obtained as the main products. In CD_3CO_2D these adducts appeared to be more stable towards H_2O elimination than the 5-fluoro-6-hydroxy adduct of 1-methylcytosine,[1] but immediately exchanged 5-H for D. For this reason the chemical shifts of 5-H and their corresponding $J_{5F,5H}$ and $J_{5H,6H}$ coupling constants are missing (Table 3).

As was also observed for the adducts of 1-methylcytosine,[1] all the adducts (**19**)—(**24**) [compounds (**23**) and (**24**) were obtained by fluorination of (**16**) in water] appeared to deaminate in water, yielding the corresponding pairs of uracil analogues (**5**)—(**10**). The chemical half life of the difluoro adducts (**21**)—(**24**) was about 2—4 h, that of the 5-fluoro-6-hydroxy adducts (**19**) and (**20**) about 15—50 h. Although compounds (**23**) and (**24**) could not be isolated, their assignment is somewhat more than only tentative. On h.p.l.c. analysis these compounds were detected at 254 nm and were converted into compounds with different h.p.l.c. R_t values; the latter compounds could only be detected at 210 nm and were found to be the corresponding 5,5-difluoro-6-hydroxy uracil counterparts (**9**) and (**10**).

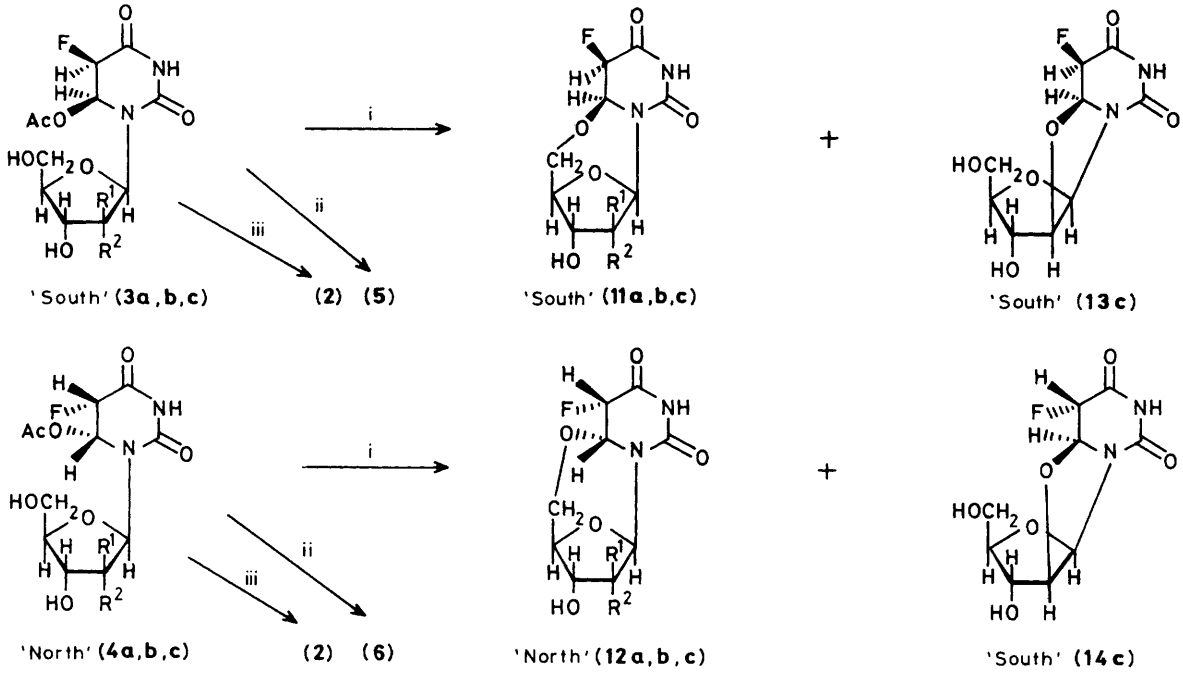
Assignment of the Diastereoisomers.—As already mentioned, as with uracil and its *N*-1-alkyl derivatives, the $J_{5H,6H}$ and $J_{5F,6H}$ coupling constants point to the *cis*-stereochemistry of the F and OR groups, with an axial OR at C-6 due to a favourable anomeric effect with N-1.[1,3]

Pyrimidine nucleosides are known to exist in an *anti*-conformation,[10–13] the 5,6-double bond lying above the sugar

Table 1. Main characteristics of the uracil nucleosides (**1**)—(**14**)[a]

		δ(CD$_3$CO$_2$D)													*b*		
Compound	R_f (min)	6-H	5-H	1′-H	2′-H$_a$	2′-H$_b$	3′-H	4′-H	5′-H$_a$	5′-H$_b$	$J_{5F,5H}$	$J_{5H,6H}$	$J_{5F,6H}$	$J_{5F,1'H}$	6-H	1′-H	$J_{5F_{1,2},6H}$
(**1a**)	7.4	7.93	5.92	6.27	2.47	2.31	4.55	4.08	3.91	3.84	—	8.1	—	—			
(**2a**)	10.1	8.11	—	6.27	2.46	2.29	4.57	4.08	3.94	3.86	—	—	6.6	1.3			
(**3a**) South	22.0	6.83	5.47	6.14	2.38	2.33	4.46	3.99	3.86	3.79	45.3	4.0	3.0	—	6.75	6.09	2.9; 5.9
(**4a**) North	17.7	6.76	5.49	6.16	2.41	2.31	4.47	3.92	3.76	3.69	45.2	4.0	3.0	1.5	6.69	6.19	3.0; 5.7
(**5a**) South	2.8	5.68	5.29	6.18	2.46	2.26	4.51	3.99	3.87	3.80	46.3	3.8	3.6	—	5.62	6.14	3.5; 5.2
(**6a**) North	2.6	5.58	5.29	6.26	2.37	2.30	4.57	3.95	3.88	3.81	46.0	3.7	3.1	1.1	5.48	6.29	2.8; 5.6
(**11a**) South	12.3	5.36	5.18	6.23	2.70	2.35	4.54	4.46	4.13	3.79	46.4	3.8	13.0	—			
(**12a**) North	12.6	5.22	5.11	6.54	2.51	2.44	4.59	4.42	4.26	3.81	46.9	8.9	10.6	2.5			
(**1b**)	5.4	7.94	5.91	5.89	4.41		4.36	4.14	3.98	3.86	—	8.1	—	—			
(**2b**)	8.0	8.17	—	5.91	4.37		4.36	4.15	4.00	3.88	—	—	6.7	0.4			
(**3b**) South	6.7	6.78	5.47	5.70	4.27		4.27	4.04	3.88	3.80	45.3	3.9	3.3	—	6.71	5.69	2.9; 5.9
(**4b**) North	18.7	6.77	5.58	5.78	4.31		4.31	4.01	3.80	3.72	45.2	4.0	3.2	1.3	6.72	5.77	3.0; 5.8
(**5b**) South	2.3	5.67	5.27	5.76	4.50		4.35	4.03	3.94	3.83	46.3	3.9	3.5	—	5.63	5.75	3.5; 5.6
(**6b**) North	1.7	5.57	5.34	5.84	4.36		4.35	4.04	3.92	3.81	46.0	3.8	3.2	1.4	5.50	5.84	3.0; 5.6
(**11b**) South	4.1	5.38	5.27	5.81	4.64		4.41	4.49	4.09	3.79	45.2	4.2	10.5	—			
(**12b**) North	6.4	5.19	5.15	6.15	4.54		4.43	4.49	4.24	3.81	47.0	9.0	11.8	1.7			
(**1c**)	7.7	7.91	5.88	6.19	4.40		4.29	4.10	3.97	3.90	—	8.1	—	—			
(**2c**)	10.1	8.04	—	6.16	4.40		4.30	4.09	3.99	3.91	—	—	6.8	1.5			
(**3c**) South	7.2	6.92	5.44	5.87	4.31		4.23	3.95	3.92	3.86	45.7	3.9	3.1	—	6.83	5.96	2.9; 5.7
(**4c**) North	25.2	6.93	5.57	6.11	4.28		4.23	3.91	3.89	3.81	45.6	4.0	3.3	2.1	7.04	6.06	3.0; 5.2
(**5c**) South	3.0	5.69	5.29	6.07	4.34		4.26	3.90	3.95	3.87	46.0	3.7	3.0	—	5.59	6.06	2.7; 5.5
(**6c**) North	1.9	5.62	5.38	6.19	4.40		4.32	3.88	3.92	3.86	46.4	3.7	3.3	1.8	5.70	6.16	2.8; 4.9
(**11c**) South	3.3	5.37	5.15	6.45	4.50		4.35	4.34	4.25	3.85	47.2	3.2	18.1	—			
(**12c**) North	5.4	5.23	5.28	6.40	4.50		4.38	4.33	4.27	3.81	47.2	8.6	10.1	1.7			
(**13c**) South	8.6	5.64	5.01	6.20	4.88		4.38	4.12	3.83	3.74	49.5	1.8	22.5	—			
(**14c**) South	3.5	5.53	5.13	6.13	4.96		4.40	4.03	3.86	3.75	48.6	8.2	10.1	1.4			
(**1d**)	9.4	7.62	5.95	5.81	4.36		3.97	4.12	1.40	—		8.1	—	—			
(**2d**)	13.5	7.68	—	5.80	4.34		3.96	4.12	1.42	—		—	6.3	1.2			
(**3d**) South	19.5	6.72	5.48	5.64	4.20		3.88	3.99	1.36	45.1		3.8	3.2	—	6.60	5.64	2.7; 6.1
(**4d**) North	24.7	6.70	5.56	5.73	4.36		3.90	3.92	1.34	45.1		3.9	3.5	0.7	6.55	5.74	2.9; 5.9
(**5d**) South	3.6	5.48	5.28	5.71	4.45		3.98	3.99	1.36	46.2		3.9	3.6	—	5.38	5.70	3.5; 5.5
(**6d**) North	1.8	5.47	5.35	5.65	4.35		4.00	4.01	1.35	46.0		3.7	2.8	0.7	5.34	5.65	3.1; 5.2

[a] δ_{OAc} (**3**), (**4**), (**7**), (**8**): 2.09—2.16. [b] Chemical shift and J_{FH} of the corresponding difluoro compound. The chemical shifts of the protons 2′-H—5′H are those of the monofluoro compound and are therefore omitted. The h.p.l.c. retention times of (**7**) and (**8**) are in the order of 40—100 min, those of (**9**) and (**10**) 4—15 min.



Scheme 2. *Reagents and conditions:* i, $FeCl_3$-MeCN(anh.); ii, $FeCl_3$-MeCN(wet); iii, TEA, 70 °C

Table 2. Chemical yield (%) of the fluorination products of (**1**), (**2**) and (**15**), (**16**) using gaseous AcOF

Substrate	Solvent	(**2**)	(**3**) + (**4**) (S/N)	(**5**) + (**6**) (S/N)	(**7**) + (**8**) (S/N)	(**9**) + (**10**) (S/N)
(**1a**,**b**,**d**)	AcOH	1—5	60—70 (2.0)	1—5 (1.4)	—	—
(**1c**)	AcOH	1—5	40—50[a] (1.0)	1—5 (1.2)	—	—
(**1a**,**b**,**d**)	H_2O	1—5	25—30 (2.5)	40—45 (1.4)	—	—
(**1c**)	H_2O	1—2	20—25 (1.1)	45—55 (1.2)	—	—
(**2a**,**b**,**d**)	AcOH	—	—	—	60—75 (2.0)	1—5 (0.6)
(**2c**)	AcOH	—	—	—	50—60[b] (1.0)	1—5 (0.5)
(**2a**,**b**,**d**)	H_2O	—	—	—	25—30 (2.5)	35—45 (0.6)
(**2c**)	H_2O	—	—	—	15—20 (3.0)	50—55 (0.4)

		(**16**) (S/N)	(**17**) + (**18**) (S/N)	(**19**) + (**20**) (S/N)	(**21**) + (**22**) (S/N)	(**23**) + (**24**) (S/N)
(**15a**—**c**)	CD_3CO_2D	50—60	—	1—5	10—15[c] (2.0)	—
(**15a**—**c**)	H_2O	1—5	—	60—70 (0.9)	—	—
(**16a**—**c**)	CD_3CO_2D	—	—	—	60—70[c] (2.0)	—
(**16a**—**c**)	H_2O	—	—	—	15—25 (3.0)	40—50 (0.4)

[a] Compound (**13c**) was formed as a by-product; (**3c**):(**4c**):(**13c**) = 1:1:1. [b] A 5,5-difluoro-O^6,2′-cyclonucleoside was formed as a by-product: 6-H 5.67, 1′-H 6.23, 2′-H 4.93, 3′-H 4.41, 4′-H 4.19, 5′-H_a 3.84, 5′-H_b 3.74; $J_{5F_1,6H}$ 15.9 Hz, $J_{5F_2,6H}$ 2.7 Hz, $J_{1'H,2'H}$ 4.2 Hz, $J_{2'H,3'H}$ 1.5 Hz, $J_{3'H,4'H}$ 3.7 Hz, $J_{4'H,5'Ha}$ 4.9 Hz, $J_{4'H,5'Hb}$ 6.0 Hz $J_{5'Ha,5'Hb}$ 11.9 Hz. [c] N.m.r. analysis of the crude reaction mixture indicated the formation of a 5,5-difluoro-O^6,2′-cyclonucleoside. In water this compound rapidly deaminated to its ARA-U analogue.

Table 3. Characteristics of the cytosine nucleosides (**15**)—(**22**)[a]

		δ(CD_3CO_2D)[b]										
Compound	R_f (min)	6-H	5-H	1′-H	2′-H_a	2′-H_b	3′-H	4′-H	5′-H_a	5′-H_b	$J_{5H,6H}$	$J_{5F,6H}$	$J_{5F,1'H}$
(**15a**)	6.3	8.19	6.20	6.22	2.53	2.31	4.55	4.13	3.93	3.85	7.8	—	—
(**16a**)	8.5	8.20	—	6.22	2.53	2.26	4.52	4.09	3.95	3.86	—	6.5	0.8
(**19a**) South	2.0	5.74	—	6.15	2.45	2.25	4.50	3.99	3.88	3.81	—	3.8	—
(**20a**) North	1.8	5.64	—	6.22	2.41	2.29	4.54	3.96	3.88	3.80	—	3.4	0.7
(**21a**) South	23.6	6.73	—	6.09	2.21	2.19	4.42	3.99	3.84	3.78	—	2.9; 5.9	—
(**22a**) North	35.9	6.67	—	6.18	2.23	2.19	4.45	3.94	3.76	3.70	—	2.9; 5.9	0.6
(**15b**)	3.8	8.19	6.18	5.87	4.38		4.34	4.17	4.00	3.88	7.7	—	—
(**16b**)	5.6	8.26	—	5.82	4.31		4.31	4.16	4.03	3.89	—	6.7	0.3
(**19b**) South	1.7	5.73	—	5.72	4.47		4.35	4.05	3.93	3.83	—	4.0	—
(**20b**) North	1.5	5.66	—	5.78	4.35		4.35	4.05	3.92	3.81	—	2.7	1.0
(**21b**) South	7.2	6.67	—	5.62	4.24		4.24	4.04	3.88	3.79	—	3.0; 5.6	—
(**22b**) North	14.3	6.70	—	5.69	4.30		4.25	4.02	3.82	3.73	—	2.9; 6.3	0.8
(**15c**)	5.8	8.08	6.14	6.19	4.42		4.29	4.14	3.97	3.90	7.2	—	—
(**16c**)	7.4	8.12	—	6.16	4.47		4.26	4.10	3.99	3.91	—	6.7	1.5
(**19c**) South	2.1	5.72	—	6.06	4.35		4.25	3.89	3.96	3.88	—	3.3	—
(**20c**) North	1.6	5.67	—	6.16	4.38		4.29	3.87	3.90	3.84	—	3.4	2.0
(**21c**) South	17.2	6.85	—	5.89	4.27		4.14	3.93	3.90	3.84	—	3.2; 5.3	—
(**22c**) North)	21.6	6.97	—	6.04	4.27		4.20	3.93	3.85	3.79	—	3.2; 5.1	1.1

[a] The magnitude of the sugar coupling constants of each cytosine nucleoside was within the range of its corresponding uracil nucleoside and is therefore not included. [b] δ_{OAc}(**21**), (**22**):2.09—2.15.

ring. When the chemical shifts of 6-H in the adducts are compared with those of uracil and its *N*-1-alkyl derivatives,[1,3] it is clear that in each diastereoisomer, 6-H is deshielded. Thurber[14] reported that this deshielding is due to an interaction of 6-H with the ring oxygen of the sugar moiety, which means that each individual diastereoisomer still possesses the *anti*-conformation. In addition, the 2′-H and 3′-H protons have been reported[10,11] to undergo a significant deshielding when going from the *anti*- to the *syn*-conformation; this is not observed for our adducts.

Given the *anti*-conformation with the C-6 substituent in an axial position with respect to the pyrimidine ring, models show

Table 4. Coupling constants of the uracil nucleoside sugar protons

Compound	$J_{1'H,2'H}$	$J_{2'H,3'H}$	$J_{3'H,4'H}$	$J_{4'H,5'H_a}$	$J_{4'H,5'H_b}$	$J_{5'H_a,5'H_b}$	$J_{1'H,2'H_a}$	$J_{1'H,2'H_b}$	$J_{2'H_a,3'H}$	$J_{2'H_b,3'H}$	$J_{2'H_a,2'H_b}$
(**1a**)—(**10a**)	—	—	2.8—4.3	2.9—3.4	3.5—4.8	12.0—12.3	6.1—6.8	6.6—7.8	6.2—6.9	3.3—5.4	13.9—14.0
(**11a**)	—	—	0	0	2.4	12.8	1.7	7.1	7.1	3.1	14.7
(**12a**)	—	—	0	0	2.3	12.8	2.2	8.4	7.1	1.4	14.8
(**1b**)—(**10b**)	3.0—5.9	3.4—6.2	2.5—4.9	2.5—3.2	3.2—4.1	12.2—12.6					
(**11b**)	0	5.9	1.9	0	2.2	12.7					
(**12b**)	1.6	6.1	1.9	0	2.1	12.9					
(**1c**)—(**10c**)	4.6—5.9	3.3—4.3	4.2—5.8	3.0—4.8	3.4—5.3	12.1—12.4					
(**11c**)	6.7	1.7	1.1	0	1.9	12.8					
(**12c**)	6.7	2.5	1.4	0	2.0	12.8					
(**13c**)	4.4	1.6	3.9	4.2	6.0	12.2					
(**14c**)	4.8	2.6	5.4	3.8	5.2	12.4					
(**1d**)—(**10d**)	3.6—4.7	4.7—5.8	5.3—6.6	5.1—6.3		—					

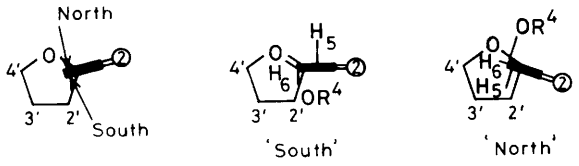


Figure 1. Simplified presentation of the conformation of the nucleoside adduct after the attack of AcOF from the South and the North side

that attack of AcOF from the front side [*i.e.*, over C-2′, further called South attack, (Figure 1)] results in a conformation where 6-H lies over the C-1′–O bond, whilst 5-H lies outside the sugar ring. Therefore, 5-H will not undergo any deshielding. Furthermore, as a consequence of the substituent at C-6, the pyrimidine ring is pushed into a slightly more equatorial position, driving 1′-H into a more axial position and resulting in an upfield shift for this proton.

In the case of an attack of AcOF from the back side (*i.e.*, over the sugar ring oxygen, further called North attack), the pyrimidine ring carries out a tiny clockwise rotation around the glycosidic bond as a consequence of the oxygen-oxygen repulsion between the sugar oxygens and the substituent at C-6. As a result, 5-H lies slightly over the sugar ring (pointing towards C-2′), 6-H lies over the sugar ring oxygen, but less ideally than in the case of the South attack, whilst 1′-H is kept more or less in its original position. Therefore, for the North attack a small deshielding of 5-H is expected; for 6-H a high deshielding but less than in the case of the South attack, while the chemical shift of 1′-H will be more or less unaltered. On the basis of these model studies the diastereoisomers are assigned as given in Tables 1 and 3, and in Figure 1 the situation is visualized; in this figure the dihedral angle between the C-6–N-1 and the C-5–C-4 bond (the 'ring puckering') is omitted.

It appears that in contrast to the South diastereoisomers, the North diastereoisomers all exhibit a detectable $J_{5F,1'H}$ long range coupling[15] probably as a consequence of the 'ideal' W-conformation between F and 1′-H. As already mentioned in the North diastereoisomers 5-H points towards C-2′. This implies that the amount of deshielding of 5-H for the North adducts is dependent on the differences in the sugar moiety and will descend in the order (**1c**) (OH up) > (**1b, d**) (OH down) > (**1a**) (no OH), which actually is found. In turn, for the South adducts of (**1b**), (**1d**), and (**15b**) it can be expected that the substituent at C-6 will have an effect on 2′-H. It appears that consequently for their corresponding mono- and di-fluorohydroxy adducts a small downfield shift of 2′-H is observed in comparison with their North counterparts, and for their corresponding acetoxy adducts a small upfield shift. Proton 2′-H_a of compounds (**1a**) and (**15a**) follows the same trend.

For all South adducts the chemical shift of 1′-H is lower than that of the North counterparts with one exception—the hydroxy adducts of (**1d**). Obviously this is a consequence of the absence of 5′-OH, by preference lying over the sugar moiety,[10] in combination with the fact that the hydroxy group is small compared to the acetoxy group.

As in the work of Schweizer,[11] the differences in the individual sugar proton coupling constants of each adduct might be used to draw conclusions on the conformation of the sugar moiety, for instance on shifts in the equilibrium away from the 2′-*endo* conformation or changes in several rotamer populations. However, we feel that this would become too speculative especially because in order to remove all hydroxy and amine chemical shifts our spectra were recorded in CD_3CO_2D instead of DMSO, and therefore lacked good references. For that reason in Table 4 only the range of the sugar proton coupling constants found for all adducts from a specific substrate are given. However, it is of note that in all cases $J_{4'H,5'H_a}$ was about 0.4—1 Hz smaller than $J_{4'H,5'H_b}$. In line with Dugas[10] this might indicate for all adducts a preference of the exocyclic CH_2OH for the non-classical gauche-gauche rotamer (Figure 2).

Difference in Chemical Properties of the Diastereoisomers.—Once the different diastereoisomers are assigned it can be concluded that in most of the cases AcOF shows a distinct preference for attack from the South side. In acetic acid, the S/N ratio of the formed acetoxy isomers (**3**)/(**4**) is about 2; in water, where the formation of hydroxy adducts (**5**) and (**6**) is accompanied by the formation of acetoxy adducts (**3**) and (**4**), the S/N ratio of the hydroxy adducts (**5**)/(**6**) is about 1.4, in the latter case the ratio of the acetoxy adducts (**3**)/(**4**) is even higher (about 2—3; Table 2). Most probably the sugar ring oxygen is responsible for this preference for the South attack. There seems to be one exception—the acetoxy adducts of ARA-U (**1c**) are formed in equal amounts. However, here the formation of the South O^6,2′-cyclonucleoside (**13c**) (*vide infra*) is a side-reaction. Therefore, also for (**1c**) the South attack is preferred.

It is not unreasonable to assume that in both solvents the South attack is preferred to the same extent. Thus, from the fact that in water the S/N ratio of the hydroxy isomers is 1.4, while that of the acetoxy isomers is still 2—3, one may conclude that the North intermediary reacts relatively more efficiently with water than its South counterpart. This line is even more pronounced on reaction of AcOF with the 5-fluoro compounds (**2**), yielding the difluoro adducts (**7**)—(**10**). Here the S/N ratio of the acetoxy diastereoisomers is again about 2—3 in acetic acid as well as in water, but that of the hydroxy diastereoisomers is 0.6—0.9, which means that the North hydroxy adducts (**10**) are formed in favour of (**9**). These findings imply that during reaction an open carbocation is more easily formed in the case

of a North attack. Or, as proposed recently in the single-electron-transfer (SET) concept,[1,3,16] it means that the acetoxy radical after the North attack favours a second SET instead of recombination within the intimate ion radical pair. As far as we know such an observation is without precedence and proves once again that the reaction pathways by which AcOF reacts are strongly dependent on the substrate and its environment. In line with these observations, we found that in water at 60 °C, the solvolytic elimination reaction is about twice as fast for the North adducts (**4a**, **b**, **d**) as for their South counterparts, again implying that in the former case a carbocationic intermediary is more easily formed.

For ARA-U (**1c**) the results are slightly different. It appears that in the case of the South attack the open carbocation pathway is favoured, probably due to the upstanding 2′-OH; this is reflected in the formation of a relatively large amount of the South O^6,2′-cyclonucleoside (**13c**), in the higher ratio of OH:OAc adducts in water (2.2 instead of 1.5), and in the fact that for the South acetoxy adduct (**3c**) the solvolysis at 60 °C is a factor of about 1.5 faster than its North counterpart (**4c**).

Another interesting observation with respect to differences in the properties of the diastereoisomers concerns the different stability of cytosine adducts with respect to the deamination at C-4. The chemical half-life for the spontaneous rate of hydrolysis of cytidine (**15b**) in neutral solution at room temperature is calculated to be in the order of years.[17] In water in 8 days 66% hydrolysis of 5,6-dihydrocytidine was found,[18] while the chemical half-life of the same compound in phosphate buffer was *ca.* 105 min;[19] inorganic anions increase the speed of deamination.[20] For the 5-fluoro-6-hydroxy adducts (**19**) and (**20**) we found chemical half-lives of 15—50 h in water. Under the same conditions for the difluoro adducts (**21**)—(**24**) half-lives of 2—4 h were found. This difference can be explained by the electronic effect of the additional fluorine atom.[21] Furthermore, because 5-H is absent in the latter compounds, it is clear that tautomerisation of this proton[1] does not play a crucial role in the deamination. Instead the protonation of N-3[20]—as is also proposed for cytidine deaminase[22]—seems to be the important step. The acetoxy adducts (**21**) and (**22**) did not show an observable difference in the deamination rate. However, the chemical half-lives of the North cytosine hydroxy adducts (**20**) and (**24**) were higher than those of their corresponding South adducts (**19**) and (**23**) respectively; for cytidine (**15b**) there was a small difference, for deoxycytidine (**15a**) and ARA-C (**15c**) the difference was more pronounced (*ca.* a factor of 2). Since within the corresponding diastereoisomers the substituent at C-6 and the steric hindrance around C-4, if any, is the same, it transpires that somehow the electron density at N-3 of the South hydroxy diastereoisomers is higher than their corresponding North adducts. In our opinion this difference in electron density can only originate from a slight difference in the position of the pyrimidine ring with respect to the sugar moiety (Figure 1).

Conformation of the Cyclonucleosides.—The 6-hydroxy-O^6,5′-anhydrocyclonucleosides (**11**) and (**12**). From model studies it can be concluded that for the South acetoxy adducts (**3**) the exocyclic CH_2OH group has to shift to a more axial position for ring closure to occur. After ring closure, the C-5′–O bond points to C-2′. Carrying out the same ring closure for the North acetoxy adducts (**4**) as for the South adducts, the 5′-O atom would lie over the sugar ring oxygen resulting in a strong oxygen-oxygen repulsion. For this reason the pyrimidine ring carries out an anti-clockwise rotation around the glycosidic bond before ring closure. As a result, the pyrimidine ring also has to shift to a fully axial position, while the C-5′–O bond points to C-3′, and 5′-H_a now nearly lies over the sugar ring oxygen, so that in this rigid system a higher downfield shift of

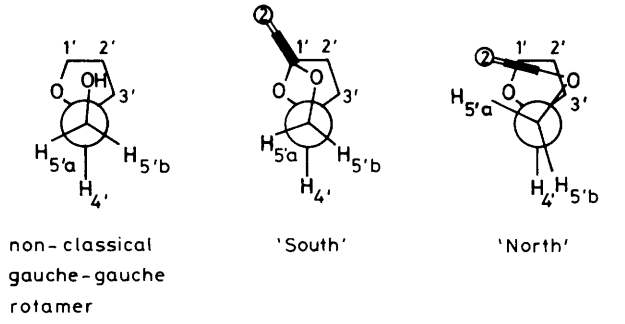


Figure 2. Conformation of the exocyclic CH_2O-group of the South and the North O^6,5′-cyclonucleosides (**11**) and (**12**)

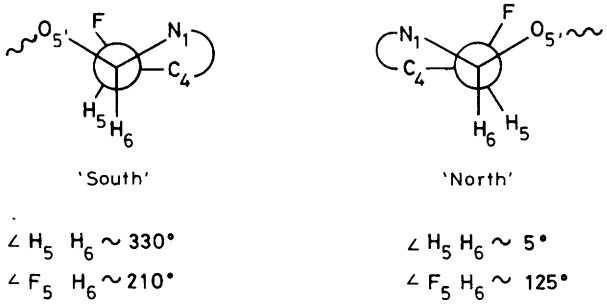


Figure 3. Conformation of the pyrimidine ring of the South and North O^6,5′-cyclonucleosides (**11**) and (**12**)

1′-H and of 5′-H_a than in its corresponding South analogue can be expected (Figure 2).

The n.m.r. spectra confirm this expectation. In both cases 5′-H_a shows the high downfield shift, observed for O^6,5′-cyclonucleosides[7,8], but the O^6,5′-rings (**12**) formed from the North acetoxy adducts (**4**) show the highest deshielding for 5′-H_a. In both cases 2′-H and 3′-H undergo deshielding effects as a consequence of the 5′-O lying over the sugar ring, but for the North O^6,5′-rings (**12a**) and (**12b**) the deshielding of 2′-H (2′-H_a) is lower and that of 3′-H higher than in its South counterpart. The downfield shift of 4′-H originates from its nearly equatorial position. In the North rings (**12**) the nearly equatorial position of 1′-H is reflected in a very high δ-value, the highest of all adducts from a specific substrate (Table 1).

As a result of the 5′-O-ring closure, the conformation of the sugar ring becomes nearly fixed, resulting in the coupling constants shown in Table 4. From the changes in δ_{6H}, δ_{5H} and the $J_{5F,6H}$ and $J_{5H,6H}$ coupling constants, it can be concluded that the preferred conformation of the pyrimidine ring [the substituent at C-6 axial to N-1 (anomer effect)] is changed to another more fixed situation. In this rigid system any conformational change of the pyrimidine ring is linked with a conformational change of the sugar ring. Using Karplus relations, corrected for substituent effects (CAGPLUS),[3] and combining the coupling constants of $J_{5F,6H}$ and $J_{5H,6H}$ with those of the sugar protons it appears that in the case of the South ring (**11**) the sugar ring forms an envelope conformation with C-4′ pointing upwards (4′-*endo* position), while in the case of the North rings (**12**) the sugar ring forms an envelope with the ring oxygen pointing downwards. In this way in both cases the pyrimidine ring is nearly non-puckered while the position of the C-6 substituent (5′-O) is between an axial and equatorial position; the position focussed on 6-H is given in Figure 3. The fact that the position of 6-H has become more axial is reflected in the observation that the δ-values of 6-H are both lower than those of the corresponding hydroxy adducts (**5**) and (**6**); the

South rings (**11**) have the higher δ_{6H} value because of the deshielding effect of the sugar ring oxygen. The same holds for the chemical shifts of 5-H. The more equatorial position of 5-H results in a lower δ-value,[3] while—as is confirmed by the model—in the South rings (**11**) 5-H now undergoes a small deshielding effect of the sugar ring oxygen.

The O⁶,5′-ring (**11c**) shows a small deviation from (**11a**) and (**11b**) with respect to the positions of 5-H and 1′-H. This difference is due to the oxygen-oxygen repulsion between the upstanding 2′-OH group and 5′-O pointing towards the 2′-position. As a result the substituent at C-6(5′-O) takes in a more equatorial position than in (**11a**) and (**11b**) resulting in a somewhat higher $J_{5F,6H}$ (F atom more axially) and a somewhat lower $J_{5H,6H}$ coupling constant, and an upfield shift of 5-H (5-H more equatorially). Other consequences of this small conformational change in (**11c**) are a higher deshielding of 5′-H_a with respect to its (**11a**) and (**11b**) counterparts and a more equatorial position of 1′-H. Interestingly, also for (**12c**) a certain oxygen-oxygen repulsion between the upstanding 2′-OH and 5′-O is reflected in a slight lowering of the $J_{5F,6H}$ and $J_{5H,6H}$ coupling constants and a small downfield shift of 5-H when compared with those of (**12a**) and (**12b**).

The 6-hydroxy-*O*⁶,5′-anhydrocyclonucleosides (**13c**) and (**14c**). For O⁶,2′-ring closure, the pyrimidine ring has to perform an anti-clockwise rotation of some 90° around the glycosidic bond. Furthermore, it appears that the favourable anomeric effect of an axial substituent at C-6 with N-1 has to be given up for the formation of such a five-membered ring. As a result, the pyrimidine ring is rather puckered and conformationally more or less fixed with the F-atom pseudoaxial, leading to a high $J_{5F,6H}$ coupling constant; (**13c**) shows *J* 22.5 Hz. The O⁶,2′-ring (**13c**) shows a high δ-value for 2′-H which is also observed for O²,2′-cyclonucleosides.[23] Most probably because of the loss of the anomeric effect, the spontaneous formation of the O⁶,2′-ring is not a strongly favoured process: in water it is not formed, while in acetic acid the acetoxy adducts are still predominantly formed. Interestingly, only one O⁶,2′-ring is spontaneously formed; no detectable amounts of a second O⁶,2′-ring isomer were observed. With respect to its assignment, ring closure experiments revealed its identity. Upon treatment with $FeCl_3$ in MeCN, the South acetoxy adduct (**3c**) gave the O⁶,2′-ring (**13c**) and the O⁶,5′-ring (**11c**) in nearly equal amounts. For this reason (**13c**) is identified as the South O⁶,2′-ring so the O⁶,2′-ring originating from the North attack does not form spontaneously.

On similar treatment of the North acetoxy adduct (**4c**) two rings were also obtained in nearly equal amounts. The n.m.r. data of the first (R_F 5.60) completely corresponded with those of the North O⁶,5′-rings (**12a**) and (**12b**). The second resembled the O⁶,2′-ring (**13c**) especially with respect to the high δ-value of 2′-H. It still shows the $J_{5F1'H}$, long range coupling characteristic for North adducts, but not the expected high $J_{5F,6H}$ coupling constant; instead a relatively high $J_{5H,6H}$ coupling constant is found. The coupling constants $J_{5F,6H}$ 8.2 Hz and $J_{5H,6H}$ 10.1 Hz fit in with a *trans*-conformation. Therefore, this compound is identified as the South *trans*-O⁶,2′-ring (**14c**). This means that after elimination of the acetoxy group, the *cis*-incorporation of 5′-O [resulting in the North O⁶,5′-ring (**12c**)] is in competition with the *trans* incorporation of 2′-O [resulting in the South trans O⁶,2′-ring (**14c**)]. These results indicate that under these anhydrous conditions new bond formation at C-6 after acetoxy elimination does not proceed with the energetically favourable cisoid (gauche) formation only, as is observed for all the other fluoro adducts.

Experimental

For most of the experiments ^{18}F was used as a tracer which permitted a simple determination of the yields of the different fluorinated products as radiochemical yields using h.p.l.c. techniques. Gaseous [^{18}F]AcOF was produced by passing [^{18}F]F_2 through a column of KOAc–HOAc.[24]

The β-D-pyrimidines (**1**), (**15**) and most of (**2**) and (**16**) were purchased from Sigma; compound (**16a**) was from Calbiochem; compound (**1d**) was synthesized from 2′-3′-isopropylidene-uridine according to Cook *et al.*[9b]; compound (**2d**) was a gift from the Free University hospital. H.p.l.c. was performed on a 20 cm CPTM-Spher C18 column (Chrompack), eluant MeOH–0.1M $NH_4H_2PO_4$ (2:98 v/v), flow rate 2 ml min^{-1}. For (**1d**) and its derivatives MeOH–0.1M $NH_4H_2PO_4$ (5:95, v/v) was the eluant of choice. Prior to injection all products were dissolved in water. Peaks were detected by a radioactivity monitor and u.v. detector (254 nm for the cytidine and 210 nm for the uridine derivatives; Diode Array detector); fractions of 500 μl were collected and assessed for radioactivity. Solvents were evaporated under reduced pressure at 50 °C using a rotary evaporator. ^1H n.m.r. were measured on a Bruker WM-250 spectrometer with CD_3CO_2D as solvent. Chemical shifts are reported in δ (p.p.m.) relative to δ(CD_2HCO_2D) 2.04 p.p.m.

General Procedure for the Fluorination of the Pyrimidines.—Gaseous [^{18}F]AcOF (40—60 μmol) was bubbled through a solution of 100 μmol of substrate in 12 ml of AcOH, CD_3CO_2D or H_2O; CD_3CO_2D was used for direct ^1H n.m.r. analyses of the reaction mixtures. The solvent (acetic acid or water) was evaporated and the unchanged starting material was removed by column chromatography on silica (Lobar Lichroprep Si-60, size A, 40—63 μm, Merck) using the organic phase of a 6:0.5:2 mixture of ethyl acetate, propanol, and water (eluant A) as eluant; the reaction mixtures were applied to the column in 1.5 ml of the organic phase of a 3:2:2 mixture of the same solvents. Separation of the diastereoisomers was performed by reversed-phase column chromatography (Lobar Lichroprep RP-8, size A, 40—63 μm, Merck) using 0.001M $NH_4H_2PO_4$ (eluant B); for the difluoro compounds (**7**) and (**8**) MeOH–0.001M $NH_4H_2PO_4$ (5:95, v/v) was the eluant of choice. After collection of the fractions, the products were desalted by removal of the solvent, dissolution in 2 ml of the organic phase of a 4:1:2 mixture of ethyl acetate, propanol, and water, absorption on a Seppak-SiO_2 cartridge column (Waters), and elution with 10 ml of eluant A. In order to suppress enhanced solvolytic elimination reactions of compounds (**3**), (**4**), (**7**) and (**8**) and deamination of compounds (**19**)—(**24**), the separation and the desalting procedure has to be carried out as quickly as possible.

Uracil Nucleosides.—The acetoxy adducts (**3**), (**4**), (**7**), *and* (**8**). The individual diastereoisomers (**3**) and (**4**) were converted into their corresponding 5-fluoro compounds (**2**) by treatment with triethylamine (TEA) at 70 °C for 30 min as described previously.[25] After purification by column chromatography on silica Si-60 (eluant A), the products (**2a**—**d**) were identified by comparison of their ^1H n.m.r. spectra and h.p.l.c. R_t values (Table 1) with those of authentic samples of (**2**). Solvolytic elimination reactions of (**3**), (**4**), (**7**), and (**8**) in water were measured either at room temperature by a daily h.p.l.c. analysis or at 60 °C every hour.

The hydroxy adducts (**5**), (**6**), (**9**), *and* (**10**). After reversed-phase column chromatography (RP-8) compounds (**9**) and (**10**) were obtained diastereoisomerically pure. However, due to their small difference in retention, compounds (**5**) and (**6**) were not fully separated; only fractions enriched in (**5**) or (**6**) respectively were obtained. Pure diastereoisomers (**5**) and (**6**) were obtained starting from the individual diastereoisomers (**3**) and (**4**) by dissolution of the latter compounds in 2 ml of 0.5M $FeCl_3$ in wet acetonitrile. After 10 min Fe^{3+} was precipitated by the addition of a 10% (w) NaH_2PO_4 aqueous solution, after which the precipitate was removed by centrifugation. The compounds (**5**)

and (**6**) were further purified by column chromatography (RP 8, eluant B). An alternative method involved the dissolution of each individual diastereoisomer (**3**) and (**4**) in 2 ml of 0.5M $NH_4H_2PO_4$ solution and heating at 60 °C for 48 h; compounds (**5**) and (**6**) were then separated from formed (**2**) (RP-8, eluant B).

The cyclonucleosides (**11**), (**12**), (**13c**), *and* (**14c**). The individual diastereoisomers (**3**) and (**4**) were dissolved in an anhydrous solution of 0.5M $FeCl_3$ in acetonitrile. After precipitation and centrifugation (*vide supra*), the compounds were isolated by column chromatography (RP 8, eluant B); overall yield based on (**3**)/(**4**) 70—80%. Their slow conversion in water into (**2**) was measured by daily h.p.l.c. analysis and subsequent ^1H n.m.r. analysis; in Na_2HPO_4 solution the latter conversion appeared to be nearly instantaneous.

The Cytosine Nucleosides.—The directly formed compounds (**16**) from (**15**) were isolated by column chromatography on silica (Si-60, eluant A), and identified by comparison of their ^1H n.m.r. spectra and h.p.l.c. R_t values with those of authentic samples (Table 3). The by-products (**21**) and (**22**) (Table 2) were compared with the products obtained from the reaction of gaseous [^{18}F]AcOF with (**16**) in acetic acid. The compounds (**19**), (**20**), (**21**), and (**22**) were obtained according to the general procedure. As with compounds (**5**) and (**6**), compounds (**19**) and (**20**) were not fully separated; only fractions enriched in (**19**) or (**20**) respectively were obtained. The compounds (**23**) and (**24**) could not be isolated at all. They appeared to be completely converted into the corresponding uracil derivatives (**9**) and (**10**) during the work-up procedure.

For a measurement of the deamination rates of compounds (**19**)—(**24**) in water, the solutions were analysed by h.p.l.c. immediately after reaction and further after each hour; detection at 254 nm was for the measurement of the amount of deamination, detection at 210 nm was for the measurement of the formation of their corresponding uracil derivatives; this conversion was also measured during the first eight hours using ^{18}F as a tracer. In addition, deamination was confirmed by ^1H n.m.r.

Acknowledgements

The authors wish to thank the personnel of the Free University for performing the irradiations, B. W. van Halteren (NIKHEF-K), R. Wedzinga, A. Bijma, and J. Dijksman for technical assistance, and B. van der Berg for drawing the figures.

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Received 9th *October* 1987; *Paper* 7/1803