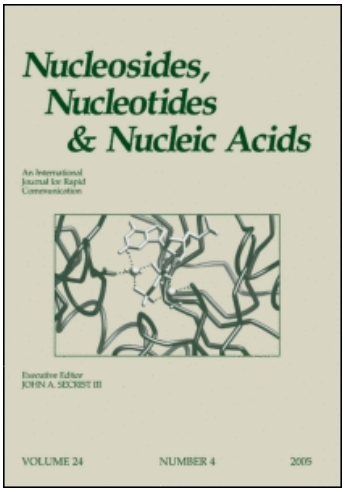


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Biological Activities of Unsaturated Ketohexopyranosyl Nucleosides of Pyrimidines

Arun P. Sharma[a]; Abraham P. Ollapally[a]; Willishea Jones[a]; Tracey Lemon[a]
[a] Department of Chemistry, Florida Agricultural and Mechanical University, Tallahassee, Florida

To cite this Article Sharma, Arun P. , Ollapally, Abraham P. , Jones, Willishea and Lemon, Tracey(1992) 'Synthesis and Biological Activities of Unsaturated Ketohexopyranosyl Nucleosides of Pyrimidines', Nucleosides, Nucleotides and Nucleic Acids, 11: 5, 1009 — 1038
To link to this Article: DOI: 10.1080/07328319208021165
URL: http://dx.doi.org/10.1080/07328319208021165

NUCLEOSIDES & NUCLEOTIDES, 11(5), 1009-1038 (1992)

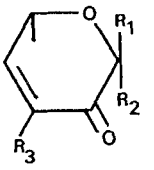
SYNTHESIS AND BIOLOGICAL ACTIVITIES OF UNSATURATED KETO-HEXOPYRANOSYL NUCLEOSIDES OF PYRIMIDINES

Arun P. Sharma, Abraham P. Ollapally*, Willishea Jones[+] and Tracey Lemon[+]
Department of Chemistry
Florida Agricultural and Mechanical University
Tallahassee, Florida 32307

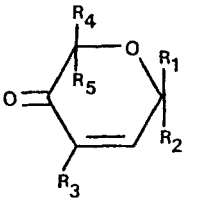
Abstract: Synthesis of unsaturated ketohexopyranosyl nucleosides of a few pyrimidines is described. The results of their bioevaluation for anticancer and antiviral activity are also discussed.

Medicinal interest in unsaturated ketopyranosyl nucleosides has been generated in our laboratory owing to the presence of significant *in vitro* and *in vivo* anticancer activity in some theophylline nucleosides (2, 3, 9 and 10).[1,2,3] Their unconventional mode of action has further enhanced our interest in this class of compounds.[4] *In vitro*, these nucleosides have been shown to react as Michael acceptor with nucleophilic thiol groups of physiologically active molecules, e.g., glutathione, cysteine and lactate dehydrogenase.[4] *In vivo*, their reaction with plasma membrane surface thiols and soluble intracellular thiols has also been clearly demonstrated.[4] Furthermore, there exists a good correlation between the extent of *in vitro* plasma membrane alkylation and *in vivo* anticancer activity.[4] It is also noteworthy that they do not show genotoxicity.[5] Thus, the anticancer activity could be due to alkylation of thiol groups of key proteins involved in cell metabolism.[4]

[+]Undergraduate students worked under Minority Biomedical Research Support Program.



1. R_1 = H, R_2 = -1-(5-fluorouracil), R_3 = OBz
2. R_1 = H, R_2 = -7-theophylline, R_3 = OAc
3. R_1 = -7-theophylline, R_2 = H, R_3 = Br



4. R_1 = H, R_2 = -1-thymine, R_3 = R_4 = R_5 = H
5. R_1 = -1-thymine, R_2 = H, R_3 = H, R_4 = CH_2OH, R_5 = H
6. R_1 = H, R_2 = -1-thymine, R_3 = H, R_4 = CH_2OH, R_5 = H
7. R_1 = -1-(5-fluorouracil), R_2 = H, R_3 = OAc, R_4 = H, R_5 = CH_3
8. R_1 = -1-(5-fluorouracil), R_2 = H, R_3 = OBz, R_4 = H, R_5 = CH_3
9. R_1 = -7-theophylline, R_2 = H, R_3 = OBz, R_4 = CH_3, R_5 = H
10. R_1 = -7-theophylline, R_2 = H, R_3 = OBz, R_4 = CH_2OH, R_5 = H

Chart - I

Very recently some unsaturated ketopyranosyl nucleosides of thymine (4, 5, and 6) have been found to possess anti-AIDS and anti-HSV activities.[6] Their mode of action is yet to be explained.

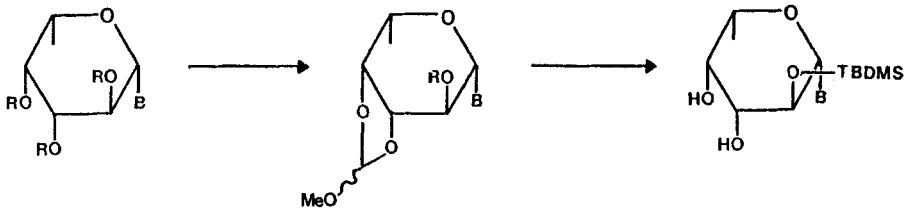
The study of structure-activity relationship of unsaturated ketopyranosyl nucleosides has shown that presence of α,ß-unsaturated keto system in the sugar ring is a necessary prerequisite for biological activities.[4] The compounds with hydroxyl groups in place of keto group in the sugar ring have been found inactive.

Encouraged by the significant anticancer and antiviral properties as well as novel mode of action of unsaturated ketopyranosyl nucleosides, we also undertook synthesis and bioevaluation of similar nucleosides of a few pyrimidines. The pyrimidines chosen are especially those which are biologically active per se or in the nucleoside form.

Earlier, we have reported the synthesis of 3'-O-acyl-2', 3'/3',4'-unsaturated-2'/4'-keto- α/ß-L-hexopyranosyl nucleosides of 5-fluorouracil (1,7, and 8).[7,8] Moreover, on in vitro anticancer activity evaluation, 3'-O-acyl-2',3'- unsaturated-4'- keto-analogs (7 and 8) have been found to show anticancer activity as good as that of 5-fluorouracil in four different cell lines.[8] This paper illustrates subsequent details of our continuing investigation in the direction of synthesis and biological activity evaluation of pyrimidine unsaturated ketopyranosylnucleosides.

Chemistry:

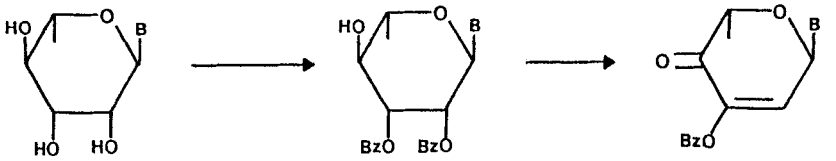
Tribenzoylnucleoside of 5-fluorocytosine 17 was synthesized according to the earlier procedure described for 5-fluorouracil (scheme-I).[7] As expected, coupling of 5-fluorocytosine with tetra-O-acetyl-L-rhamnose catalyzed by $SnCl_4$ gave only ß-isomer of tri-O-acetylnucleoside 11 in 50% yield. Nucleoside 11 was then converted into



11 R = OAc
12 R = H
13 R = H
14 R = TBDMS
15
B = -1-(5-fluorocytosine)

16 R = TBDMS
17 R = H
18
B' = -1-(N-benzoyl-5-fluorocytosine)
TBDMS = t-butyldimethylsilyl

Scheme - I



19, 20
21, 22
23, 24

19, 21, 23 B = -1-uracil
20, 22, 24 B = -1-(5-chlorouracil)

Scheme - II

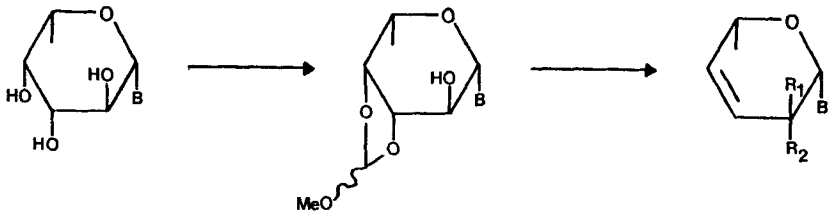
tribenzoylnucleoside 17 in six steps using a sequential protection-deprotection strategy. ^{1}H NMR and elemental analyses data of all compounds were found fully consistent with their assigned structures.

Di-O-benzoylnucleosides of uracil 21 and 5-chlorouracil 22 were prepared by benzoylation of unprotected nucleosides 19 and 20 respectively (scheme-II). Benzoylation was effected with benzoyl chloride and pyridine in dichloroethane.[9] A mixture of mono, di and tri-O-benzoyl nucleosides was obtained in each case. Column chromatography of the mixture, furnished the required nucleosides 21 and 22 in 39% and 41% yield respectively.

Structures of 21 and 22 were confirmed by comparison of their ^{1}H NMR spectral data with those reported earlier for 2', 3'-di-O-benzoyl-α-L-rhamnopyranosyl nucleosides of 5-fluorouracil and adenine.[8,10]

Synthesis of unsaturated nucleosides (27, 35 and 37) was accomplished as shown in scheme-III and IV. Recently reported procedure for conversion of 2-methoxy-1,3-dioxolanes into olefins, by heating with Ac_2O was used to generate unsaturation in the sugar moiety.[11]

Synthesis of 25 and 31 has already been reported[7,8] and similar procedure was used to synthesize 32. Elaboration of cis-hydroxyls of 25, 31 and 32 into 2-methoxy -1,3,-dioxolanes was achieved by reacting nucleosides with $(CH_3O)_3CH$ in DMF using p-toluenesulfonic acid or its pyridinium salt as catalyst. Subsequently nucleosides dioxolanes were heated with Ac_2O at 140°C and the progress of the reaction was carefully monitored by TLC. The free hydroxyl of nucleosides rapidly underwent acetylation to afford the corresponding acetates which were then gradually transformed into the unsaturated nucleoside acetates. Apart from the desired products, acetylated bases were also isolated from the reaction mixtures, indicating, as reported earlier,[11] deglycosylation of the unsaturated nucleosides, because of their sensitivity to acid particularly at high temperature.

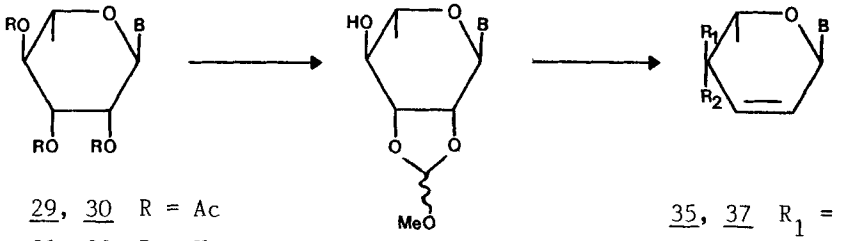


25 26

27 R_1 = OH, R_2 = H
28 R_1 = R_2 = O

B = -1-(5-fluorouracil)

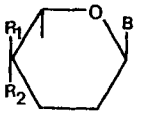
Scheme - III



29, 30 R = Ac
31, 32 R = H

33, 34

35, 37 R_1 = OH, R_2 = H
38, 40 R_1 = R_2 = O



36 R_1 = OH, R_2 = H
39 R_1 = R_2 = O

29, 31, 33, 35, 36, 38, 39 B = -1-(5-fluorouracil)
30, 32, 34, 37, 40 B = -1-(5-trifluoromethyluracil)

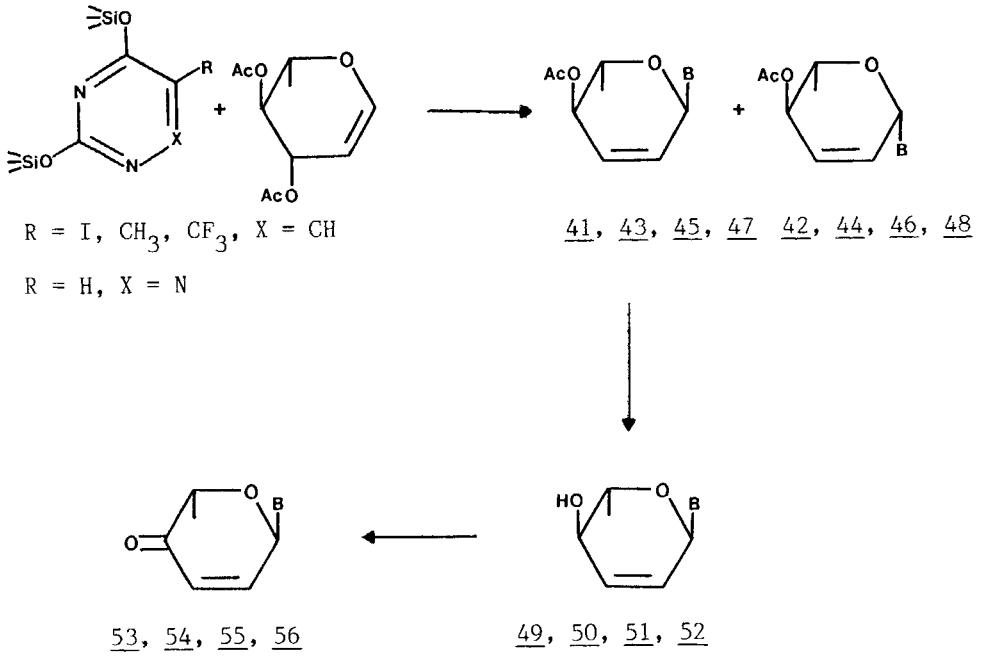
Scheme - IV

Hydrolysis of these acetates with MeOH/NH_3 furnished the nucleosides 27 and 35 in about total 55% and 37 in 35% yield. Rather low yields could be attributed to substantial deglycosylation. Further, 35 was reduced with H_2 and Pd/C to obtain 2',3'-dihydronucleoside 36.

In the ^1H NMR spectra of 27, 35 and 37, presence of a pair of doublets of about 10 Hz strongly indicates the presence of unsaturation in the sugar ring. Further, comparison of ^1H NMR spectral data of the unsaturated nucleosides with the data of the starting nucleosides 25, 31 and 32 was used to confirm the position of double bond. In case of 25 and 31, H-1' appears as a doublet of doublets of 1.8 and 9.0 Hz, and in case 32 as a doublet of 9.0 Hz. Upon unsaturation, in case of 35 and 37, signal for H-1' collapsed into a broad singlet, which is attributed to change of H-2' into an olefinic proton and thereby loss of its trans-diaxial relationship with H-1'. In contrast, in case of 27, H-1' stayed as such with a similar J value of 1.5 and 8.6 Hz. Morever, H-6' appears as a doublet of 7.0 Hz in case of both 25 and 27 indicating against the loss of H-5' in 27, had double bond migrated to C-5', 6' upon unsaturation. All these observations taken together strongly support the C-3', 4' unsaturation in 27 and C-2', 3' in 35 and 37.

Because of overall low yields of 2', 3'-unsaturated nucleosides by the method described above, an alternate short method was subsequently explored to synthesize them. The unsaturated nucleosides were prepared by condensation of 3', 4'-di-O-acetyl-L-rhamnal with silylated bases (scheme-V). The catalyst tried were $SnCl_4$, trimethylsilyl trifluoromethanesulfonate (TMS-triflate) and trityl perchlorate[12]. Among these, TMS-triflate was found to be a better catalyst, while trityl perchlorate did not work at all, in our hands. Using this method, both α and ß-anomers of the unsaturated nucleosides of 5-iodouracil, thymine, 5-trifluoromethyluracil and 6-azauracil were obtained in about combined 70% yield and in 1:1 ratio. Initially, in case of 5-iodouracil, 1 molar equivalent of the catalyst to the diacetylrhamnal was used, but later on, 0.5 to 0.75 equivalent was found to be effective.

In case of 5-trifluoromethyluracil and 6-azauracil nucleosides (45, 46, 47, and 48), α and ß-anomers were found to be inseparable on TLC and by column chromatography or



R = I, CH_3, CF_3, X = CH

R = H, X = N

41, 43, 45, 47 42, 44, 46, 48

53, 54, 55, 56 49, 50, 51, 52

41, 42, 49, 53 B = -1-(5-iodouracil)

43, 44, 50, 54 B = -1-thymine

45, 46 B = -1-(5-trifluoromethyluracil)

47, 48, 51, 52 (β), 55, 56 (β) = -1-(6-azauracil)

Scheme - V

crystallization. In contrast to this, anomers of 5-iodouracil and thymine nucleosides (41, 42 and 43, 44) showed good R_f difference on TLC and were separated by fractional crystallization.

Formation of 4'-O-acetyl-2', 3'-unsaturated nucleosides was supported by presence of a pair of doublets of about 10 Hz attributed to olefinic protons and a broad singlet attributed to H-1' in the downfield region (5.70 to 6.50 ppm) of their ^1H NMR spectra. Assignment of α and ß-anomers was done on the basis of pattern of the ^1H NMR chemical shifts of their sugar ring protons. As evident from the table-1 as well as reported

Table - I

No	H-5/6	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'	Acetyl-H 5-CH_3	
35(α, acetate)	7.55 (d)	6.40 (brs)	5.85 (brd, 10.0)	6.30 (ddd, 0.9, 2.7, 10.0)	5.00 (m)	4.00 (quin, 7.0)	1.33 (d, 7.0)	2.15 (s)	
41 (α)	7.85 (s)	6.20-6.40 (m)	5.85 (brd, 10.0)	6.20-6.40 (m)	5.00 (m)	4.00 (quin, 7.0)	1.35 (d, 7.0)	2.15 (s)	
42 (β)	7.40 (s)	6.45 (brs)	5.75 (dt, 1.6, 10.0)	6.20 (dt, 1.6, 10.0)	5.20 (dq, 1.6 7.0)	3.85 (m)	1.25 (d, 7.0)	2.13 (s)	
43 (α)	7.25 (s)	6.40 (brs)	5.85 (brd, 10.0)	6.25 (ddd, 0.9, 2.7, 10.0)	5.00 (m)	3.97 (quin, 7.0)	1.33 (d, 7.0)	2.18 (s)	1.95 (s)
44 (β)	7.27 (s)	6.50 (brs)	5.75 (dt, 1.6, 10.0)	6.15 (dt, 1.6, 10.0)	5.20 (dq, 1.6 7.0)	3.87 (m)	1.27 (d, 7.0)	2.13 (s)	1.95 (s)
45 (α)	7.95 (s)	6.45 (brs)	5.90 (brd, 9.45)	6.35 (ddd, 0.9, 2.7, 9.45)	5.00 (m)	4.00 (m)	1.33 (d, 7.0)	2.15 (s)	
46 (β)	7.75 (s)	6.50 (brs)	5.75 (dt, 1.6, 9.45)	6.20 (dt, 1.6, 9.45)	5.20 (dm, 8.1)	3.90 (m)	1.25 (d, 7.0)	2.15 (s)	
47 (α)	6.44 (s)	6.45 (brs)	5.80 (dm, 10.0)	6.15 (dt, 2.4, 10.0)	5.10 (dm, 8.0)	4.10 (m)	1.35 (d, 7.0)	1.13 (s)	
48 (β)	6.48 (s)	6.50 (brs)	5.80 (dm, 10.0)	6.05 (dt, 2.4, 10.0)	5.20 (dm, 8.0)	3.95 (m)	1.25 (d, 7.0)	1.12 (s)	

Solvent: $CDCl_3$; number in parantheses: J values in Hz

earlier,[12, 13] H-2' and 3' of the α-anomers resonate more downfield than those of corresponding ß-anomers. Reverse is the case with H-1' and 4'. Therefore, 41, 43, 45 and 47 have tentatively been assigned as α-anomers and 42, 44, 46 and 48 as ß-anomers,

Earlier, in case of pyrimidine 4', 6'-di-O-acetyl-2',3'-unsaturated nucleosides obtained from 3, 4, 6-tri-O-acetyl-D-glucal, ß-anomers have been reported to show higher R_F value than the corresponding α-anomers.[13] Similar case has been found here too for well-resolving anomers of 5-iodouracil and thymine.

α-Anomers of 5-iodouracil and thymine were subsequently deacetylated with $MeOH/NH_3$ to their 4'-hydroxylanalogs. However, in case of 6-azauracil, the mixture of anomers was deacetylated because of inseparability of anomers.

Finally, the synthesis of unsaturated ketonucleosides was achieved by oxidation of hydroxyl group of 2', 3'/3',4' -unsaturated nucleosides with pyridinium dichromate/molecular sieves in CH_2Cl_2[14]. Similar oxidation of 2'/4'- hydroxyl of di-O-benzoyl nucleosides 17, 21 and 22 gave directly α-benzoyloxy-α, ß-unsaturated ketonucleosides 18, 23 and 24 as a result of subsequent *in situ* ß-benzoyloxy elimination in intermediate dibenzoylketonucleosides. In comparison to all other unsaturated ketonucleosides which showed good stability during silica gel column chromatography 18 and 23 were found quite prone to deglycosylation during this process.

Absence of signal for one sugar ring proton and substantial downfield shifts for signals of olefinic protons in the ^1H NMR spectra of unsaturated ketonucleosides 28, 38, 40, 53, 54, 55 and 56, in comparison to those of their precursors well support the presence of α , ß-unsaturated-keto system in them. In case of 18, 23 and 24, absence of signals for one benzoyloxy group and two sugar ring protons in their ^1H NMR spectra in comparison to those of their precursors strongly favors the introduction of unsaturation as well as keto group in them. Rest of the features of ^1H NMR

spectra of all unsaturated ketonucleosides were also found consistent with their assigned structures.

For the 4'-keto-2', 3'-saturatednucleoside 39, homogenous on TLC, a pair of signals for each proton was obtained in its ^{1}H NMR spectra. This could be due to the presence of its two conformers.

As the anomers of 6-azauracil unsaturated nucleosides 47 and 48 could not be separated, they were deacetylated as a mixture and the mixture of 4'-hydroxylnucleosides 51 and 52 was then oxidized to afford two keto analogs 55 and 56. These ketoanalogs resolved very well on TLC with 55 having higher R_f than 56 and were separated by fractional crystallization.

The stereochemical assignments for 55 and 56 were made by reducing their 4'-keto group respectively with $NaBH_4$ and then acetylating the resultant alcohols. In case of 55, this reaction sequence afforded only one isomer, while in case of 56 two isomers were obtained. The ^{1}H NMR chemical shifts of isomer from 55 were found to be identical to those of 47 and for major isomer from 56, they were identical to chemical shifts of 48. Based on this observation, 55 has been assigned as α-anomer and 56 as ß-anomer.

Biological Activities:

As mentioned in the introduction, the unsaturated keto nucleosides show biological activity due mainly to the presence of α, ß- unsatuarated keto system in the sugar moiety, however the role of aglycon in modifying the activity has not been extensively investigated. So far only theophylline among purines and thymine among pyrimidines have been found to be suitable aglycons whose nucleosides show biological activity. Our aim has been to investigate how different pyrimidine analogs as aglycons influence the biological activity. To this end, we synthesized a number of pyrimidine unsaturated ketonucleosides with both α and ß-anomeric configuaration, and with or without substitution in the sugar moiety. Subsequently these nucleosides were tested for their anti-cancer and -viral activities at NCI and NIAID of NIH respectively.

Anticancer Activity: Nucleosides 1, 7, 18, 23, 24, 28, 35, 36, 38, 39, 39, 40, 53, 54, 55, and 56 all were evaluated for their in vitro anticancer activities in 8 different types of cancer cell panels each consisting of 5 to 8 different cell lines. Nucleosides 7 and 8 were also tested in vitro in four different cell lines in De Clercq's laboratory in Belgium. None of the nucleosides showed any significant anticancer activity (anti-cell growth activity or cell toxicity under the dose range (-4 to -9 $\log_{10}$ molar concentration of test compounds) used routinely at NCI for initial screening. It thus indicates that pyrimidines are not a suitable aglycon moiety for anticancer activity. Interestingly nucleosides 7 and 8 were found to possess anti-cell growth activity comparable to that of 5-fluorouracil and 5-fluorodeoxyuridine in murine leukemia L1210, murine mammary carcinoma FM3A, human B-lymphoblast Raji, and human T-lymphoblast Molt/4F, upon testing in Belgium.[8] The reason for discrepancy in the results from two testing centers is not completely clear. Perhaps the activity is due to 5-fluorouracil which is generated from 7 and 8 by deglycosylation. Then the difference in capabilities of different cancer cell lines to deglycosylate the 7 and 8 may account for this discrepancy.

Antiviral Activity: Nucleoside 1, 7, 28, 35, 36, 37, 38, 39, 40, 49, 53, 54, 55, and 56, all were evaluated for their anti-HIV activity. None of them was however found active against HIV virus. It is interesting to note that 4, a 5' nor anolog of 54 has recently been found to show very good activity against this virus (ED_{50} 2 μg/mL; HIV-MT4),[6] while, 54 is inactive. Furthermore even 5'-hydroxymethyl analogs 5 and 6 are also inactive.[6] Apparently, 5'-position of 4 is very sensitive to any substitution for anti-HIV activity.

Compound 1 to 56 (mentioned above) were also tested for their detailed antiviral activities against HSV, CMV, VZV, Flu, measles, RSV, PIV-3 and AD-5 viruses. Only 5-trifluoro-methyluracil and 6-azauracil-nucleosides 40 and 56 showed some

significant activity. Nucleoside 40 was found active against. HSV-1 and 2 (ED_{50} 0.31 and 2 μg/mL; ACV 0.04 and 0.06 μg/mL and 56 against Flu B (ED_{50} 2 μg/mL; rivarin 6 μg/mL). However, both 40 and 56 as well as all other nucleosides assayed for antiviral activities were also found to possess very high cell toxicity or anti-cell growth activity or both at very low doses. Therefore the selectivity index for both 40 and 56 is very low which renders them very poor antiviral candidates.

As most of the unsatuarated ketonucleosides assayed do not show any significant anticancer or antiviral activity, it is not possible to draw any conclusion about SAR in this class of nucleosides. It appears that only purines are suitable aglycons for anticancer activity, because so far significant anticancer activity is confined only in purine (more precisely theophylline) unsaturated ketonucleosides. Furthermore only 5-fluorouracil nuclosides 7 and 8 show anticancer activity in some cell lines. Since 5-fluorouracil itself has anticancer activity, more biological studies are required to know whether unsaturated keto system is also contributing to the activity.

So far as their potential as antiviral agents is concerned, it is first necessary to achieve good differentiation between antiviral activity and toxicity. Whether it could be accomplished by modifying base or sugar moiety, can be determined only by further investigations.

Experimental

Melting points (uncorrected) were recorded using Mel-Temp apparatus. Elemental analyses were performed by Gailbraith Laboratories, Inc. ^1H NMR spectra were reordered on a Brucker/IBM-SY200 spectrometer at 270 Mz (Me_4Si as internal standard) and are expressed in ppm. Optical rotations were recorded on a quick polarimeter, model 52 of Rudolph Instruments at 25°C. Thin layer chromatography (TLC) was performed on a precoated silica gel plastic TLC sheets 60 F_{254} (0.2mm) EM Reagents. Compound visualization was effected with a UV lamp (254 nM) or 5% solution of H_2SO_4 in EtOH . Silica

gel 60(70-230 mesh ASTM) was used for column chromatography. All solvents were evaporated in vacuo below 40°C. All products which were obtained as an oil and could not be crystallized, were dried in vacuo at 40°C for several hours before using them in the next reaction.

5-Fluoro-1-(6-deoxy-2,3,4-tri-O-acetyl-ß-L-galacto-pyranosyl)cytosine (11). A mixture of 5-fluorocytosine (4.65 g, 36 mmol), hexamethyldisilazane (HMDS 26.7 mL), and saccharin (100 mg) in 26 mL of $(CH_2Cl)_2$ was heated with stirring under reflux to dissolve all the base and for an additional 0.5 h. The solution was then cooled in an ice-bath, and a solution of tetra-O-acetyl-L-fucose (13.5 g, 40.7 mmol) in 30 mL of $(CH_2Cl)_2$ was added. This was followed by portionwise addition of a solution of $SnCl_4$ (6 mL, 51.4 mmol) in 6 mL of $(CH_2Cl)_2$. On completing the addition of $SnCl_4$, the reaction mixture was heated at 84°C for 3.5 h. The reaction mixture was then cooled and neutralized with methanolic ammonia solution. The solvents were evaporated and the residue obtained was mixed with EtOAc. The precipitate was filtered and washed with a large amount of EtOAc. The combined filtrate was concentrated and the residue obtained was chromatographed (EtOAc) to afford 11 (7.2 g, 50%): mp 270-272°C (EtOAc); $[\alpha]_D$ - 40.0° (c 0.25, MeOH); ^{1}H NMR (Me_2CO-d_6) 1.20 (d, 3 H, H-6', J = 6.6 Hz), 1.90, 1.95 and 2.20 (3 x s, 3 H each, $-COCH_3$), 4.34 (q, 1 H, H-5', J = 6.6 Hz), 5.20 to 5.40 (m, 3 H, H-2', 3' and 4'), 5.97 (dd, 1 H, H-1', J = 1.5 and 9.0 Hz), 6.25 (d, 1 H, H-6, J = 6.6 Hz). Anal. calcd. for $C_{10}H_{20}FN_3O_8$: C, 47.88; H, 4.98; N, 10.47. Found: C, 47.90; H, 5.23; N, 10.10.

5-Fluoro-1-(6-deoxy-ß-L-galactopyranosyl)cytosine (12). 11 (10 g, 25 mmol) was deacetylated by dissolving it in MeOH (1 L) saturated with NH_3 gas. After 24 h, the solution was evaporated and the residue obtained was crystallized from MeOH to afford 12 (6.2 g, 90%): mp 294-295°C (MeOH); $[\alpha]_D$ - 88.0° (c 0.25, MeOH); ^{1}H NMR (D_2O) 1.25 (d, 1 H, H-6', J= 7.0 Hz), 3.75 to 3.85 (m, 3 H, H-2', 3' and 4'), 4.00 (q, 1 H, H-5', J

= 7.0 Hz), 5.55 (br s, 1 H, H-1'), 7.95 (d, 1 H, H-6, J = 7.0 Hz). Anal. calcd. for $C_{10}H_{14}FN_3O_5$: C, 43.63; H, 5.09; N, 15.27. Found: C, 44.04; H, 5.19; N, 15.25.

5-Fluoro-1-(6-deoxy-3,4-di-O-methoxymethylene-ß-L-galactopyranosyl)cytosine (13). A mixture of 12 (6.9 g, 25 mmol), $(CH_3O)_3CH$ (14 mL, 137 mmol) and pyridinium p-toluenesulfonate (100 mg, 0.4 mmol) in dry DMF (25 mL) was stirred for 16 h and then basified with Na_2CO_3 solution. The solvents were evaporated, the residue obtained was mixed with 100 mL of Me_2CO and then stirred for 15 min. The precipitate was filtered and washed with Me_2CO. The combined filtrate was concentrated and the oily residue obtained was column chromatographed (2:1 EtOAc -hexane) to afford 13 (6.34 g, 80%) as a mixture of two diastereomers (1:2, ^1H NMR). Small amount of major isomer was separated by crystallization from Me_2CO. Rest of the mixture was used as such in the next reaction. Major isomer: mp 238-240°C; $[\alpha]_D$ - 120.0° (c 0.1, MeOH); ^1H NMR (Me_2CO-d_6) 1.35 (d, 3 H, H-6', J = 6.70 Hz), 3.30 (s, 3 H, $-OCH_3$), 3.80 (br m, 1 H, H-3'), 4.15 to 4.25 (m, 2 H, H-4' and 5'), 4.75 (t, 1 H, H-2', J = 8.1 Hz), 5.65 (dd, 1 H, H-1', J = 2.0 and 8.1 Hz), 5.90 (s, 1 H, $-CHOCH_3$), 7.72 (d, 1 H, H-6, J = 8.0 Hz). Anal. calcd. for $C_{12}H_{13}FN_3O_6$: C, 45.42; H, 5.05; N, 13.24. Found: C, 44.94; H, 5.55; N, 13.57.

5-Fluoro-1-(2-O-t-butyldimethylsilyl-6-deoxy-3,4-O-methoxymethylene-ß-L-galactopyranosyl)cytosine (14). To a mixture of t-butyldimethylsilyl chloride (3 g, 20 mmol), imidazole (3 g, 4.4 mmol) and 4-dimethylaminopyridine (200 mg, 1.64 mmol) was added a solution of 13 (5 g, 15.8 mmol) in dry DMF (10 mL). After stirring for 20 h, DMF was evaporated and the residue obtained was chromatographed (1:1, EtOAc-hexane) to afford 14 (4.76 g, 70%). 14 was crystallized from EtOAc and crystalline compound was then used in the next reaction.

5-Fluoro-1-(2-O-t-butyldimethylsilyl-6-deoxy-ß-L-galactopyranosyl)cytosine (15). To a stirred solution of 14 (4 g, 93 mmol) in dry MeOH (10 mL) was added p-toluenesulfonic

acid until the solution became acidic up to pH 2. After 1 h, the solution was neutralized with IR-45 resin and the resin was then filtered off. The filtrate was concentrated and the residue obtained was crystallized from EtOAc to afford 15 (3.25 g, 90%): mp 224-226°C; $[\alpha]_D$-88.0° (c 0.25, MeOH); ^{1}H NMR (Me_2SO-d_6) -0.10 and + 0.05 (2xs, 3 H each, -$SiCH_3$), 0.70 (s, 9 H, -t-butyl), 1.15 (d, 3 H, H-6', J = 6.75 Hz), 3.40 to 3.55 and 3.65 to 3.85 (2xm, 4 H, H-2', 3', 4' and 5'), 5.45 (br s, 1 H, H-1'), 7.95 (d, 1 H, H-6, J = 8.0 Hz). Anal. calcd. for $C_{16}H_{28}FN_3O_5Si$: C, 49.35; H, 7.19; N, 10.79. Found: C, 49.20; H, 7.59; N, 10.45.

N-Benzoyl-5-fluoro-1-(2-O-t-butyldimethylsilyl-3,4-di-O-benzoyl-6-deoxy-ß-L-galactopyranosyl)cytosine (16). A mixture of 15 (3.89 g, 10 mmol), Bz_2O (18.0 g, 80 mmol) and 4-dimethylaminopyridine (200 mg, 1.64 mmol) in dry pyridine (20 mL) was stirred for 24 h. The pyridine was evaporated and the residue obtained was column chromatographed (2:1 hexane-EtOAc) to afford 16 with some benzoic acid. Benzoic acid was removed by washing the solution of the mixture in EtOAc with Na_2CO_3 solution. Later, washing (H_2O), drying (Na_2SO_4) and concentration of the organic layer afforded 16 as an oil, which upon drying changed into an amorphous solid (5.61 g, 80%): mp 146°C; $[\alpha]_D$ -88.0° (c 0.25, MeOH); ^{1}H NMR (Me_2CO-d_6) -0.15 and + 0.05 (2xs, 3 H each, -$SiCH_3$), 0.70 (s, 9 H, -t-butyl), 1.35 (d, 3 H, H-6', J = 7.0 Hz), 4.55 to 4.80 (m, 2 H, H-2' and 5'), 5.65 (d, 1 H, H-3', J = 8.0 Hz), 5.75 (d, 1 H, H-4', J = 1.5 Hz), 6.00 (br s, 1 H, H-1'), 8.25 (d, 1 H, H-6, J= 8.0 Hz), 7.40, 7.50 to 7.65, 7.75, 8.15 and 8.30 (15 H, benzoyl H). Anal. calcd. for $C_{37}H_{40}FN_3O_8Si$: C, 63.34; H, 5.71; N, 5.99. Found: C, 62.99; H, 5.98; N, 5.85.

N-Benzoyl-5-fluoro-1-(3,4-di-O-benzoyl-6-deoxy-ß-L-galactopyranosyl)cytosine (17). To a stirred solution of 16 (2.8 g, 4 mmol) in dry THF was added a solution of tetrabutylammonium fluoride in THF (5.5 mL, 1 M). After 5 h, the solution was concentrated and the residue obtained was column chromatographed (1:1 EtOAc: hexane) to afford 17 as an

oil which upon drying changed into a viscous mass (1.9 g, 80%). ^{1}H NMR (Me_2CO-d_6) 1.25 (d, 3 H, H-6', J = 7.0 Hz), 4.20 to 4.35 (m, 2 H, H-2' and 5'), 5.60 (dd, 1 H, H-3', J = 4.0 and 11.0 Hz), 5.75 (d, 1 H, H-4', J = 4.0 Hz), 5.95 (d, 1 H, H-1', J = 9.5 Hz), 7.25, 7.30 to 7.75, 7.85, 8.10 and 8.25 (16 H, benzoyl H and H-6).

1-(2,3-Di-O-benzoyl-6-deoxy-α-L-mannopyranosyl)uracil (21). To a stirred mixture of 19 (10 g, 38.8 mmol) and dry pyridine (120 mL) in 400 mL of $(CH_2Cl)_2$ was added, BzCl (40 mL). After 3 min, reaction was stopped by adding MeOH (400 mL). The solvents were evaporated and the residue obtained was mixed with EtOAc. The precipitate was filtered and washed. The combined filtrate was concentrated. The oily residue obtained was column chromatographed. Elution first with EtOAc - hexane (1:5) gave methyl benzoate and then on gradually increasing the polarity of the eluting solvent 21 was obtained as an oil. The oil was first dried by coevaporation twice with toluene and then dried in vacuo to give 7 g (39%) of 21: ^{1}H NMR ($CDCl_3$) 1.60 (d, 3 H, H-6', J = 7.65 Hz), 4.10 (brs, 1 H, H-4'), 4.35 to 4.45 (m, 1 H, H-5'), 5.75 to 5.85 (m, 3 H, H-2', 3' and 5), 6.55 (d, 1 H, H-1', J = 10.12 Hz), 7.30, 7.40 to 7.70, 7.85 and 8.05 (11 H, benzoyl H and H-6).

5-Chloro-1-(2,3-di-O-benzoyl-6-deoxy-α-L-mannopyranosyl) uracil (22). Following the procedure as described for the benzoylation of 20, 22 (10 g, 34.2 mmol) was benzoylated with BzCl (40 ml) in a mixture of pyridine (120 ml) and $(CH_2Cl)_2$ (400 ml). 7 g (41%) of 22 was obtained as an oil. It was dried like 21 before oxidation. ^{1}H NMR ($CDCl_3$) 1.60 (d, 3 H, H-6', J = 6.75 Hz), 4.10 (m, 1 H, H-4'), 4.35 to 4.45 (m, 1 H, H-5'), 5.75 to 5.85 (m, 2 H, H-2' and 3'), 6.50 (d, 1 H, H-1', J = 8.1 Hz), 7.25 to 7.70, 7.82, 7.85 and 8.05 (11 H, benzoyl H and H-6).

5-Fluoro-1-(3,4,6-trideoxy-ß-L-erythro-hex-3-eno-pyranosyl)uracil (27). A mixture of 26 (960 mg, 3 mmol) in

Ac_2O (12 mL) was heated at 140°C with stirring until the disappearance of acetate of 26 (TLC, 12 to 16 h). After cooling the solution, it was basified with Et_3N and the solvents were evaporated. The residue obtained was column chromatographed (1:1 EtOAc-hexane) to obtain acetate of 27 as an oil (520 mg). It was then hydrolyzed with MeOH (50 mL) saturated with NH_3 gas to afford 27 (398 mg, 55%): mp 217-219°C (EtOAc-hexane); $[\alpha]_D$ + 124.0° (c 0.25, MeOH); ^1H NMR (Me_2CO-d_6) 1.25 (d, 3 H, H-6', J = 6.75 Hz), 4.35 and 4.65 (2xm, 2 H, H-2' and 5'), 5.65 (dd, 1 H, H-1', J = 1.5 and 8.60 Hz), 5.75 (d, 1 H, H-4', J = 10.0 Hz), 5.85 (d, 1 H, H-3', J = 10.0 Hz), 7.55 (d, 1 H, H-6, J = 8.0 Hz). Anal. calcd. for $C_{10}H_{11}FN_2O_4$: C, 49.58; H, 4.54; N, 11.57. Found: C, 49.43; H, 4.51; N, 11.48.

5-Fluoro-1-(6-deoxy-2,3-O-methoxymethylene-α-L-manno-pyranosyl)uracil (33). A mixture of 29 (19.3 g, 70 mmol), $(CH_3O)_3CH$ (38 mL, 348 mmol) and pyridinium p-toluenesulfonate (0.5 g, 2 mmol) in dry DMF (40 mL) was stirred for 6 h and then basified with Na_2CO_3 solution. The solvents were evaporated and the residue obtained was column chromatographed (3:2 EtOAc-hexane) to afford 33 (18.47 g, 83%) as a mixture of two diastereomers (4:5, ^1H NMR). Small amount of major isomer was separated by fractional crystallization (EtOAc-hexane). Rest of the mixture was used as such in the next reaction. Major isomer: mp 196-198°C; $[\alpha]_D$ - 12.0° (c 0.25, MeOH); ^1H NMR (d, 3 H, H-6', J = 7.0 Hz), 3.35 (s, 3 H, $-OCH_3$), 3.90 (brs, 2 H, H-4' and 5'), 4.40 (t, 1 H, H-3', J = 7.70 Hz), 4.75 (t, 1 H, H-2', J = 7.70 Hz), 5.80 (s, 1 H, $-HCOCH_3$), 5.95 (brd, 1 H, H-1', J = 7.70 Hz), 7.95 (d, 1 H, H-6, J = 8.0 Hz). Anal. calcd. for $C_{12}H_{15}FN_2O_7$:C, 45.28; H, 4.71; N, 8.80. Found: C, 45.63; H, 4.84; N, 8.88.

5-Fluoro-1-(2,3,6-trideoxy-α-L-erythro-hex-2-eno- pyranosyl) uracil (35). A solution of 33 (960 mg, 3 mmol) in Ac_2O (12 mL) was heated with stirring at 140°C. On complete disappearance of acetate of 33 (TLC), the solution was cooled and basified with Et_3N. The solvents were evaporated and the

residue obtained was filtered through a silica gel column (3:2 EtOAc-hexane) to afford a mixture of two products (O-acetate and N, O diacetate of 35). The mixture was dissolved in MeOH (50 mL) saturated with NH_3 gas. After 24 h, the solution was concentrated and the residue obtained was column chromatographed (2:1 EtOAc-hexane) to afford 35 (429mg, 55%): mp 105-108°C (EtOAc-hexane); $[\alpha]_D$ -88.0° (c 0.25, MeOH); ^{1}H NMR (Me_2CO-d_6) 1.25 (d, 3 H, H-6, J = 8.0 Hz), 3.65 (m, 1 H, H-5'), 4.35 (d, 1 H, H-4', J = 7.5 Hz), 5.85 (dm, 1 H, H-2', J = 9.9 Hz), 6.30 (brs, 1 H, H-1'), 6.35 (dm, 1 H, H-3', J = 9.9 Hz), 7.85 (d, 1 H, H-6, J = 8.0Hz). Anal. Calcd. for $C_{10}H_{11}FN_2O_4H_2O$: C, 46.15; H, 5.00; N, 10.76. Found: C, 46.50; H, 5.03; N, 10.68.

5-Fluoro-1-(2,3,6-trideoxy-α-L-erythro-hexopyranosyl) uracil (36). A mixture of 35 (2.0 g, 7.7 mmol), and Pd/C (10%, 460 mg) in Aq. EtOH (90%, 200 mL) was shaken with H_2 gas at 20 psi for 5 h in a parr hydrogenator. The mixture was then filtered through celite and the celite was washed with EtOH (50 mL). The combined filtrate was concentrated to afford 36 (1.8 g, 96%): mp 164-166°C (EtOAc-hexane); $[\alpha]_D$-72.0° (c 0.25, MeOH); ^{1}H NMR (Me_2CO-d_6) 1.33 (d, 3 H, H-6', J = 8.0 Hz), 1.70 and 1.80 (2 x brs, 1 H each, H-3'), 2.10 to 2.20 (m, 2 H, H-2'), 3.55 (brs, 1 H, H-4'), 4.15 (m, 1 H, H-5'), 5.85 (brs, 1 H, H-1'), 7.90 (d, 1 H, H-6, J= 8.0 Hz). Anal. Calcd. for $C_{10}H_{13}FN_2O_4$: C, 49.18; H, 5.33; N, 11.47. Found: C, 49.07; H, 5.42; N, 11.75.

5-Trifluoromethyl-1-(6-deoxy-2,3,6-tri-O-acetyl-α-L-mannopyranosyl)uracil (30). A mixture of 5-trifluoromethyl-uracil (6.48 g, 36 mmol), HMDS (18.2 mL) and saccharin (100 mg) in 50 mL of dry $(CH_2Cl)_2$ was heated with stirring under reflux to dissolve all the base and for an additional 0.5 h. The solution was then cooled in an ice-bath and a solution of tetra-O-acetyl-L-rhamnose (13.5 g, 40.7 mmol) in 30 mL of $(CH_2Cl)_2$ was added. This was followed by portion wise addition of a solution of $SnCl_4$ (6 mL, 54.6 mmol) in 6mL of $(CH_2Cl)_2$. After stirring for 0.5 h at 25°C, the reaction mixture was neutralized with methanolic ammonia

solution. The solvents were evaporated and the residue obtained was mixed with EtOAc. The precipitate was filtered and washed with a large amount of EtOAc to dissolve all the nucleoside. The combined filtrate was evaporated and the oily residue obtained was column chromatographed (1:1 hexane -EtOAc) to afford 30 (13.8 g, 85%): mp 194-196°C (EtOAc-hexane); $[\alpha]_D$ - 8.0° (c 0.25, MeOH); ^{1}H NMR ($CDCl_3$) 1.50 (d, 3 H, H-6', J = 7.10 Hz), 2.00 and 2.25 (2 x s, 3 H and 6 H respectively, $COCH_3$), 4.35 (q, 1 H, H-5', J = 7.10 Hz), 4.85 (dd, 1H, H-3', J = 1.5 and 3.6 Hz), 5.25 (dd, 1 H, H-2', J = 3.6 and 9.0 Hz), 5.50 (t, 1 H, H-4', J = 1.5 Hz), 6.25 (d, 1 H, H-1', J = 9.0 Hz), 7.85 (s, 1 H, H-6). Anal. calcd. for $C_{17}H_{19}F_3N_2O_9$: C, 45.13; H, 4.20; N, 6.18. Found: C, 45.00; H, 4.34; N, 6.25.

5-Trifluoromethyl-1-(6-deoxy-α-L-mannopyranosyl)uracil (32). 30 (13.56 g, 30 mmol) was dissolved in MeOH (1 L) saturated with NH_3 gas. After 24 h, MeOH was evaporated and the residue obtained was dried in vacuo. The crude 32 was used as such in the next reaction.

5-Trifluoromethyl-1-(6-deoxy-2,3-O-methoxymethylene-α-L-mannopyranosyl)uracil (33). A mixture of 32 (from the preceding reaction), $(CH_3O)_3CH$ (16.4 mL, 150 mmol) and pyridinium p-toluenesulfonate (1.5 g, 6 mmol) in dry DMF (25 mL) was stirred and the progress of the reaction was monitored by TLC. After 25 h, TLC indicated only 75% transformation of the substrate and the reaction did not proceed upon further stirring. The reaction mixture was then basified with Na_2CO_3 solution and the solvents were evaporated. The residue obtained was column chromatographed (1:1 EtOAc-hexane) to afford 33 (6.0 g, 54%) as a mixture of two diastereomers (1:8, ^{1}H NMR). The small amount of major isomer was separated from the mixture by fractional crystallization (EtOAc-hexane). Rest of the mixture was used as such in the next reaction. Major isomer: 211-212°C; $[\alpha]_D$ -24.0° (c 0.25, MeOH); ^{1}H NMR (Me_2CO-d_6) 1.30 (d, 3 H, H-6', J = 9.0 Hz), 3.35 (s, 3 H,

$-OCH_3$), 3.80 to 4.00 (m, 2 H, H-4' and 5'), 4.45 (t, 1 H, H-3', J = 7.70 Hz), 4.85 (t, 1 H, H-2', J = 7.70 Hz), 5.80 (s, 1 H, $\underline{H}COCH_3$), 6.05 (d, 1 H, H-1' J = 7.70 Hz), 8.30 (s, 1 H, H-6, J= 8.0 Hz). Anal. calcd. for $C_{13}H_{15}F_3N_2O_7$: C, 42.39; H, 4.08; N, 7.60. Found: C, 42.66; H, 4.10; N, 7.90.

5-Trifluoromethyl-1-(2,3,6-trideoxy-α-L-erythro-hex-2-enopyranosyl)uracil (37). A solution of 33 (1.1 g, 3 mmol) in Ac_2O (12 mL) was heated with stirring at 140°C. The progress of the reaction was monitored by TLC. On about 70% transformation of acetate of 33 into product, the reaction mixture was cooled, since further heating was found to cause more decomposition of the product and less transformation of the substrate. The reaction mixture was then basified with Et_3N and the solvents were evaporated. The oily residue obtained was column chromatographed (1:1, EtOAc-hexane) to afford a mixture of acetates of 33 and 37. The mixture was dissolved in MeOH (100 mL) saturated with NH_3 gas. After 24 h, the solution was concentrated and the oily residue obtained was column chromatographed (EtOAc-hexane) to afford 37 as an oil. Upon drying, the oil changed into an amorphous solid (0.3 g, 35%). $[\alpha]_D$-40.0° (c 0.20, MeOH); ^{1}H NMR (Me_2CO-d_6) 1.30 (d, 3 H, H-6', J = 8.0 Hz), 3.65 (m, 1 H, H-5'), 3.80 (br d, 1 H, H-4', J = 8.0 Hz), 5.90 (dq, 1 H, H-2', J = 2.0, 3.0 and 10.0 Hz), 6.35 (brs, 1 H, H-1'), 6.45 (dt, 1H, H-3', J = 2.0 and 10.0 Hz), 8.13 (s, 1 H, H-6). Anal. calcd. for $C_{11}H_{11}F_3N_2O_4$: C, 45.20; H, 3.76; N, 9.59. Found: C, 45.16; H, 4.36; N, 8.94.

5-Iodo-1-(4-O-acetyl-2,3,6-trideoxy-L-erythro-hex-2-eno-pyranosyl)uracil (41, α) and (42, ß). A mixture of 5-iodouracil (2.38 g, 10 mmol) and saccharin (50 mg) in HMDS (15 mL) was heated under reflux for 2.5 h to get a clear solution. Excess HMDS was removed by evaporation under vacuum and the reaction assembly was flushed with dry N_2 gas. A solution of 3,4-di-O-acetyl-L-rhamnal (1.93 g, 9 mmol) in dry MeCN (9 mL) was then added to the silylated base. The reaction mixture was then cooled in an ice-bath for 5 min. The stirred and cooled reaction mixture was finally treated

dropwise with TMS-triflate (1.74 mL, 9 mmol). After complete addition of TMS-triflate, the reaction mixture was stirred at 5-10°C for 30 min, and then neutralized with methanolic ammonia solution below 5°C. The solvents were evaporated and the resultant residue was mixed with EtOAc. The precipitate separated was filtered and washed with EtOAc. The combined filtrate was concentrated. The residue obtained was column chromatographed (1:1 EtOAc-hexane) to afford many fractions having both isomers in varying proportions. The isomers were finally separated by fractional crystallization (EtOAc-hexane) to give both α-anomer (1.5 g) and ß-anomer (1.5 g) in total 85% yield. α-Anomer: mp 183-185°C; R_F 0.45 (2:3 hexane-EtOAc); $[\alpha]_D$ - 16.0° (c 0.25, MeOH). Anal. calcd. for $C_{12}H_{13}IN_2O_5$: C, 36.73; H, 3.31; N, 7.14; I, 32.39. Found: C, 36.63; H, 3.47; N, 6.74; I, 32.05. ß-Anomer: mp 204-208°C; R_F 0.50 (2:3 hexane-EtOAc); $[\alpha]_D$ -160.0° (c 0.25, MeOH). Anal. calcd. for $C_{12}H_{13}IN_2O_5$: C, 36.73; H, 3.31; N, 7.14; I, 32.39. Found: C, 36.90; H, 3.55; N, 6.90; I, 32.65.

5-Iodo-1-(2,3,6-trideoxy-α-L-erythro-hex-2-eno-pyranosyl)uracil (49). 41 (2.35 g, 6 mmol) was deacetylated with MeOH (500 mL) saturated with NH_3 gas using the procedure as described for deacetylation of 11, to afford 49 (1.89 g, 90%): mp 200°C (decomp., MeOH); $[\alpha]_D$ -96.0° (c 0.25, MeOH); ^1H NMR ($CDCl_3$ + Me_2SO-d_6) 1.25 (d, 3 H, H-6', J = 7.0 Hz); 3.45 (m, 1 H, H-5'), 3.70 (m, 1 H, H-4'), 5.70 (dt, 1 H, H-2', J = 1.9 and 10.0 Hz), 6.15 (brs, 1 H, H-1'), 6.30 (brd, 1 H, H-3', J = 10.0 Hz), 8.00 (s, 1 H, H-6). Anal. calcd. for $C_{10}H_{11}IN_2O_4$: C, 34.28; H, 3.14; N, 8.00; I, 36.28. Found: C, 34.76; H, 3.14; N, 8.00; I, 36.60.

1-(4-O-Acetyl-2,3,6-trideoxy-L-erythro-hex-2-eno-pyranosyl)thymine (43, α) and (44, ß). Following the procedure as described for synthesis of 41, thymine (3.15 g, 25 mmol) was silylated with HMDS (35 mL) and saccharin (100mg) and the resultant silylated base was reacted with

2,3-di-O-acetyl-L-rhamnal (4.92 g, 23 mmol) in presence of TMS- triflate (3.5 mL, 18 mmol) in dry MeCN (23 mL). After addition of the catalyst, the reaction mixture was further stirred for 4 h at 25°C and then worked up. Products were purified by column chromatography (1:1 hexane-EtOAc) to give a mixture of 43 and 44 (4.5 g, 70%; 1:1, ^{1}H NMR). Fractional crystallizations (EtOAc-hexane) were performed to resolve the mixture into pure anomers. α-Anomer: mp 110-113°C (Lit.[12] mp 87°C); R_F 0.30 (2:1 EtOAc-hexane); $[\alpha]_D$ - 56.0° (c 0.25, MeOH), [Lit. $[\alpha]_D^{20}$ - 82.5° (c 0.1, MeOH)]. ß-Anomer: mp 209-210°C (Lit.[12] mp 205°C); R_F 0.35 (2:1 EtOAc-hexane); $[\alpha]_D$ - 124.0° (c 0.25, MeOH) [Lit.[12] $[\alpha]_D^{20}$ - 115.0° (c 0.1, MeOH)].

1-(2,3,6-Trideoxy-α-L-erythro-hex-2-enopyranosyl)thymine (50). 43 (11.2 g, 4 mmol) was deacetylated with MeOH (100 mL) saturated with ammonia gas, using the procedure as described for deacetylation of 11, to afford 50. The crude 50 was then dried in vacuo.

5-Trifluoromethyl-1-(4-O-acetyl-2,3,6-trideoxy-L-erythro-hex-2-enopyranosyl)uracil (45, α) and (46, ß). Following the procedure as described for the synthesis of 41, 5-trifluoromethyluracil (2.7 g, 22.5 mmol) was silylated with HMDS (22.5 mL) and saccharin (100 mg), and then silylated base was condensed with 2,3-di-O-acetyl-L-rhamnal (3.21 g, 15 mmol) in presence of TMS-triflate (1.5 mL, 7.8 mmol) in dry MeCN (15 mL). Workup followed by column chromatography (2:1 EtOAc-hexane) of the reaction mixture afforded an oily mixture (4.5 g) of 45, 46 and a side product (9:11:5, ^{1}H NMR). The mixture was found inseparable by TLC, column chromatography or crystallization.

6-Aza-1-(4-O-acetyl-2,3,6-trideoxy-L-erythro-hex-2-enopyranosyl)uracil (47 α) and (48 ß). Following the procedure as described for the synthesis of 41, 6-azauracil (4.75 g, 42 mmol) was silylated with HMDS (45 mL) and saccharin (100mg) and the silylated base was condensed with 2,3-di-O- acetyl-L-rhamnal (6.7 g, 31.46 mmol) in presence of TMS- triflate (3.6 mL, 18.65 mmol) in dry MeCN (33 mL). Workup followed by column chromatography (1:1, EtOAc-hexane) of the reaction

mixture afforded a mixture of α and ß-anomers (7 g, 83.3%; 1:1, ^{1}H NMR) which was crystallized from EtOAc- hexane. Isomers were found inseparable by TLC, column chromatgraphy or crystallization.

6-Aza-1-(2,3,6-trideoxy-L-erythro-hex-2-enopyranosyl)-uracil (51) and (52). A mixture of 47 and 48 (6.67 g, 25 mmol) was deacetylated with MeOH (500 mL) saturated with NH_3 gas using the procedure as described for deacetylated of 11, to afford a mixture of 51 and 52. The crude mixture was then dried in vacuo.

General Method for Oxidation: 3 Å molecular sieves (MS) was powdered and then dried at 350° C over P_2O_5 in vacuo. After cooling to room temperature, MS was added to a mixture of pyridinium dichromate (PDC) and the compound to be oxidized. The mixture was then stirred in dry CH_2Cl_2. After 6 h the reaction mixture was diluted with equal volume of EtOAc and stirred further for 30 min. The reaction mixture was then filtered through a bed of silica gel and celite, and the bed was washed with EtOAc. The combined filtrate was concentrated and the residue obtained was column chromatographed to obtain the pure compound.

5-Fluoro-1-(3,4,6-trideoxy-ß-L-glycero-hex-3-enopyranos-2-ulosyl)uracil (28). 27 (605 mg, 2.5 mmol) was oxidized with PDC (1.4 g, 3.75 mmol) and MS (1.5 g) in CH_2Cl_2 (50 mL). Workup followed by column chromatography (2:1 $CHCl_3$-EtOAc) of the reaction mixture afforded 28 (420 mg, 70%): mp 178-180° C; (EtOAc-hexane); $[\alpha]_D$ - 24.0° (c 0.25, MeOH); ^{1}H NMR (Me_2CO-d_6) 1.45 (d, 3 H, H-6', J = 7.0 Hz), 4.95 to 5.10 (m, 1 H, H-5'), 6.20 (dd, 1 H, H-3', J = 1.92 and 11.57 Hz), 6.25 (brs, 1 H, H-1'), 7.25 (dd, 1 H, H-4', J = 1.92 and 11.57 Hz), 7.65 (d, 1 H, H-6, J = 8.0 Hz). Anal. calcd for $C_{10}H_9FN_2O_4$: C, 50.00; H, 3.75; N, 11.66. Found: C, 50.27; H, 3.86; N. 11.55.

N-Benzoyl-5-fluoro-1-(3-O-benzoyl-4,6-dideoxy-ß-L-glycero-hex-3-enopyranos-2-ulosyl)cytosine (18). 17 (1.76 g, 3 mmol) was oxidized with PDC (1.8 g, 4.78 mmol) and MS (1.75 g) in CH_2Cl_2 (100 mL). After stirring for 6 h, the

reaction mixture was diluted with CH_2Cl_2 (100 mL) and then stirred again for 0.5 h. Workup followed by column chromatography (CH_2Cl_2) of the reaction mixture afforded 18 (694 mg, 50%): mp 204-206°C (benzene-hexane); $[\alpha]_D$ - 120.0° (c 0.1, CH_2Cl_2); ^1H NMR ($CDCl_3$) 1.60 (d, 3 H, H-6', J = 8.0 Hz), 3.15 (m, 1 H, H-5'), 6.45 (br s, 1 H, H-1'), 6.85 (d, 1H, H-4', J = 1.5 Hz), 7.30, to 7.70, 8.10 and 8.20 (11 H, benzoyl H and H-6). Anal. calcd. for $C_{24}H_{18}FN_3O_6$: C, 62.20; H, 3.89; N, 9.07. Found: C, 61.91; H, 3.90; N, 9.04.

1-(3-O-Benzoyl-2,6-dideoxy-α-L-glycero-hex-2-enopyranos-4-ulosyl)uracil (23). 21 (2.5 g, 5.36 mmol) was oxidized with PDC (3.0 g, 8 mmol) and MS (2.5 g) in CH_2Cl_2 (150 mL). Workup followed by column chromatography (2:1 EtOAc-hexane) of the reaction mixture afforded 23 (1.1 g, 60%): mp 158-160°C (benzene-hexane); $[\alpha]_D$ - 136.0° (c 0.25, MeOH); ^1H NMR (Me_2CO-d_6) 1.55 (d, 3 H, H-6', J = 6.75 Hz), 4.60 (q, 1 H, H-5', J = 6.75 Hz), 5.85 (d, 1 H, H-5, J = 8.0 Hz), 6.65 (d, 1 H, H-1', J = 2.0 Hz), 6.95 (d, 1 H, H-2', J = 2.0 Hz), 7.45 to 7.70 and 8.10 (6 H, benzoyl H and H-6). Anal. calcd. for $C_{17}H_{14}N_2O_6$: C, 59.64; H, 4.09; N, 8.19. Found: C, 59.88; H, 4.16; N, 7.95.

5-Chloro-1-(3-O-benzoyl-2,6-dideoxy-α-L-glycero-hex-2-enopyranos-4-ulosyl)uracil (24). 22 (2.5 g, 5 mmol) was oxidized with PDC (3.0 g, 8 mmol) and MS (2.5 g) in CH_2Cl_2 (150 mL). Workup followed by column chromatography (3:2 EtOAc-hexane) of the reaction mixture afforded 24 (1.22 g, 65%): mp 181-182°C (EtOAc-hexane); $[\alpha]_D$ - 136.0° (c 0.25, MeOH); ^1H NMR (Me_2CO-d_6) 1.60 (d, 3 H, H-6', J = 6.75 Hz); 4.65 (q, 1 H, H-5', J = 6.75 Hz), 6.65 (d, 1 H, H-1', J = 2.0 Hz), 6.85 (d, 1 H, H-2', J = 2.0 Hz), 7.55, 7.70, 7.82 and 8.15 (6 H, benzoyl H and H-6). Anal. calcd. for $C_{17}H_{13}ClN_2O_6$: C, 54.18; H, 3.45; N, 7.43; Cl, 9.43. Found: C, 53.95; H, 3.43; N, 7.59; Cl, 9.29.

5-Fluoro-1-(2,3,6-trideoxy-α-L-glycero-hex-2-enopyranos-4-ulosyl)uracil (38). 35 (484 mg, 2 mmol) was

oxidized with PDC (1.13 g, 3 mmol) and MS (1g) in CH_2Cl_2 (60 mL). After 6 h, the reaction mixture was diluted with a mixture of EtOAc and Me_2CO (1:1, v/v), and then stirred for 30 min. After usual work up, the residue obtained was dissolved in CH_2Cl_2 and the solution was applied onto a fine silica gel pad (2 cm). The pad was washed with CH_2Cl_2 (100 mL). The eluant obtained was concentrated to reduce the volume up to 20 mL and then stored in a fridge over night to afford the crystalline 38 (300 mg, 62.5%): mp 226-230°C; $[\alpha]_D$ - 48.0° (c 0.25, MeOH); ^1H NMR (Me_2CO-d_6 + Me_2SO-d_6) 1.30 (d, 3 H, H-6', J = 9.0 Hz), 4.50 (q, 1 H, H-5', J = 9.0 Hz), 6.35 (d, 1 H, H-3', J = 10.0 Hz), 6.50 (brd, 1 H, H-1'), 7.10 (d, 1 H, H-2', J = 10.0 Hz), 8.10 (brd, 1 H, H-6, J = 8.0 Hz). Anal. calcd. for $C_{10}H_9FN_2O_4$: C, 50.00; H, 3.75; N, 11.56. Found: C, 49.65; H, 3.79; N, 11.56.

5-Fluoro-1-(2,3,6-trideoxy-α-L-glycero-hexopyranos-4-ulosyl)uracil (39). 36 (1.84 g, 7.54 mmol) was oxidized with PDC (5 g, 13.3 mmol) and MS (4.5 g) in CH_2Cl_2 (150 mL). Workup followed by column chromatography (1:1, EtOAc-hexane) of the reaction mixture afforded 39 (1.2 g, 65%) as an oil. 39 was stored under high vacuum for several hours to obtain it as a fluffy material: $[\alpha]_D$ - 48.0° (c 0.25, MeOH); ^1H NMR ($CDCl_3$) 1.37 and 1.38 (2 x d, 3 H each, H-6', J = 7.0 Hz), 1.15 to 1.35 and 1.45 to 1.65 (2 x m, 1 H each, H-3'), 1.80 (m, 2 H, H-2'), 4.45 and 4.55 (2 x q, 1 H each, H-5', J = 7.0 Hz), 6.50 and 6.60 (2 x br d, 1 H each, H-1', J = 10.0 Hz), 7.55 and 7.65 (2 x d, 1 H each, H-6, J = 8.0 Hz). Anal. calcd. for $C_{10}H_{11}FN_2O_4$: C, 49.58; H, 4.54; N, 11.57. Found: C, 49.50; H, 4.86; N, 11.22.

1-(2,3,6-Trideoxy-α-L-glycero-hex-2-enopyranos-4-ulosyl) thymine (54). 50 (800 mg, 3.36 mmol) was oxidized with PDC (2 g, 5.32 mmol) and MS (1.8 g) in CH_2Cl_2 (100 mL). Workup followed by column chromatography (1:1 EtOAc-hexane) of the reaction mixture afforded 54 (500 mg, 63%): mp 126-127°C (EtOAc- hexane); $[\alpha]_D$ - 20.0° (c 0.25, MeOH); ^1H NMR ($CDCl_3$)

1.45 (d, 3 H, H-6', J = 7.0 Hz), 1.90 (s, 3 H, 5-CH_3), 5.50 (q, 1 H, H-5', J = 7.0 Hz), 6.40 (dd, 1 H, H-3', J = 2.23 and 10.5 Hz), 6.70 (t, 1 H, H-1', J = 2.23 Hz), 6.85 (dd, 1 H, H-2', J = 2.23 and 10.5 Hz), 7.1 (s, 1 H, H-6). Anal. calcd. for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.08; N, 11.86. Found: C, 56.32; H, 5.46; N, 11.55.

5-Iodo-1-(2,3,6-trideoxy-α-L-glycero-hex-2-enopyranos-4-ulosyl)uracil (53). 50 (1.05 g, 3 mmol) was oxidized with PDC (1.7 g, 4.5 mmol) and MS (1.5 g) in CH_2Cl_2 (100 mL). Workup followed by column chromatography (3:2 EtOAc- hexane) of the reaction mixture afforded 53 (815 mg, 78%): mp 126-128° C (EtOAc-hexane); $[\alpha]_D$ - 264.0° (c 0.25, MeOH); ^1H NMR ($CDCl_3$) 1.50 (d, 3 H, H-6', J = 7.0 Hz), 4.50 (q, 1 H, H-5', J = 7.0 Hz), 6.45 (dd, 1 H, H-3', J = 2.23 and 10.5 Hz), 6.65 (t, 1 H, H-1', J=2,23 Hz), 6.90 (dd, 1 H, H-2', J = 2.23 and 10 Hz), 7.70 (s, 1 H , H-6). Anal. calcd. for $C_{10}H_9IN_2O_4$: C, 34.48; H, 2.59; N, 8.04; I, 36.49. Found: C, 34.89; H, 2.50; N, 7.68; I, 36.51.

5-Trifluoromethyl-1-(2,3,6-trideoxy-α-L-glycero-hex-2-enopyranos-4-ulosyl)uracil (40). 36 (876 mg, 3 mmol) was oxidized with PDC (1.7 mg, 4.5 mmol) and MS (1.5 g) in CH_2Cl_2 (100 mL). Workup followed by column chromatography (1:1 EtOAc-hexane) of the reaction mixture afforded 40 (592 mg, 68%) as a waxy material. $[\alpha]_D$ - 40.0° (c 0.10, MeOH); ^1H NMR 1.55 (d, 3 H, H-6', J = 7.0 Hz), 4.50 (q, 1 H, H-5', J = 7.0 Hz), 6.45 (dd, 1 H, H-3', J = 2.9 and 10.0 Hz), 6.70 (t, 1 H, H-1', J = 2.9 Hz), 6.85 (dd, 1 H, H-2', J = 2.9 and 10.0 Hz), 7.75 (s, 1 H, H-6). Anal. calcd. for $C_{11}H_9F_3N_2O_4$: C, 45.51; H, 3.10; N, 9.65. Found: C, 45.67; H, 3.52; N, 9.73.

6-Aza-1-(2,3,6-trideoxy-L-glycero-hex-2-enopyranos-4-ulosyl)uracil (55 and 56). A mixture of 51 and 52 (3.1 g, 13.8 mmol) was oxidized with PDC (7.9 g, 21.2 mmol) and MS (7 g) in CH_2Cl_2 (300 mL). Workup followed by column chromatography (3:2 EtOAc-hexane) of the reaction mixture

afforded a mixture of 55 and 56 (2.4 g, 77.9%) which were separated by fractional crystallization from EtOAc-hexane. 55: mp 196-197°C; R_f 0.55 (2:1 EtOAc-hexane); $[\alpha]_D$ + 50.0° (c 0.10, MeOH); ^1H NMR ($CDCl_3$) 1.45 (d, 3 H, H-6', J = 7.0 Hz), 5.65 (q, 1 H, H-5', J = 7.0 Hz), 6.40 (dd, 1 H, H-3', J = 2.0 and 10.0 Hz), 6.70 (dd, 1 H, H-1', J = 2.0 and 3.5 Hz), 6.85 (dd, 1 H, H-2', J = 3.5 and 10.0 Hz), 7.45 (s, 1 H, H-5). Anal. calcd. for $C_9H_9N_3O_4$: C, 48.43; H, 4.03; N, 18.83. Found: C, 48.85; H, 4.18; N, 19.30. 56: mp 168-171°C; R_f 0.5 (2:1 EtOAc-hexane); $[\alpha]_D$ - 26.7° (c 0.15, MeOH); ^1H NMR 1.45 (d, 3 H, H-6', J = 7.0 Hz), 4.45 (qd, 1 H, H-4' J = 2.1 and 7.0 Hz), 6.35 (dd, 1 H, H-3', J = 2.9 and 11.0 Hz), 6.70 (dd, 1 H, H-1', J = 1.5 and 4.0 Hz), 6.95 (dd, 1 H, H-2', J = 1.5 and 11 Hz), 7.50 (s, 1 H, H-5). Anal. calcd. for $C_9H_9N_3O_4$: C, 48.43; H, 4.03; N, 18.83. Found: C, 48.30; H, 4.30; N, 19.25.

Sodium borohydride reduction of 55 and 56 and subsequent acetylation of the resultant products: To a stirred solution of 55 (223 mg, 1 mmol) in ethanol (5 ml) was added $NaBH_4$ (38 mg, 1 mmol). After 0.5 h, the solution was neutralized with NH_4Cl solution and then evaporated. The residue was mixed with water and extracted with EtOAc. The organic layer was separated, dried (Na_2SO_4) and concentrated. The residue obtained was dried by twice coevaporation with benzene and then dissolved in Et_3N (1 mL). Ac_2O (1 mL) was then added to this stirred solution. After 1 h, 5 mL of MeOH was added to the reaction mixture and the reaction mixture was concentrated. The residue obtained was then partitioned between EtOAc and water. The aqueous layer was acidified with 2N HCl and then extracted with EtOAc. The combined organic layer was washed first with 2N HCl and then with water. Separation, drying (Na_2SO_4) and evaporation of the organic layer gave a residue which was purified by column chromatography (2:1 EtOAC-hexane) to afford a nucleoside acetate (224 mg, 85%): mp 161°C (EtOAc-hexane): $[\alpha]_D$ - 80.0° (c 0.10, MeOH); ^1H NMR similar to that of 47.

Similarly, 56 was reduced with $NaBH_4$ in ethanol and the resultant products were acetylated to afford a mixture of two nucleoside acetates which were separated by column chromatography (2:1 EtOAc-hexane): major isomer: yield 181 mg (68%) mp 86°C (EtOAc-hexane); $[\alpha]_D$ - 30.0° (C 0.10, MeOH); ^1H NMR ($CDCl_3$) similar to that of 48. Minor isomer: yield 45 mg (oil); ^1H NMR ($CDCl_3$) 1.25 (d, 3 H, H-6', J = 6.7 Hz), 2.10 (s, 3 H, $COCH_3$), 4.10 (qd, 1 H, H-5', J = 2.5 and 6.7 Hz), 5.10 (brm, 1 H, H-4'), 6.00 (d, 1 H, H-2', J = 10.0 Hz), 6.30 (ddd, 1 H, H-3', J=2.5, 5.0 and 10.0 Hz), 645 (brs, 1 H, H-1'), 7.50 (s, 1 H, H-6).

ACKNOWLEDGEMENT: We are thankful to the National Institute of Health for financial assistance through Minority Biomedical Research Support Program grant number NIGMS GM0811.

REFERENCES

1. K. Antonakis and I. Chouroulinkov, Biochem. Parmacol., 23 (1974) 2095.

2. I. Chouroulinkov and K. Antonakis, C. R. Acad. Sci. Paris, 285, Se'r D. (1977) 1021.

3. K. Antonakis, T. Halmos, J. Bach and I. Chouroulinkov, Eur. J. Med. Chem., 15 (1980) 237.

4. T. Halmos, A. Cardon and K. Antonakis, Chem. Biol. Interactions, 46 (1983) 11.

5. M. A. Alaoui-Jamali, Cl. Lasnes, K. Antonakis, and I. Chouroulinkov, Mutagenesis, 1, (1986) 411.

6. M. Bessodes, M-J Egron, J. Filippi and K. Antonakis J. Chem. Soc. Perkin-I (1990), 3035.

7. A. P. Sharma, M. Blair and A. P. Ollapally, Nucleosides and Nucleotides, 9 (1990) 713.

8. S. Delatre, D. Komiotis, L. Holt, A. P. Ollapally, J. Balzarini, E. De Clercq and M. Iigo, Nucleosides and Nucleotides, 10 (1991) 431.

9. J. Herscovici and K. Antonakis, J. Chem. Soc. Perkin-I (1979) 2682.

10. M. Bessodes, R. Lakaf and K. Antonakis, Carbohydr. Res. 148 (1986) 148.

11. M. M. Mansuri, J. E. Starett, Jr., J. A. Wos, D. R, Tortolani, P. R. Brodfuehrer and J. C. Martin, J. Org. Chem. 54 (1989) 4780.

12. J. Herscovici, R. Montserret and K. Antonakis, Carbohydr. Res., 176 (1988) 219.

13. T. Ueda and S. Watanabe, Chem. Pharm. Bull., 33 (1985) 3689.

14. J. Herscovici and K. Antonakis, J. Chem. Soc. Chem. Commun. (1980) 561.

Received 5/20/91
Accepted 10/25/91