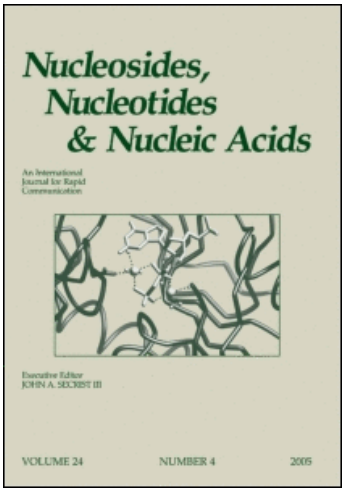

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Synthesis and Antiretroviral Evaluation of Various 5-Alkyl-6-AZA-5,6-Dihydrouridine

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NOTE

SYNTHESIS AND ANTIRETROVIRAL EVALUATION OF VARIOUS 5-ALKYL-6-AZA-5,6-DIHYDROURIDINE

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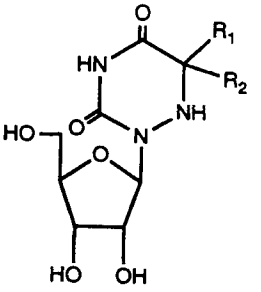
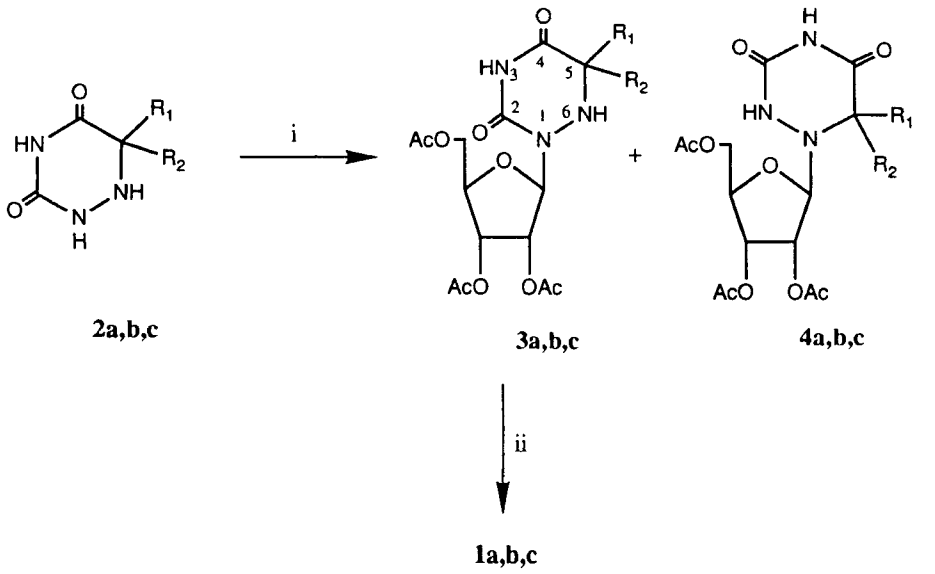
ABSTRACT : Various 5-alkyl-6-aza-5,6-dihydrouridine were synthesized from the corresponding triazinic heterocycles and acetylated ribose. These nucleosides were tested for inhibitory activity on lentivirus Visna-Maedi.

Although the chemistry and pharmacology of nucleosides have been widely and thoroughly studied, little has been undertaken in the case of dihydroazauracil derivatives despite promising biological activities.[1] As a part of our continuing effort to acquire new antiviral agents,[2] we report here the synthesis and preliminary antiretroviral activity of the modified nucleosides **1a,b,c**.



1a : R_1 = -H ; R_2 = $-CH_3$
1b : R_1 = -H ; R_2 = $-(CH_2)_2CH_3$
1c : R_1 = R_2 = $-CH_3$

The bases **2a,b,c** have been prepared according to a method developed previously in our laboratory.[3]



a : R_1 = -H , R_2 = $-CH_3$; **b** : R_1 = -H , R_2 = $-(CH_2)_2CH_3$; **c** : R_1 = R_2 = $-CH_3$.
i : 1,2,3,5-tetra-O-acetyl-ribofuranose (1 eq), TMSCl (0.8 eq), HMDS (0.8 eq), TMSTf (2.2 eq); ii : NH_3 in MeOH.

FIGURE 1

Among the different methods of coupling, the modified Vorbrüggen procedure[4,5,6] was found to be the most effective. Thus, condensation of **2a,b,c** with 1,2,3,5-tetra-O-acetyl-ribofuranose in 1,2-dichloroethane at 60°C during 15 mn led to protected dihydroazauracil nucleosides **3a,b,c** and **4a,b,c**[7] (for experimental conditions see Fig. 1). The most efficient quantity of Lewis acid was found to be 2.2 eq of TMSTf.

The difficult separation of the acetylated N_1-nucleosides **3a,b,c** and N_6-nucleosides **4a,b,c** was achieved by silica gel column chromatography using as eluants AcOEt/pet.ether for compounds **a** and **c** and $CHCl_3$/EtOH for compounds **b** .

The N-linkage between the peracetylated ribose and bases reported here was assigned using ^1H nmr by observation of the anomeric proton of the ribose moiety and the H-1, H-3 and H-6 protons of the coupled dihydroazauracil (Table 1).

The shift of the anomeric proton of the N_1-nucleosides **3a,b,c** was assigned at 6 ppm as with acetylated dihydrouridine.[8] In the case of the N_6-nucleosides **4a,b,c**, it appeared at 4.8 ppm as with ribofuranosylamines.[9]

These assignments were supported by irradiation of the H-2' proton leading to the decoupling of H-1' (and H-3' at 5.2 ppm).

Moreover, the observation of the protected N6-nucleosides **4a,b,c** showed the NH protons H-1 and H-3 while H-6 was not present. In the peracetylated N_1-nucleosides **3a,b,c**, H-3 and H-6 were observed while H-1 was absent.

Compounds **3** and **4** were obtained from bases **2a,b,c** with 15 %, 6 %, and 40 % overall yields respectively (with **3/4a** = 2.75, **3/4b** = 1, and **3/4c** = 0.7).

We obtained chromatographically purified mixtures of diastereoisomers for compounds

TABLE 1 . Selected values of ^1H nmr data (200 MHz in $CDCl_3$).

Compounds	H-1' (ppm)	H-1 (ppm)	H-3 (ppm)	H-6 (ppm)
3a	6.0 d(J1',2' = 5.9 Hz)	-	7.55	4.4 m
3b	6.1 d(J1',2' = 6.8 Hz)	-	7.65	4.5 m
3c	6.0 d(J1',2' = 5.8 Hz)	-	7.4	4.45 s
4a	4.7 d(J1',2' = 7.5 Hz)	7.1	7.6	-
4b	4.8 d(J1',2' = 6.9 Hz)	7.1	7.65	-
4c	4.9 d(J1',2' = 7.1 Hz)	7.1	7.4	-

TABLE 2 : Cytotoxicity and EC50 of **1a,b,c**, AZT, ddC and ddU.

Compounds	EC50 (μM)		MCC (μM)	S I	
	Visna	Maedi		Visna	Maedi
1a	109.2	89.9	500	4.6	5.6
1b	/	/	400	/	/
1c	/	/	200	/	/
AZT	0.37	0.26	100	270	385
ddC	0.3	0.28	250	833	893
ddU	126.6	133.8	500	3.95	3.7

3a,b and **4a,b** due to their asymetric carbon C-5. We were unsuccessful in the separation of the individual diastereoisomers.

The protecting groups of **3a,b,c** were removed by treatment with a saturated solution of ammonia in dry methanol (at 0°C) to give the desired nucleosides **1a,b,c** in nearly quantitative yields.[10]

The antiretroviral activities of **1a**, **1b**, and **1c** were compared to those of known nucleosides AZT,ddC and an inactive parent molecule of **1a,b,c** : ddU, for *in vitro* replication of the Visna-Maedi lentivirus (Visna virus : strain K796 and Maedi virus : strain WLC1) in Sheep Choroïd Cells (SCP) (Table 2).

The minimal cytotoxic concentration (MCC) of both **1a** and ddU for actively dividing SCP cells was determined to be 500 μM; **1c** was the most cytotoxic with a MCC of 200μM.

50 % effective concentration values (EC50) were determined by cytopathic effect inhibition assays (CPE).[11] EC50 values of **1b** and **1c** could not be determined because these compounds were not effective below their MCC.

A comparable inhibition to that of AZT and ddC, which are recognized to be the most active agents against Visna-Maedi virus, was not obtained for compounds **1a,b,c**. Modifications of the carbohydrate moiety of these dihydroazauracil nucleosides[12] are under investigation.

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For selected ^1H nmr data, see TABLE 1
3a : Rf 0.60 (AcOEt/pet. ether 4:1); MS *m/z* (EI) : 314 (M^+ - $AcOCH_2$).
3b : Rf 0.20 ($HCCl_3$/EtOH 95:5); MS *m/z* (EI) : 417 $(M + 2H)^+$.
3c : Rf 0.63 (AcOEt/pet. ether 4:1); MS *m/z* (EI) : 402 $(M + H)^+$; $[\alpha]_D$ -8.4° (c 0.4, $HCCl_3$).
4a : Rf 0.42 (AcOEt/pet. ether 4:1); MS *m/z* (EI) : 389 $(M + 2H)^+$.
4b : Rf 0.17 ($HCCl_3$/EtOH 95:5); MS *m/z* (EI) : 417 $(M + 2H)^+$.
4c : Rf 0.46 (AcOEt/pet. ether 4:1); MS *m/z* (EI) : 403 $(M + 2H)^+$; $[\alpha]_D$ -80.4° (c 0.3, $HCCl_3$).
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10 . Selected data for **1a,b,c**.
1a : Rf 0.45 ($HCCl_3$/EtOH 7:3); MS *m/z* (FAB) : 262 $[(M + H)^+]$.
1b : Rf 0.55 ($HCCl_3$/EtOH 7:3); MS *m/z* (FAB) : 290 $[(M + H)^+]$.
1c : Rf 0.63 ($HCCl_3$/EtOH 7:3); MS *m/z* (FAB) : 276 $[(M + H)^+]$.
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