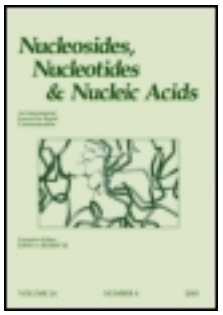


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# Synthesis of C-6 Pyrimidine Acyclic Nucleoside Analogs as Potential Antiviral Agents

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NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 563-584 (1994)

# SYNTHESIS OF C-6 PYRIMIDINE ACYCLIC NUCLEOSIDE ANALOGS AS POTENTIAL ANTIVIRAL AGENTS

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**Abstract.** A number of pyrimidine acyclic nucleosides in which the acyclic moiety is attached to the C-6 position rather than N-1 of the pyrimidine ring have been prepared. This was accomplished via treatment of lithiated 2,4-dimethoxy-5,6-dimethylpyrimidine, or, 2,4-dimethoxy-6-methylpyrimidine with 1,3-bis-(benzyloxy)-2-propanone, benzyl chloromethyl ether or oxirane, respectively, to give the corresponding key intermediates 6-[3-benzyloxy-2-[(benzyloxy)methyl]-2-hydroxypropyl]-2,4-dimethoxy-5-methylpyrimidine (**2a**), 6-[3-benzyloxy-2-[(benzyloxy)methyl]-2-hydroxypropyl]-2,4-dimethoxypyrimidine (**2b**), 6-(2-benzyloxyethyl)-2,4-dimethoxy-5-methylpyrimidine (**3**), and 2,4-dimethoxy-6-(3-hydroxypropyl)-5-methylpyrimidine (**4a**). After acidic hydrolysis, followed by debenzylation with boron trichloride these key intermediates were converted to the target C-6 pyrimidine acyclic derivatives. Compounds **6-8b**, **11-13**, **15**, **16**, **20**, **22**, **26**, and **29-32** were evaluated for activity against herpes viruses and human immunodeficiency virus. None of the compounds were active against the viruses nor were they cytotoxic at the highest concentration tested.

## INTRODUCTION

Acyclovir (ACV)[1-3] is the drug of choice for treatment of herpes simplex virus type 1 and type 2 infections. The mechanism of action of ACV and its antiviral selectivity is a consequence of biochemical specificity on two levels: (1) ACV is a substrate for the herpes-virus-encoded thymidine kinase (TK) but it is not a substrate for the host TK;[4] (2) as the triphosphate derivative, ACV is both a selective inhibitor of,[5] and a substrate for[6] the viral DNA polymerase. Since the successful demonstration that replacement of the

**This manuscript is dedicated to the memory of Roland K. Robins**

ribose moiety by an acyclic chain gave an active drug, a large number of acyclic nucleoside analogs have been synthesized as potential antivirals. Some purine and purine-type acyclic nucleosides have shown potent antiviral activity.[7]

In contrast to the purine acyclic nucleosides, pyrimidine acyclic nucleosides such as acyclic thymidine and related compounds have not shown significant antiviral activity[8] because they are surprisingly poor substrates for viral TK.[9] Nonetheless, a pyrimidine acyclic nucleoside, 1-[(hydroxyethoxy)methyl]-6-(phenylthio)thymine, has been found which is active against HIV.[10] This encouraged us to extend our work with anti-restricted acyclic pyrimidines[11] and synthesize selected pyrimidine acyclic nucleosides as potential antiviral agents. In this paper, we report the synthesis and evaluation of a new type of pyrimidine acyclic nucleoside in which the acyclic sugar is attached to the C-6 position of thymine, uracil and related pyrimidines.

## RESULTS AND DISCUSSION

A common aspect of the acyclic moieties of antivirals such as acyclovir is that they mimic some structural features of the intact ribofuranose ring of naturally-occurring nucleosides. Thus, conformation of the acyclic moieties must play some role in interaction with the enzyme systems involved in the antiviral activity of the molecule. This prompted us to investigate pyrimidine acyclic nucleoside analogs with an acyclic sugar mimic on the C-6 position of pyrimidine as opposed to the N-1 position. Superimposition of **8a**, one of the proposed target compounds in this study, on thymidine indicated a very close fit of the hydroxyl groups on the C-6 acyclic side chain of **8a** to the positions of the O-3', O-4', and O-5' oxygen and hydroxyl moieties of the furanose ring of thymidine (FIGURE 1). Based upon this fit, we hypothesized that the acyclic moiety of these pyrimidines might mimic the sugar moiety of thymidine during biological processing via single-single bond rotation thereby leading to biological activity.

To prepare the target compounds, the required key intermediates **2a**, **2b**, **3** and **4a** were synthesized via an addition reaction of the lithiated 2,4-dimethoxy-6-methylpyrimidine derivatives with 1,3-bis(benzyloxy)-2-propanone ,[11] benzyl chloromethyl ether, or oxirane respectively (Scheme I). Attempts to hydrolyze the 2- and 4-methoxyl groups of **2a** by stirring in methanolic hydrogen chloride, or, a mixture of aqueous sodium hydroxide in dioxane were unfruitful. However, treatment of **2a** and **2b** with acetyl chloride, containing several drops of water, effected a conversion to the acetylated thymine derivatives, 6-[2-acetoxy-3-benzyloxy-2-[(benzyloxy)methyl]propyl]thymine (**5a**), and the uracil derivative **5b**, respectively (Scheme 2). To prepare the thymine analog, 6-[2,3-dihydroxy-2-(hydroxymethyl)propyl]thymine (**8a**), the benzyl groups of **5a** were

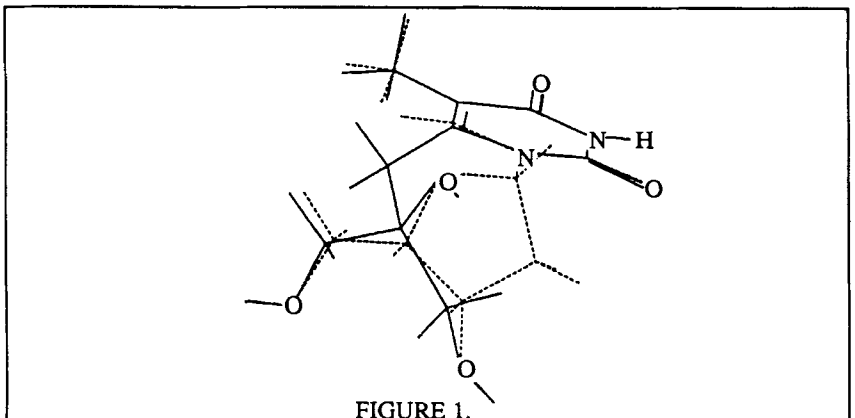
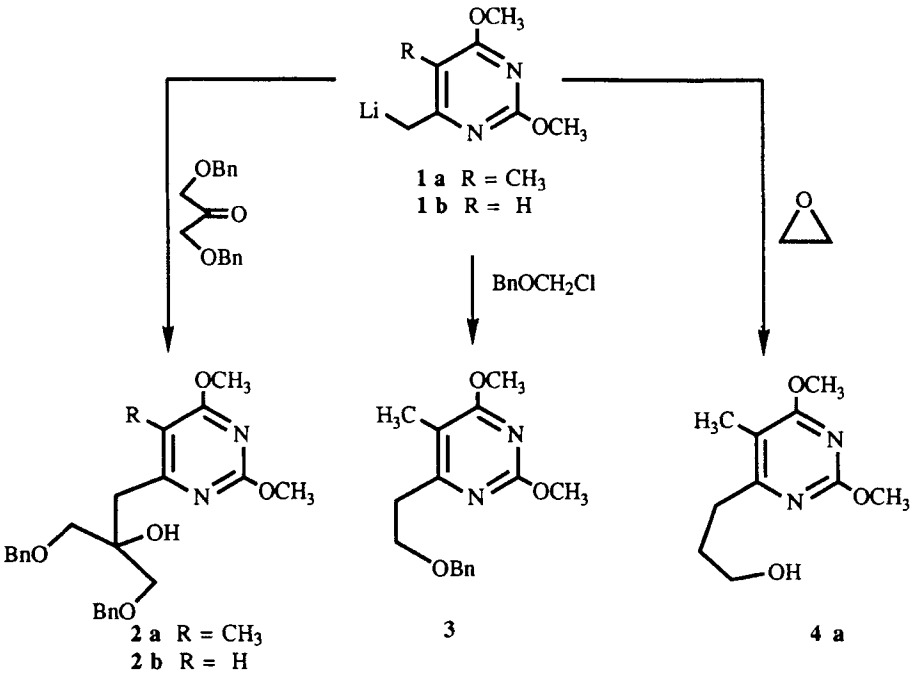
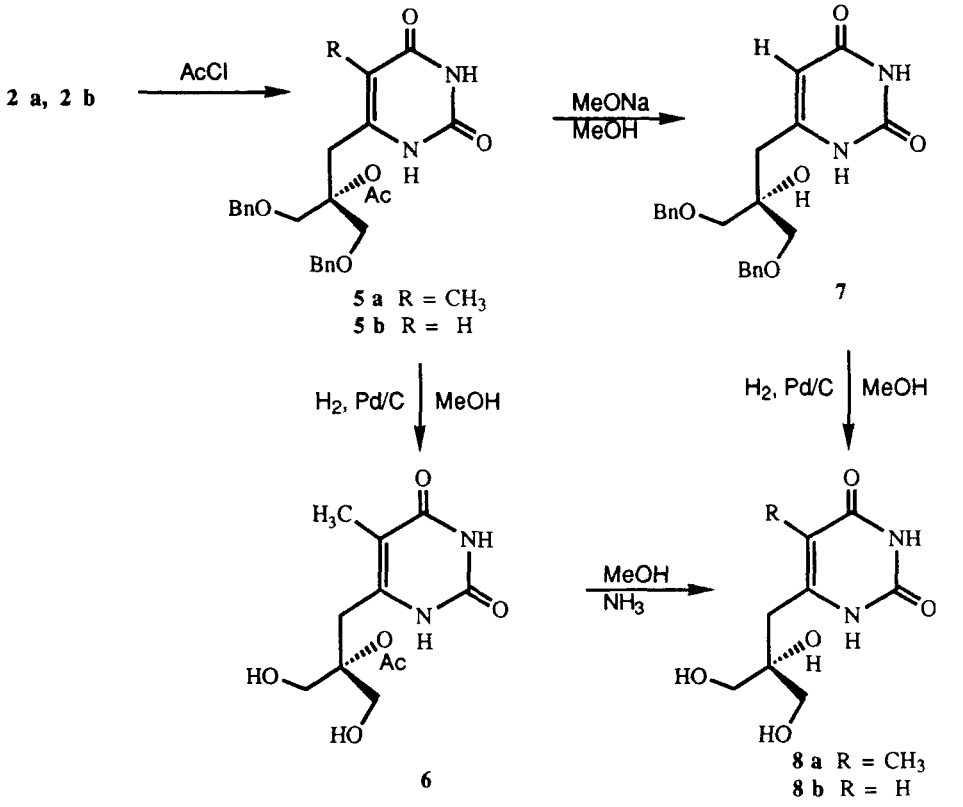


FIGURE 1.
Superimposition of the structures of thymidine (.....) and compound **8a** (——).




**SCHEME I**



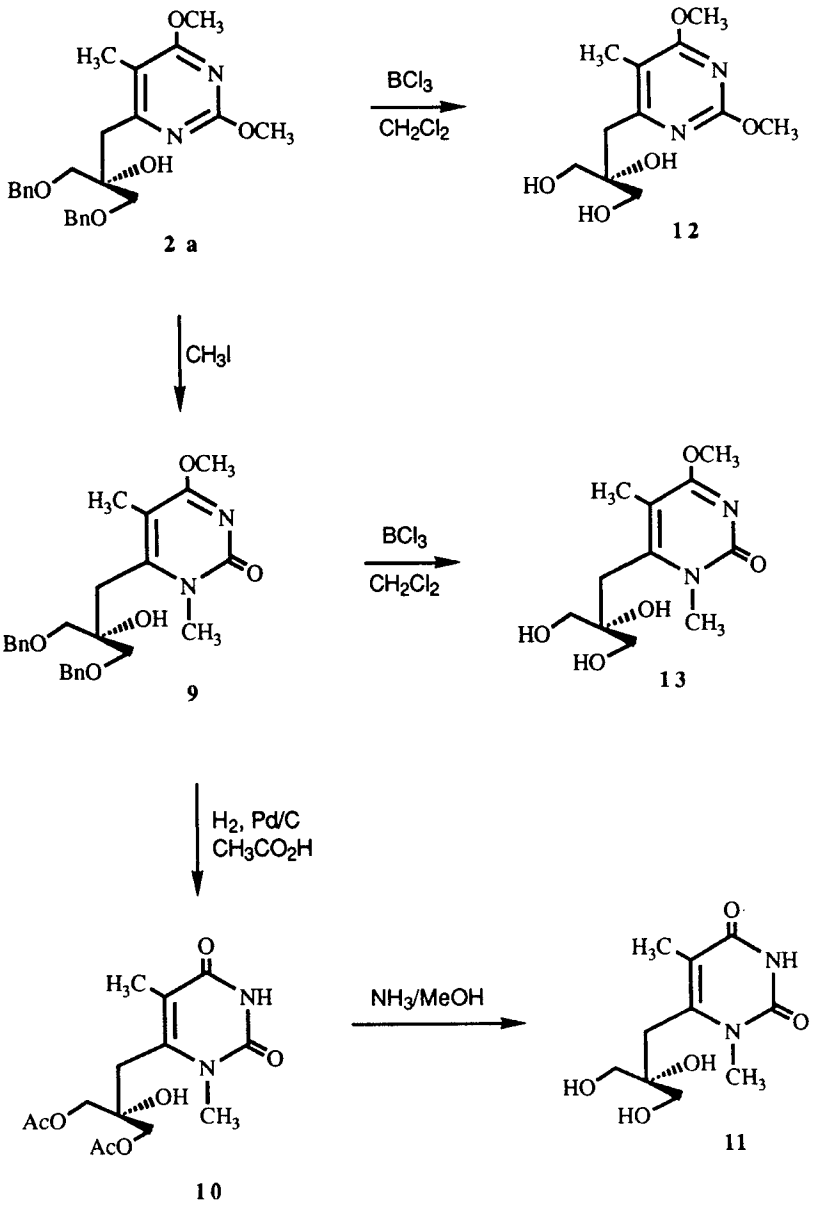

SCHEME II

removed by hydrogenolysis in the presence of 20% palladium on carbon to afford **6** in 25% yield. Subsequent deacetylation of **6** with methanolic ammonia gave the target compound **8a** in 75% yield. Compound **8b** was prepared by an initial deacetylation of **5b**, followed by debenzylation to give the uridine derivative, **8b**, however, no improvement in overall yield was observed by using this reverse order of deblocking.

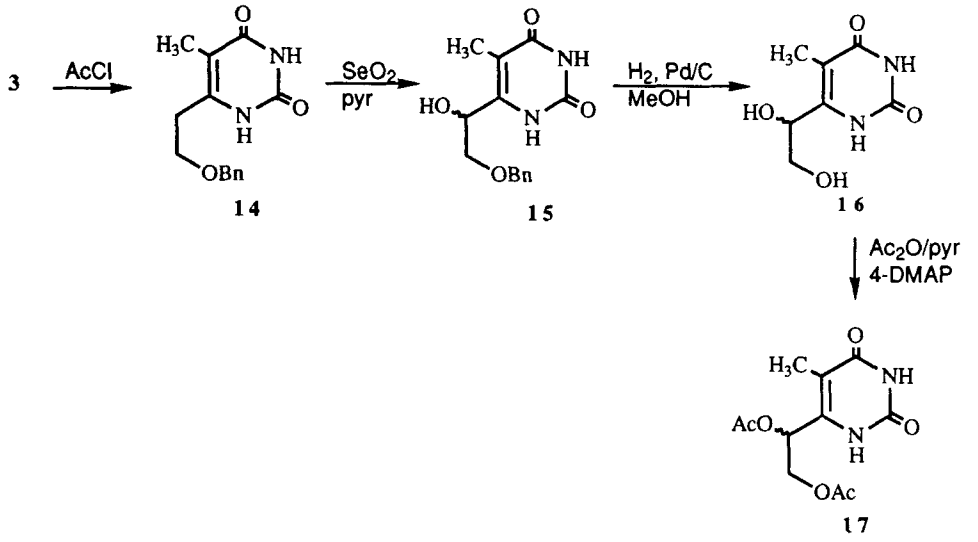
Since these derivatives were designed as analogs of thymidine it was thought that the thymine heterocycle might require substitution at the N-1 nitrogen atom, as in thymidine, in order to better mimic the heterocyclic system of thymidine, and, thus be able to participate in classical Watson-Crick complementary base pair hydrogen-bonding with adenine nucleotides. We chose to place a methyl group at the N-1 nitrogen based upon a molecular modeling study which indicated that the size of the methyl group did not interfere with the molecules ability to attain a conformation in which the oxygen atoms of the acyclic moiety may overlap with those of thymidine. The introduction of the methyl

group was accomplished by treatment of **2a** with methyl iodide under classical Hilbert-Johnson conditions at rt, affording the N-1 methylated derivative, 6-[3-benzyloxy-2-[(benzyloxy)methyl]-2-hydroxypropyl]-4-methoxy-1-methylpyrimidin-2-one (**9**) in a 14% yield, which could be moderately increased to 21%, if the reaction was allowed to proceed under reflux for 14 hours. Debenzylation and concurrent hydrolysis of the 4-methoxy group of compound **9** was accomplished by hydrogenolysis of **9** at 50 psi of hydrogen in acetic acid in the presence of palladium on carbon to give the acetylated derivative **10** in 56% yield. The structure of **10** was confirmed by the absence of a peak in the $^1$H NMR spectra for the 4-methoxyl group, and the existence of a singlet at $\delta$ 11.26, which was exchangeable upon addition of $D_2O$, and the presence of a singlet at $\delta$ 2.03, for the two acetoxy groups. The presence of the acetyl signals indicates that the two primary hydroxyl groups were readily acetylated by acetic acid under these reaction conditions. 6-[2,3-Dihydroxy-2-(hydroxymethyl)propyl]-1-methylthymine (**11**) was obtained by the reaction of **10** with saturated methanolic ammonia. Debenzylation of **2a** and **9** with $BCl_3$ at -78 °C furnished 6-[2,3-dihydroxy-2-(hydroxymethyl)propyl]-2,4-dimethoxy--5-methyl-pyrimidine (**12**), and 6-[2,3-dihydroxy-2-(hydroxymethyl)propyl]-1,5-dimethyl-4-methoxypyrimidine (**13**), respectively (Scheme III).

A series of C-6 acyclic pyrimidines with sugar mimics other than the [2,3-dihydroxy-2-(hydroxymethyl)propyl]- moiety were also prepared from the intermediates **3** and **4a**. Treatment of **3** with acetyl chloride containing several drops of water effected a hydrolysis of the methoxy group to give 6-(2-benzyloxyethyl)thymine (**14**). Allylic oxidation of **14** using selenium dioxide in pyridine at reflux temperature produced a racemic mixture of **15**. Debenzylation of **15** was accomplished by treatment with hydrogen in the presence of palladium on carbon for 14 h at 50 psi to afford (±) 6-[1,2-(dihydroxyethyl)]thymine (**16**). An acetylated derivative of compound **16** was prepared by acetylation with acetic anhydride in pyridine in the presence of a catalytic amount of 4-(dimethylamino)pyridine to give **17** in 80% yield (Scheme IV). The N-1 methyl derivative, 6-(2-benzyloxyethyl)-1,5-dimethyl-4-methoxypyrimidin-2-one (**18**), was prepared in 72% yield by the treatment of compound **3** with iodomethane at 50 °C for 15 h. The 4-methoxyl group of **18** was hydrolyzed by using methanolic hydrogen chloride to furnish **19**, in 72% yield. Debenzylation of **19** gave the target compound 6-(2-hydroxyethyl)-1-methylthymine (**20**) in 85% yield. Allylic oxidation of **19** under acidic conditions by treatment with selenium dioxide in a solvent mixture of acetic acid/dioxane (1/11, v/v) at reflux temperature gave a racemic mixture of 6-[2-benzyloxy-1-hydroxy-ethyl]-1-methylthymine (**21**). Debenzylation of **21** with hydrogen in the presence of palladium on carbon at 50 psi afforded (±) 6-[1,2-dihydroxyethyl]-1-methylthymine (**22**). Acetylation of **22** with acetic anhydride in pyridine with a catalytic amount of 4-




**SCHEME III**

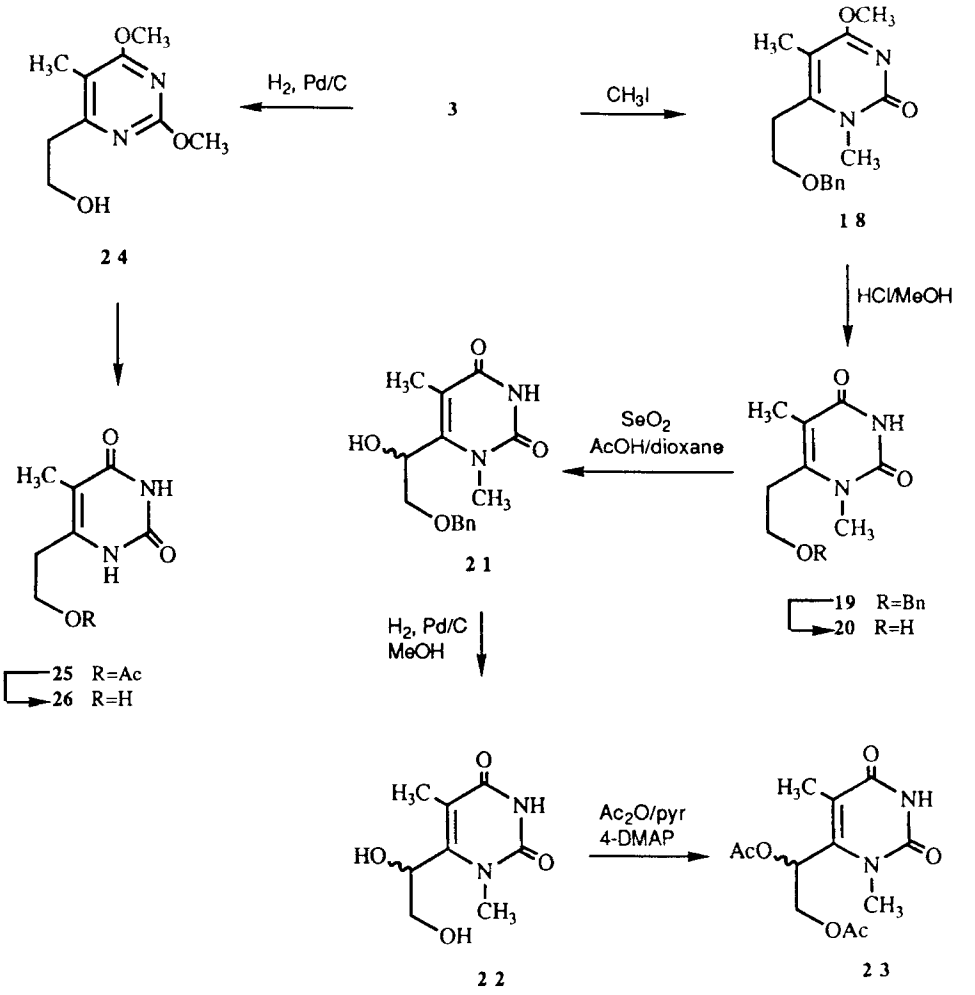



SCHEME IV

(dimethylamino)pyridine furnished **23** (Scheme V). The thymine derivative, 6-(2-hydroxyethyl)thymine (**26**) was prepared by debenzylating **3** to give **24**, and subsequently treating **24** with acetyl chloride containing a few drops of water to afford 6-(2-acetoxyethyl)thymine (**25**) which was deacetylated with ammonia to give **26**.

To prepare the target compounds **28** and **32**, a similar series of reactions was employed. The N-1 methyl derivative, 6-(3-hydroxypropyl)-1-methylthymine (**28**), was prepared by heating compound **4b**, prepared by acetylation of **4a**, at reflux temperature in neat iodomethane for 36 h to give 6-(3-acetoxypropyl)-4-methoxy-1,5-dimethylpyrimidin-2-one (**27**) in 69% yield. Subsequent deblocking and hydrolysis of the methoxy group of **27** was accomplished by heating in methanolic hydrogen chloride to furnish **28** in 51% yield. Treatment of **4a** with acetyl chloride and several drops of water effected a hydrolysis of the methoxyl group to give 6-(3-acetoxypropyl)thymine (**29**) which was deacetylated by treatment with methanolic ammonia to give **30**. Allylic oxidation of **29** using selenium dioxide in pyridine at reflux temperature gave a racemic mixture of **31**. Deacetylation of **31** was accomplished by treatment with saturated methanolic ammonia to afford (±) 6-(1,3-dihydroxypropyl)thymine (**32**) (Scheme VI).

Selected compounds from Schemes II-VI were evaluated for cytotoxicity and for activity against herpes simplex virus type 1 (HSV-1), human cytomegalovirus (HCMV), and/or human immunodeficiency virus (HIV). Compounds **7**, **8a**, **8b**, **15**, **16**, **20**, **22**,

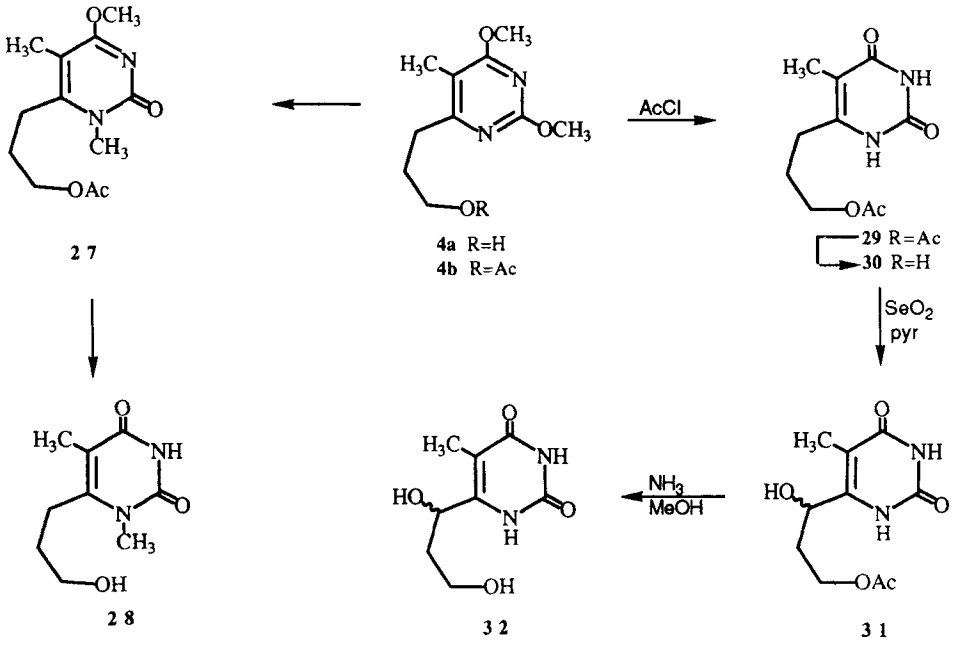



SCHEME V

**26** and **29-32** were tested against HSV-1 and HCMV but were inactive and non-toxic in human foreskin fibroblasts at concentrations as high as 100 μM. Likewise, compounds **6**, **8a**, **8b**, **11-13**, **15**, **16**, **20**, **22** and **26** were inactive against HIV up to the highest concentration tested (100 μg/mL). Neither were they cytotoxic to uninfected CEM or MT2 cells, the human lymphoid cell lines used to propagate the virus.

## EXPERIMENTAL SECTION

**General Methods.** Melting Points are uncorrected. The silica gel used for chromatography was silica gel 60 230-400 mesh (E. Merck, Darmstadt, West Germany),




**SCHEME VI**

TLC was performed on prescored SilicAR 7GF plates (Analtech, Newark, DE, USA). Compounds were visualized by illuminating under UV light (254 nm). Evaporations were carried out at < 50 °C using a rotary evaporator at reduced pressure (water aspirator). Solvent ratios reported are v/v ratios. $^1$H NMR spectra were obtained at 270 MHz. Where necessary, deuterium exchange, and homonuclear decoupling experiments were used to obtain proton shift assignments. IR spectra were recorded on a Nicolet 5 DXB FT spectrophotometer. UV spectra were obtained on a Hewlet-Packard UV 8450 spectrometer. Analytical samples were dried under reduced pressure at 78 °C in the presence of $P_2O_5$ for at least 12 hours unless otherwise specified. Elemental analyses were obtained from M-H-W Laboratories, Phoenix, AZ.

**6-(2-Benzyloxyethyl)-2,4-dimethoxy-5-methylpyrimidine (3).** Lithium diisopropylamide (LDA) (1.5 M in tetrahydrofuran (THF), 37 mL, 55 mmol) was added dropwise to a solution of 2,4-dimethoxy-5,6-dimethylpyrimidine[12] (8.41 g, 50 mmol) in anhydrous THF (200 mL) at -70 °C. The temperature was warmed to -55 °C and the solution was stirred for 30 min. Benzyl chloromethyl ether (21 mL, 0.15 mol) was added dropwise to the solution and the stirring was continued for 1 h. The solution was neutralized by the addition of acetic acid, then the temperature was raised to rt, and the

solvent was removed under reduced pressure. The residue was partitioned between $CHCl_3$ and water, and the organic layer was separated and dried over $Na_2SO_4$. After removing the drying agent, the solvent was evaporated to afford a residue. The residue was chromatographed on silica gel (600 g, 15 x 20 cm column) and eluted with n-hexane/EtOAc (6/1). The fractions ($R_f$ = 0.24, n-hexane/EtOAc, 5/1) containing product were collected and evaporated to give 13.19 g (90%) of **3** as an oily product; IR (neat): 2957, 2864, 1582, 1450, 1370, 1211, 1118, 1085 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 7.29-7.25(brs, 5 H, Ph); 4.52(s, 2 H, Ph-$CH_2$); 3.95(s, 3 H, $OCH_3$); 3.91(s, 3 H, $OCH_3$); 3.86(t, 2 H, J=7 Hz, $CH_2O$); 2.96(t, 2 H, J=7 Hz, $CH_2$-$C_6$); 2.05(s, 3 H, $CH_3$). Anal. Calcd. for $C_{16}H_{20}N_2O_3$ (288.34): C, 66.64; H, 6.99; N, 9.70. Found: C, 66.70; H, 7.02; N, 9.68.

**2,4-Dimethoxy-6-(3-hydroxypropyl)-5-methylpyrimidine (4a).** Oxirane (2 M in diethyl ether, 10 mL, 20 mmol) dissolved in diethyl ether (30 mL) was added rapidly at -70 °C to lithiated 2,4-dimethoxy-5,6-dimethylpyrimidine (1.68 g, 10 mmol) prepared by the addition of LDA (1.5 M in THF, 10 mL, 15 mmol) to 2,4-dimethoxy-5,6-dimethylpyrimidine (1.68 g, 10 mmol) in THF. The mixture was allowed to warm to rt and then hydrolyzed with saturated ammonium chloride solution. The solvent was removed under reduced pressure. The residue was partitioned between $CHCl_3$ and water. The organic layer was washed with saturated NaCl solution (3 X 15 mL) and water (3 X 15 mL) and then dried over $MgSO_4$. The solvent was removed and the residue was chromatographed on silica gel (50 g, 3 x 20 cm column) eluting with $CHCl_3$/EtOAc (9/1). The desired fractions ($R_f$ = 0.31, $CHCl_3$/EtOAc, 1/1) were collected and concentrated under reduced pressure to give an oil like product (1.21 g, 57%); IR (neat): 3376, 2957, 2871, 1583, 1477, 1463, 1377, 1211, 1111, 1058, 799 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ 3.93(s, 3 H, $OCH_3$); 3.90(s, 3 H, $OCH_3$); 3.64(t, 2 H, J=6 Hz, $CH_2O$); 3.44(brs, 1 H, OH); 2.78(t, 2 H, J=7 Hz, $CH_2$-$C_6$); 2.02 (s, 3 H, $CH_3$-$C_5$); 1.90(m, 2 H, C-$CH_2$-C). Anal. Calcd. for $C_{10}H_{16}N_2O_3$ (212.25): C, 56.58; H, 7.60; N, 13.20. Found: C, 56.57; H, 7.64; N, 13.09.

**6-(3-Acetoxypropyl)-2,4-dimethoxy-5-methylpyrimidine (4b).** To a solution of **4a** (1.90 g, 9 mmol) in pyridine (20 mL) was added acetic anhydride (4 mL, 42 mmol) and the mixture was stirred at rt for 5 h. Methanol (10 mL) was then added, and the mixture was stirred for an additional 10 min. The solution was then partitioned between a mixture of $CHCl_3$/$H_2O$ (2/1). The $CHCl_3$ layer was separated and dried over $MgSO_4$. Evaporation of the solvent afforded the product **4b** as an oil (2.19g, 96%). IR (neat): 2959, 1742, 1583, 1463, 1377, 1244, 1211, 1118, 1045, 793 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ 4.09 (t, 2 H, J=6.2 Hz, $CH_2OCO$); 3.93, 3.91 (2s, 6 H, $OCH_3$): 2.69 (d, 2

H, J=7.2 Hz, $CH_2$-$C_6$); 2.08 - 1.98 (m, 8 H, $CH_2$, $CH_3CO$, $CH_3$-$C_5$). Anal. Calcd. for $C_{12}H_{18}N_2O_4$ (254.28): C, 56.68; H, 7.13; N, 11.02. Found: C, 56.72; H, 7.13; N, 11.05.

**6-[2-Acetoxy-3-benzyloxy-2-[(benzyloxy)methyl]propyl]thymine (5a).** A solution of **2a**[11] (1.35 g, 3 mmol) in acetyl chloride (4.26 mL) containing three drops of water was stirred at rt for 3 days. The solvent was removed under reduced pressure. The residue was applied onto a silica gel column (20 g, 3 x 10 cm) and eluted with $CHCl_3$/EtOAc (9/1). The desired fractions ($R_f$ = 0.2, $CHCl_3$/EtOAc, 3/1) were concentrated to give the product, 0.41 g (33%). $^1H$ NMR ($CDCl_3$): δ 8.69(s, 1 H, NH); 8.68(s, 1 H, NH); 7.38-7.25(m, 10 H, Ph); 4.51(s, 4 H, $PhCH_2$); 3.96-3.74(m, 4 H, $OCH_2$); 3.00(s, 2 H, $CH_2$-$C_6$); 1.99(s, 3 H, $CH_3$); 1.89(s, 3 H, $CH_3CO$). Anal. Calcd. for $C_{25}H_{28}N_2O_6$ (452.49): C, 66.36; H, 6.24; N, 6.19. Found: C, 66.67; H, 6.34; N, 6.00.

**6-[2-Acetoxy-3-benzyloxy-2-[(benzyloxy)methyl]propyl]uracil (5b).** To a solution of **2b**[11] (4.95 g, 11.7 mmol) dissolved in $CHCl_3$ (15 mL) was added acetyl chloride (7.5 mL) containing three drops of water. The mixture was heated at 35 °C for 2 weeks. The reaction was quenched with crushed ice (7.25 mL) and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (400 g, 12 x 14 cm column) with $CHCl_3$/ EtOAc (9/1). The desired fractions ($R_f$ = 0.26, $CHCl_3$/EtOAc, 1/1) were concentrated to give the product, 4.24 g (83%); $^1H$ NMR ($CDCl_3$): δ 8.95, 8.80(s, 2 H, NH); 7.36-7.27(m, 10 H, Ph); 5.50(s, 1 H, H-5); 4.51(s, 4 H, $PhCH_2$); 3.83(d, 2 H, J=10 Hz, $OCH_2$); 3.73(d, 2 H, J=10 Hz, $OCH_2$); 2.94(s, 2 H, $CH_2$-$C_6$); 2.01(s, 3 H, $CH_3CO$). Anal. Calcd. for $C_{24}H_{26}N_2O_6$ (438.47): C, 65.74; H, 5.98; N, 6.40. Found: C, 65.66; H, 6.19; N, 6.28.

**6-[2-Acetoxy-3-hydroxy-2-(hydroxymethyl)propyl]thymine (6).** To a solution of **5a** (0.363 g, 0.8 mmol) in MeOH (15 mL) was added 20% Pd/C (150 mg). The solution was subjected to hydrogenolysis at 50 psi of hydrogen for 24 h. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The residue was crystallized from MeOH/$H_2O$ to give pure product, 55.1 mg (25%). Mp 210-212 °C; $R_f$: 0.32 ($CHCl_3$/EtOAc /MeOH, 5/1/1); IR (KBr): 3444, 3388, 3269, 3142, 1743, 1714, 1644, 1384, 1229, 1047 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 10.97(s, 1 H, NH); 9.81(s, 1 H, NH); 4.97(m, 2 H, OH); 3.87(s, 2 H, $OCH_2$); 3.32(s, 2 H, $OCH_2$); 2.56 (s, 2 H, $CH_2$-$C_6$); 2.00(s, 3 H, $CH_3$); 1.74(s, 3 H, $CH_3CO$). Anal. Calcd. for $C_{11}H_{16}N_2O_6$ (272.26): C, 48.52; H, 5.92; N, 10.29. Found: C, 48.69; H, 6.06; N, 10.19.

**6-[3-Benzyloxy-2-[(benzyloxy)methyl]-2-hydroxypropyl]uracil (7).** To a stirred solution of **5b** (5.18 g, 11.8 mmol) in MeOH (20 mL) was added a 1 M solution of sodium methoxide in MeOH (20 mL, 20 mmol). After stirring at rt for 20 h, the solution was neutralized with Dowex (50 W x 2) to pH 7. The resin was removed and washed with MeOH. The combined filtrate and washing were concentrated to give **7**, 3.28 g (70 %). Rf: 0.19 ($CHCl_3$/EtOAc, 1/1). Mp 143-144 °C; IR (KBr): 3395, 3276, 3022, 2917, 1743, 1652, 1328, 1124, 1103, 723, 688 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 9.32(s, 1 H, NH); 9.19(s, 1 H, NH); 7.37-7.25(m, 10 H, Ph); 5.46(s. 1 H, H-$C_5$); 4.51(s, 4 H, $PhCH_2$); 3.42(s, 4 H, $OCH_2$); 3.39(s, 1 H, OH); 2.62(s, 2 H, $CH_2$-$C_6$). Anal. Calcd. for $C_{22}H_{24}N_2O_5$ (396.43): C, 66.65; H, 6.10; N, 7.10. Found: C, 66.60; H, 6.14; N, 7.01.

**6-[2,3-(Dihydroxy)-2-(hydroxymethyl)propyl]thymine (8a).** Compound **6** (37.5 mg, 0.14 mmol) was dissolved in rt saturated methanolic ammonia and the solution was stirred for 4 h at 0 °C and evaporated to dryness. The product was collected and washed with EtOAc/MeOH (95/5) to obtain pure **8a**, 24.3 mg (75 %). Mp 209-211 °C; Rf: 0.32 ($CHCl_3$/MeOH, 3/1); IR (KBr): 3451, 3346, 3248, 3142, 3008, 2889, 2819, 1715, 1687, 1476, 1047, 780, 765 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 10.92(brs, 1 H, NH); 9.67(brs, 1 H, NH); 4.73(brs, 3 H, OH); 3.34-3.39(m, 4 H, $OCH_2$); 2.53 (s, 2 H, $CH_2$-$C_6$); 1.79(s, 3 H, $CH_3$). Anal. Calcd. for $C_9H_{14}N_2O_5$ (230.22): C, 46.95; H, 6.13; N, 12.17. Found: C, 46.65; H, 6.27; N, 12.07.

**6-[2,3-(Dihydroxy)-2-(hydroxymethyl)propyl]uracil (8b).** Compound **8b** was prepared in a manner similar to the preparation of compound **6**. Reagents: **7** (1.76 g, 4.4 mmol), MeOH (150 mL), 20% Pd/C (0.6 g). Product: 0.211 g, 22%. Mp 223-225 °C; Rf: 0.18 ($CHCl_3$/ MeOH, 4/1); IR (KBr): 3340, 3080, 1650, 1628, 1462, 1110, 1075 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 10.90(s, 1 H, NH); 10.17(s, 1 H, NH); 5.37(s, 1 H, H-$C_5$); 4.73(t, 2 H, OH); 4.49(s, 1 H, t-OH); 3.29(d, 2 H, J=11 Hz, $OCH_2$); 3.23(d, 2 H, J=11 Hz, $OCH_2$); 2.42(s, 2 H, $CH_2$-$C_6$). Anal. Calcd. for $C_8H_{12}N_2O_5$ (216.20): C, 44.44; H, 5.60; N, 12.96; Found: C, 44.42; H, 5.44; N, 13.06.

**6-[3-Benzyloxy-2-[(benzyloxy)methyl]-2-hydroxypropyl]-4-methoxy-1-methylpyrimidin-2-one (9).** A solution of **2a** (1.74 g, 4 mmol) in methyl iodide (9 mL) was heated at reflux for 14 h. The excess methyl iodide was removed under reduced pressure to give an oily product. The oil was triturated with n-hexane (25 mL). The hexane was decanted and the residue was triturated with ethyl ether (25 mL). At that time, a precipitate appeared which was collected and washed with n-hexane/EtOAc (5/1) to give 362 mg (21%) of product. Mp 130-131 °C; Rf:

0.29($CHCl_3$/EtOAc /MeOH, 5/1/1); IR (KBr): 3402, 3255, 2362, 1659, 1644, 1616, 1560, 1384, 1117, 1096, 737, 695 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 7.35-7.24(m, 10 H, Ph); 4.52(s, 4 H, Ph-$CH_2$); 3.96(s, 3 H, $OCH_3$); 3.59(s, 3 H, $NCH_3$); 3.49(d, 2 H, J=8.9 Hz, $CH_2O$); 3.40(d, 2 H, J=8.9 Hz, $CH_2O$); 3.03 (s, 2 H, $CH_2$-$C_6$); 2.71(s, 1 H, OH); 1.95(s, 3 H, $CH_3$). Anal. Calcd. for $C_{25}H_{30}N_2O_5$ (438.51): C, 68.47; H, 6.90; N, 6.39. Found: C, 68.57; H, 6.77; N, 6.35.

**6-[3-Acetoxy-2-[(acetoxy)methyl]-2-hydroxypropyl]-1-methylthymine (10).** A solution of compound **9** (0.25 g, 0.57 mmol) dissolved in glacial acetic acid (30 mL) and concentrated HCl (0.5 mL) was subjected to hydrogenolysis in a Parr apparatus at 50 psi of hydrogen in the presence of 20% Pd/C (200 mg) for 46 h. The Pd/C was removed by filtration and the filtrate was concentrated. The residue was chromatographed on silica gel (15 g, 3 x 10 cm column) using $CHCl_3$/EtOAc /MeOH (8/1.5/0.5) as the eluant. The desired fractions ($R_f$ = 0.47, $CHCl_3$/EtOAc /MeOH, 6/1/1) were collected and concentrated to give pure **10**, 104 mg (56%). Mp 202-204 °C; IR (KBr): 3459, 3156, 3023, 1743, 1722, 1687, 1666, 1265, 1223, 1047 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 11.26(s, 1 H, NH); 5.47(s, 1 H, OH); 4.01(d, 2 H, $CH_2O$); 3.88(d, 2 H, $CH_2O$); 3.38(s, 3 H, $NCH_3$); 2.93(s, 2 H, $CH_2$-$C_6$); 2.03(s, 6 H, $CH_3CO$); 1.79(s, 3 H, $CH_3$). Anal. Calcd. for $C_{14}H_{20}N_2O_7$ (328.32): C, 51.21; H, 6.14; N, 8.53. Found: C, 51.31; H, 6.16; N, 8.36

**6-[2,3-(Dihydroxy)-2-(hydroxymethyl)propyl]-1-methylthymine (11).** Compound **10** (85 mg, 0.26 mmol) was dissolved in rt saturated methanolic ammonia (30 mL). The mixture was stirred at rt for 4 h and then the solvent was removed under reduced pressure. The product was crystallized with EtOAc/MeOH to give pure **11**, 45 mg (72%). Mp 201-203 °C: $R_f$: 0.21($CHCl_3$/MeOH, 5/1); UV $l_{max}$ nm (log ε): MeOH: 277(4.24); pH 1: 276(4.24); pH 11: 219(4.66); 342(2.48); 365(2.23); IR (KBr): 3529, 3360, 3149, 1687, 1659, 1420, 1061, 1026, 709 $cm^{-1}$;. $^1H$ NMR (DMSO-$d_6$): δ 11.14(s, 1 H, NH) 4.78(t, 2 H, OH) 4.46(s, 1 H, OH), 3.38(s, 3 H, $NCH_3$), 3.29(d, 4 H , $CH_2O$) 2.82(s, 2 H, $CH_2$-$C_6$), 1.84(s, 3 H, $CH_3$). Anal. Calcd. for $C_{10}H_{16}N_2O_5$ (243.24): C, 49.38; H, 6.63; N, 11.52. Found: C, 49.26; H, 6.57; N, 11.34.

**6-[2,3-(Dihydroxy)-2-(hydroxymethyl)propyl]-2,4-dimethoxy-5-methylpyrimidine (12).** To a solution of compound **2a** (0.66 g, 1.5 mmol) dissolved in $CH_2Cl_2$ (10 mL) at -78 °C under argon was added a 1M solution of $BCl_3$ in $CH_2Cl_2$ (8 mL, 8 mmol) *via* a syringe. The mixture was stirred at -78 °C for 3h then the temperature was raised to -40 °C. A mixture of $CH_2Cl_2$/MeOH (1/1, 30 mL) was added and the cooling bath was removed. The solution was neutralized with saturated $NaHCO_3$ solution. The organic layer was separated and dried over $MgSO_4$. The solvent was removed under reduced pressure. Water (2 mL) was added to the residue and the solvent

was removed. The residue was applied to a silica column (12 g, 2.5 x 10 cm ) and eluted with $CHCl_3$/*i*-PrOH (95/5). The desired fractions ($R_f$ = 0.34, $CHCl_3$/*i*-PrOH, 9/1) were concentrated to give the product, 97 mg (25%). Mp 81-83 °C; IR (KBr): 3374, 3318, 3191, 1581, 1482, 1448, 1337, 1356, 1209, 1131, 1075, 1033, 787 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 5.33(brs, 1 H, t-OH); 3.91(s, 3 H, $OCH_3$); 3.85(s, 3 H, $OCH_3$); 3.72(brs, 2 H, OH); 3.46(s, 4 H, $OCH_2$); 2.84(s, 2 H, $CH_2$-$C_6$); 2.01(s, 3 H, $CH_3$). Anal. Calcd. for $C_{11}H_{18}N_2O_5$ (258.27): C, 51.15; H, 7.02; N, 10.85. Found: C, 51.18; H, 7.02; N, 10.77.

**6-[2,3-(Dihydroxy)-2-(hydroxymethyl)propyl]-1,5-dimethyl-4-methoxypyrimidine (13).** Compound **13** was prepared in a manner similar to the preparation of compound **12**. Reagents: **9** (0.38 g, 0.87 mmol) in $CH_2Cl_2$ (10 mL), $BCl_3$ in $CH_2Cl_2$ (1M, 5 mL, 5 mmol), $CH_2Cl_2$/MeOH (1/1, 20 mL). Purification was accomplished by chromatography on silica (12 g, 2.5 x 10 cm column) using $CHCl_3$/MeOH (9/1) as the eluting solvent. Product: 113.6 mg (51%). Mp 169-170 °C; $R_f$: 0.23 (MeOH/$CHCl_3$, 1/7); IR (neat): 3369, 3190, 1636, 1609, 1550, 1384, 1058 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 4.79(t, 2 H, OH); 4.45(s, 1 H, OH); 3.79(s, 3 H, $OCH_3$); 3.47(s, 3 H, $NCH_3$); 3.32-3.25(m, 4 H, $OCH_2$); 2.90(s, 2 H, $CH_2$-$C_6$); 1.94(s, 3 H, $CH_3$). Anal. Calcd. for $C_{11}H_{18}N_2O_5$ (258.27): C, 51.15; H, 7.02; N, 10.85. Found: C, 51.19; H, 7.12; N, 10.77.

**6-(2-Benzyloxyethyl)thymine (14).** A mixture of **3** (1.25 g, 4.3 mmol) and acetyl chloride (5.7 mL) was heated at reflux temperature for 14 h. Several drops of water were added and the mixture was stirred for an additional 0.5 h. The resulting precipitate was collected and washed with EtOAc to give the product, 0.438 g. The washings and mother liquid were combined and the mixture was concentrated under reduced pressure to provide additional product, 0.334 g (total yield: 0.772 g, 69%) of **14**. Mp 149-151 °C; $R_f$: 0.54 ($CHCl_3$/MeOH, 9/1); IR(KBr): 3227, 3163, 3093, 1715, 1638, 1455, 1117, 744 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 10.94(s, 1 H, NH); 10.56(s, 1 H, NH); 7.32-7.25(m, 5 H, Ar); 4.47(s, 2 H, $CH_2Ph$); 3.60(t, 2 H, J=6.5 Hz, $CH_2O$); 2.66(t, 2 H, $CH_2$-$C_6$); 1.73( s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_{14}H_{16}N_2O_3$ (260.29): C, 64.60; H, 6.20; N, 10.76. Found: C, 64.40; H, 6.34; N, 10.68.

**(±) 6-[2-Benzyloxy-1-(hydroxy)ethyl]thymine (15).** A mixture of **14** (0.52 g, 2 mmol) and selenium dioxide (0.28 g, 2.5 mmol) in pyridine (10 mL) was heated at reflux temperature for 24 h. The mixture was filtered through a Celite bed and the bed was washed thoroughly with $CHCl_3$ and the filtrate was evaporated to afford a red residue. The residue was coevaporated with MeOH and silica gel. This material was applied to a silica column (15 g, 3 x 10 cm) and eluted with $CHCl_3$/MeOH (95/5). The

desired fractions ($R_f$ = 0.27, $CHCl_3$/MeOH, 9/1) were collected and concentrated under reduced pressure to give the product (148 mg, 27%). Mp 148-149 °C; IR(KBr): 3360, 3100, 3001, 2826, 1729, 1652, 1194, 1124, 695 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 11.03(s, 1 H, NH); 9.94(s, 1 H, NH); 7.52-7.26(m, 5 H, Ar); 5.83(d, 1 H, OH); 4.79(t, 1 H, CH-$C_6$); 4.50(s, 2 H, $CH_2Ph$); 3.58(m, 2 H, $CH_2O$); 1.74(s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_{14}H_{16}N_2O_4$(276.29): C, 60.86; H, 5.84; N, 10.14. Found: C, 61.00; H, 6.00; N, 9.95.

(±) **6-[1,2-Dihydroxyethyl]thymine (16).** Compound **15** (0.36 g, 1.3 mmol) in MeOH (30 mL) was subjected to hydrogenolysis under 50 psi of hydrogen in the presence of 20% Pd/C (80 mg) for 12 h. The mixture was filtered through a Celite bed on a filter paper to remove the catalyst and the filtrate was concentrated under reduced pressure. The solid product was collected by filtration and washed with MeOH to give 198.5 mg (84%). Mp 216-217 °C; $R_f$: 0.33 ($CHCl_3$/MeOH, 5/1); IR(KBr): 3456, 3376, 3316, 3150, 3004, 2831, 1749, 1663, 1503, 1443, 793, 759 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 10.99(s, 1 H, NH); 9.75(s, 1 H, NH); 5.63(d, 1 H, OH); 4.90(t, 1 H, OH); 4.59(t, 1 H, J=4.6 Hz, CH-$C_6$); 3.50(d, 2 H, J=5.8 Hz, $CH_2O$) ;1.74(s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_7H_{10}N_2O_4$ (186.17): C, 45.16; H, 5.41; N, 15.05. Found: C, 45.36; H, 5.65; N, 14.89.

(±) **6-[1,2-(Diacetoxy)ethyl]thymine (17).** A solution of **16** (66.2 mg, 0.35 mmol) in acetyl chloride (2 mL) was stirred at rt for 2 h. The reaction was quenched with crushed ice and extracted with EtOAc (5 mL x 3). The organic solvent was removed and the residue was dried under reduced pressure to give 76 mg (80%) of **17**. Mp 236-238 °C. IR (KBr): 3262, 3163, 3022, 1756, 1750, 1700, 1652, 1230, 1209, 1054, 773 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 11.18(s. 1 H, NH); 10.67(s, 1 H, NH); 5.72(brs, 1 H, CH-$C_6$); 4.33(d, 2 H, J=3.5 Hz, $CH_2O$); 2.10(s, 3 H, $CH_3CO$); 2.09(s, 3 H, $CH_3CO$); 1.81(s, 3 H, Me-$C_5$). Anal. Calcd. for $C_{11}H_{14}N_2O_6$ (270.24): C, 48.89; H, 5.22; N, 10.37. Found: C, 48.87; H, 5.27; N, 10.14.

**6-(2-Benzyloxyethyl)-1,5-dimethyl-4-methoxypyrimidin-2-one (18).** A solution of **3** (4.35 g, 15 mmol) in methyl iodide (25 mL) was heated under reflux for 15 h. After evaporation, the mixture was chromatographed on silica (200 g, 12 x 14 cm) eluting with $CHCl_3$/EtOAc (3/1). The desired fractions ($R_f$ = 0.3) were collected and concentrated under high vacuum to give 3.13 g (72%) of **18** as an oily product, $R_f$: 0.3 ($CHCl_3$/EtOAc, 3/1). IR (neat): 2952, 2875, 1666, 1546, 1377, 1356, 1096, 787, 737, 702 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 7.31-7.22(m, 5 H, Ar); 4.46 (s, 2 H, $CH_2Ph$); 3.79(s, 3 H, $OCH_3$); 3.65(t, 2 H, J=6.6 Hz, $CH_2O$); 3.41 (s, 3 H, $NCH_3$); 3.02(t, 2 H, $CH_2$-$C_6$); 1.88(s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_{16}H_{20}N_2O_3$ (288.34): C, 66.64; H, 6.99; N, 9.72. Found: C, 66.54; H, 6.88; N, 9.51.

**6-(2-Benzyloxyethyl)-1-methylthymine (19).** To compound **18** (3.13 g, 11 mmol) dissolved in MeOH (20 mL) was added MeOH saturated with dry hydrogen chloride gas (20 mL) and the mixture was stirred at rt for 16 h. The solvent was removed and the residue was applied to a silica column (200 g, 12 x 14 cm) and eluted with $CH_2Cl_2$/EtOAc (1/1). The desired fractions ($R_f$ = 0.23) were collected and concentrated under reduced pressure to afford 2.19 g (72%) of **19**. Mp 125-126 °C; $R_f$: 0.23 ($CHCl_3$/EtOAc, 1/1); IR (KBr): 3170, 3030, 2861, 1701, 1666, 1483, 723 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ 9.14(s, 1 H, NH); 7.36-7.27(m, 5 H, Ar); 4.52(s, 2 H, $CH_2Ph$); 3.66(t, 2 H, J=6.7 Hz, $CH_2O$); 3.44(s, 3 H, $NCH_3$); 2.94(t, 2 H, $CH_2$-$C_6$); 1.95(s, 3 H, $CH_3$-$C_5$); Anal. Calcd. for $C_{15}H_{18}N_2O_3$ (274.31): C, 65.67; H, 6.61; N, 10.21. Found: C, 65.81; H, 6.75; N, 10.20.

**6-(2-hydroxyethyl)-1-methylthymine (20).** To a solution of **19** (0.274 g, 1 mmol) in MeOH (15 mL) was added 20% Pd/C (50 mg). The mixture was subjected to hydrogenolysis at 50 psi of hydrogen for 24 h. The catalyst was removed by filtration and the filtrate evaporated to give a solid residue. The residue was recrystallized from MeOH to give 1.2 mg (85%) of **20**; mp 237-238 °C. IR (KBr): 3416, 3163, 3022, 1694, 1652, 1476, 1412, 1054, 878, 730 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 11.20 (s, 1 H, NH); 4.96 (t, 1 H, OH); 3.59 (q, 2 H, $CH_2O$); 3.30 (s, 3 H, $NCH_3$); 2.78 (t, 2 H, $CH_2$-$C_6$); 1.82 (s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_8H_{12}N_2O_3$ (184.2): C, 52.16; H, 6.57; N, 15.21. Found: C, 52.27; H, 6.62; N, 15.41.

**(±) 6-[2-Benzyloxy-1-hydroxyethyl]-1-methylthymine (21).** A suspension of **19** (0.29 g, 1.1 mmol) and selenium dioxide (0.35 g, 3.17 mmol) in dioxane/acetic acid (11/1, v/v, 5 mL) was heated at reflux temperature for 1 day. The mixture was filtered through a filter paper covered with a Celite pad and the pad was washed thoroughly with EtOAc. The filtrate was evaporated to give a red residue. Methanol was added to the residue and the mixture was coevaporated with MeOH and silica gel. The material was applied to a silica column (20 g, 3 x 10 cm) and eluted with benzene/EtOAc (1/1). The desired fractions ($R_f$ = 0.14) were collected and concentrated under reduced pressure to give the product (121.3 mg, 39%). Mp 147-148 °C; $R_f$: 0.14 (benzene/EtOAc, 1/1); IR(KBr): 3388, 3149, 3008, 1694, 1668, 1419, 1103, 1075, 744, 716 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 11.33(s, 1 H , NH); 7.32-7.26(m, 5 H, Ar); 6.09(m, 1 H, OH); 5.13(m, 1 H, CH-$C_6$); 4.51(m, 2 H, $CH_2Ph$); 3.70(m, 2 H, $CH_2O$); 3.36-3.32(brs, 3 H, $NCH_3$); 1.87-1.84(brs, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_{15}H_{18}N_2O_4 \cdot 3/4H_2O$ (303.81): C, 59.30; H, 5.97; N, 9.20. Found: C, 59.42; H, 6.03; N, 9.08.

**(±) 6-[1,2-Dihydroxyethyl]-1-methylthymine (22).** A solution of compound **21** (164.2 mg, 0.54 mmol) dissolved in AcOH (15 mL) and HCl (conc.) (0.3

mL) was subjected to hydrogenolysis at 50 psi of hydrogen in the presence of 20% Pd/C (100 mg) for 24 h. The reaction mixture was passed through a Celite bed to remove the catalyst and the filtrate was concentrated to dryness. Methanolic ammonia (10 mL) was added to the residue and the solution was stirred at rt for 14 h. After evaporation, the solid product was collected to give 80 mg (72%) of **22**. Mp 201-203 °C; $R_f$: 0.33 ($CHCl_3$/MeOH, 5/1); IR(KBr): 3349, 3170, 3017, 1696, 1649, 1483, 1417, 1071, 865, 753 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 11.29(s, 1 H, NH); 5.92(d, 1 H, OH); 5.02(t, 1 H, OH); 4.93(m, 1 H, J=4.6 Hz, CH-$C_6$); 3.63(m, 2 H, $CH_2O$); 3.34(s, 3 H, $NCH_3$); 1.89(s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_8H_{12}N_2O_4$ (200.2): C, 47.99; H, 6.04; N, 14.00. Found: C, 48.04; H, 6.04; N, 14.05.

(±) **6-[1,2-Diacetoxyethyl]-1-methylthymine (23).** To a solution of compound **22** (70 mg, 0.29 mmol) dissolved in acetic acid (3 mL) was added acetyl chloride (1 mL) , and the mixture was stirred at rt for 1 h. At that time additional acetyl chloride (1 mL) in chloroform (10 mL) was added to the reaction, and the mixture was stirred for an additional 18 h. The reaction mixture was absorbed onto a small amount of silica gel by coevaporation and then applied to an open-bed silica gel column (12 g, 2.5 x 10 cm). The column was eluted with CHCl3/MeOH (95/5). The desired fractions ($R_f$ = 0.23, CHCl3/EtOAc, 1/1) were collected and evaporated under reduced pressure to give the product (50 mg, 63%). Mp 116-118 °C; IR (KBr): 3170, 3037, 1750, 1701, 1659, 1223, 1054, 871, 751 $cm^{-1}$. $^1H$ NMR (CDCl3): δ 8.95(s, 1 H, NH); 6.12(t, 1 H, J=6 Hz, CH-$C_6$); 4.42(d, 2 H, J=6 Hz, $CH_2O$); 3.57(s, 3 H, $NCH_3$); 2.14 (s, 3 H, Me-$C_5$); 2.08(s, 6 H, $CH_3CO$). Anal. Calcd. for $C_{12}H_{16}N_2O_6$ (284.27): C, 50.79; H, 5.67; N, 9.85. Found: C, 50.74; H, 5.53; N, 10.04.

**2,4-Dimethoxy-6-(2-hydroxyethyl)-5-methylpyrimidine (24).** A solution of compound **3** (3.16 g, 11 mmol) in MeOH (50 mL) was subjected to hydrogenolysis at 50 psi of hydrogen in the presence of 20% Pd/C (0.8 g) for 36 h. After removing the catalyst by filtration, the solution was concentrated under reduced pressure to a residue. The residue was applied to a silica column (12 g, 2.5 x 10 cm) and eluted with CHCl3/MeOH (9/1). The fractions containing product were pooled and evaporated to afford pure **24** (2.1g, 95%) as a hard glass. IR (KBr): 3668, 2957, 2871, 1583, 1437, 1390, 1211, 1125, 739, 699 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 4.62 (t, 1 H, OH); 3.87, 3.82 (s, 6 H, $OCH_3$); 3.69 (g, 2 H, $CH_2O$); 2.75 (t, 2 H, $CH_2$-$C_6$); 2.00 (s, 3 H, $CH_3$). Anal. Calcd. for $C_9H_{14}N_2O_3$ (198.22): C, 54.53; H, 7.12; N, 14.14. Found: C, 54.30; H, 7.17, N, 13.95.

**6-(2-Acetoxyethyl)thymine (25).** A mixture of **24** (0.4 g, 2 mmol) in acetyl chloride (2.5 mL) was stirred at rt for 35 h. The excess acetyl chloride was evaporated

and the resulting residue was triturated with EtOAc (10 mL) and recrystallized from MeOH to give **25** (99 mg, 23%), mp 219-221 °C. IR (KBr): 3227, 3135, 3001, 1750, 1721, 1659, 1419, 1230, 765 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 10.98, 10.63 (s, 2 H, NH); 4.19 (t, 2 H, $CH_2O$); 2.67 (t, 2 H, $CH_2$-$C_6$); 1.97 (s, 3 H, $CH_3$-$C_5$); 1.74 (s, 3 H, $CH_3CO$). Anal. Calcd. for $C_9H_{12}N_2O_4$ (212.21): C, 50.94; H, 5.70; N, 13.20. Found: C, 50.76; H, 5.77; N, 12.96.

**6-(2-Hydroxyethyl)thymine (26).** A solution of **25** (74 mg, 0.35 mmol) in 0 °C saturated methanolic ammonia (20 mL) was stirred for 8 h at 0 °C. The solvent was evaporated and the resulting solid was recrystallized from MeOH to give pure **26** (16.6 mg, 28%), mp 240-241 °C. IR (KBr): 3374, 3226, 3121, 2994, 2952, 2805, 1708, 1688, 1420, 1166, 1054, 857, 765, 625 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 10.92, 10.50 (s, 2 H, NH); 4.81 (s, 1 H, OH); 3.57 (t, 2 H, $CH_2O$); 2.51 (t, 2 H, $CH_2$-$C_6$); 1.73 (s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_7H_{10}N_2O_3$ (170.17): C, 49.40; H, 5.92; N, 16.47. Found: C, 49.49; H, 6.02; N, 16.35.

**6-(3-Acetoxypropyl)-1,5-dimethyl-4-methoxypyrimidin-2-one (27).** A solution of **4** (1.7 g, 6.6 mmol) in methyl iodide (10 mL) was heated under reflux for 36 h. After evaporation of the excess methyl iodide, the resulting residue was dissolved in EtOAc (~15 mL). Upon cooling to -78 °C the product crystallized and was collected by filtration (1.15 g, 69%). Mp 102-103 °C; IR (KBr) 3003, 1742, 1602, 1244, 1045, 786 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ 4.11 (t, 1 H, J=6 H, $CH_2OCO$); 3.91 (s, 3 H, $OCH_3$); 3.50 (s, 3 H, N-$CH_3$); 2.70 (m, 2 H, $CH_2$-$C_6$); 2.05 (s, 3 H, $CH_3CO$); 1.93 (s, 3 H, $CH_3$-$C_5$); 1.83 (m, 2 H, $CH_2O$). Anal. Calcd. for $C_{12}H_{18}N_2O_4$ (254.28): C, 56.68; H, 7.13; N, 11.02. Found: C, 56.50; N, 7.32; H, 10.85.

**6-(3-Hydroxypropyl)-1-methylthymine (28).** Compound **27** (0.26 g, 1 mmol) was dissolved in MeOH (5 mL), which was previously saturated with HCl gas at rt, and stirred at rt for 18 h. The solvent was evaporated and the resulting solid was eluted through a short silica column (2.5 x 3 cm) with $CHCl_3$/MeOH (4/1). The appropriate fractions were evaporated and the resulting solid was recrystallized from an EtOAc/MeOH solvent mixture to give 101 mg (51%). Mp 186-187 °C; $^1H$ NMR (DMSO-$d_6$): δ 11.19 (s, 1 H, NH); 4.7 (s, 1 H, OH); 3.47 (t, 2 H, $CH_2O$); 3.28 (s, 3 H, N-$CH_3$); 2.61 (t, 2 H, $CH_2$-$C_6$); 1.81 (s, 3 H, $CH_3$-$C_5$); 1.60 (m, 2 H, $CH_2$). Anal. Calcd. for $C_9H_{14}N_2O_3$ (198.22): C, 54.53; H, 7.12; N, 14.14. Found: C, 54.64; H, 6.96; N, 13.94.

**6-(3-Acetoxypropyl)thymine (29).** A solution of **4** (2.45 g, 11.5 mmol) in acetyl chloride (5 mL) was stirred at rt until a precipitate appeared (about 17 h). The reaction mixture was added to crushed ice (25 mL) and MeOH (5 mL) and then stirred at rt

for 5 min. The product was collected by filtration and washed with MeOH. The compound was recrystallized from MeOH to yield 1.62 g (62%) of product. Mp 170-171 °C; Rf: 0.22 ($CH_2Cl_2$/MeOH, 20/1); IR (KBr): 3223, 3163, 3130, 3023, 2798, 1735, 1722, 1656, 1244, 1045, 872, 759 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 10.95(s, 1 H, NH); 10.61(s, 1 H, NH); 3.98(t, 2 H, J=6.2 Hz, $CH_2O$); 2.40(d, 2 H, J=7.1 Hz, $CH_2$-$C_6$); 1.98(s, 3 H, $CH_3CO$); 1.82(m, 2 H, $CH_2$); 1.71(s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_{10}H_{14}N_2O_4$ (226.23): C, 53.09; H, 6.24; N, 12.39. Found: C, 53.01; H, 6.29; N, 12.42.

**6-(3-Hydroxypropyl)thymine (30).** A solution of **29** (0.23g, 1 mmol) dissolved in 0 °C saturated methanolic ammonia (10 mL) was stirred at rt in a sealed vessel for 18h. The solvent was removed and the resulting residue was dissolved in MeOH (25 mL) and co-evaporated with silica gel (3g). This material was applied to the top of a silica column (2.5 x 10 cm) and eluted with $CHCl_3$/MeOH (14/1). The fractions containing product were pooled and evaporated. The resulting residue was crystallized from MeOH (116 mg, 63%). Mp 178-179 °C; IR(KBr): 3437, 3220, 3100, 2945, 2819, 1701, 1652, 1462, 1435, $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 10.91, 10.54 (s, 2 H, NH); 4.57 (s, 1 H, OH); 3.37 (t, 2 H, $CH_2O$); 2.36 (t, 2 H, $CH_2$-$C_6$); 1.71 (s, 3 H, $CH_3$-$C_5$); 1.60 (m, 2 H, $CH_2$). Anal. Calcd. for $C_8H_{12}N_2O_3$ (184.3): C, 52.16; H, 6.57; N, 15.21. Found: C, 52.32; H, 6.45; N, 15.19.

**(±) 6-(3-Acetoxy-1-hydroxypropyl)thymine (31).** A suspension of **29** (0.23 g, 1 mmol), selenium dioxide (0.2 g, 1.8 mmol) in pyridine (5 mL) was heated at reflux temperature for 10 h. The selenium was removed by filtration through a Celite bed and the filtrate was concentrated under reduced pressure. The residue was absorbed on silica gel and applied to a silica column (20 g, 3 x 10 cm) and eluted with $CH_2Cl_2$/MeOH (95/5). The desired fractions ($R_f$ = 0.13, $CH_2Cl_2$/MeOH, 20/1) were collected and concentrated under reduced pressure to give the product (102 mg, 42%). Mp 168-169 °C; IR (KBr): 3449, 3250, 3150, 3090, 1742, 1702, 1636, 1370, 1271, 1065, 832, 766 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 11.01(s, 1 H, NH); 10.02(s, 1 H, NH); 5.71(s, 1 H, OH); 4.68(brs, 1 H, CH-$C_6$); 4.05(m, 2 H, $CH_2O$); 1.97(s, 3 H, $CH_3CO$); 1.94-1.83(m, 2 H, $CH_2$); 1.73(s, 3 H, $CH_3$-$C_5$). Anal. Calcd. for $C_{10}H_{14}N_2O_5$ (242.23): C, 49.58; H, 5.83; N, 11.57. Found: C, 49.73; H, 5.84; N, 11.46.

**(±) 6-(1,3-Dihydroxypropyl)thymine (32).** A solution of **31** (242 mg, 1 mmol) dissolved in 0 °C saturated methanolic ammonia (15 mL) was stirred in a sealed vessel for 14 h. The solvent was removed and EtOAc was added to precipitate the product (147 mg, 73%). Mp 202-204 °C; Rf: 0.16 ($CHCl_3$/MeOH, 9/1); IR (KBr): 3329, 3183, 3103, 3030, 1716, 1656, 1503, 1403, 1198, 1065, 859, 779 $cm^{-1}$. $^1H$ NMR (DMSO-

d6): δ 10.97(s, 1 H, NH); 9.94(s, 1 H, NH); 5.48(s, 1 H, OH); 4.74(m, 1 H, CH-C6); 4.55(s, 1 H, OH); 3.47(m, 2 H, $CH_2O$); 1.73(s, 3 H, $CH_3$-$C_5$); 1.67-1.64 (m, 2 H, $CH_2$). Anal. Calcd. for $C_8H_{12}N_2O_4$ (200.2): C, 47.99; H, 6.04; N, 14.00. Found: C, 48.19; H, 6.13; N, 14.12.

***In Vitro* Antiviral Evaluation. (a) Cells and Viruses.** Diploid human foreskin fibroblasts (HFF cells) were grown in minimal essential medium (MEM) with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. BSC-1 (African green monkey kidney) cells were grown and passaged in MEM(E) supplemented with 10% calf serum. Cells were passaged according to conventional procedures as detailed previously.[13] A plaque-purified isolate, $P_O$, of the Towne strain of HCMV was used and was a gift of Dr. M. F. Stinski, University of Iowa. The S-148 strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corporation. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[13] The HTLV-$III_B$ strain of HIV-1 was propagated in the human T-lymphocyte cell line, H9 as detailed elsewhere.[14] The virus inoculum consisted of supernatant fluids from H9-$III_B$ producer cultures.

**(b) Antiviral Assays for Herpesviruses.** HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above[13] for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. HSV-1 was grown in BSC-1 cells and was assayed using an enzyme immunoassay described by Prichard and Shipman.[15]

**(c) Antiviral Assay for HIV.** HIV was assayed using the XTT colorimetric technique as detailed previously.[16-18] Briefly, drugs were dissolved in DMSO or in sterile deionized water and diluted in culture medium using log or 0.5 log series dilutions. Each dilution was added to 96-well culture plates and tested in triplicate wells per dilution with infected cells, and in duplicate wells per dilution with uninfected cells for evaluation of cytotoxicity. The highest drug concentration was 100 μg/mL.

The desired total number of cells was placed in a 50 mL conical centrifuge tube and virus was added to give a multiplicity of infection of 0.03 $TCID_{50}$/cell in MT-2 cells and approximately 0.12 $TCID_{50}$/cell in CEM cells. Fresh medium was added to adjust the cell density to 1 x $10^5$ cells/mL, and the virus-cell suspension was incubated at 37 °C for 1-2 hrs until ready for plating. Uninfected cells were prepared in the same manner but without the addition of virus. Cell pellets were collected by low speed centrifugation and supernatants were discarded. Infected and uninfected cells were resuspended in an appropriate volume of medium and added to plates in the amount of 100 μL/well to give a starting cell number of 1 x $10^4$ cells/well. Plates were incubated for 7 days in a humidified

atmosphere of 5% $CO_2$ in air. On day 7 post-infection, viable cells were measured by addition of a tetrazolium salt (XTT) to the test plates. The resulting XTT formazan was dissolved and the optical density was determined at a wave-length of 570 nm on a plate reader.

**(d) Cytotoxicity Assays.** Two basic tests for cellular toxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Drug-induced cytopathology was estimated at 30-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration.[13] Cytotoxicity in CEM and MT2 cells was determined colorimetrically using the XTT assay[16] as described above for HIV except uninfected cells were used.

**(e) Data Analysis.** Dose-response relationships were constructed by linear regression analysis of the percent inhibition of parameters derived in the preceding sections against log of drug concentration. Fifty-percent inhibitory ($IC_{50}$) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, zidovudine for HIV) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

**Molecular Modeling.** Modeling was performed on a Silicon Graphics Personal IRIS using the molecular similarities program within Polygen's Quanta molecular modeling package.



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