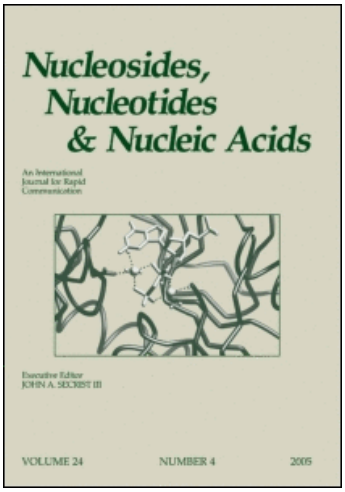

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A DETAILED INVESTIGATION OF THE METHYLATION REACTION OF A 5-BROMOPYRIMIDINE NUCLEOSIDE

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Abstract 5-Bromo-2',2-anhydrouridine was methylated using NaH in DMSO and methyl iodide as methylating reagent. Applying two or more equivalents of the methylating reagent a Br to CH_3 substitution took place at C5 of the pyrimidine ring and besides the N,O-dimethyl-epoxide 9 the N-methyl-5-methyl-dianhydro compound 16 could be isolated[1]. Under drastic conditions a N,O,C-trimethyl-epoxide 15 was formed, the structure of which was determined by X-ray crystallography. Compounds 9, 15 and 16 were formed in different ratios depending on the reaction conditions applied. Based on these facts a mechanism is suggested for this reaction.

In our previous paper it was mentioned[1], that a rearrangement and a bromo-methyl substitution took place, when 5-bromo-2',2-anhydrouridine 1 was treated with methyl iodide in the presence of a base, yielding 5',6-anhydro-1(β-D-2',3'-anhydro-ribofuranosyl)-3,5-dimethyluracil 16 besides the corresponding methylated epoxide 9. For studying the mechanism of this unusual reaction the reaction conditions were systematically altered to determine their influence upon the course of the reaction. In every experiment DMSO was used as solvent, NaH as base, and methyl iodide as reagent, the two latter in equivalent amounts. The following conditions were altered:

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A) the ratio of the nucleoside and the reagents,
B) the time interval between the application of the nucleoside and methyl iodide to the NaH-DMSO solution.

RESULTS AND DISCUSSION

When 1 equivalent of base was applied (a), and 15 minutes elapsed before adding methyl iodide, the N-methylated 2',3'-epoxide 8 could be isolated as the main product (49%) and the corresponding N,O-dimethyl compound 9 was the only byproduct (4%). Increasing the amount of the reagents to 1.5 equivalents (b) led to a dramatic shift in the product distribution, as the amount of the monomethyl derivative 8 was reduced to 9%, and besides the N,O-dimethyl-epoxide 9 (15%) an equal amount of the N-methyl-5-methyl-dianhydro compound 16 (14.4%) could be isolated as a result of the bromo-methyl substitution reaction. When the amount of the reagents was further increased to 2 equivalents (c), the monomethyl derivative 8 could no longer be detected and the ratio of 9 and 16 was shifted towards the latter (1:3). This ratio remained essentially unchanged when 3 or 4 equivalents of the reagents were applied (d,e).

In our further experiments the ratio of the nucleoside and the reagents was kept constant (1:4), but the time of the basic treatment (i.e. the addition of methyl iodide) was changed. When the suspension of the starting material 1 was stirred only for 1 minute (g) in the NaH-DMSO solution a very exothermic reaction took place when the methyl iodide was added, and from the reaction products neither of the epoxides 8 and 9 could be isolated and the C-methyl compound 16 was only formed in traces (1%). Nevertheless under this rather uncontrolled conditions a new C-methyl derivative 15 was formed and isolated (7%), the structure of which was proved by mass spectroscopy and X-ray crystallography. When the methyl iodide was added after 5 min (f) the reaction was somewhat less exothermic and anhydride 16 could already be separated as the main component (12.5%) being accompanied by traces of epoxide 9 (1%) and some of the new C-methyl derivative 15 (3.5%).

Keeping the basic solution of nucleoside 1 for 1 hour (h) before the addition of methyl iodide led to no significant change compared to the 15 min interval, as 9 and 16 were the only products formed in ratio 1:2. DMSO as solvent is not essential for the reaction and could be replaced by DMF giving similar results (i).

TABLE 1

Methylation of 5-bromo-2',2-anhydrouridine

	NaH and CH_3I		Products %			
	quantity (equivalent) (A)	time interval (minute) (B)	8	9	16	15
a)	1.06	15	49	4	-	-
b)	1.59	15	9	15	14.4	-
c)	2.12	15	-	6	16	-
d)	3.18	15	-	9	28	-
e)	4.24	15	-	5	20	-
f)	4.24	5	-	1	12.5	3.5
g)	4.24	1	-	-	1	7
h)	4.24	60	-	8.6	14	-
i)	4.24	15 (DMF)	-	6.8	18	-

From these data the following conclusions could be drawn regarding the reaction mechanism: (Fig. 1). In the presence of a base nucleoside 1 is converted via anion 2 into epoxide 5, which is in equilibrium with the dianhydro intermediate 4 and probably with the double charged anion 6, too.

This equilibrium is base dependent and in the presence of 1 equivalent of base (a) 5 is the dominant anion, which gives on methylation 8. Increasing the amount of the base (b,c,d,e) the equilibrium is shifted towards 6, yielding on methylation 9.

Nevertheless, 9 can be formed via another pathway as well, since 8 formed in the primary process can undergo a fast deprotonation at OH5', yielding ion 11 which would give in a subsequent methylation step 9. The probability of this latter process is backed by the fact, that formation of dianhydride 16 can only be explained via a hypothetic intermediate 10, which can be formed either from 4 via 7 or from 8 via 11.

For supporting this theoretical possibility both N-methyl derivatives 7[1] and 8[1], which were prepared previously, were submitted to further methylation using one equivalent of reagents and following the reaction by t.l.c. Independent of the starting material 9 and 16 were formed in both cases proving the existence of the equilibrium 10 $\rightleftarrows$ 11. It is worthwile mentioning, that the anions formed from 1 under basic conditions are much more stable (4-5 hours) than those, formed from the N-methylated derivatives 7 and 8 (10-15 min) therefore methyl iodide

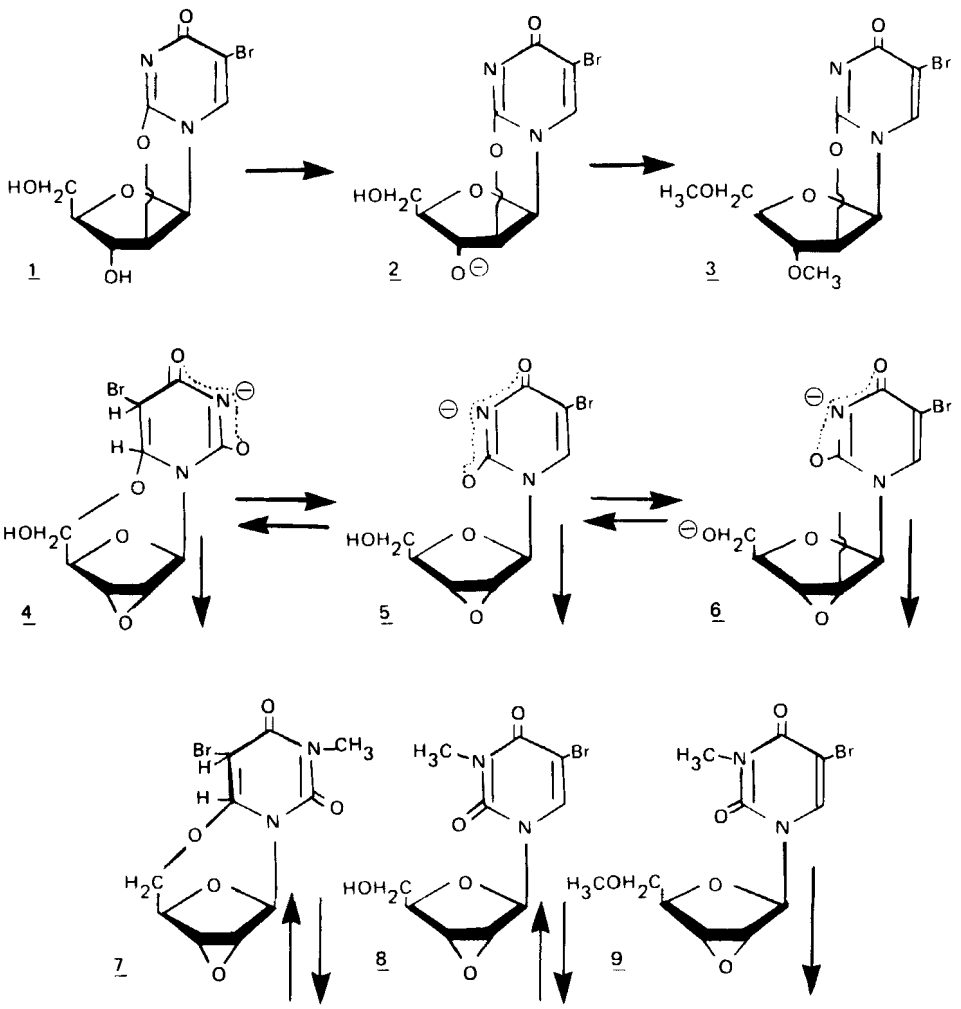

FIG. 1

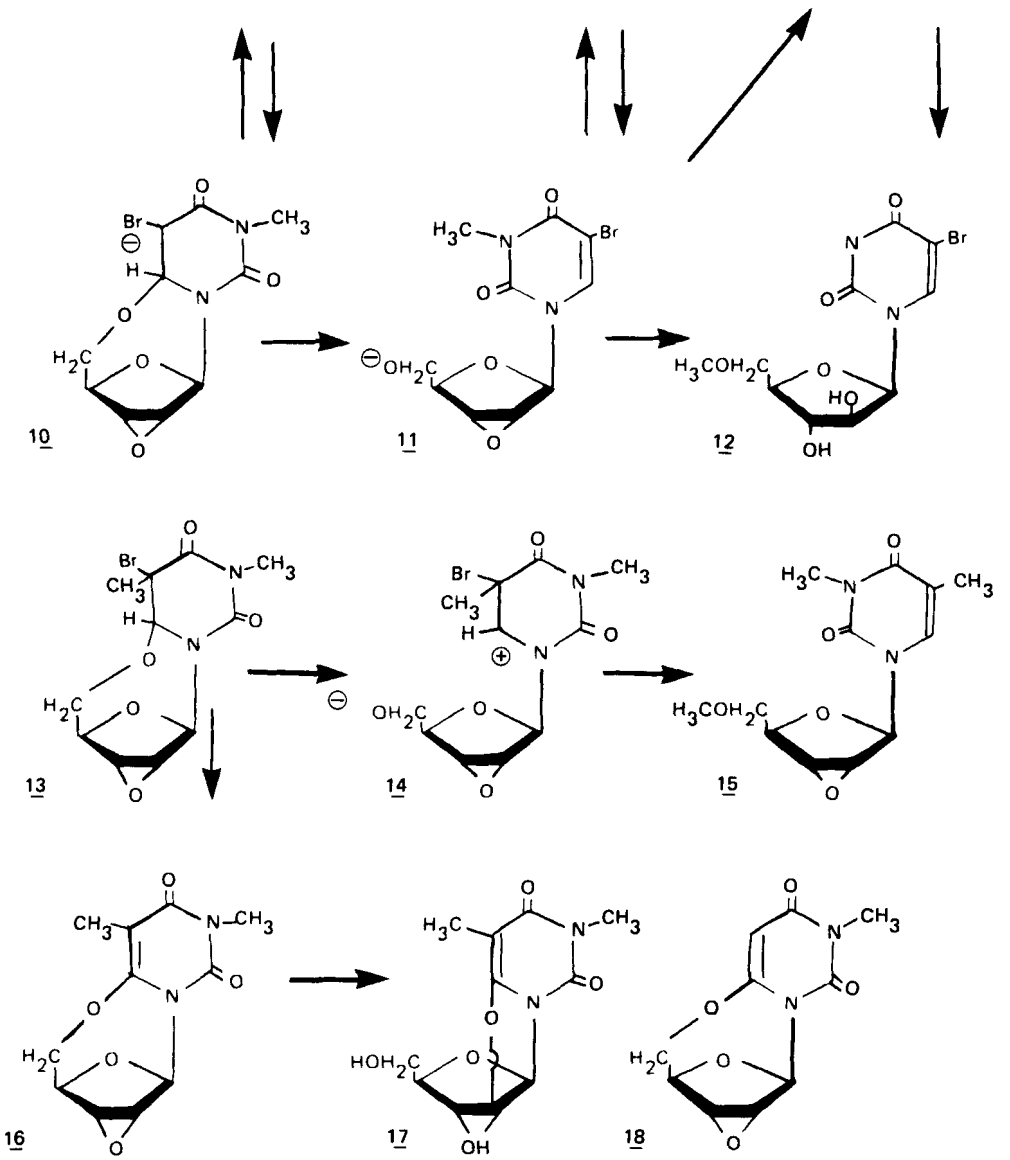

Fig. 1b

had to be given immediately after dissolution. When the amount of the base was increased to 3 equivalents, 9 could no more be detected. That means, that the 10 ⇌ 11 equilibrium is base dependent and is shifted towards 10 when the amount of base is increased.

The fact, that in the case of nucleoside 1 compound 9 was always formed, even in the presence of 4 equivalents of base, suggests, that 9 can be formed not only via 11, but via the hypothetical dianion 6, too, which can exist under strong basic conditions.

A further analysis of this latter reaction afforded two other components, the D-arabinose derivative 12, which can be formed during the workup via a hydrolytic cleavage of epoxide 9, and the 5',6-anhydro derivative 18 which can be formed from 7 by elimination of hydrobromic acid. The 3,5-di-O-methyl derivative 3, could neither be isolated, nor its presence proved by MS-investigation of the crude reaction mixture. For the formation of the N,O,C,-trimethylated epoxide 15 under the applied very drastic conditions (f,g), it was presumed that 13 undergoes a heterolytic ring fission at the O5'-C6 bridge yielding an ion pair which is immediately attacked by a hydride ion as well as by a methyl cation.

In our further experiments we submitted the methylated epoxide 9 and the dianhydro compound 16 to the conditions of methylation mentioned above. While 16 remained unchanged, 9 underwent a rearrangement process and further methylation, affording the methylated 2',6-anhydroarabinoside 25, in which the bromide was exchanged by a methyl group, too. The proposed mechanism of this reaction (Fig. 2) is the following: The carbonyl group of the pyrimidine attacks the oxirane at C2', and the formed zwitterion is immediately methylated at O3', affording cation 19 which might be stable in the presence of the dimethyl-sulphoxonium anion. A slow hydrolysis yields via ketal 20 arabinoside 21, the free OH2' group of which can undergo an addition reaction to the 5,6-double bond of the pyrimidine ring (22) which is activated by the presence of the bromine at C5. This intermediate is methylated at the same carbon atom analogously to the 5',6-anhydro compound 10 giving 24 from which hydrobromic acid is eliminated yielding 25. The oxazolidine ring in 25 is an energetically favored arrangement and is readily formed not only from 5-bromopyrimidine-arabinosides under basic conditions[2], but even under acidic conditions, as treatment of the 5',6-anhydroribo-epoxide 16 with

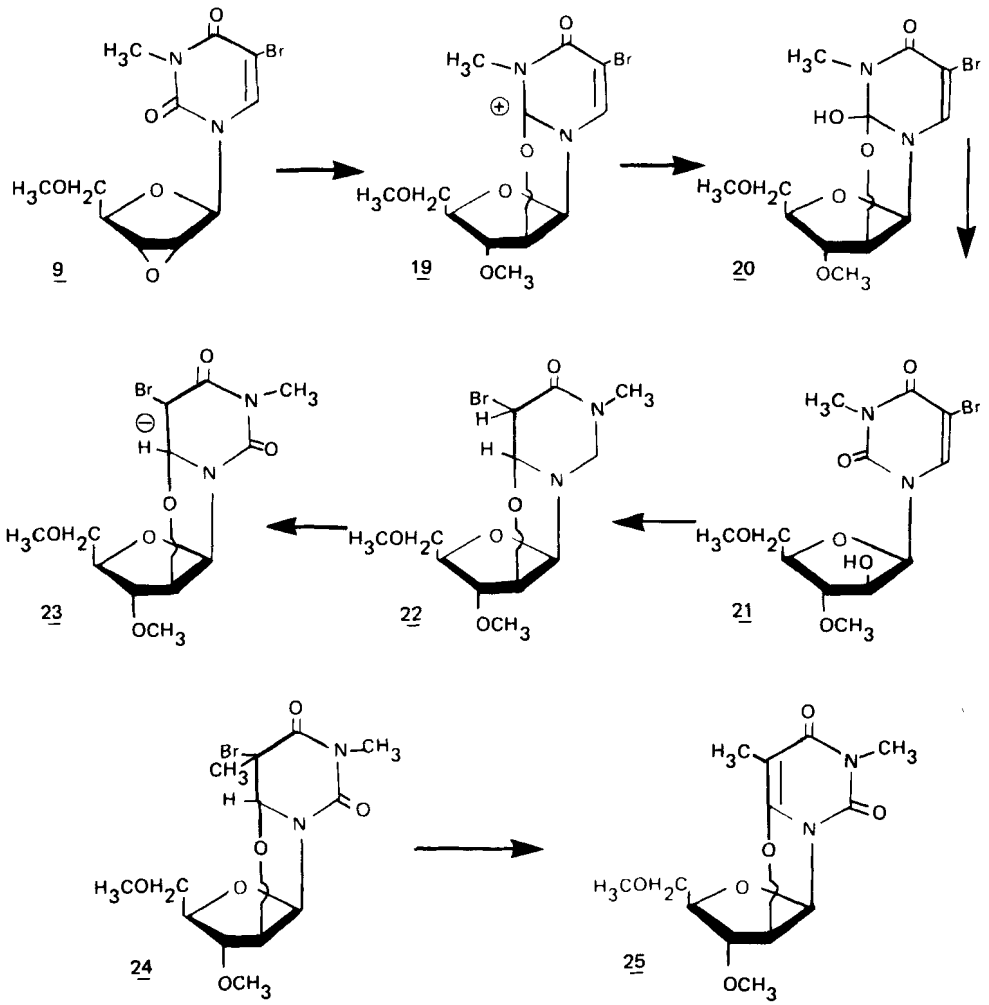

FIG. 2.

1N hydrochloric acid yielded the corresponding 2',6-anhydro-arabinoside 17 via hydrolysis and subsequent cyclization.

Figure 3 shows a view of the X-ray structure of compound 15 computed from the final relative atomic coordinates given with their e.s.d.'s in Table 2. The majority of the bond lengths and angles listed in Tables 3 and 4 agree within experimental error with those observed recently[1] for 3-methyl-2',3'-anhydrouridine and 3,5-dimethyl-2',3':5',6-dianhydro-6-hydroxyuridine (16). The fused oxirane ring shortens both C2'-C3' and C3'-C4' bonds, resulting in a quasi planar sugar ring. The mean deviation of the ring atoms from the least-squares plane (-0.43390X + 0.82628Y -

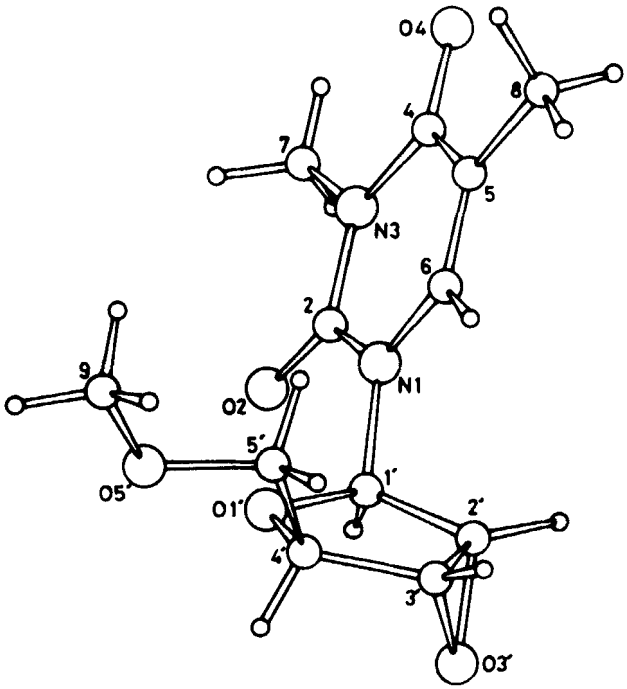

FIG. 3. A perspective view of molecule 15 showing atomic labelling. Bare numbers are for carbon atoms unless indicated otherwise. The H atoms are shown but not labelled.

- 0.35916Z = - 1.23466) is 0.035 Å. The endocyclic bond angles especially at O1' are increased relative to those found in thymidine[3] and related compounds[4]. It is worth noting that the conformation (χ = 86.7(4)^{o}) about the glycosidic C1'-N1 bond is practically identical with that (χ = 88.1(3)^{o}) observed in 3-methyl-2',3'-anhydrouridine[1]. The dihedral angles between the best planes of the pyrimidine base and the planar sugar ring are also similar (71.5^{o} vs 72.0^{o}). This suggests that the sugar ring with planar conformation prefers a torsion angle χ in a narrow range about 87^{o} independently from the substituents on the pyrimidine base and the sugar residue. Even the hydrogen bond O5'-H5'...O4(x-1/2,y+1/2,$\bar{z}$-1/2) formed in the unit cell of 3-methyl-2',3'-anhydrouridine does not influence this arrangement. However, it has an impact on the torsion angles about the C5'-C4' bond. In 15 Ψ = = - 169.6(4)^{o} and O5'-C5'-C4'-O1' = 70.2(3) (-t,+g) while in 3-methyl-

TABLE 2[12]

Atomic coordinates ($\times 10^4$) for non-hydrogen atoms and ($\times 10^3$) for hydrogen atoms with their e.s.d.'s

N1	5471(4)	5744(2)	9727(1)	C9	- 208(8)	2492(3)	8250(2)
C2	4681(5)	6125(2)	10346(1)	C1'	4380(5)	6289(2)	9136(1)
O2	2942(4)	6802(2)	10410(1)	O1'	2228(3)	5688(2)	8867(1)
N3	5968(5)	5676(2)	10889(1)	C2'	6312(5)	6373(2)	8576(1)
C4	7986(5)	4903(2)	10853(1)	C3'	5294(6)	5759(2)	8007(1)
O4	9087(4)	4604(2)	11366(1)	O3'	5159(4)	6956(2)	8017(1)
C5	8567(5)	4486(2)	10190(1)	C4'	2786(5)	5248(2)	8209(1)
C6	7340(5)	4925(2)	9668(1)	C5'	2739(6)	4004(2)	8207(1)
C7	5127(8)	6054(3)	11558(1)	O5'	105(4)	3664(2)	8291(1)
C8	10604(6)	3612(2)	10129(1)				
H6	768(5)	465(2)	921(1)	H9B	70(8)	220(3)	789(2)
H7A	601(9)	566(3)	1185(2)	H9C	60(8)	221(3)	865(2)
H7B	328(8)	592(3)	1158(2)	H1'	387(6)	704(2)	931(1)
H7C	550(8)	683(3)	1162(2)	H2'	816(7)	664(3)	866(2)
H8A	1083(8)	336(3)	964(2)	H3'	636(8)	537(3)	773(2)
H8B	1020(6)	295(2)	1038(1)	H4'	139(7)	551(2)	790(1)
H8C	1218(7)	389(3)	1025(1)	H5'A	355(7)	371(2)	779(1)
H9A	- 205(8)	230(3)	823(2)	H5'B	383(6)	367(2)	857(1)

TABLE 3

Bond lengths (Å) with e.s.d.'s

N1-C6	1.382(3)	C4 -O4	1.224(3)	C2'-O3'	1.445(3)
N1-C2	1.378(3)	N3 -C2	1.380(3)	C3'-C4'	1.486(4)
N1-C1'	1.461(3)	N3 -C7	1.475(3)	C3'-O3'	1.440(3)
C6-C5	1.327(3)	C2 -O2	1.217(3)	C4'-O1'	1.444(3)
C5-C4	1.446(3)	C1'-C2'	1.499(3)	C4'-C5'	1.495(3)
C5-C8	1.489(4)	C1'-O1'	1.428(3)	C5'-O5'	1.427(4)
C4-N3	1.396(4)	C2'-C3'	1.452(3)	O5'-C9	1.420(4)

TABLE 4

Bond angles (°) with e.s.d.'s

C6-N1-C2	121.2(4)	C4 -N3 -C7	118.1(4)	C2'-C3'-C4'	108.2(4)
C6-N1-C1'	121.2(4)	C2 -N3 -C7	116.7(4)	C2'-C3'-O3'	59.9(3)
C2-N1-C1'	117.4(3)	N1 -C2 -N3	115.6(4)	C4'-C3'-O3'	111.5(4)
N1-C6-C5	123.2(4)	N1 -C2 -O2	122.2(4)	C3'-C4'-O1'	105.6(3)
C6-C5-C4	118.8(4)	N3 -C2 -O2	122.2(4)	C3'-C4'-C5'	115.3(4)
C6-C5-C8	123.5(4)	N1 -C1'-C2'	112.1(3)	O1'-C4'-C5'	111.4(3)
C4-C5-C8	117.7(4)	N1 -C1'-O1'	112.0(3)	C1'-O1'-C4'	111.9(3)
C5-C4-N3	115.6(4)	C2'-C1'-O1'	105.6(3)	C2'-O3'-C3'	60.4(3)
C5-C4-O4	124.6(4)	C1'-C2'-C3'	108.0(3)	C4'-C5'-O5'	107.5(4)
N3-C4-O4	119.8(4)	C1'-C2'-O3'	109.6(3)	C5'-O5'-C9'	112.7(4)
C4-N3-C2	125.3(4)	C3'-C2'-O3'	59.6(3)		

2',3'-anhydrouridine $\Psi = 47.7(3)^{o}$ O5'-C5'-C4'-O1' $= -70.9(3)^{o}$ (+g,-g). Ψ' assumes in both related structures 179.5^{o} within experimental error. The dihedral angles between the best planes of the sugar rings and the epoxy triangles are also rather similar (-79.2^{o} vs -77.5^{o}).

EXPERIMENTAL

Melting points are uncorrected. TLC was carried out on Kieselgel HF_{254} coated microscope slides using EtOAc (A), EtOAc/CCl_4 1:1 (B) and EtOAc/CCl_4 7:3 (C) for elution. Detection was effected by UV light and with 0.1N $KMnO_4$ and 2N H_2SO_4 (1:1) and heating to 105^{o}. For column chromatography Kieselgel 40(0.063-0.200 mm) was used. ^{1}H-NMR spectra were recorded with a Jeol C-60-HL (60 MHz) and a Varian EM-390 (90 MHz) spectrometer, using DMSO-d_6 as solvent and DSS as the internal standard. The low resolution (R = 1250) mass spectra were recorded using a MAT SM-1 spectrometer operating at a 70 eV electron energy, an ion source temperature of 250^{o}, accelerating voltage 8 kV, electron current 300 μA. Evaporation temperatures direct inlet: 15, 150^{o}, 25, 170^{o}, 18, 160^{o}.

All evaporations were carried out in a rotary evaporator under diminished pressure.

General method for methylation of pyrimidine nucleoside derivatives (Ratio and time are given in Table 1)

To a slurry of NaH (53%, washed 3x with light petroleum) in DMSO (10x vol/nucleoside) 1 was added and the mixture was stirred at room temperature for 15 min. Then MeI was added (see Table 1), when an exothermic reaction took place raising the temperature to 60^{o}. The mixture was kept at room temperature for 24h, and was then evaporated at 60^{o}. The residue was separated by column chromatography. For recrystallizations EtOH was used.

1-(β-D-2',3'-Anhydro-5'-O-methyl-ribofuranosyl)-3,5-dimethyl-uracil (15) was prepared according to the general method (see Table 1, g) starting from 1. Purification was carried out by column chromatography using for elution solvent (B). Yield 7%, m.p. 145-146^{o}, Rf (B) 0.35, Rf (A) 0.7, $[\alpha]_D^{20}$ 13.5^{o} (c1DMF)

Anal. Calcd. for $C_{12}H_{16}N_2O_5$: C,53.7;H,6.0;N,10.4

Found: C,53.6;H,6.1;N,10.0

IR (KBr): νC=O 1705, 1665 cm^{-1}: νring 1640, 1470 cm^{-1}.

^{1}H-NMR data (DMSO-d_6): δ7.68 (s, 1H, ^{6}CH); 5.9 (s, 1H, $^{1'}$CH); 4.1-4.4 (m, 3H, $^{2'}$CH, $^{3'}$CH, $^{4'}$CH), 3.58 (d, 2H, $^{5'}CH_2$); 3.28 (s, 3H, OCH_3);

3.17 (s, 3H, N-CH_3) ; 1.85 (s, 3H, C-CH_3) MS[5,6,7]; m/z: 129 $[S]^+$; 140 $[B+H]^+$; 141 $[B+2H]^+$; 251 $[M-OH]^+$; 223 $[M-CH_2OCH_3]^+$.

Crystal data: $C_{12}H_{16}N_2O_5$, MW = 268.27, orthorhombic, a = 5.150(1), b = 12.018(2), c = 19.947(3) Å, U = 1234.6(6) $Å^3$, D_c = 1.443 $g.cm^{-3}$, Z = 4, space group $P2_12_12_1$ (from systematic absences) F(000) = 568. Intensities of 2097 symmetry independent reflections were collected in the range $2\theta \leq 50^o$ by an ω - 2θ scan on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated MoK_α (λ = 0.71073 Å) radiation. Cell constants were determined by least squares refinement from the setting angles of 25 reflections. After data reduction 1401 reflections with I - δ(I) > 0 were taken as observed. The phase problem was solved by the MULTAN program. Full-matrix least-squares refinement of the positional and anisotropic vibrational parameters of non-hydrogen atoms resulted in a final R = 0.047 (R_w = 0.043). The H positions were generated from assumed geometries and refined isotropically in the final stage of the least squares procedure. Scattering factors were taken from ref. 9. All calculations were performed on a PDP 11/34 minicomputer with the use of the SDP-34 system of Enraf-Nonius with local modifications.

2',6-Anhydro-1-(β-D-arabinofuranosyl)-3,5-dimethyl-6-hydroxyuracil (17) The mixture of 16 (1 mmol, 0.25g) in M HCl (10x vol) was heated on a water bath for 3 h. The dark-brown solution was evaporated to dryness, and the brown residue was crystallized with EtOH (2mL). Yield 0.13g (50%), m.p. 225-226°, Rf (A) 0.25, $[\alpha]_D^{20}$ - 182.0° (c1DMF)

Anal. Calcd. for $C_{11}H_{14}N_2O_6$: C,49.0; H,5.2; N,10.4; Found: C,48.6; H,5.5; N,10.1.

IR (KBr): νOH 3480 cm^{-1}; νC=O 1720, 1690 cm^{-1}; νring 1650, 1490 cm^{-1}.

^{1}H-NMR data (DMSO-d_6): δ6.32 (d, 1H, $^{1'}$CH); 5.85 (d, 1H, $^{3'}$C-OH); 5.25 (d, 1H, $^{2'}$CH); 4.92 (t, 1H, $^{5'}$C-OH); 4.35 (dd, 1H, $^{3'}$CH); 4.05 (dt, 1H, $^{4'}$GH); 3.32 (m, 2H, $^{5'}CH_2$) 3.16 (s, 3H, N-CH_3); 1.70 (s, 3H, C-CH_3).

5',6-Anhydro-1-(β-D-2',3'-anhydroribofuranosyl)-3-methyl-6-hydroxyuracil (18) was prepared from the methylation reaction mixture of 1 besides 9 and 16, using column chromatography with solvent (C). Yield 5%, m.p. 200-205° subl., Rf (C) 0.4.

Anal. Calcd. for $C_{10}H_{10}N_2O_5$: C,50.5; H,4.2; N,11.8;
Found: C,49.8; H,4.5; N,11.4.

IR (KBr): ν^5CH 3100 cm^{-1}; νC=O 1700, 1655 cm^{-1}; νring 1615, 1440 cm^{-1}.

^{1}H-NMR data (DMSO-d_6): δ6.50 (s, 1H, ^{5}CH); 5.35 (s, 1H, $^{1'}$CH); MS[10]: m/z: 238 $[M^\cdot]^+$

2',6-Anhydro-1-(β-D-3',5'-di-O-methyl-arabinofuranosyl)-3,5-dimethyl-6-hydroxyuracil (25) was prepared as described in general method (see Table 1,e) starting from 9. Purification was carried out by column chromatography using solvent (B). The crude material was recrystallized with EtOH, yielding 25 (17%), m.p. 166-170° subl. Rf (B) 0.3, $[\alpha]_D^{20}$ -156.3° (c1DMF)

Anal. Calcd. for $C_{13}H_{18}N_2O_6$: C,52.4; H,6.1; N,9.4;
Found: C,51.9; H,6.3; N,9.3;
MS[7,11,12]: m/z: 298 $[M]^+$; 253 $[M-\dot{C}_2H_5O]^+$; 225 $[M-\dot{C}_3H_5O_2]^+$; 196 $[M-\dot{C}_5H_{10}O_2]^+$.

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