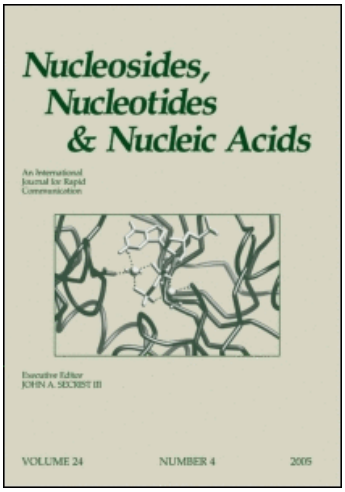

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Biologically Important Nucleosides: A General Method for the Synthesis of Unsaturated Ketonucleosides of Uracil and its Analogs

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BIOLOGICALLY IMPORTANT NUCLEOSIDES: A GENERAL METHOD FOR THE SYNTHESIS OF UNSATURATED KETONUCLEOSIDES OF URACIL AND ITS ANALOGS.

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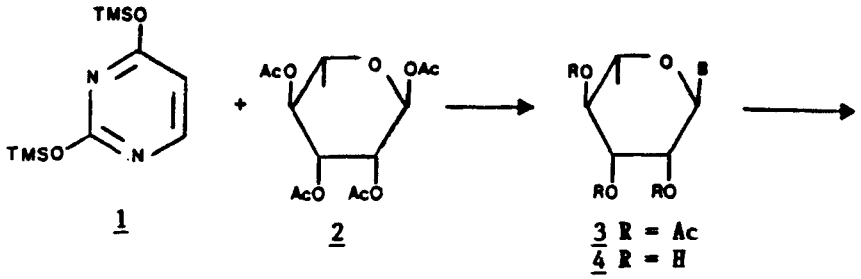
Abstract: A general method for the synthesis of unsaturated keto- nucleosides of α-L-rhamnose with 5-fluorouracil and their in vitro and in vivo anticancer activity evaluation results are discussed. The synthesis was effected by selective protection and deprotection of the sugar hydroxyls with acid and base labile groups following standard procedure. In the final step, oxidation of the 2',3' acylated nucleoside afforded the 4'-ketoenonucleoside. Two unsaturated ketonucleosides, 3'-O-benzoyl and 3'-O-acetyl were synthesized in this way and both these compounds show inhibition of tumor cell growth of murine leukemia L1210, murine mammary carcinoma FM3A, human B-lymphoblast Raji, human T-lymphoblast Molt/4F. Also one compound shows antitumor activity in the adenocarcinoma 755 model in BDF_1 mice.

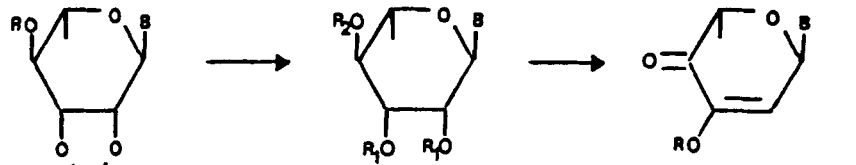
Antonakis and his associates have reported about a decade and a half ago that unsaturated keto nucleosides of α-L-rhamnose and α-L-fucose with purine and its analogs show significant in vitro and in vivo growth inhibitory activity against a variety of cancer cells.[1,2] Based on these observations we have undertaken the synthesis of unsaturated ketonucleosides of 4,5 and 6-substituted pyrimidine bases,

to investigate whether these compounds also show anticancer activity analogous to their purine counterparts. We describe in this paper the syntheses and detailed anticancer evaluation of 3'-O-acyl-2',3'- unsaturated-4'- ketorhamnopyranosyl nucleosides of 5-fluorouracil.

On attempting to synthesize larger quantity of the unsaturated ketonucleosides of 5-fluorouracil, following the route used in the purine class, ie, by cleaving the 2',3'-O-isopropylidene group from the 4'-keto-2',3'-O-isopropylidenerhamnosyl purine and then acylation of the ketonucleoside to effect spontaneous elimination to afford the unsaturated keto nucleoside, we failed to get the pure 4'-keto- α -L-rhamnosyl pyrimidine nucleoside during the cleavage of the isopropylidene group. We have already reported the synthesis of 4'-keto-2',3'-O-isopropylidene rhamnosyl nucleoside of uracil and its 5-chloro analog,[3,4] also that of 5-fluoro-1-(3-O- benzoyl-4,6-dideoxy-β-L-glycero-hex-3-enopyranos-2-ulosyl)uracil[5]. Although several methods were tried to cleave the isopropylidene group, none of them was satisfactory. Each method gave a mixture of several degradation products with close R_f values making it very difficult to separate them. This led us to search for an alternative approach for the synthesis of unsaturated ketonucleosides of pyrimidine analogs. Although the new method is time-consuming and arduous, it gave excellent yield in each step. In this method we have used 5-fluorouracil as the uracil analog and α-L-rhamnose as the sugar moiety. It is certain that this method will also be applicable for other pyrimidine bases (Scheme 1).



1 2 3 R = Ac, 4 R = H



5 R = H, 6 R = Bz

7 R_1 = H, R_2 = Bz
8 R_1 = THP, R_2 = Bz
9 R_1 = THP, R_2 = H
10 R_1 = THP, R_2 = TBDMS
11 R_1 = H, R_2 = TBDMS
12 R_1 = Ac, R_2 = TBDMS
13 R_1 = Ac, R_2 = H
14 R_1 = Bz, R_2 = H

15 R = Ac, 16 R = Bz

B = -1-(5-fluorouracil)
THP = 2-tetrahydropyranyl
TBDMS = t-butyldimethylsilyl

Scheme I

In the new approach, the protected nucleoside was synthesized using the standard procedure by coupling the silylated base with peracetylated α-L-rhamnose to yield 3[6]. It was then deacetylated using methanolic ammonia to give 4. This product was then converted to 2',3'-O-isopropylidene derivative 5, by reacting 4 with 2,2-dimethoxypropane and sulfuric acid in a nitrogen atmosphere. 5 was then benzoylated in the usual manner to yield 4'-O-benzoyl-2', 3'-O-isopropylidene nucleoside 6. In the next step the isopropylidene group was cleaved using trifluoroacetic acid/ methanol mixture to yield the 4'-O-benzoyl nucleoside 7. In the following step 7 was reacted with 2,3-dihydro-4H-pyran to give 2',3'-bis-O-tetrahydropyranyl-4'-O-benzoyl nucleoside 8. Next the benzoyl group was cleaved to afford the 2',3'-bis-O-tetrahydropyranyl nucleoside 9. This was then treated with tert-butyldimethylsilyl chloride (TBDMSCl) to yield 2',3'-bis-O-tetrahydropyranyl-4'-O-TBDMS nucleoside 10. In the next step the tetrahydropyranyl groups were removed using p-toluenesulfonic acid to yield the 4'-O-TBDMS nucleoside 11. In the ensuing step, the 2',3'-hydroxyl groups were acetylated in dry pyridine/acetic anhydride at 0 °C in presence of a catalytic amount of dimethylaminopyridine to yield the 2',3'-di-O-acetyl-4'-O-TBDMS nucleoside 12. Subsequently the 4'-O-TBDMS group in compound 12 was cleaved off using trifluoroacetic acid in methanol to yield the 2',3'-di-O-acetyl derivative 13. In the final step, the nucleoside was oxidized using pyridinium dichromate (PDC) in presence of molecular sieves 3Å to

afford 5-fluoro-1-(3-O- acetyl-2,6-dideoxy- α-L-glycero-hex-2-enopyranos-4-ulosyl)uracil 15, by spontaneous β-acetoxy elimination. A shorter route was followed in the synthesis of 5-fluoro-1-(3-O-benzoyl-2,6-dideoxy- α-L-glycero-hex-2-enopyranos-4- ulosyl)uracil 16. 5-Fluoro-1-(α-L-rhamnopyranosyl) uracil 4 was reacted with 2.5 molar equivalent of benzoyl chloride in mixture of pyridine/dichloroethane. A 2', 3'-di-O-benzoyl nucleoside 14 was isolated and purified by silica gel column chromatography in 40% yield from a mixture of several benzoylated compounds (TLC). Oxidation of the 2',3'-di-O-benzoyl nucleoside 14 using PDC/MS yielded the compound 16 by concomitant β-debenzoyloxygenation of 14. Biological evaluation of 3'-O-acetyl and 3'-O-benzoyl unsaturated compounds showed significant anticancer cell activity.

The ^{1}H NMR spectrum of 15 clearly showed the H-1' proton resonating at 6.1 ppm as a doublet of doublets (J1',F = 1.5 Hz, J1',2'= 3.7 Hz) whereas in the case of parent 13 this proton resonates at 6.1 ppm as a doublet of doublets (J1',F= 1.5 Hz, J1',2'= 8.5 Hz). The signal for H-2' in 13 appeared at 5.5 ppm whereas in 15 it appeared as a doublet at 5.9 ppm (J1',2'= 3.7 Hz) characteristic of an olefinic proton. Moreover, the H-5' proton which resonated as a multiplet at 4.3 ppm in 13 (J4',5'= 2.5 Hz, J5',6'= 7.56 Hz) appeared only as a quartet at 3.75 ppm in the case of 15 (J5',6'= 7.56 Hz) thus showing the disappearance of the H-4' proton confirming the oxidation of 4'-hydroxyl group. In addition the disappearance of the peaks corresponding to

one of the acetyl groups in 15 confirmed the β-elimination reaction occurring during the oxidation step. Similar arguments hold for 16 also.

Biological Activity

The compounds 15, 16, 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FdUrd) were evaluated for *in vitro* antitumor cell activity in four different tumor cell systems [i.e. murine leukemia (L1210), murine mammary carcinoma (FM3A), human B-lymphoblast (Raji) and human T-lymphoblast (Molt/4F)] (Table 1). Both 15 and 16 showed growth inhibitory activity against all four cell lines. The acetyl nucleoside 15 appeared to be a better tumor cell growth inhibitor than the benzoyl derivative 16. The ID_{50}'s of both 15 and 16 were comparable to those of FU against L1210 and FM3A, but quite higher than those for Raji and Molt/4F cells. In comparison to FdUrd, both 15 and 16 showed a lower growth inhibitory activity (higher ID_{50}) against all the cell systems assayed, except for L1210/TK^-. Unlike FdUrd, both 15 and 16 inhibited growth of L1210/TK^- cells to a similar extent as the wild-type L1210/0 cells.

Addition of deoxynucleosides (i.e. dUrd, dThd and dCyd) did not markedly affect the growth inhibitory effects of 15, 16 and FU (Table 2). In contrast, dThd but not dUrd and dCyd completely reversed the effect of FdUrd. It was also found that 15, 16, FU and FdUrd inhibited the incorporation of ^{3}H-dUrd more than that of ^{3}H-dCyd in L1210 cell DNA, but incorporation of ^{3}H-dThd remained unaffected (Table 3).

Table 1. Inhibition of tumor cell growth

Compounds	ID_{50} (μg/ml)*				
	Murine Leukemia L1210		Murine mammary carcinoma FM3A	Human B-lymphoblast Raji	Human T-lymphoblast Molt/4F
	L1210/0 (TK^+)	L1210/BdUrd (TK^-)			
15	0.58 ± 0.12	0.6 ± 0.16	0.35 ± 0.04	21 ± 2	36 ± 4
16	2.5 ± 0.3	2.9 ± 0.9	1.3 ± 0.3	52 ± 15	67 ± 12
FU	0.25 ± 0.03	0.45 ± 0.01	0.16 ± 0.04	6.6 ± 4.8	7.5 ± 1.9
FdUrd	0.003 ± 0.0002	2.9 ± 0.3	0.003 ± 0.0002	0.11 ± 0.07	0.05 ± 0.006

* 50% inhibitory dose (mean values ± standard deviation for 3 to 4 separate experiments).

TK^+: thymidine kinase-positive; TK^-: thymidine kinase-negative (or deficient).

ND: not determined.

Table 2. Inhibition of L1210 cell growth in the presence of dUrd, dThd or dCyd

Compound	ID_{50} (g/ml)*			
	As such	Upon addition of dUrd (125 g/ml)@	Upon addition of Thd (5 g/ml)@	Upon addition of dCyd (500 g/ml)@
15	0.56 ± 0.11	2.5 ± 0.3	4.2 ± 0.8	1.6 ± 0.3
16	1.8 ± 0.3	4.8 ± 0.8	8.4 ± 2	3.9 ± 0.75
FU	0.25 ± 0.03	0.41 ± 0.03	0.52 ± 0.04	0.37 ± 0.04
	0.36 ± 0.09	0.60 ± 0.13	0.58 ± 0.07	0.44 ± 0.07
FdUrd	0.003 ± 0.0001	0.049 ± 0.006	221 ± 28	0.031 ± 0.0004
	0.002 ± 0.0004	0.09 ± 0.01	423 ± 397	0.02 ± 0.003

* 50% inhibitory dose (mean values ± standard deviation for 3 to 4 separate experiments).
@ Highest concentration at which dUrd, dThd or dCyc were themselves not inhibtory to the growth of L1210 cells

The acetyl nucleoside 15 was evaluated for antitumor activity against adenocarcinoma 755 tumors in BDF_1 mice in which 15 showed an antitumor activity profile similar to FU (Table 4). In fact, compound 15 proved about 2.5-fold less effective that FU in inhibiting tumor growth in vivo.

The biological results discussed above point to almost similar activity profiles of unsaturated ketonucleosides and FU. This in turn indicates that the nucleosides (15 and 16) and FU may have similar mode of action. Thymidylate synthase may not be the principal target of antitumor action

Table 3. Inhibition of Incorporation of ^{3}H-dUrd, dThd and dCyd in L1210 cell DNA

Compound	ID_{50} (g/ml)*		
	[1', 2'- ^{3}H]dUrd incorporation	[methyl-^{3}H]Thd incorporation	[5-^{3}H]dCyd incorporation
15	1.3 ± 0.5	> 100	20 ± 5
16	4.2 ± 0.5	> 100	27 ± 9
FU	0.33 ± 0.03	> 100	2.2 ± 1.0
	0.57 ± 0.02	> 100	7.9 ± 2.1
FdUrd	0.001 ± 0.0001	> 100	0.003 ± 0.001
	0.002 ± 0.0004	> 100	0.003 ± 0.001

* 50% inhibitory dose (mean values ± standard deviation for 3 to 4 separate experiments).

of FU, 15 and 16. In contrast, FdUrd appears to differ from the nucleosides 15 and 16 in that thymidylate synthase is the most likely target for antitumor activity. 15 and 16 do not depend upon thymidine kinase as they show similar activity in both TK^{+} and TK^{-} L1210 cell lines. In contrast, FdUrd appears to need this enzyme for cell growth inhibition. Also dThd can completely reverse the effect of FdUrd, but has little effect on the activity of 15 and 16, pointing further to the differences in the molecular modes of action of FdUrd versus FU, 15 and 16.

Table 4. Inhibition of growth of adenocarcinoma 755 tumors in BDF_1 mice by 5-flurouracil (FU) and compound

Compound	Dose* (mg/kg/day)	Tumor weight on day 12		Survival rate on day 12
		Mean ± S.D. (mg)	T/C (%)	
Control		2215 ± 1212		6/6
FU	5	3398 ± 1048	153	6/6
FU	10	2692 ± 1570	122	6/6
FU	20	1213 ± 950	55	6/6
FU	30	314 ± 344	14	6/6
FU	50	20 ± 9	1	4/6
15	5	2799 ± 1227	126	6/6
15	10	2285 ± 1269	103	6/6
15	20	3346 ± 1060	151	6/6
15	30	3063 ± 1079	138	6/6
15	50	2199 ± 806	99	6/6
15	100	44 ± 5	2	4/6

* Administered intraperitoneally on days 1 till 5.

The similarity in the modes of action of the compounds (15, 16, and FU) suggests that the FU moiety in the unsaturated ketonucleosides may be responsible for the antitumor activity. FU may be released in the cells upon deglycosylation of the nucleosides 15 and 16. However, in case of purine unsaturated ketonucleosides altogether

different mode of action has been suggested.[2] The unsaturated keto system has been found indispensable for their activity. This raises the question whether 5-fluorouracil alone is responsible for the antitumor activity or the unsaturated keto system also imparts activity to the nucleosides. Obviously further studies are warranted to resolve this issue.

Experimental Section

Melting points (uncorrected) were determined using Mel-Tem apparatus. Thin layer chromatography (TLC) was done on precoated silica gel plastic sheets 60 F_{254} (0.2 mm) EM Reagents. Compounds were detected under short wave UV light, and by spraying with 3% conc. sulfuric acid in ethanol (w/v) and heating the developed TLC plastic strips. Six solvent systems were used for TLC: (A) ethyl acetate, (B) hexane/ethyl acetate 2:3 (v/v), (C) hexane/ethyl acetate 1:1 (v/v), (D) hexane/ethyl acetate 1:4 (v/v), (E) hexane/-ethyl acetate 1:3 (v/v), (F) methanol/ethyl acetate 1:4 (v/v). Optical rotations were determined by "Quick" polarimeter and are expressed in degrees. Nuclear magnetic resonance (NMR) spectra were recorded using Bruker/IBM SY 200 and are expressed in ppm. Dichloromethane used in the oxidation step was first distilled over phosphorus pentoxide (P_2O_5) and stored in a glass container over molecular sieves (MS) 4 Å, at least two to three days before use. Molecular sieves 3 Å, used for oxidation, was finely powdered, and then heated to about 375°C *in vacuo* in the vicinity of P_2O_5 in a sleeve of a specially designed tube just before the

experiment. MS was cooled to ambient temperature before adding to the reaction mixture. Column chromatography was done using silica gel (70-230 mesh ASTM. E. Merck. Darmstadt). Methodology involved in the *in vitro* antitumor cell activity evaluation was described by De Clercq *et al*.[7]

5-Fluoro-1-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl) uracil 3.

5-Fluorouracil 30.0 g (0.2306 mol) was silylated in hexamethyldisilazane (HMDS) under anhydrous conditions using catalytic amount of saccharin. The silylated base was coupled at 60 °C with peracetylated α-L-rhamnose, 36.07 g (0.2196 mol) in presence of anhydrous stannic chloride 38 mL (0.35 mol), diluted with equal volume of dry 1,2-dichloroethane. Coupling reaction was monitored by TLC in solvent (E). It was complete in 5 h. The mixture was diluted with about 250 mL 1,2-dichloroethane and neutralized with saturated sodium bicarbonate solution. The precipitated stannic oxide was vacuum filtered and was washed with 1,2-dichloroethane (200 mL). The aqueous layer was separated and was washed three times with 75 mL of 1,2-dichloroethane. The organic layers were pooled together, dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo*. The product 3 was recrystallized from absolute ethanol. Yield 85%; mp 170 °C; R_f 0.42 (solvent E); $[\alpha]_D^{20}$ +31.0 (c 0.5, chloroform); ^{1}H NMR 270 MHz (chloroform-d) 1.60 (d, 3 H, H-6′, $J_{5',6'}$ = 7.0 Hz), 2.00 and 2.20 (2 s, 3 H and 6 H, 3 x-$COCH_3$), 4.30 (q, 1 H, H-5′, $J_{5',6'}$ = 7.0 Hz), 4.85 (broad s, 1 H, H-4′), 5.25

(dd, 1 H, H-2′, $J_{1',2'}$ = 9.0 and $J_{2',3'}$ = 3.5 Hz), 5.45 (d, 1 H, H-3′, $J_{2',3'}$ = 3.5 Hz), 6.20 (broad d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 7.5 (d, 1 H, H-6′, $J_{6,F}$ = 7.0 Hz). Anal. Calcd. for $C_{16}H_{19}FN_2O_9$: C, 47.76; H, 4.76; N, 6.96; F, 4.72. Found: C, 48.24; H, 4.77; N, 6.84; F, 4.64.

5-Fluoro-1-(α-L-rhamnopyranosyl)uracil 4.

A liter of dry methanol was placed in a 2 L flask, which was cooled to 0°C in an ice bath. Methanol was then saturated with NH_3 gas. 10.0 g of 1 (24.8 mmol) was then added into the flask. The compound dissolved immediately. The solution was kept well stirred mechanically. Deacetylation was followed by TLC in solvent (F). The reaction was complete overnight. Methanol was removed in vacuo, at about 40°C. Product 4 was obtained as a fluffy mass. Yield 97%; R_f 0.42 (solvent F); $[\alpha]_D^{20}$-35.0 (c 0.5 methanol); ^1H NMR 270 MHz (acetone-d_6) 1.5 (d, 3 H, H-6′, $J_{5',6'}$= 6.75 Hz), 3.75 (dd, 1 H, H-4′), 4.15 (m, 3 H, H-2′, 3′ and 5′), 6.05 (dd, 1 H, H-1′, $J_{1',2'}$= 9.0 Hz and $J_{1',F}$ = 1.5 Hz), 7.85 (d, 1 H, H-6, $J_{6,F}$ = 6.75 Hz). Anal. Calcd. for $C_{10}H_{13}FN_2O_6$: C, 43.48; H, 4.71; N, 10.14. Found: C, 40.40; H, 4.69; N, 10.09.

5-Fluoro-1-(2,3-O-isopropylidene-α-L-rhamnopyranosyl)-Uracil 5.

In a 250 mL flask which was dried over a flame in a nitrogen atmosphere and cooled to room temperature, 10.0 g (36.2 mmol) of product, 4, was placed. It was then dissolved in 50mL of dry dimethylformamide (DMF). The solution was kept stirred mechanically. To this was added

30 mL of 2,2-dimethoxypropane and 1 mL of conc. H_2SO_4. The reaction was allowed to continue under nitrogen atmosphere. In one to two hours, the reaction was complete as tested in the solvent (A). The reaction mixture was neutralized with saturated sodium carbonate solution. The solvent was then evaporated in vacuo. The product was then dissolved in ethyl acetate and washed with a saturated solution of sodium chloride. The aqueous phase was separated and washed four times with 20-25 mL of ethyl acetate. The combined organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness in vacuo. The product was purified by column chromatography eluting with hexane/ethyl acetate (1:4 v/v). Compound 5 was obtained as an oil. Yield 85%; R_f 0.62 (solvent A); $[\alpha]_D^{22}$-23.75 (c 0.1, methanol); ^1H NMR 270 MHz (acetone-d_6) 1.3 (d, 3 H, H-6′, $J_{5',6'}$ = 6.48 Hz), (s, 3 H, CH_3- isopropyl), 1.45 (s, 3 H, CH_3-iso- propyl), 3.7 (ddd, 1 H, H-4′, $J_{4',5'}$= 6.48 Hz, $J_{4',3'}$= 6.48 Hz, $J_{4',OH}$= 6.48 Hz), 3.95 (dq, 1 H, H-5′, $J_{5',4'}$= 6.48 Hz, $J_{5',6'}$= 6.48 Hz), 4.45 (dd, 1 H, H-3′, $J_{3',4'}$= 6.48 Hz), 4.6 (dd, 1 H, H-2′, $J_{2',3'}$= 6.48 Hz, $J_{2',1'}$= 7.56 Hz), 4.7 (d, 1 H, -OH, $J_{OH,4'}$= 6.48 Hz), 5.8 (d, 1 H, H-1, $J_{1',2'}$= 7.56 Hz), 7.95 (d, 1H, H-6, $J_{6,F}$= 6.75 Hz). Anal. Calcd. for $C_{13}H_{17}O_6N_2FH_2O$: C, 47.27; H, 5.76; N, 8.48; F, 5.76. Found: C, 47.31; H, 5.54; N, 8.33; F, 5.91.

5-Fluoro-1-(4-O-benzoyl-2-3-O-isopropylidene-α-L-rhamnopyranosyl) uracil 6.

A 250 mL flask was dried over a flame in a nitrogen atmosphere and cooled to room temperature. To this flask

were added 38.8 mmol (12.7 g) of 5 and 100 mL of pyridine (distilled and dried over MS 4Å). After dissolution of 3 the reaction mixture was cooled to 0 °C, 12 mL of benzoyl chloride was added to the flask and then the reaction was allowed to continue at room temperature. In 0.5 h the reaction was complete as shown by the TLC in the solvent (B). The solvents were removed in vacuo and the product obtained was dissolved in chloroform. The organic layer was washed first with water, then with saturated $NaHCO_3$ solution until being neutral and then with saturated NaCl solution. It was then dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness in vacuo. The product 6 was crystallized from ethyl acetate. Yield 92%; mp 236 °C; R_f 0.61 (solvent B); $[\alpha]_D^{20}$-24.0 (c 0.5 methanol); ^1H NMR 270 Hz (acetone-d_6) 1.35 (d, 3 H, H-6′, $J_{5',6'}$ = 6.48 Hz), (s, 3 H, CH_3- isopropyl), 1.55 (s, 3 H, CH_3-isopropyl), 4.3 (dq, 1 H, H-5′, $J_{5',6'}$= 6.48 Hz, $J_{5',4'}$= 6.48 Hz), 4.8 (m, 2 H, H-2′ and 3′), 5.25 (dd, 1 H, H-4′, $J_{4',5'}$= 6.48 Hz, $J_{4',3'}$= 4.86 Hz), 5.9 (d, 1 H, H-1′, $J_{1',2'}$= 5.4 Hz), 7.5 (m, 2 H, benzoyl), 7.65 (m, 1 H, benzoyl), 7.95 (d, 1 H, H-6, $J_{6,F}$= 6.75 Hz), 8.1 (m, 2 H, benzoyl). Anal. Calcd. for $C_{20}H_{21}O_7N_2F$: C, 57.14; H, 5.00; N, 6.67; F, 4.52. Found: C, 57.07; H, 5.31; N, 6.33; F, 4.56.

5-Fluoro-1-(4-O-benzoyl-α-L-rhamnopyranosyl)uracil 7.

In a 250 mL flask fitted with a drying tube, 14.0g (33.34mmol) of compound 6 was placed. To this was added a mixture of 14mL of methanol and 126 ml of trifluoroacetic acid. The solution was kept well stirred at ambient

temperature. The deacetalation was monitored by TLC in the solvent (B) and was complete in 10 to 20 min. Then the solvents were evaporated <u>in vacuo</u>, at room temperature. The solid product obtained was dissolved in ethyl acetate. The solution was washed carefully with a saturated solution of $NaHCO_3$ until being neutral, then with a saturated solution of NaCl. The organic layer was then separated and dried over anhydrous sodium sulfate, filtered and evaporated <u>in vacuo</u> to dryness. The product <u>7</u> was crystallized from methanol. Yield 96%; mp 136 °C, R_f 0.22 (solvent E); $[\alpha]_D^{22}$-12.93 (c 0.1, methanol), ^1H NMR 270 MHz (acetone-d_6) 1.6 (d, 3 H, H-6′, $J_{5',6'}$= 6.75Hz), 4.35 (m, 4 H, H-2′, H-3′, H-5′ and OH), 5.15 (dd, 1 H, H-4′), 6.2 (dd, 1 H, H-1′, $J_{1',2'}$= 8.82 Hz, $J_{1',F}$= 1.26 Hz), 7.5 (m, 2 H, benzoyl), 7.65 (m, 1 H, benzoyl), 7.9 (d, 1 H, H-6, $J_{6,F}$= 6.75 Hz), 8.15 (m, 2 H, benzoyl). <u>Anal.</u> Calcd. for $C_{17}H_{17})_7N_2FH_2O$: C, 51.26; H, 4.78; N, 7.03; F, 4.77. Found: C, 51.34; H, 4.76; N, 6.64; F, 5.10.

5-Fluoro-1-(4-<u>O</u>-benzoyl-2,3-bis-<u>O</u>-tetrahydropyranyl-α-L-rhamnopyranosyl)uracil <u>8</u>.

To a 250 mL flask dried in an atmosphere of nitrogen, was added 5.0g (13.16 mmol) of compound <u>7</u>, and 3 mL (32.9 mmol) of 3,4-dihydro-2<u>H</u>-pyran. The mixture was then dissolved in 50 mL of dry CH_2Cl_2. To the stirred solution was added 6 mg of 4-dimethylaminopyridine (DMAP) and 97 mg of <u>p</u>-toluenesulfonic acid monohydrate. The progress of the reaction was monitored by TLC in the solvent (C). On completion of the reaction, 200 mL of CH_2Cl_2 was added to

the mixture. This was washed with a saturated solution of $NaHSO_4$ and Na_2HPO_4 1:1 (v/v). The aqueous layer was separated and washed three times with about 50 mL of CH_2Cl_2. The combined organic layer was again washed with a saturated solution of NaCl. The organic layer was separated and dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness in vacuo. The product was purified by column chromatography using graded eluents hexane/ethyl acetate 8:2 (v/v), 6:4 (v/v), 1:1 (v/v). The final product 8 was crystallized from ethyl ether. Yield 93%; mp 108°C; R_f 0.53 (solvent C); $[\alpha]_D^{20}$+22.1 (c 0.1, methanol); ^1H NMR 270 MHz (chloroform-d) 1 to 2 (m, 15 H, H-3, 4 and 5 of THP, and H-6′), 3.4 to 5.2 (m, 7 H, H-2′, 3′, 4′, 5′, and H-2′ and H-6 of THP), 6 to 6.3 (m, 1 H, H-1′), 7.5 (m, 3 H, benzoyl), 7.65 (d, 1 H, H-6, $J_{6,F}$= 6.75 Hz), 8.05 (m, 2 H, benzoyl). Anal. Calcd. for $C_{27}H_{33}O_9N_2F$: C, 59.12; H, 6.02; N, 5.11; F, 3.47. Found: C, 59.07; H, 6.34; N, 6.51; F, 3.51.

5-Fluoro-1-(2,3-bis-O-tetrahydropyranyl-α -L-rhamnopyranosyl) uracil 9.

To a 1 L flask dried well over a flame in a nitrogen atmosphere was added 21.4g (48.21 mmol) of compound 8. This was dissolved in 600 mL of dry methanol. The solution was kept well stirred mechanically under nitrogen. To this solution was added 40.15 mL of 2 N sodium methoxide solution. The reaction was followed by TLC in solvent (C). On completion of the reaction, it was neutralized using IR 101 (H^+) resin. The resin was filtered off, washed with dry methanol and the filtrate was concentrated to dryness in

vacuo. The product 9 was purified by column chromatography using a gradient mixture of ethyl acetate 5:5 (v/v); 4:6 (v/v) and 3:7 (v/v) to afford 7 as foam. Yield 92%, R_f 0.59 (solvent E); $[\alpha]_D^{22}$ +40.21 (c 0.1, methanol); ^{1}H NMR 270 MHz (acetone-d_6) 1.5 (m, 15 H, H-3′, 4′ and 5′ of THP, and H-6′), 3.5 to 5.0 (m, 10 H, H-2′, 3′, 4′and 5′, and H-2′ and 6′ of THP), 6.0 to 6.2 (m, 1 H, H-1′), 7.8 to 7.9 (m, 1 H, H-6). Anal. Calcd. for $C_{20}H_{29}O_8N_2F$: C, 54.05; H, 6.53; N, 6.31; F, 4.28. Found: C, 53.91; H, 6.73; N, 6.1; F, 4.25.

5-Fluoro-1-(4-O-tert-butyldimethylsilyl-2,3-bis-O-tetrahydropyranyl-α-L-rhamnopyranosyl)uracil 10.

Product 7 was dried overnight in a vacuum desiccator over P_2O_5. To a 100 mL flask, dried over a flame under N_2 atmosphere and cooled to room temperature, was added 5.5g (12.38 mmol) of 9, followed by 8 mL of DMF under N_2 atmosphere. Then 1.76 g of imidazole followed by 2.7 g of tert-butyldimethylsilylchloride were added. Further, a catalytic amount of 4-DMAP was also added. The mixture was kept stirred mechanically under N_2 atmosphere with exclusion of moisture. The reaction was followed by TLC in solvent (C). Upon completion of the reaction, DMF was evaporated in vacuo and the solid product obtained was dissolved in ethyl acetate. This solution was washed with a saturated solution of $NaHSO_4$ and Na_2HPO_4 1:1 (v/v). The aqueous layer was separated and washed three times with about 50 ml of ethyl acetate. The combined organic layer was again washed with a saturated solution of NaCl. The organic layer was separated and dried over anhydrous Na_2SO_4, filtered, and

evaporated to dryness under reduced pressure. The product was purified by column chromatography using graded eluents hexane/ethyl acetate 8:2 (v/v) and 4:6 (v/v) to yield 10 as a fluffy mass. Yield 93%; R_f 0.68 (solvent C); $[\alpha]_D^{22}$ -15.62 (c 0.1 methanol); ^{1}H NMR 270 MHz (acetone-d_6) 0.10 (s, 3 H, CH_3-Si), 0.15 (s, 3 H, CH_3-Si), 0.9 (s, 9 H, $(CH_3)_3C$-), 1.2 to 1.8 (m, 15 H, H-3, 4 and 5 of THP and H-6′), 3.3 to 5.0 (m, 10 H, H-2 and 6 of THP, and H-2′, 3′, 4′, 5′), 6.0 to 6.2 (m, 1 H, H-1′), 7.5 to 7.7 (m, 1 H, H-6). Anal. Calcd. for $C_{26}H_{43}O_8N_2FSi$: C, 55.91; H, 7.71; N, 5.02; F, 3.40. Found: C, 55.98; H, 7.79; N, 4.84; F, 3.78.

5-Fluoro-1-(4-O-tert-butyldimethylsilyl-α-L-rhamno-pyranosyl) uracil 11.

To a 500 mL flask dried over a flame under N_2 atmosphere, was added 24.15 g (43.90 mmol) of product 10. To this was added 175 mL of dry methanol followed by 1.66 g of p-toluenesulfonic acid. The reaction mixture was stirred mechanically under N_2 atmosphere. The progress of the reaction was followed by TLC in solvent (D). Upon completion of the reaction, the mixture was neutralized using 1R45(OH^-) resin. The resin was filtered off, washed with methanol and then filtrate was evaporated to dryness in vacuo. The final product was purified by column chromatography with eluents of varying polarity using hexane/ethyl acetate (4:6/ v:v). The final product 11 was crystallized from methanol. Yield 88%; mp 103°C; R_f 0.46 (solvent D); $[\alpha]_D^{22}$+4.58 (c 0.1, methanol); ^{1}H NMR 270 MHz (chloroform-d) 0.1 (s, 6 H, $(CH_3)_2Si$-), 0.9 (s, 9 H, $(CH_3)_3C$-), 1.5 (d, 3

H, H-6′, $J_{5',6'}$= 6.75 Hz), 3.8 to 4.2 (m, 4 H, H-2′, 3′, 4′ and 5′), 6.05 (d, 1 H, H-1′, $J_{1',2'}$= 8.82 Hz), 7.5 (d, 1 H, H-6, $J_{6,F}$= 6.75 Hz). Anal. Calcd. for $C_{16}H_{27}O_6N_2FSi$ 1/2 H_2O: C, 48.12; H, 7.01; F, 4.76. Found: C, 48.26; H, 7.02; N, 6.82; F, 4.87.

5-Fluoro-1-(2,3,-di-O-acetyl-4-O-tert-butyldimethy-silyl-α-L- rhamnopyranosyl)uracil 12.

To a 250 mL flask dried over a flame under N_2 atmosphere, was added 5.5 g (14.58 mmol) of product 11. This was carefully dissolved in 68 mL of dry pyridine. The solution was cooled to 0 °C and then 34 mL of acetic anhydride and a catalytic amount of 4-DMAP were added to the solution. The mixture was kept at 0 °C for 15 min and then kept at ambient temperature. Acetylation was complete in 2 reaction, the pyridine was evaporated in vacuo. Complete removal of pyridine and acetic anhydride was ensured by coevaporation with 25 ml toluene three times. The solid product obtained was dissolved in ethyl acetate and was washed with a saturated solution of sodium chloride. The organic layer was then dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The final product 13 was purified by column chromatography using hexane/ethyl acetate 7:3 (v/v)) as eluent. Product 10 was obtained as a fluffy mass. Yield 94%; R_f 0.56 (solvent B); $[\alpha]_D^{22}$+7.92 (c 0.1, methanol); ^1H NMR 270 MHz (chloroform-d) 0.15 (s, 3 H, CH_3-Si), 0.17 (s, 3 H, CH_3-Si), 0.95 (s, 9 H, $(CH_3)_3C$-), 1.45 (d, 3 H, H-6′, $J_{5',6'}$= 7.56 Hz), 2.00 (s, 3 H, -$OCCH_3$), 2.15 (s, 3 H, -$OCCH_3$), 3.75 (dd, 1 H, H-4′, $J_{4',5'}$= 1.26 Hz

and $J_{4',3'}$= 3.78Hz), 4.15 (qd, 1 H, H-5', $J_{5',6'}$= 7.56 Hz), 5.25 to 5.35 (m, 2 H, H-2', H-3'), 6.15 (dd, 1 H, H-1', $J_{1',2'}$= 8.82 Hz and $J_{1',F}$= 1.5 Hz), 7.4 (d, 1 H, H-6, $J_{6,F}$= 6.75 Hz). Anal. Calcd. for $C_{20}H_{31}O_8N_2FSi$: C, 50.63; H, 6.54; N, 5.91; F, 4.01. Found: C, 50.56; H, 6.79; N, 5.86; F, 4.01.

5-Fluoro-1-(2,3-di-O-acetyl-α-L-rhamnopyranosyl)uracil 13.

To a dry 250 mL flask protected by a drying tube, 10.0 g (21.1 mmol) of 10 was added which was then dissolved in 8.27 mL of dry methanol. To this well stirred solution was added 75 mL of trifluoroacetic acid. The progress of the reaction was monitored by TLC in solvent (B). On completion of the reaction (25 h), the solvents were evaporated to dryness in vacuo at room temperature. The solid product was dissolved in ethyl acetate and then neutralized with a saturated solution of $NaHCO_3$. Subsequently, the mixture was washed with a saturated NaCl solution. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated. The final product was purified by silica gel column chromatography, initially using the solvent system hexane/ethyl acetate 8:2 (v/v) and then 3:7 (v/v). The final product was foamy. Yield 88%; R_f 0.32 (solvent B); $[\alpha]_D^{22}$+26.45 (c 0.1, methanol); ^{1}H NMR 270 MHz (chloroform-d) 1.5 (d, 3 H, H-6', $J_{6',5'}$= 7.56 Hz), 2.0 (s, 3 H, -OCCH$_3$), 3.85 (dd, 1 H, H-4', $J_{4',5'}$= 2.5 Hz and $J_{4',3'}$= 3.5 Hz), 4.3 (dq, 1 H, H-5', $J_{4',5'}$= 2.5 Hz and $J_{5',6'}$= 7.56 Hz), 5.4 to 5.5 (m, 2 H, H-2', H-3'), 6.1 (dd, 1 H, H-1', $J_{1',F}$= 1.5

Hz and $J_{1',2'}$= 8.5 Hz), 7.65 (d, 1 H, H-6, $J_{6,F}$= 6.75 Hz). Anal. Calcd. for $C_{14}H_{17}O_8N_2F$: C, 46.67; H, 4.72; N, 7.78; F, 5.28. Found: C, 46.40; H, 5.18; N, 7.25; F, 4.84.

5-Fluoro-1-(3-O-acetyl-2,6-dideoxy-α-L-glycero-hex-2-enopyranos- 4-ulosyl)uracil 15.

6.41 g (17.5 mmol) of 11 was placed in 500 ml flask dried well over a flame under N_2 atmosphere. 77.0 mL of dry CH_2Cl_2 was added to the flask and the mixture was vigorously stirred mechanically. The contents of the flask were meticulously protected from any trace of moisture. To this solution was added 19.23 g (51.1 mmol) pyridinium dichromate (PDC)[8] and 17.82 g of MS 3 Å. The oxidation was followed by TLC in the solvent system (B). Although the unsaturated keto-nucleoside was clearly visible under uv light, no dark color was observed with H_2SO_4 spray and heating. The reaction was complete in 5 h. The solution was diluted with 100 mL of dry ethyl ether and stirred for 0.5 h. This was vacuum filtered through a bed of (40) silica gel mixed with $CaSO_4$ (1:1). The filter was washed once with 200 mL of ethyl ether and then with 1500 mL of diethyl ether/ethyl acetate (1:1). The filtrate was concentrated in vacuo to dryness and crystallized from ethanol to afford 15. Yield 50%; mp 274 °C; R_f 0.43 (solvent B); $[\alpha]_D^{22}$+22.9 (c 0.1, methanol); ^{1}H NMR 270 MHz (acetone-d_6) 0.4 (d, 3 H, H-6′, $J_{5',6'}$= 7.56 Hz), 1.25 (s, 3 H, -OCCH$_3$), 3.75 (q, 1 H, H-5′, $J_{5',6'}$ = 7.56 Hz), 5.8 (dd, 1 H, H-1′, $J_{1',2'}$= 3.7 Hz and $J_{1',F}$= 1.5 Hz), 5.9 (d, 1 H, H-2′, $J_{1',2'}$= 3.7 Hz), 7.0 (d, 1 H, H-6, $J_{6,F}$= 6.75 Hz). Anal. Calcd. for $C_{12}H_{11}O_6N_2F$: C,

48.32; H, 3.69; N, 9.40; F, 6.38. Found: C, 48.28; H, 3.75; N, 9.28; F, 6.12.

5-Fluoro-1-(2,3-di-O-benzoyl α-L-rhamnopyranosyl)uracil 14.

10.0 g (36.2 mmol) of 4 was dissolved in a mixture of pyridine (120 mL) and dichloroethane (400 mL). To this well stirred solution, 40 mL of benzoyl chloride was added quickly, keeping the reaction flask free from moisture.[9] The reaction mixture was stirred for three more min. The reaction was then stopped by adding methanol (300 mL) and the resultant mixture was concentrated. The solid product was suspended in ethyl acetate (300 mL) and filtered. The filter was washed with ethyl acetate and the combined filtrate was evaporated. The residue was chromatographed over a silica gel column. Elution with ethyl acetate-hexane (1:1, v/v) afforded 2′,3′- di-O-benzoyl compound 14 as a major product (7.0 g, 40%), with other benzoyl nucleosides. The viscous 2′,3′-di-O-benzoyl nucleoside was dried in a vacuum desiccator over P_2O_5 for several hours. This was then crystallized from ethyl acetate-hexane. R_f 0.3 (solvent C); mp 125°C; $[\alpha]_D^{22}$ + 20.3 (c 0.1, Chloroform); ^1H NMR (chloroform-d) 1.50 (d, 3 H, H-6′, $J_{5',6'}$ = 7.0 Hz), 4.0 (m, 1 H, H-4′), 4.35 (q, 1 H, H-5′, $J_{5',6'}$ = 7.0 Hz), 5.65 to 5.80 (m, 2 H, H-2′ and 3′), 6.40 (broad d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 7.20, 7.40, 7.50, 7.75 and 8.0 (benzoyl H), 7.65 (d, 1 H, H-6, $J_{6,F}$ = 8.0 Hz). Anal. Calcd. for $C_{24}H_{21}O_8N_2F$: C, 59.5; H, 4.37; N, 5.78. Found: C, 58.78; H, 4.51; N, 5.55.

5-Fluoro-1-(3-O-benzoyl-2,6-dideoxy- α-L-glycero-hex-2-enopyranos-4-ulosyl)uracil 16.

7 g (14.5 mmol) of 14 was placed in a 250 mL flask (flame dried under N_2) fitted with a condenser and drying tube. 70 mL of dry CH_2Cl_2 was then added to the flask and the mixture was vigorously stirred magnetically at about 50 °C. To this solution was added 11.96 g (31.80 mmol) pyridinium dichromate (PDC) and 14.46 g well powdered MS 3Å. The progress of the oxidation was monitored by TLC. The with 200 mL of ethyl acetate/ether mixture (1:1 v/v) for 0.5 h. The mixture was filtered through a bed of fine silica gel mixed with $CaSO_4$ (1:1). The filter was washed twice with 50 mL each of ethyl acetate-ether (1:1). The filtrate was concentrated in vacuo to dryness and crystallized from ethyl acetate to afford 16 as a colorless product. Yield 80%; mp 200 C; R_f 0.41 (solvent C); $[\ ]^{22}$-164.0 (c 0.25, methanol); ^1H NMR (acetone-d_6) 1.45 (d, 3 H, H-6', $J_{5',6'}$ = 9.0 Hz), 4.80 (q, 1 H, H-5', $J_{5',6'}$ = 9.0 Hz), 6.90 (dd, 1 H, H-1', $J_{6',F}$ = 1.35 and $J_{1',2'}$ = 2.7 Hz), 7.10 (d, 1 H, H-2', $J_{1',2'}$ = 2.70 Hz), 7.60, 7.75 and 8.15 (benzoyl H), 8.07 (d, 1 H, H-6, $J_{6,F}$ = 8.0 Hz,) Anal. Calcd. for $C_{17}H_{13}O_6N_2F$: C, 56.67; H, 3.64; N, 7.80; F, 5.27. Found: C, 56.70; H, 3.57; N, 7.68; F, 4.94.

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