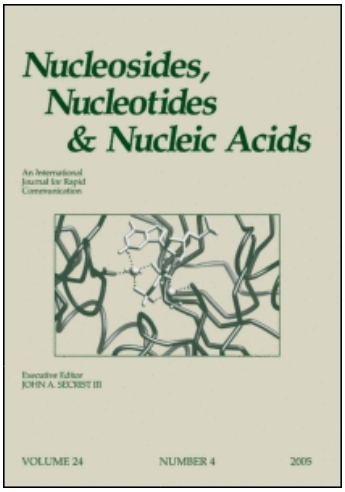

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Synthesis, Conformation, and Antiviral Activity of 5-Methoxymethyl-2′-deoxycytidine Analogs

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ABSTRACT

Analogs of 5-methoxymethyl-2′-deoxycytidine, MMdCyd (**1**) by substitution at N^4 were synthesized to impart resistance against deamination. The anti HSV-1 activity and solution conformation of analogs were determined. N^4-Butanoyl-MMdCyd (**10**) was a potent inhibitor of HSV-1 replication while N^4-hexanoyl-MMdCyd (**11**), N^4-propanoyl-MMdCyd (**9**) and N^4-acetyl-MMdCyd (**8**) had good activity against HSV-1 replication. All other analogs were devoid of activity against HSV-1.

Key Words: Herpes Simplex Virus; 5-methoxymethyl-2′-deoxycytidine; Conformation.

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INTRODUCTION

The antimetabolite 5-methoxymethyl-2′-deoxycytidine (MMdCyd) is a selective antiherpes agent with low cytotoxicity.[1,2] The antiviral activity of MMdCyd (**1**) is influenced by the cytidine/deoxycytidine (Cyd/dCyd) deaminase and deoxycytidilate (dCMP) deaminase content of the cell lines used for antiviral assays.[1] When deamination is prevented, MMdCyd (**1**) is a potent inhibitor of Herpes Simplex Virus type 1 (HSV-1). The IC_{99} (concentration required to reduce the yield of infectious virus obtained 72 h after infection by 99% relative to control cultures) was 1.6 μM when MMdCyd (**1**) was used in combination with tetrahydrodeoxyuridine (H_4dUrd; an inhibitor of both dCyd and dCMP deaminases).[1] MMdCyd (**1**) is also a good inhibitor of Varciella Zoster Virus (VZV) replication.[3]

A major drawback for the therapeutic use of cytidine analogs is their tendency to undergo deamination in the presence of deaminating enzymes. These enzymes are ubiquitously present in blood and mammalian cells and catalyze the deamination of cytidine compounds to the corresponding uridine analogs, which are either less active or do not display selectivity for HSV-infected cells.[4]

Previous studies have shown that resistance to deamination for cytidines can be accomplished by structural modifications of the cytidine molecule.[5–7] Therefore, systematic investigations on the development of 5-substituted deoxycytidine analogs resistant to deamination were initiated. The rationale is that deoxycytidines resistant to deamination would retain selectivity and metabolic stability thus simplifying treatment regimens.

In this communication, the preparation, solution conformation and antiviral activity of the novel analogs N^4-phenyl-MMdCyd (**3**), N^4-benzyl-MMdCyd (**4**), N^4-methoxy-MMdCyd (**5**), N^4-hydroxy-MMdCyd (**6**), 3,4-etheno-MMdCyd (**7**), N^4-acetyl-MMdCyd (**8**), N^4-propanoyl-MMdCyd (**9**), N^4-butanoyl-MMdCyd (**10**), N^4-hexanoyl-MMdCyd (**11**), N^4-pivaloyl-MMdCyd (**12**) and 2-imino-MMdUrd (**13**) are described. Analog (**13**) was prepared as a precursor of 2-imino-MMdCyd. Cytidine analogs with an imino group at position 2 of the pyrimidine ring were reported to be deaminase resistant.[8] The synthesis of N^4-methyl-MMdCyd (**2**), its X-ray crystal structure and biological activity have been reported.[7] Chemical structures for compounds (**1**–**13**) are shown in Fig. 1.

RESULTS

Biological Activity

The effectiveness of compounds (**2**–**13**) as inhibitors of HSV-1 replication was determined by the plaque reduction assay using A549 cells. MMdCyd (**1**) was used as a positive control. N^4-Butanoyl-MMdCyd (**10**) was significantly more active ($ED_{50} = 0.87\,\mu M$) than MMdCyd (**1**)($ED_{50} = 6.69\,\mu M$). N^4-Butanoyl-MMdCyd (**10**) was comparable in its anti HSV-1 activity ($ED_{50} = 0.87\,\mu M$) to (E)-5-(2-bromovinyl)-2′-deoxycytidine, BrVdCyd ($ED_{50} = 0.61\,\mu M$). The antiviral activity data indicates that the optimum chain length of the acyl moiety at N^4-position for anti HSV activity is, four carbon atoms when the substituent at C (5) position of the

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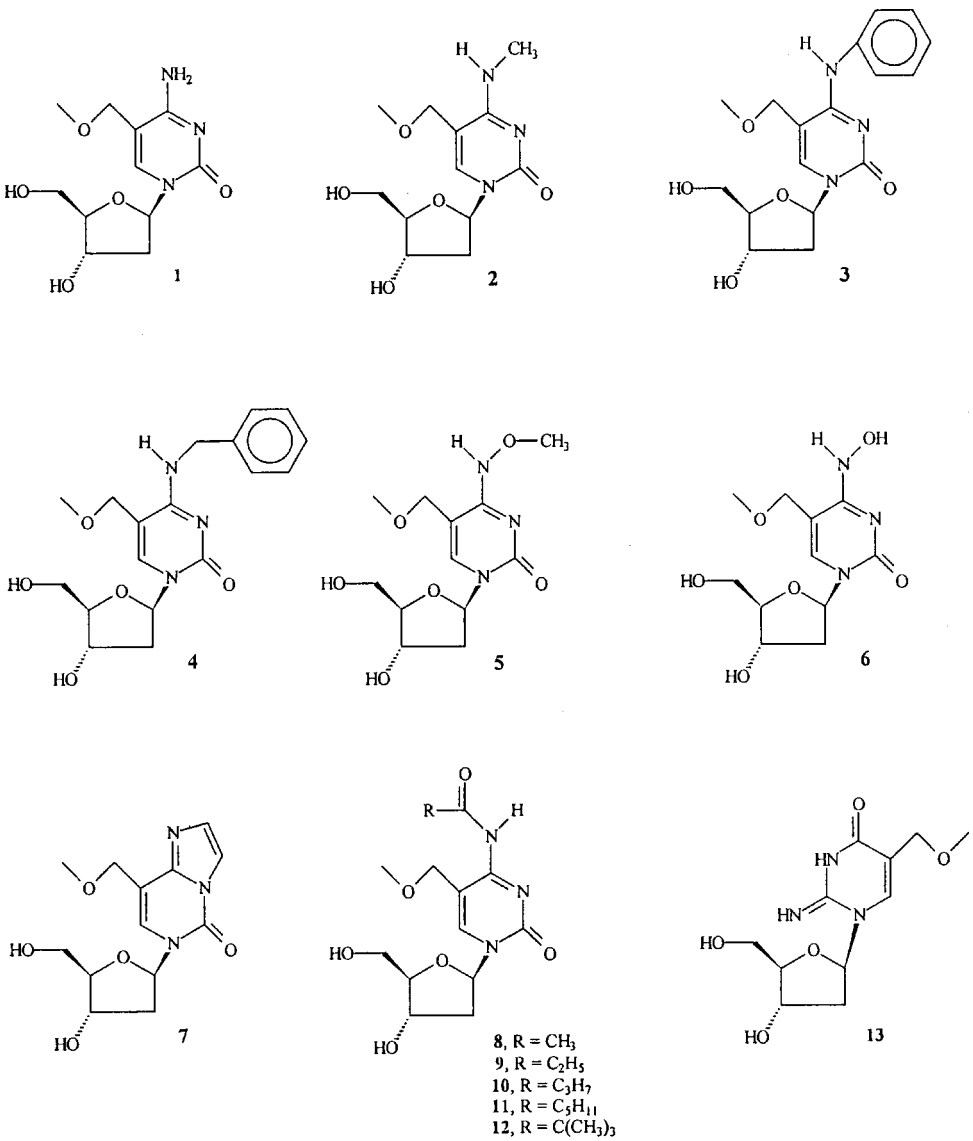

Figure 1. Chemical structures of compounds (**1–13**): MMdCyd (**1**), N^4-methyl-MMdCyd (**2**), N^4-phenyl-MMdCyd (**3**), N^4-benzyl-MMdCyd (**4**), N^4-methoxy-MMdCyd (**5**), N^4-hydroxy-MMdCyd (**6**), 3,4-etheno-MMdCyd (**7**), N^4-acetyl-MMdCyd (**8**), N^4-propanoyl-MMdCyd (**9**), N^4-butanoyl-MMdCyd (**10**), N^4-hexanoyl-MMdCyd (**11**), N^4-pivaloyl-MMdCyd (**12**), and 2-imino-MMdUrd (**13**).

pyrimidine ring is methoxymethyl. The lipophilicity increased as the length or number of carbon atoms increased.

The CC_{50} (cytotoxic concentration required to reduce cell growth by 50% using confluent monolayers of A549 cells) were greater than 2700 μM, for MMdCyd (**1**)

Table 1. Antiviral activity of the compounds listed against HSV type-1 in A549 cells.[a]

Compound	ED_{50} (μM)[b]	Minimum toxic concentration (MCC)[c] (μM)	Cell growth (CC_{50})[d] (μM)	Selectivity index (SI)[e]
MMdCyd	6.69 ± 0.70	>738	>3690	>551
BrVdCyd	0.61 ± 0.27	>604	>3020	>4950
N^4-methyl-MMdCyd	>1024[f]	>1024[f]		
N^4-phenyl-MMdCyd	>2920[f]	>2920[f]		
N^4-benzyl-MMdCyd	>2800[f]	>2800[f]		
N^4-methoxy-MMdCyd	>3370[f]	>3370[f]		
N^4-hydroxy-MMdCyd	1475	>3000		
3,4-etheno-MMdCyd	>3440[f]	>3440[f]		
N^4-acetyl-MMdCyd	4.79 ± 0.35	>640	>3195	>667
N^4-propanoyl-MMdCyd	3.06 ± 0.76	>612	>3060	>1000
N^4-butanoyl-MMdCyd	0.87 ± 0.54	>586	>2933	>3371
N^4-hexanoyl-MMdCyd	4.89 ± 0.16	>544	>2717	>555
N^4-pivaloyl-MMdCyd	>1024[f]	>1024[f]		
2-imino-MMdUrd	>3740[f]	>3740[f]		

[a]HSV-1 McIntyre strain was used. Virus input was 50 PFU.
[b]ED_{50}: Inhibitory concentration required to reduce viral plaques by 50% (mean ± SD, n = 12).
[c]MCC: Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[d]CC_{50}: Cytotoxic concentration required to reduce cell growth by 50%.
[e]SI: Ratio of CC_{50}/ED_{50}.
[f]Highest concentration tested.

and its N^4-acyl derivatives (**8**, **9**, **10** and **11**). The selectivity index (CC_{50}/ED_{50}) for N^4-butanoyl-MMdCyd (**10**) > 3371 was better than that for MMdCyd (**1**) > 551 in A549 cells.

Compounds **2**, **3**, **4**, **5**, **6**, **7**, **12** and **13** were devoid of antiherpes activity up to 3 mM (highest concentration tested). The minimum cytotoxic concentration, MCC (concentration that causes a microscopically detectable alteration of cell morphology) of these compounds for monolayers of A549 cells was greater than 544 μM.[9] These results are summarized in Table 1.

Conformational Analysis

The in-solution conformation of compounds (**1**–**10, 12 & 13**) was initially determined by NMR spectroscopy (spectroscopic data for N^4-hexanoyl-MMdCyd (**11**) was not obtained in full and thus only biological data on (**11**) is presented). The proton-proton coupling constants are given in Tables 2, 3 and 4, conformational parameters are summarized in Tables 5 and 6.

The conformation of the sugar ring was obtained form the relationship between the proton-proton coupling constants and the pseudorotational properties (Table 5) of the ring using the computer program PSEUROT.[10] The population of the three

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Table 2. Vicinal coupling constants (experimental and calculated in Hz) of MMdCyd, N^4-methyl-MMdCyd, N^4-phenyl-MMdCyd, and N^4-benzyl-MMdCyd.

	MMdCyd		N^4-methyl-MMdCyd		N^4-phenyl-MMdCyd		N^4-benzyl-MMdCyd	
	J exp[a]	J cal[b]	J exp[a]	J cal[b]	J exp[a]	J cal[b]	J exp[a]	J cal[b]
$J_{1'2'}$	7.1	7.2	7.2	7.3	7.2	7.0	7.0	7.2
$J_{1'2''}$	6.7	6.8	6.8	6.8	6.7	6.9	6.7	6.8
$J_{2'2''}$	−14.1		−14.7		−13.1		−13.1	
$J_{2'3'}$	6.7	6.4	6.7	6.5	6.8	6.4	6.8	6.3
$J_{2''3'}$	4.3	4.4	4.1	4.3	4.1	4.4	4.1	4.3
$J_{3'4'}$	4.0	4.1	4.1	4.1	4.0	4.1	4.0	4.0
$J_{4'5'}$	4.7		4.7		3.5		3.5	
$J_{4'5''}$	4.6		3.9		4.5		4.4	
$J_{5'5''}$	−12.3		−12.6		−11.8		−11.9	

[a]J experimental; precision 0.1 Hz.
[b]J calculated using PSEUROT with $\tau_m = 36°C$.

rotamers ($\mathbf{g}^+$, $\mathbf{g}^-$ and **t**) about the exocyclic C (4′)–C (5′) bond (Table 5) was determined from the $J_{4'5'}$ and $J_{4'5''}$ coupling constants.[11,12] The *syn*/*anti* glycosidic preference (orientation of the pyrimidine ring relative to the deoxyribose moiety) was determined by nOe, the enhancement of protons $H_{1'}$, $H_{2'}$ and $H_{3'}$ were observed on H_6 using the method of Davies[13] and the data is summarized in Table 6. The position of the N^4-substituents (*proximal* to C (5) or *proximal* to N (3)) was elucidated, by observing the nOe of the N^4-substituent protons or lack of it on C (5,1) (methylene) and C (5, 3) (methoxy) protons. The various conformational modes are shown in Fig. 2.

Table 3. Vicinal coupling constants (experimental and calculated in Hz) of N^4-methoxy-MMdCyd, N^4-hydroxy-MMdCyd, 3,4-etheno-MMdCyd, and 2-imino-MMdUrd.

	N^4-methoxy-MMdCyd		N^4-hydroxy-MMdCyd		3,4-etheno-MMdCyd		2-imino-MMdUrd	
	J exp[a]	J cal[b]	J exp[a]	J cal[b]	J exp[a]	J cal[b]	J exp[a]	J cal[b]
$J_{1'2'}$	7.0	6.8	7.0	6.8	7.0	6.8	7.0	7.1
$J_{1'2''}$	6.0	6.3	6.2	6.4	6.3	6.4	6.7	6.7
$J_{2'2''}$	−13.8		−13.8		−13.2		−14.2	
$J_{2'3'}$	6.4	6.4	6.1	6.3	5.8	6.2	6.6	6.4
$J_{2''3'}$	4.8	4.6	4.8	4.6	4.8	4.7	4.3	4.4
$J_{3'4'}$	4.1	4.0	4.2	4.1	4.2	4.0	4.1	4.2
$J_{4'5'}$	3.9		4.0		3.7		4.4	
$J_{4'5''}$	4.4		4.8		4.6		3.3	
$J_{5'5''}$	−12.2		−12.4		−12.6		−12.6	

[a]J experimental; precision 0.1 Hz.
[b]J calculated using PSEUROT with $\tau_m = 36°C$.

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Table 4. Vicinal coupling constants (experimental and calculated in Hz) of N^4-acetyl-MMdCyd, N^4-propanoyl-MMdCyd, N^4-butanoyl-MMdCyd, and N^4-pivaloyl-MMdCyd.

	N^4-acetyl-MMdCyd		N^4-propanoyl-MMdCyd		N^4-butanoyl-MMdCyd		N^4-pivaloyl-MMdCyd	
	J exp[a]	J cal[b]	J exp[a]	J cal[b]	J exp[a]	J cal[b]	J exp[a]	J cal[b]
$J_{1'2'}$	6.2	6.3	6.3	6.2	6.3	6.2	6.3	6.4
$J_{1'2''}$	6.2	6.4	6.1	6.3	6.0	6.2	6.3	6.4
$J_{2'2''}$	−13.8		−13.7		−12.9		−13.8	
$J_{2'3'}$	6.4	6.6	6.2	6.4	6.2	6.4	6.3	6.6
$J_{2''3'}$	5.0	5.1	5.2	5.2	5.1	5.1	4.9	5.0
$J_{3'4'}$	4.8	4.7	4.5	4.3	4.4	4.2	4.8	4.6
$J_{4'5'}$	3.4		3.4		3.7		3.5	
$J_{4'5''}$	4.7		4.5		4.8		4.8	
$J_{5'5''}$	−12.3		−12.5		−12.3		−12.1	

[a]J experimental; precision 0.1 Hz.
[b]J calculated using PSEUROT with $\tau_m = 36°C$.

Table 5. Conformation populations (%): South (S) and North (N) conformers of the furanose ring and the three rotamers (g^+, g^- and t) of the exocyclic C(5′) side chain of MMdCyd, its analogs, and 2-imino-MMdUrd.

	S[a]	**N**[a]	$\mathbf{g}^{+b}$	$\mathbf{g}^{-b}$	$\mathbf{t}^b$
MMdCyd	60 (162°)[c]	40 (27°)[c]	45	27	28
N^4-methyl-MMdCyd	60 (161°)[c]	40 (30.0°)[c]	52	27	21
N^4-phenyl-MMdCyd	63 (157°)[c]	37 (9.0°)[c]	50	16	34
N^4-benzyl-MMdCyd	62 (161°)[c]	38 (11°)[c]	51	17	32
N^4-methoxy-MMdCyd	62 (162°)[c]	38 (−3.0°)[c]	53	18	29
N^4-hydroxy-MMdCyd	61 (161°)[c]	39 (3.0°)[c]	48	19	33
3,4-etheno-MMdCyd	61 (161°)[c]	39 (2.0°)[c]	52	16	32
N^4-acetyl-MMdCyd	55 (148°)[c]	45 (−3.0°)[c]	55	11	34
N^4-propanoyl-MMdCyd	53 (157°)[c]	47 (−7.0°)[c]	57	11	32
N^4-butanoyl-MMdCyd	54 (158°)[c]	46 (−8.0°)[c]	51	15	34
N^4-pivaloyl-MMdCyd	56 (148°)[c]	44 (−0.0°)[c]	53	13	34
2-imino-MMdUrd	60 (160°)[c]	40 (24°)[c]	72	13	15

[a]In the PSEUROT calculations, τ_m was constrained to 36.0°. The rms deviation for each calculation was: N^4-methyl-MMdCyd: 0.112 E; N^4,N^4-dimethyl-MMdCyd: 0.242 E; N^4-phenyl-MMdCyd: 0.202 E; N^4-benzyl-MMdCyd: 0.192 E; N^4-methoxy-MMdCyd: 0.145 E; N^4-hydroxy-MMdCyd: 0.150 E; 3,4-etheno-MMdCyd: 0.261 E; N^4-acetyl-MMdCyd: 0.204 E; N^4-propanoyl-MMdCyd: 0.166 E; N^4-butanoyl-MMdCyd: 0.181 E; N^4-pivaloyl-MMdCyd: 0.256 E; 2-imino-MMdUrd: 0.110 E.
[b]C(5′) Exocyclic orientation at 37°C.
[c]Numbers in brackets are the calculated pseudorotational phase angles P_S and P_N.

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Table 6. *Syn/anti* glycosidic preference of MMdCyd, its analogs, and 2-imino-MMdUrd.

Compound	$\eta_6\{1'\}$[a]	$\eta_6\{2'\}$	$\eta_6\{3'\}$	Glycosicidic preference[b]
MMdCyd	4	8	5	anti
N^4-methyl-MMdCyd	3	7	4	anti
N^4-phenyl-MMdCyd	8	13	10	anti
N^4-benzyl-MMdCyd	7	13	9	anti
N^4-methoxy-MMdCyd	5	11	7	anti
N^4-hydroxy-MMdCyd	4	10	6	anti
3,4-etheno-MMdCyd	8	15	11	anti
N^4-acetyl-MMdCyd	4	9	5	anti
N^4-propanoyl-MMdCyd	5	11	6	anti
N^4-butanoyl-MMdCyd	3	7	4	anti
N^4-pivaloyl-MMdCyd	4	8	5	anti
2-imino-MMdUrd	12	9	2	syn

[a]The common notation used for nOe is $\eta_i\{s\}$ which indicates that this is the nOe of nucleus i when nucleus {s} is saturated.

[b]Glycosidic preference refers to a dynamic solution equilibrium that is biased towards either **syn** or **anti**. For **syn** orientations, H_6 is closest to $H_{1'}$ and the nOe to H_6 will be mainly from $H_{1'}$. For **anti** orientations, the nOe to H_6 will be mainly from $H_{2'}$ and $H_{3'}$.

Compounds **1–7** and **13** displayed an approximate 60/40 South/North equilibrium for the deoxyribose conformation. However, N^4-acyl-MMdCyd analogs (**8–10 & 12)**, displayed South/North equilibrium in the range 53/47 for the deoxyribose conformation.

All compounds have a predominant $\mathbf{g}^+$ rotamer for the C (5′) exocyclic side chain with 2-imino-MMdUrd having a very high $\mathbf{g}^+$ contribution (Table 5).

The *syn/anti* glycosidic equilibrium in compounds **1–10** & **12** is biased towards the *anti* region. 2-Imino-MMdUrd **(13)** is the only exception where the *syn* conformation is predominant (Table 5).

The N^4 substituent in N^4-methyl-MMdCyd (**2**), N^4-phenyl-MMdCyd (**3**), N^4-benzyl-MMdCyd (**4**), N^4-methoxy-MMdCyd (**5**) and N^4-hydroxy-MMdCyd (**6**) was *distal* from C (5) (*proximal* to N (3)). The N^4-acyl group in N^4-acetyl-MMdCyd (**8**), N^4-propanoyl-MMdCyd (**9**), N^4-butanoyl-MMdCyd (**10**), N^4-pivaloyl-MMdCyd (**12**) was *proximal* to C (5). The analog 3, 4-etheno-MMdCyd (**7**) has a rigid geometry with the N^4 substituent bonded covalently to N (3). Figure 2 shows the major conformational modes.

The structure of MMdCyd (**1**), N^4-methyl-MMdCyd (**2**) and 3,4-etheno-MMdCyd (**7**) has been solved by X-ray crystallography.[2,7,14] The conformational parameters of **1**, **2** and **7** are compared in Table 7.

Stability

The stability of MMdCyd and its N^4-acyl derivatives under physiological conditions (pH 7.0, 37°C) was monitored by UV absorption spectra and by identification

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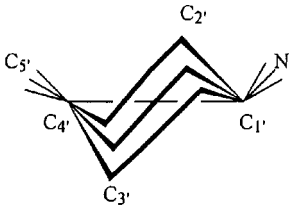
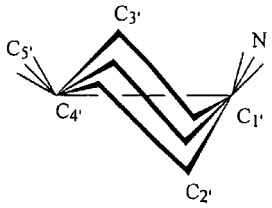
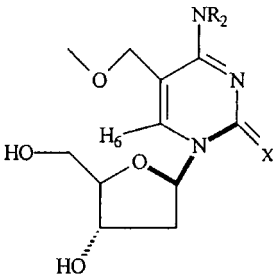


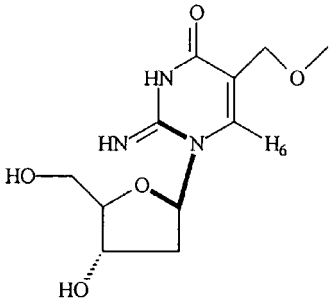
South (S) conformations



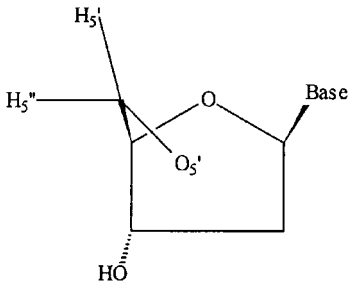
North (N) conformations



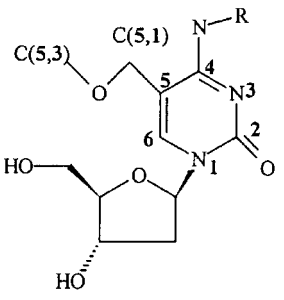
Anti glycosidic conformation



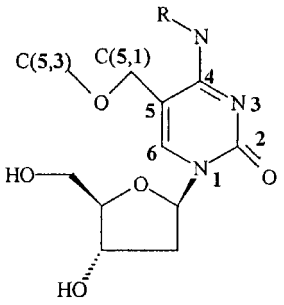
Syn glycosidic conformation



g^+ Exocyclic sidechain conformation



N^4 Substituent proximal to N_3



N^4 Substituent proximal to C_5

Figure 2. The four conformational modes in pyrimidine deoxyribonucleoside analogs discussed in this study.

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Table 7. X-ray parameters for MMdCyd and two of its analogs.

	MMdCyd (A)	MMdCyd (B)	N^4-methyl-MMdCyd	3, 4-etheno-MMdCyd
χ	213.7°	222.2°	193.8°	−93.2°
Puckering mode	$_3$E	^{2}E	E_1	$_2T^3$
P	185.5°	174.5°	130.9°	2.54°
τ_m	32.3°	36.3°	39.4°	27.8°
γ	g^+	g^+	t	g^+

of the compounds using HPLC. All compounds were stable under these conditions for up to 72 h.

DISCUSSION

The conformation of the deoxyribose moiety is important in determining the biological activity of 5-substituted 2′-deoxyribonucleosides against Herpes Simplex virus and Varciella Zoster virus.[2,5,15] Molecular conformation studies by X-ray crystallography (solid state) and NMR spectroscopy (in solution) indicate that the conformation of the 5′-exocyclic side chain [γ torsion angle C (3′)-C (4′)-C (5′)-O (5′)] is important in determining the *activation* of 5-substituted pyrimidine-2′-deoxynucleosides by HSV- induced thymidine kinase (HSV-TK). The $\mathbf{g}^+$ conformer seems to be the preferred orientation required by HSV-TK; whereas the **t** conformer appears to be an unfavored conformation.[7] Four major modes of conformational flexibility for pyrimidine 2′-deoxyribonucleosides are shown in Fig. 2. The C (5′) -hydroxyl group in MMdCyd (**1**), and its N^4 substituted analogs (compounds **2–10** & **12**) exists predominantly (about 50%) in a $\mathbf{g}^+$ rotamer conformation in solution. The X-ray data for compounds **1** and **7** are in agreement with the NMR in-solution data. In contrast, for compound **2**, the C (5′) -hydroxyl group has a **t** conformation in the solid state. The predominant conformation of the glycosidic bond in MMdCyd (**1**), and its N^4 substituted analogs (compounds **2–10 & 12**) is *anti* in both solid and in-solution data, Table 7.

On the basis of the rotamer population of C (5′)-hydroxyl group (predominantly $\mathbf{g}^+$) and preferred glycosidic conformation (*anti*) in solution, one would expect that compounds **2–12** should exhibit anti HSV-1 activity. However, antiviral assays indicated that only N^4-acyl-MMdCyd derivatives **(8–11)** were inhibitors of HSV-1 replication. The anti HSV-1 potency increases from N^4-acetyl-MMdCyd (**8**) to N^4-propanoyl-MMdCyd (**9**) to N^4-butanoyl-MMdCyd (**10**) with the latter being the most potent. Antiviral activity decreases with N^4-hexanoyl-MMdCyd (**11**) and N^4-pivaloyl-MMdCyd (**12**) is not active (Table 1).

Interestingly, the orientation of the N^4 substituent, in N^4-acetyl-MMdCyd, (**8**), N^4-propanoyl-MMdCyd (**9**), N^4-butanoyl-MMdCyd (**10**) and N^4-hexanoyl-MMdCyd (**11**) is *proximal* to C (5). It appears from antiviral data in Table 1 that an optimal linear chain of four carbon atoms (compound **10**) yields the highest anti

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HSV-1 activity. The analog N^4-pivaloyl-MMdCyd (**11**) has the N^4 substituent *proximal* to C (5) yet shows no anti HSV-1 activity.

The glycosidic preference of the compound 2-imino-MMdUrd (**13**) is *syn,* which might be due to strong hydrogen bonding between the NH group at C (2) and the 5′ (OH).

In conclusion, results of this investigation show an enhanced activity against HSV-1 of compounds **8**, **9**, **10** and **11** (with an N^4-acyl moiety) compared to the parent compound **1**, with the optimum linear acyl chain length being four carbons (compound **10**). Modeling studies on the ease of docking of all these newly synthesized analogs to the established three dimensional structure of the active site of HSV-1 thymidine kinase [16,17] should give an idea on the structure activity relation correlation.

EXPERIMENTAL SECTION

^{1}H Nuclear magnetic resonance (^{1}H NMR) spectra and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra were measured on a Brucker AM-300 spectrometer. For ^{1}H NMR, residual HDO in D_2O was employed as the internal standard and assigned as 4.67 ppm down- field from TMS. The ^{1}H NMR assignments were made on the basis of chemical shift and were necessary, confirmed by homonuclear decoupling.^{1}H NMR spectra were obtained with a digital resolution of 0.224 Hz/pt (sweep width = 4000 Hz, FID = 16 K).

Ultraviolet spectra were obtained using a Kontron Uvikon 860 spectrophotometer. Samples were prepared using phosphate buffer (pH 7.2). Extinction coefficients were rounded to the nearest zero.

Mass spectra were obtained on a FISONS VG70SE high-resolution mass spectrometer with FAB capacity. The matrix used to dissolve the samples was 3-nitrbenzylalcohol.

Chemical Synthesis

Compounds **3–6** were prepared in a manner similar to the preparation of **2** reported earlier.[2] The protected compound, 3′,5′-diacetyl-MMdUrd was transformed to the intermediate 4-triazolyl-3′, 5′-diacetyl-MMdUrd for **3** and **4** [7] or 4-tosyl-3′, 5′-diacetyl-MMdUrd for **5** and **6**.[18] Crude 4-triazolyl-3′, 5′-diacetyl-MMdUrd was treated with the proper base to yield the protected 3′, 5′-diacetyl form of **3** and **4** and crude 4-tosyl-3′, 5′-diacetyl-MMdUrd was treated with methoxyl or hydroxylamine hydrochloride to furnish the protected 3′, 5′-diacetyl form of **5** and **6**.[18] Deprotection of the 3′ and 5′ hydroxyl groups provided the desired analogs.

3, 4-Etheno-MMdCyd (**7**) was prepared by treating MMdCyd (**1**), with aqueous chloroacetaldehyde followed by methanolic sodium hydroxide.[19]

N^4-Acyl-MMdCyd analogs (**8–12**) were prepared by dissolving MMdCyd (**1**), in DMF and treating the mixture with the proper acyl anhydride.[20]

2-Imino-MMdUrd (**13**), was prepared from MMdUrd by installing the leaving group tosyl at position 5′ of the deoxyribose. The compound 5′-tosyl-MMdUrd

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was treated with ethanolic sodium hydroxide to produce the intermediate 2-O-5′-anhydro-MMdUrd which when treated with ammonia in methanol gave the product 2-imino-MMdUrd.[21]

N^4-Aryl-MMdCyd, Compounds 3 and 4, General Procedure. 3′, 5′-Diacetyl-5-methoxymethyl-2′-deoxyuridine (1 mmol) in acetonitrile (5 mL), was added to a mixture of Et_3N (25 mol. eq.), 1, 2, 4-triazole (27 mol. eq.) and $POCl_3$ (6 mol. eq.) at 0°C. The reaction mixture was stirred for 2 h at 25°C, concentrated in vacuo and the residue was dissolved in ethyl acetate (150 mL). The organic layer was washed with saturated $NaHCO_3$ (50 mL) followed by water (50 mL), and finally with brine (50 mL), dried and concentrated under vacuum. The crude triazolyl intermediate was dissolved in dioxane (5 mL), the solution was cooled in an ice water bath, and the appropriate amine was added or bubbled into it. After 14 h, the crude product was dissolved in methanol (5 mL) and saturated aqueous NH_4OH (2 mL) was added. After 14 h, the final product was purified by flash column chromatography using 10% methanol in dichloromethane.

N^4-Phenyl-5-methoxymethyl-2′-deoxycytidine (3) (Yield 73%). **^{1}H NMR** (D_2O): 7.70 (1H, s, H_6), 7.30–7.0 (5H, m, C_6H_5), 5.95 (1H, dd, $H_{1'}$), 4.25 (1H, m, $H_{3'}$), 4.15 (2H, s, CH_2), 3.85 (1H, m, $H_{4'}$), 3.65 (1H, dd, $H_{5'}$), 3.55 (1H, dd, $H_{5''}$), 3.18 (3H, s, OCH_3), 2.23 (1H, m, $H_{2''}$), 2.10 (1H, m, $H_{2'}$).

^{13}C NMR (D_2O): 88.6 (d, $C_{1'}$), 42.0 (t, $C_{2'}$), 72.5 (d, $C_{3'}$), 89.1 (d, $C_{4'}$), 63.3 (t, $C_{5'}$), 159.0 (s, C_2), 163.7 (s, C_4), 106.9 (s, C_5), 148.4 (d, C_6), 70.2 (t, CH_2), 59.5 (q, OCH_3), 139.0, 131.3, 128.2 and 126.1 (C_6H_5).

UV: λ_{min}: 253 nm, λ_{max}: 290 nm (ε, 9250)

MS: $(M^+ + 1)$ Calculated: 348.1521 FAB-MS $(M^+ + 1)$: 348.1552

N^4-Benzyl-5-methoxymethyl-2′-deoxycytidine (4) (Yield 70%). **^{1}H NMR** (D_2O): 7.55 (1H, s, H_6), 7.10–6.90 (5H, m, C_6H_5), 5.90 (1H, dd, $H_{1'}$), 4.37 (2H, s, NCH_2), 4.15 (1H, m, $H_{3'}$), 4.00 (2H, s, OCH_2), 3.75 (1H, m, $H_{4'}$), 3.60 (1H, dd, $H_{5'}$), 3.50 (1H, dd, $H_{5''}$), 3.05 (3H, s, OCH_3), 2.13 (1H, m, $H_{2'}$), 1.92 (1H, m, $H_{2'}$).

^{13}C NMR (D_2O): 88.3 (d, $C_{1'}$), 41.8 (t, $C_{2'}$), 72.6 (d, $C_{3'}$), 88.9 (d, $C_{4'}$), 63.3 (t, $C_{5'}$), 159.5 (s, C_2), 164.9 (s, C_4), 106.6 (s, C_5), 142.4 (d, C_6), 70.1 (t, CH_2O), 59.2 (q, OCH_3), 46.1 (t, NCH_2), 130.9, 129.6, 129.4 and 126.1 (C_6H_5).

UV: λ_{min}: 254 nm, λ_{max}: 276 nm (ε, 8950)

MS: $(M^+ + 1)$ Calculated: 362.1681 FAB-MS $(M^+ + 1)$: 362.1712

N^4-Methoxy and N^4-hydroxy-MMdCyd, Compounds 5 and 6 General Procedure. To a solution of 3′,5′-diacetyl-5-methoxymethyl-2′-deoxyuridine (1 mmol) in dry acetonitrile (5 mL) was added K_2CO_3 (4 mol. eq.) and TsCl (2 mol. eq.) at room temperature. The reaction mixture was refluxed for 3 h and concentrated to dryness in vacuo. To the crude reaction product, a solution of methoxylamine for **5** or hydroxylamine hydrochloride for **6** (20 mol. eq.) in pyridine (25 mL) was added and the solution was stirred at 25°C for 18 h. The solvent was removed and residue dissolved in ethyl acetate (75 mL). The organic phase was washed with water (2×25 mL) followed with brine (15 mL) and dried. The solvent was removed the crude product dissolved in methanol (5 mL) and the solution was saturated with ammonia. After 14 h at

25°C, solvent was removed and the final product was purified by flash column chromatography using 10% methanol in dichloromethane.

N^4-Methoxy-5-methoxymethyl-2′-deoxycytidine (5) (Yield 70%). **^{1}H NMR** (D_2O): 7.09 (1H, s, H_6), 6.11 (1H, dd, H_{15}), 4.30 (1H, ddd, $H_{3'}$), 4.01(2H, d, CH_2), 3.83 (1H, ddd, $H_{4'}$), 3.73 (1H, dd, $H_{5'}$), 3.75 (3H, s, $NOCH_3$), 3.60 (1H,dd, $H_{5''}$) 3.21 (3H, s, OCH_3) 2.3–2.1 (2H, m, $H_{2',2''}$).

^{13}C NMR ($CDCl_3$): 85.7(d, $C_{1'}$), 39.2 (t, $C_{2'}$), 71.5 (d, $C_{3'}$), 86.6 (d-$C_{4'}$), 62.5 (t, $C_{5'}$), 142.7 (s, C_2), 149.4 (s, C_4), 107.9 (s, C_5), 128.9 (d, C_6) 66.7 (t, CH_2), 58.8 (q, CH_3), 61.9 (q, $NOCH_3$).

UV: λ_{min}: 253 nm, λ_{max}: 282 nm (ε, 9300)

MS: ($M^+ + 1$) Calculated: 302.1342 FAB-MS ($M^+ + 1$): 302.1329

N^4-Hydroxy-5-methoxymethyl-2′-deoxycytidine (6) (Yield 85%). **^{1}H NMR** (D_2O): 7.05 (1H, s, H_6), 6.1 (1H, dd, $H_{1'}$), 4.3 (1H, ddd, $H_{3'}$), 4.0(2H, s, CH_2), 3.83 (1H, ddd, $H_{4'}$), 3.5–3.7 (2H, 2dd, $H_{5'}$,$H_{5''}$), 3.2 (3H, s, OCH_3), 2.18 (2H, m, $H_{2',2''}$).

^{13}C NMR (D_2O): 86.7 (d, $C_{1'}$), 40.2 (t, $C_{2'}$), 73 (d, $C_{3'}$), 88.5 (d-$C_{4'}$), 63.6 (t, $C_{5'}$), 147.7(s, C_2), 153.2 (s, C_4), 109.4 (s, C_5), 133.8 (d, C_6) 69.4 (t, CH_2), 59.6 (q, CH_3).

UV: λ_{min}: 259 nm, λ_{max}: 283 nm (ε, 9300)

MS: ($M^+ + 1$) Calculated: 288.1176 FAB-MS ($M^+ + 1$): 288.1191

3,4-Etheno-5-methoxymethyl-2′-deoxycytidine (7) (Yield 42%). Chloroacetaldehyde in water (1161 mg, 7.4 mmol), 50% w/w was added slowly to MMdCyd (100 mg, 0.37 mmol) with stirring till the reaction mixture was homogeneous. The reaction pH was adjusted to 3.5 using a solution of 1M HCl and the reaction was left at room temperature for 48 h. The pH of the reaction was raised to 7.0 using a saturated solution of methanolic NaOH and the mixture was co-evaporated with ethanol. The crude was purified by FCC using 15% MeOH in dichloromethane.

^{1}H NMR (D_2O): 7.60 and 7.15 (2H, 2d, etheno), 7.49 (1H, s, H_6), 6.30 (1H, dd, $H_{1'}$), 4.40–4.30 (3H, m, CH_2 and $H_{3'}$), 3.90 (1H, m, $H_{4'}$), 3.70 (1H, dd, $H_{5'}$), 3.62 (1H, dd, $H_{5''}$), 3.23 (3H, s, OCH_3), 2.42–2.20 (2H, m, $H_{2'}$ and $H_{2''}$).

^{13}C NMR (D_2O): 88.5 (d, $C_{1'}$), 41.6 (t, $C_{2'}$), 72.7 (d, $C_{3'}$), 89.2 (d, $C_{4'}$), 63.4 (t, $C_{5'}$), 147.1 (s, C_2), 48.9 (s, C_4), 110.5 (s, C_5), 134.1 (d, C_6), 69.7 (t, CH_2), 59.9 (q, OCH_3), 130.3 and 115.8 (d, etheno).

UV: λ_{min}: 232 nm, λ_{max}: 271 nm (ε, 9050)

MS: ($M^+ + 1$) Calculated: 296.1227 FAB-MS ($M^+ + 1$): 296.1249

N^4-Acetyl-5-methoxymethyl-2′-deoxycytidine (8) (Yield 70%). MMdCyd (**1**). (150 mg, 0.55 mmol) was dissolved in anhydrous DMF (1.5 mL) at 22°C. The solution was cooled in an ice water bath under argon and acetic anhydride (63 μL, 0.66 mmol) was added. The reaction mixture was left stirring at room temperature for 24 h. The solvent was removed under high vacuum. The gummy residue was dissolved in methanol and layered on a silica gel plug (3 cm in depth). The column was eluted using 5% methanol in dichloromethane, fractions were collected, pooled and the solvent was evaporated in vacuo.

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^{1}H NMR (D_2O): 8.25 (1H, s, H_6), 6.08 (1H, dd, $H_{1'}$), 4.30 (3H, m, CH_2 and $H_{3'}$), 4.00 (1H, ddd, $H_{4'}$), 3.75 (1H, dd, $H_{5'}$), 3.65 (1H, dd, $H_{5''}$), 3.28 (3H, s, OCH_3) 2.48 (1H, m, $H_{2''}$), 2.19 (4H, m, acetyl CH_3 and $H_{2'}$).

^{13}C NMR (D_2O): 89.6 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 72.1 (d, $C_{3'}$), 89.8 (d, $C_{4'}$), 63.0 (t, $C_{5'}$), 158.6 (s, C_2), 163.7 (s, C_4), 109.0 (s, C_5), 147.1 (d, C_6), 69.9 (t, CH_2), 59.6 (q, OCH_3), 175.7 (s, acetyl CO) 26.9 (q, CH_3).

UV: λ_{min}: 266 nm, λ_{max}: 298 nm (ε, 7500)

MS: ($M^+ + 1$) Calculated: 314.1335 FAB-MS ($M^+ + 1$): 314.1347

N^4-Propanoyl-5-methoxymethyl-2′-deoxycytidine (9) (Yield 74%). The compound 5-methoxymethyl-2′-deoxycytidine (100 mg, 0.37 mmol) was dissolved in DMF (1.0 mL) at room temperature. The solution was cooled in an ice water bath under argon and propanoic anhydride (53 μL, 0.41 mmol) was added using a syringe. The reaction mixture was stirred at room temperature for 24 h. A TLC (20% MeOH in CH_2C1_2) indicated complete reaction. One mL of absolute ethanol was added to the crude reaction mixture and stirring for 5 min, the reaction mixture was concentrated to dryness under high vacuum. The oily crude mixture was dissolved in chloroform and applied on a silica gel plug 3 cm in depth and eluted with several column volumes of 5% methanol in dichloromethane to yield a white solid product (88.3 mg, 0.27 mmol).

^{1}H NMR (D_2O): 8.21 (1 H, s, H_6), 6.02 (1 H, dd, $H_{1'}$), 4.33 (3H, m, CH_2O and $H_{3'}$), 3.95 (1 H, ddd, $H_{4'}$), 3.75 (1 H, dd, $H_{5',}$), 3.60 (1 H, dd, $H_{5''}$), 3.20 (3H, s, OCH_3), 2.55–2.40 (3H, m, CH_2 and $H_{2''}$), 2.15 (1 H, ddd $H_{2'}$), 0.95 (3H, t, CH_3).

^{13}C NMR (D_2O): 89.5 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 72.1 (d, $C_{3'}$), 89.8 (d, $C_{4'}$), 63.0 (t, $C_{5'}$), 158.6 (s, C_2), 163.8 (s, C_4), 108.9 (s, C_5), 147.0 (d, C_6), 70.0 (t, CH_2O), 59.5 (q, OCH_3), 178.9 (s, propanoyl CO), 33.2 (t, CH_2), 10.7 (q, CH_3).

UV: λ_{min}: 266 nm, λ_{max}: 298 nm (ε, 8040)

MS: ($M^+ + 1$) Calculated: 328.1463 FAB-MS ($M^+ + 1$): 328.1506

N^4-Butanoyl-5-methoxymethyl-2′-deoxycytidine (10) (Yield 68%). The nucleoside 5-methoxymethyl-2′-deoxycytidine (140 mg, 0.52 mmol) was dissolved in DMF (1.5 mL) at room temperature. The solution was cooled in an ice water bath under argon and butyric anhydride (93 μL, 0.57 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. A TLC (15% MeOH in CH_2Cl_2) indicated complete reaction. One mL of absolute ethanol was added to the reaction mixture and stirred for 5 min. The solution was concentrated to dryness under high vacuum, the oily crude mixture was dissolved in chloroform and applied on a silica gel plug 3 cm in depth. Elution with several column volumes of 5% methanol in dichloromethane yielded a white solid product (120 mg, 0.35 mmol).

^{1}H NMR (D_20): 8.21 (1H, s, H_6), 6.05 (1 H, dd, $H_{6'}$), 4.24 (3H, m, CH_20 and $H_{3'}$), 3.95 (1 H, ddd, H_4'), 3.75 (1 H, dd, $H_{5'}$), 3.57 (1 H, dd, $H_{5''}$), 3.18 (3H, s, OCH_3), 2.50–2.35 (3H, m, α CH_2 and $H_{2''}$), 2.15 (1 H, ddd $H_{2'}$), 1.50 (2H, m, β CH_2), 0.78 (3H, t, CH_3).

^{13}C NMR (D_2O): 87.7 (d, $C_{1'}$), 40.4 (t, $C_{2'}$), 69.5 (d, $C_{3'}$), 87.9 (d, $C_{4'}$), 61.2 (t, $C_{5'}$), 153.5 (s, C_2), 160.2 (s, C_4), 106.4 (s, C_5), 144.0 (d, C_6), 68.8 (t, CH_20), 58.2 (q, OCH_3), 170.9 (s, CO), 34.2 (t, α CH_2), 25.3 (t, β CH_2), 13.6 (q, CH_3).

UV: λ_{min}: 268 nm, λ_{max}: 300 nm (ε, 7800)

MS: $(M^+ + 1)$ Calculated: 342.1632 FAB-MS $(M^+ + 1)$: 342.1671

N^4-Trimethylacetyl-5-methoxymethyl-2′-deoxycytidine (N^4-pivaloyl-MMdCYd)-(12) (Yield 84%). The compound, 5-methoxymethyl-2′-deoxycytidine (50 mg, 0.19 mmol) was dissolved in DMF (0.5 mL) at room temperature. The solution was cooled in an ice water bath under argon and trimethylacetic anhydride (41 μL, 0.20 mmol) was added using a syringe. The reaction mixture was left at room temperature for 24 h. A TLC (10% MeOH in CH_2Cl_2) indicated complete reaction. The reaction mixture was concentrated to dryness under high vacuum. The crude mixture was dissolved in chloroform, applied on a FCC column 3 cm in length and eluted with several column volumes of 5% methanol in dichloromethane. The product was isolated as a white solid (57 mg, 0.16 mmol).

^{1}H NMR (D_2O): 8.22 (1 H, s, H_6), 6.05 (1 H, dd, $H_{1'}$), 4.40–4.20 (3H, m, CH_2 and $H_{3'}$), 4.00 (1 H, m, $H_{4'}$), 3.75 (1 H, dd, $H_{5'}$), 3.65 (1 H, dd, $H_{5''}$), 3.28 (3H, s, OCH_3), 2.45 (1 H, m, $H_{2''}$), 2.15 (1 H, m, $H_{2'}$), (9H, s, $(CH_3)_3$).

^{13}C NMR (D_2O): 89.6 (d, $C_{1'}$), 42.2 (t, $C_{2'}$), 72.2 (d, $C_{3'}$), 89.8 (d, $C_{4'}$), 63.1 (t, $C_{5'}$), 158.7 (s, C_2), 164.3 (s, C_4), 108.7 (s, C_5), 146.4 (d, C_5), 70.7 (t, CH_2), 59.7 (q, OCH_3), 182.2 (s, pivaloyl CO), 28.5 (q, pivaloyl $(CH_3)_3$).

UV: λ_{min}: 269 nm, λ_{max}: 301 nm (ε, 8300)

MS: $(M^+ + 1)$ Calculated: 356.1853 FAB-MS $(M^+ + 1)$: 356.1834

2-Imino-5-methoxymethyl-2′-deoxyuridine (13) (Yield 47%). 5′-Tosyl-MMdUrd (90 mg, 0.21 mmol), was dissolved in 95% EtOH (4 mL). One M NaOH (210 μL, 0.21 mmol) was added to the solution and refluxed for 14 h.[21] The crude reaction mixture was diluted with water (25 mL) and extracted with chloroform (25 mL × 3). The combined organic fractions were dried over anhydrous sodium sulfate and concentrated. The crude 2-O-5′-anhydro-5-methoxymethyl-2′-deoxyuridine was dissolved in MeOH (3 mL), the solution cooled to 0°C, and anhydrous ammonia gas was bubbled into the solution at a moderate rate for 10 sec. After 48 h, the crude product was purified by FCC using 20% MeOH in CH_2Cl_2.

^{1}H NMR (D_2O): 7.71 (1H, s, H_6), 5.83 (1H, dd, $H_{1'}$), 4.34 (1H, m, $H_{3'}$), 4.08 (2H, s, CH_2O), 3.83 (1H, m, $H_{4'}$), 3.73 (1H, dd, $H_{5'}$), 3.65 (1H, dd, $H_{5''}$), 3.21 (3H, s, OCH_3), 2.5–2.2 (2H, m, $H_{2',2''}$).

^{13}C NMR (D_2O): 89.3 (d, $C_{1'}$), 40.6 (t, $C_{2'}$), 72.2 (d, $C_{3'}$), 91.0 (d, $C_{4'}$), 62.9 (t, $C_{5'}$), 157.7 (s, C_2), 174.9 (s, C_4), 116.3 (s, C_5), 141.7 (d, C_6), 69.8 (t, CH_2O) and 59.8 (q, OCH_3).

UV: λ_{min}: 252 nm, λ_{max}: 280 nm (ε, 9200)

MS: $(M^+ + 1)$ Calculated: 272.1239 FAB-MS $(M^+ + 1)$: 272.1250

NMR Conformational Analysis

The NMR experiments were carried out using a Brucker 300 spectrometer. Spectra were recorded in the Fourier transform mode at 5°, 25°, 37° and 50°. Solutions were made to a concentration of 0.1 M in D_2O without adjusting the pD.^{1}H NMR spectra were simulated with the aid of the Brucker routine PANIC and final coupling constants have a precision of 0.1 Hz. Calculations of coupling constants as well as

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pseudorotational parameters were performed using PSEUROT assuming a maximum puckering amplitude $\tau_m = 36.0°$. C(5′) Exocyclic side chain populations were calculated using PANIC experimental coupling constant. The *syn*/*anti* glycosidic torsional preference was deduced from Nuclear Overhauser Effect (nOe) experiments. The enhancements of $H_{1'}$, $H_{2'}$ and $H_{3'}$ on H_6 were recorded and compared. The area under the resonance of the enhanced signal was compared to the area of the same resonance in a control spectrum.

Antiviral Activity

The A549 cell line was used in this study. The cells were grown in Eagle's Minimum Essential Medium (MEM) containing 10% Fetal Bovine Serum (FBS) as previously described.[1] The virus stocks were propagated by low multiplicity of infection of A549 cell monolayers using HSV-1 (McIntyre strain) until complete (100%) cytopathic effect and the virus titre was determined according to the procedure of Ayisi et al.[22]

The antiviral activity was determined by a plaque reduction assay using agar overlay. Briefly, confluent cell monolayers were infected with 50 plaque-forming units of virus per well in a 12-well tissue culture plate. Virus dilutions were made using serum-free MEM and 0.1 mL was added. The infected cultures were incubated at 37°C. After one hour, the unadsorbed virus was removed by washing with phosphate-buffered-saline (PBS). Each compound dissolved in growth medium at the appropriate concentration was added to each well along agarose and the plates were incubated for 72 h in a humidified CO_2 (5% atmosphere) chamber. The culture fluid was removed, monolayers fixed, the plaques were stained and enumerated. From dose response curves, the concentration required to reduce the number of plaques by 50% (ED_{50}) was determined. In each experiment, toxicity controls (test compound and medium), cell controls (medium only) and virus controls (virus and medium) were run simultaneously. The mean values reported are from 12 determinations.

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