Synthesis of Anti-Restricted Pyrimidine Acyclic Nucleosides

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A number of pyrimidine acyclic nucleosides which are constrained in the anti conformation have been prepared via treatment of 5,6-dimethyl-2,4-dimethoxypyrimidine or 6-methyl-2,4-dimethoxypyrimidine with 1,3-bis(benzyloxy)-2-propanone (3) to give 6-[[2-[1,3-bis(benzyloxy)-2-hydroxypropyl]]methyl]-2,4-dimethoxy-5-methylpyrimidine (**5a**) and **5b**, respectively. Using acetic anhydride in DMSO, these compounds were converted to a 2-[[(methylthio)methyl]oxy] intermediate which was annulated to afford 2,2-bis[(benzyloxy)methyl]-8-methoxy-9-methyl-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (**7a**) and **7b**, respectively, by using iodine in THF. Nucleophilic replacements at the 8-position and deblocking of **7a** and **7b** furnished the target compounds, 2,2-bis(hydroxymethyl)-9-methyl-(1*H*,4*H*,7*H*)-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (**9a**) and **9b**, and the cytidine derivatives, 8-amino-2,2-bis(hydroxymethyl)-9-methyl-1*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (**12a**) and **12b**. Compounds **8b**, **9a**, **9b**, **10b**, **11b**, **12a**, and **12b** were evaluated for activity against herpes viruses and human immunodeficiency virus (HIV). Compound **12a** was slightly active against HIV at noncytotoxic concentrations. All other compounds were inactive at the highest concentration tested (100 μM).

Introduction

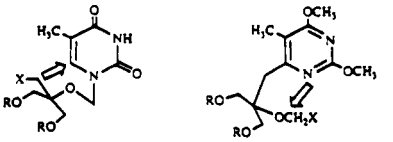
A relatively large number of C-cyclonucleosides which are bridged by a carbon linkage are known and have been used as both syn and anti fixed models in conformational studies of nucleosides and nucleotides.[1,2] Recently, the synthesis of a large number of acyclo nucleosides have been reported[3] due to the clinical success of acyclovir (ACV) in the treatment of herpes simplex virus (HSV) infections. ACV manifests its selective antiviral action in two ways: (1) it is a substrate for a viral-encoded thymidine kinase (TK) but 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. In contrast to these observations, pyrimidine acyclic nucleosides, such as acyclic thymidine, which are structurally more closely related to the natural substrate thymidine, are surprisingly poor substrates[7] for viral TK. Although it has not been demonstrated, these acyclic pyrimidine compounds, unlike acyclic purines, may exist in a constrained syn conformation stabilized by an intramolecular H-bonding between the hydrogen atom of the hydroxyl group of the aliphatic side chain and the oxygen atom at C2 of the pyrimidine base. This may account for the observed reduction in the phosphorylation rate of acyclic pyrimidine analogs and their lack of antiviral activity.

In support of the contention that the anti conformation is essential for binding to HSV-TK, X-ray analysis of acyclovir has revealed that the glycosidic torsion angle (X_{CN} = O4′–C1′–N9–C8) is in the range of 91.4–104.3°, which corresponds to the high anti conformation.[8,9] In addition, if a bulky group is present at position 8, i.e., 8-bromoacyclovir, the predominant conformation is syn.[10] 8-Bromoacyclovir was phosphorylated at only one-third of the rate of acyclovir by HSV-TK and was also 50% effective in inhibiting phosphorylation of [^{14}C]acyclovir.[7] Thus, one may hypothesize that in order for a nucleoside to be a good substrate for HSV-TK, and be active against herpes viruses, it must exist in the anti conformation rather than in the syn conformation.

We have used this hypothesis as rationale to prepare a series of conformationally restricted acyclic pyrimidine nucleosides fixing the base-sugar orientation in the anti conformation by forming a carbon bridge between the two parts of the molecule. By eliminating constraint in the syn conformation, these new compounds could be substrates for HSV or other viral kinases and thereby be active antiviral drugs.

Results and Discussion

To prepare the target anti restricted pyrimidine acyclic nucleosides, the determinative step in the synthesis was considered to be the timing in the synthetic scheme for the formation of the pyrimidine C-6 linkage to the acyclic moiety. One approach (method I) would start with the



N_1-acyclic nucleoside and have the critical pyrimidine C-6 bond formation at a near terminal step while the second approach (method II) would be antipodal to method I and have the C-6 bond connection early in the synthetic sequence and the formation of the N_1-side chain bond at the near terminal step. Since method I would involve a considerable number of protecting-group manipulations, a

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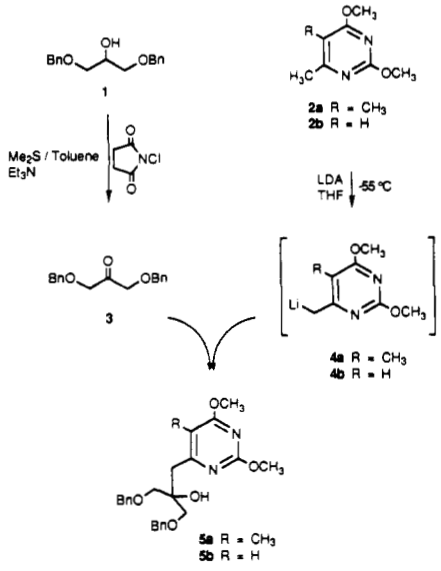
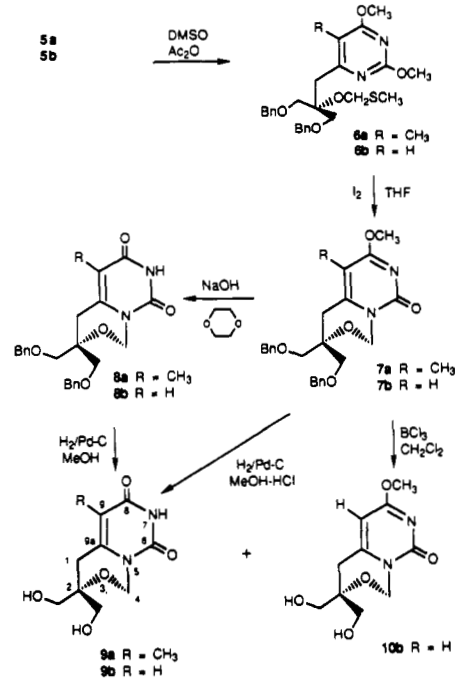
Scheme I



Scheme II



free-radical cyclization, and require a regeneration of the 5,6-double bond as a result of the addition reaction we chose to pursue the second approach.

To prepare the pyrimidine intermediates **5a** and **5b** the C-6 side chain was connected via an addition reaction of the lithiated 6-methylpyrimidine derivatives **2a** and **2b** with 1,3-bis(benzyloxy)-2-propanone (**3**). The protected pyrimidine derivatives **2a** and **2b** were synthesized from 5,6-dimethyluracil and 6-methyluracil by a known method.[11] The preparation of the acyclic keto sugar 1,3-bis(benzyloxy)-2-propanone (**3**)[12] (Scheme I) was accomplished by an oxidation of 1,3-bis(benzyloxy)-2-propanol (**1**) using the method of Corey.[13] In the literature,[12] compound **3** was purified by distillation in a kugelrohr apparatus under high vacuum to afford **3** as an oil-like product. However, we found that by cooling the concentrated reaction mixture to a temperature below −72 °C the high-vacuum distillation could be avoided and pure **3** could be isolated in a 49% yield as a solid crystalline product with a melting point of 39–40 °C. To our knowledge, this is the first report of **3** as a crystalline material.

Treatment of the 2,4-dimethoxypyrimidine bases **2a** and **2b** with LDA in tetrahydrofuran at −55 °C afforded the intermediate lithio derivatives **4a** and **4b**. These lithio derivatives, without isolation, were reacted with **3** at −55 °C to give **5a** and **5b** (Scheme I) in 60% and 78% yields, respectively. To introduce the C-1 carbon of the acyclic moiety we first attempted chloromethylation of the tertiary hydroxyl group of **5a** by treatment with paraformaldehyde and hydrogen chloride gas to prepare a reactive chloromethyl ether intermediate. However, this reaction was unsuccessful, and only starting material was recovered. However, the tertiary hydroxyl group of the side-chain group was smoothly converted to the stable **6a** and **6b** derivatives in 90% and 83% yield, respectively. This was accomplished by treating **5a** and **5b** with a mixture of acetic anhydride and anhydrous dimethyl sulfoxide at room temperature[14] for 36 h and 4 days, respectively (Scheme II). Evidence for the introduction of a (methylthio)methyl group was established by the existence of sharp singlet peaks in the ^{1}H NMR spectra corresponding to the methylthio protons and the methylene protons between the sulfur and oxygen atoms at δ 2.13 and 4.87, respectively.

Ring closure of **6a** and **6b** to the bicyclic systems **7a** and **7b** was smoothly accomplished with iodine in dry tetrahydrofuran at room temperature. This reaction most likely occurs through an initial electrophilic activation of the sulfur atom of the (methylthio)methyl group by the iodine to generate a reactive sulfonium species which promotes the nucleophilic attack at the methylene position by the N_1 nitrogen of the pyrimidine ring. Compounds **7a** and **7b** were obtained by this reaction in 67% and 54% yields, respectively. The structures of **7a** and **7b** were established by spectroscopic studies. In the ^{1}H NMR spectra, the resonances which were previously assigned to the methylthio group were no longer apparent. The peaks of the $-OCH_2N-$ methylene group were shifted downfield from δ 4.87 ppm (OCH_2S) observed in **6a** and **6b** to 5.52 ppm in **7a** and **7b**. Treatment of **7a** and **7b** with sodium hydroxide in dioxane at reflux for 14 h effected a smooth displacement of the 4-methoxy group to give **8a** and **8b**, in 82% and 80%, respectively. Evidence for the removal of the methoxy group was confirmed by the absence of the resonances in the ^{1}H NMR spectra for the methoxy groups (**7a**: δ, 3.95 ppm; **7b**: δ, 3.93 ppm). The methoxy group could also be displaced under acidic conditions by using methanolic HCl at room temperature for 20 h; however, the yield was somewhat reduced (74%). Debenzylation of **8a** was achieved by hydrogenation in the presence of 20% palladium on carbon in methanol at 50 psi of hydrogen pressure for 24 h to provide the target compound, **9a**. Since the methoxyl group of **7a** was susceptible to hydrolysis under acidic conditions, compound **9a** could also be synthesized in a one-pot reaction by treatment of **7a** in a solution of methanolic HCl with hydrogen at 50 psi in the presence of 20% palladium on carbon. This procedure furnished **9a** in an 87% yield. If the debenzylation of **7b** was carried out with boron trichloride[15] in dichloromethane at −72 °C, two products were obtained, **10b**

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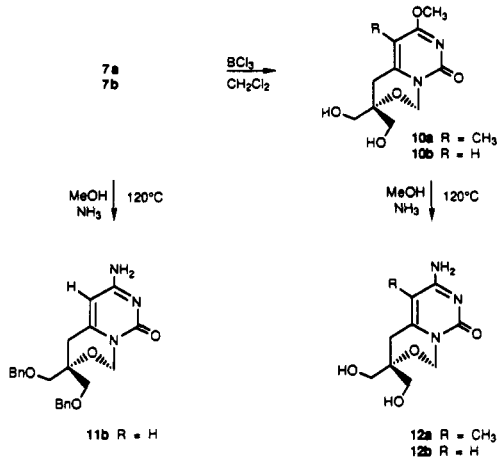
Scheme III



and the hydrolyzed product **9b**. Compound **10b** was found to be the major product.

The cytidine derivative **12a** (Scheme III) was obtained by a two-step conversion starting with debenzylation of **7a** with boron trichloride, followed by a displacement of the 8-methoxy group using saturated methanolic ammonia at room temperature. Treatment of **7b** and **10b** with saturated methanolic ammonia gave the amino derivatives **11b** and **12b**, respectively.

Target compounds **9a**, **9b**, **10b**, **11b**, **12a**, **12b**, and intermediate compound **8b** were evaluated for activity against two herpes viruses which selectively phosphorylate fraudulent nucleosides and in which acyclovir is active; namely herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). None of the compounds were active against either HSV-1 or HCMV at the highest concentration tested (100 μM). Neither were they cytotoxic to human foreskin fibroblasts (HFF cells) at this concentration. Compounds **9a**, **10b**, **11b**, **12a**, and **12b** also were tested against human immunodeficiency virus (HIV). All of these compounds except **12a** were inactive at 100 μM; compound **12a** had slight activity against HIV (IC_{50} = 36 μM) and was not cytotoxic to a human T-cell line (CEM-SS cells) at 100 μM. The lack of antiviral activity in these examples of restricted acyclic nucleosides was not totally unexpected since molecular modeling indicated that the spatial position of the "5"-hydroxymethyl group does not closely superimpose onto thymidine. Other derivatives, as directed by molecular modeling, are currently being prepared.

In summary, a synthetic strategy has been developed which successfully affords the first examples of a conformationally restricted acyclic pyrimidine nucleoside. This method is adaptable and provides a rapid entry into the formation of a variety of conformationally restricted nucleoside derivatives.

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). TLC was performed on prescored SilicAR 7GF plates (Analtech, Newark, DE). Compounds were visualized by illuminating under UV light (254 nm) and/or by spraying with 20% methanolic H_2SO_4 followed by heating (charring). Evaporations were carried out at >50 °C using a rotary evaporator at reduced pressure (water aspirator). ^1H NMR spectra were obtained at 270 MHz. ^{13}C NMR spectra were obtained at either 90 or 75 MHz. Where necessary, deuterium exchange and homonuclear decoupling experiments were used to obtain proton shift assignments. Analytical samples were dried in vacuo at 78 °C in the presence of P_2O_5 for at least 12 h unless otherwise specified.

1,3-Bis(benzyloxy)-2-propanone (3).[12] *N*-Chlorosuccinimide (17.2 g, 128 mmol) was suspended in toluene (200 mL), and the mixture was cooled in an ice bath. Dimethyl sulfide (14 mL, 190 mmol) was added, and the mixture was cooled to −25 °C in a dry ice–CCl_4 bath. 1,3-Bis(benzyloxy)-2-propanol (24 g, 88 mmol) in toluene (20 mL) was added to the mixture, and the mixture was kept under argon for 3 h. Triethylamine (90 mL, 1.052 mol) was added, and the cooling bath was removed and the reaction was allowed to warm to rt. After 25 min of stirring at rt the solution was passed through a filter paper. The residue was washed with ethyl ether (200 mL). The filtrate and washings were combined, neutralized with 5% aqueous HCl to pH 7, washed with saturated NaCl solution (50 mL × 3) and then with water (50 mL × 3), and finally dried over Na_2SO_4. The solvent was removed in vacuo, and the resulting oil was dissolved in a mixture of *n*-hexane/AcOEt = 5/1 (50 mL). The mixture was cooled to −70 °C and allowed to stand 30 min. The precipitate was collected by filtration washed with cold *n*-hexane and dried under a stream of Ar, 11.57 g (49%): mp 39–40 °C; R_f 0.32 (*n*-hexane/AcOEt = 5/1); IR (KBr) 3090–3037, 1743, 1138, 1019 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.23 (brs, 10 H, Ph), 4.46 (s, 4 H, CH_2Ph), 4.13 (s, 4 H, CH_2O). Anal. Calcd for $C_{17}H_{18}O_3$ (270.35): C, 75.52; H, 6.71. Found: C, 75.32; H, 6.54.

6-[[2-[1,3-Bis(benzyloxy)-2-hydroxypropyl]]methyl]-2,4-dimethoxy-5-methylpyrimidine (5a). LDA (1.5 M, 5 mL, 7.5 mmol) was added dropwise to a solution of **2a**[11] (0.843 g, 5 mmol) in THF (10 mL) at −70 °C. The temperature was warmed to −55 °C, and the solution was stirred for 30 min. Compound **3** (1.35 g, 5 mmol) in THF (7.5 mL) was added dropwise to the solution, and the stirring was continued for 2.5 h while the temperature was maintained at −55 °C. The solution was neutralized by the addition of AcOH to pH 7 and then the temperature was warmed to 25 °C, and the solvent removed. The residue was partitioned between AcOEt and H_2O, the organic layer was separated and dried over Na_2SO_4, and the solvent was removed. The residue was chromatographed on silica gel (100 g, 7- × 12-cm column) eluting with *n*-hexane/AcOEt = 5/1. The desired fractions (R_f = 0.36) were concentrated in vacuo to give **5a**: 1.25 g (60%); R_f 0.36 (*n*-hexane/AcOEt = 5/1); IR (neat) 3353, 3093–2861 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.28 (brs, 10 H, Ph), 5.09 (s, 1 H, OH), 4.48 (s, 4 H, $PhCH_2$), 3.87 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 3.44 (s, 4 H, OCH_2), 2.83 (s, 2 H, CH_2), 1.99 (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, 68.23; H, 6.99; N, 6.10.

6-[[2-[1,3-Bis(benzyloxy)-2-hydroxypropyl]]methyl]-2,4-dimethoxypyrimidine (5b). Compound **5b** was prepared in a manner similar to the preparation used to synthesize compound **5a**. Reagents: *n*-Butyllithium (1.6 M, 70 mL, 0.12 mol), compound **2b**[11] (15.4 g, 0.1 mol) in THF (200 mL), and compound **3** (32.44 g, 0.12 mol) in THF (10 mL). Purification of **5b** was accomplished by chromatography on silica gel (1 kg, 15- × 20-cm column) using *n*-hexane/EtOAc (9/1) as the eluting solvent. Evaporation of the fractions containing the product afforded pure **5b** as a solid foam: yield 33.1 g (78%); R_f 0.33 (*n*-hexane/AcOEt = 2/1); IR (neat) 3550, 3381, 3030, 2952, 2868, 1736, 1588, 1469, 1370, 1202, 1103, 738, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32–7.25 (brs, 10 H, Ph), 6.25 (s, 1 H, H-5), 5.29 (s, 1 H, OH), 4.51 (s, 4 H, $PhCH_2$), 3.94 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.50 (d, 2 H, J = 9.5 Hz, CH_2O), 3.38 (d, 2 H, J = 9.5 Hz, CH_2O), 2.91 (s, 2 H, CH_2). Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.48): C, 67.90; H, 6.65; N, 6.60. Found: C, 67.78; H, 6.51; N, 6.42.

6-[[2-[1,3-Bis(benzyloxy)-2-[[(methylthio)methyl]oxy]-propyl]]methyl]-2,4-dimethoxy-5-methylpyrimidine (6a). Acetic anhydride (25 mL) was added to a mixture of **5a** (4.41 g, 10 mmol) in dry DMSO (25 mL). The solution was stirred at rt for 36 h. The solution was extracted with $CHCl_3$ and washed with saturated aqueous NaCl solution and water. The organic layer was dried over $MgSO_4$ and concentrated to an oily residue. This residue was applied to a column (12 × 14 cm, 375 g) and eluted with *n*-hexane/AcOEt (6/1). The desired fractions (R_f 0.47, *n*-hexane/AcOEt = 4/1) were concentrated in vacuo to give **6a** as a solid foam: 4.49 g (90%); ^1H NMR ($CDCl_3$) δ 7.26 (brs, 10 H, Ph), 4.84 (s, 2 H, OCH_2S), 4.50 (s, 4 H, $PhCH_2$), 3.94 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.80 (d, 2 H, J = 10 Hz, CH_2O), 3.72 (d, 2 H, J = 10 Hz, CH_2O), 3.05 (s, 2 H, CH_2), 2.10 (s, 3 H, SCH_3), 2.08 (s, 3 H, CH_3). Anal. Calcd for $C_{27}H_{34}N_2O_5S$ (498.63): C, 65.03; H, 6.87; N, 5.62. Found: C, 65.24; H, 6.82; N, 5.46.

6-[[2-[1,3-Bis(benzyloxy)-2-[[(methylthio)methyl]oxy]-propyl]]methyl]-2,4-dimethoxypyrimidine (6b). Compound **6b** was prepared in a manner similar to that used for the preparation of compound **6a**. Reagents: compound **5b** (11.8 g, 27.8 mmol) in dry DMSO (35 mL) and acetic anhydride (35 mL). Reaction time: 4 days. Chromatography on silica gel (500 g, 12- × 14-cm column) with *n*-hexane/ethyl acetate (9/1). Product isolated as solid foam: yield 11.16 g (83%); R_f 0.36 (*n*-hexane/AcOEt = 4/1); IR (KBr) 2924, 2868, 1736, 1560, 1455, 1356, 1209, 1103, 1040, 737, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.31–7.26 (m, 10 H, Ph), 6.36 (s, 1 H, H-5), 4.87 (s, 2 H, OCH_2S), 4.52 (s, 4 H, $PhCH_2$), 3.92 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.66 (d, 2 H, J = 10 Hz, CH_2O), 3.64 (d, 2 H, J = 10 Hz, CH_2O), 3.64 (d, 2 H, J = 10 Hz, CH_2O), 3.00 (s, 2 H, CH_2), 2.13 (s, 3 H, SCH_3). Anal. Calcd for $C_{26}H_{32}N_2O_5S$ (484.60): C, 64.44; H, 6.66; N, 5.78. Found: C, 64.31; H, 6.69; N, 5.69.

2,2-Bis[(benzyloxy)methyl]-8-methoxy-9-methyl-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (7a). Iodine (1 g) was added to a mixture of **6a** (1.3 g, 2.6 mmol) in dry THF (15 mL), and the solution was stirred under Ar at rt for 64 h. A Na_2SO_3 solution (40 mL, 5% aq) was added, and the resulting solution was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were washed with a saturated NaCl solution and water, dried over $MgSO_4$, and evaporated. The resulting residue was chromatographed on silica gel (45 g, 3.2- × 20-cm column) eluting with $CHCl_3$/AcOEt (5/1). The fractions (R_f = 0.32, $CHCl_3$/AcOEt = 4/1) were concentrated in vacuo to give the product: 0.759 g (67%); mp 89–90 °C; IR (KBr) 2861, 1673, 1638, 1546, 1370, 1103, 752, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28 (brs, 10 H, Ph), 5.51 (s, 2 H, NCH_2O), 4.49 (s, 4 H, $PhCH_2$), 3.95 (s, 3 H, OCH_3), 3.52 (d, 2 H, J = 9.8 Hz, CH_2O), 3.41 (d, 2 H, J = 9.8 Hz, CH_2O), 2.96 (s, 2 H, CH_2), 1.84 (s, 3 H, CH_3). Anal. Calcd for $C_{25}H_{28}N_2O_5$ (436.49): C, 68.79; H, 6.47; N, 6.42. Found: C, 68.84; H, 6.45; N, 6.38.

2,2-Bis[(benzyloxy)methyl]-8-methoxy-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (7b). Compound **7b** was prepared in a manner similar to that used for the preparation of compound **7a**. Reagents: iodine (0.5 g) and **6b** (0.54 g, 1.1 mmol) in dry THF (7 mL). Reaction time: 5 h. A Na_2SO_3 solution (10 mL, 5% aq). Purification was accomplished by chromatography on silica gel (15 g, 2.5- × 10-cm column) eluting with $CHCl_3$/AcOEt (5/1): yield 0.253 g (54%); mp 117–118 °C; R_f 0.21 ($CHCl_3$/AcOEt = 9/1); IR (KBr) 3008, 2952, 2917, 2861, 2805, 1673, 1638, 1553, 1377, 1082, 744, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 –7.24 (m, 10 H, Ph), 5.69 (s, 1 H, H-9), 5.52 (s, 2 H, NCH_2O), 4.52 (s, 4 H, $PhCH_2$), 3.93 (s, 3 H, OCH_3), 3.52 (d, 2 H, J = 9.8 Hz, CH_2O), 3.43 (d, 2 H, J = 9.8 Hz, CH_2O), 2.94 (s, 2 H, CH_2). Anal. Calcd for $C_{24}H_{26}N_2O_5$ (422.47): C, 68.23; H, 6.20; N, 6.63. Found: C, 68.11; H, 6.33; N, 6.55.

2,2-Bis[(benzyloxy)methyl]-9-methyl-1*H*,2*H*,4*H*,7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (8a). A solution of **7a** (0.11 g, 0.25 mmol) in 2 N NaOH/dioxane (1/1, 6 mL) was vigorously stirred under reflux for 14 h. The organic layer (upper layer) was neutralized by Dowex-X 2 (H^+ form) to pH 7. The resin was removed by filtration, and the filtrate was concentrated to dryness. A small amount of MeOH was added to the residue, and the mixture was allowed to stand at rt for 1 h to effect precipitation of the product. The precipitate was collected and washed with a small amount of MeOH to obtain the pure product: 87.1 mg (82%); mp 111–113 °C; R_f 0.21 ($CHCl_3$/AcOEt = 3/1); IR (KBr) 1715, 1652, 1483, 1089, 765, 730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.50 (s, 1 H, NH), 7.36–7.24 (m, 10 H, Ph), 5.41 (s, 2 H, NCH_2O), 4.51 (s, 4 H, $PhCH_2$), 3.50 (d, 2 H, J = 9.7 Hz, CH_2O), 3.40 (d, 2 H, J = 9.7 Hz, CH_2O), 3.01 (s, 2 H, H_2), 1.86 (s, 3 H, CH_3). Anal. Calcd for $C_{24}H_{26}N_2O_5$ (422.47): C, 68.23; H, 6.20; N, 6.63. Found: C, 68.38; H, 6.26; N, 6.60.

2,2-Bis[(benzyloxy)methyl]-1*H*,2*H*,4*H*,7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (8b). Compound **8b** was prepared in a manner similar to that used for the preparation of compound **8a**. Reagents: compound **7b** (1.12 g, 2.6 mmol) in 2 N NaOH/dioxane (1/1, 50 mL). Reaction time: 24 h. Crystallization with MeOH: yield 0.85 g, (80%); mp 164–165 °C; R_f 0.31 ($CHCl_3$/AcOEt = 3/1); IR (KBr) 3140, 3070, 3000, 2840, 2800, 1715, 1660, 1445, 1400, 1350, 1170, 1080, 890, 745, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.22 (s, 1 H, NH), 7.35–7.26 (m, 10 H, Ph), 5.58 (s, 1 H, H-9), 5.25 (s, 2 H, NCH_2O), 4.50 (s, 4 H, $PhCH_2$), 3.49 (d, 2 H, J = 10 Hz, CH_2O), 3.41 (d, 2 H, J = 10 Hz, CH_2O), 2.91 (s, 2 H, H_2). Anal. Calcd for $C_{23}H_{24}N_2O_5$ (408.45): C, 67.63; H, 5.92; N, 6.86. Found: C, 67.69; H, 6.01; N, 6.75.

2,2-Bis(hydroxymethyl)-9-methyl-1*H*,2*H*,4*H*,7*H*-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (9a). Method A. Compound **8a** (0.239 g, 0.56 mmol) was debenzylated by 20% palladium on carbon (100 mg) in MeOH (30 mL) at 50 psi of H_2 for 24 h. After removing the catalyst by filtration, the solution was concentrated in vacuo to a residue. The residue was applied to a column (2.5 × 10 cm, 12 g of silica gel) and eluted with $CHCl_3$/MeOH (9/1). The fractions (R_f = 0.32, $CHCl_3$/MeOH = 4/1) were concentrated to give the product: 98.7 mg (73%); mp 197–198 °C; UV λ_{max} nm (log ϵ) (MeOH) 270 (4.14); (pH 1) 272 (4.14); (pH 11) 217 (4.80), 270 (4.13); IR (KBr) 3459, 3395, 3269, 3170, 1722, 1630, 1483, 1084, 1054, 772 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.22 (s, 1 H, NH), 5.23 (s, 2 H, NCH_2O), 4.92 (brs, 2 H, OH), 4.36–3.30 (d, 4 H, CH_2O), 2.84 (s, 2 H, CH_2), 1.77 (s, 3 H, CH_3). Anal. Calcd for $C_{10}H_{14}N_2O_5$ (242.23): C, 49.58; H, 5.80; N, 11.57. Found: C, 49.38; H, 5.88; N, 11.60.

Method B. To a solution of MeOH (20 mL), which was presaturated with HCl gas at rt, was added **7a** (100 mg, 0.23 mmol) and palladium on carbon (20%). The mixture was shaken in a Parr apparatus at 50 psi of H_2 for 24 h. The catalysis was removed by filtration, and the filtrate was reduced to dryness. The resulting residue was purified as in method A to furnish **9a** in a 87% yield. This material was identical in all respects with **9a** prepared by method A.

2,2-Bis(hydroxymethyl)-8-methoxy-9-methyl-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (10a). A mixture of **7a** (0.893 g, 2 mmol) in CH_2Cl_2 (10 mL) was cooled to –78 °C. BCl_3 (1 M in CH_2Cl_2, 8 mL, 8 mmol) was added via syringe and under Ar. The mixture was stirred at –78 °C for 3 h, and then the temperature was raised to –40 °C. A solution of MeOH/CH_2Cl_2 (1:1, 15 mL) was added, and the cooling bath was removed. The solution was neutralized with $NaHCO_3$ (powder) to pH 7 and stirred for an additional 30 min. After filtration, the solvent was removed under reduced pressure. Water (3 mL) was added, and the solvent was removed. This process was repeated three times, followed by the addition of absolute EtOH (5 mL) and evaporation of the solvent. The residue was dissolved in a small amount of MeOH and absorbed on silica gel (2 g). This was applied onto a silica gel column (30 g, 3.2 × 20 cm) and then eluted with *i*-PrOH/MeOH/$CHCl_3$ (0.5/0.5/9). The fractions (R_f = 0.24; $CHCl_3$/MeOH = 9/1) were concentrated in vacuo to give the product: 0.759 g (67%); mp 158–160 °C; IR (KBr) 3409, 3170, 1658, 1623, 1384, 1096, 786, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.33 (s, 2 H, NCH_2O), 4.94 (t, 2 H, OH), 3.83 (s, 3 H, OCH_3), 3.40 (dd, 2 H, CH_2O), 3.31 (dd, 2 H, CH_2O), 2.88 (s, 2 H, CH_2-1), 1.88 (s, 3 H, CH_3). Anal. Calcd for $C_{11}H_{16}N_2O_5$ (256.26): C, 51.55; H, 6.29; N, 10.93. Found: C, 51.32; H, 6.32; N, 10.69.

2,2-Bis(hydroxymethyl)-1*H*,2*H*,4*H*,7*H*-pyrimido[1,6-*c*]-[1,3]oxazine-6,8-dione (9b) and 2,2-Bis(hydroxymethyl)-8-methoxy-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (10b). Compounds **9b** and **10b** were prepared in a manner similar to that used for the preparation of compound **9a**. Reagents: **7b** (0.93 g, 2.2 mmol) in CH_2Cl_2 (15 mL), BCl_3 (1 M, 7 mL, 7 mmol), and MeOH/CH_2Cl_2 (1/1, 12 mL). Purification was accomplished using chromatography on silica gel (20 g, 3.2- × 20-cm column) with $CHCl_3$/MeOH (14/1) as the solvent. Two products **9b** (0.112 g) and **10b** (0.252 g), 70%, were obtained. **9b**: mp 214–215 °C; R_f 0.17 ($CHCl_3$/MeOH = 7/1); IR (KBr) 3460, 3420, 3210, 1690, 1450, 1060 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.54 (s, 1 H, H-9), 5.22 (s, 2 H, NCH_2O), 4.93 (brs, 2 H, OH), 3.35 (d, 2 H, J = 11.4 Hz, CH_2O), 3.28 (d, 2 H, J = 11.4 Hz, CH_2O), 2.80 (s, 2 H, CH_2-1). Anal. Calcd for $C_9H_{12}N_2O_5$ (228.21): C, 47.36; H, 5.30; N, 12.28. Found: C, 47.50; H, 5.37; N, 12.18.

10b: mp 184–185 °C; R_f 0.39 ($CHCl_3$/MeOH = 7/1); IR (KBr) 3300–3140, 1630, 1540, 1465, 1375, 1320, 1084 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.98 (s, 1 H, H-9), 5.32 (s, 2 H, NCH_2O), 4.93 (t, 2 H, J = 5.8 Hz, OH), 3.79 (s, 3 H, OCH_3), 3.36 (d, 2 H, J = 11 Hz, CH_2O), 3.29 (d, 2 H, J = 11 Hz, CH_2O), 2.87 (s, 2 H, CH_2-1). Anal. Calcd for $C_{10}H_{14}N_2O_5$ (242.23): C, 49.58; H, 5.83; N, 11.57. Found: C, 49.80; H, 5.87; N, 11.64.

8-Amino-2,2-bis(hydroxymethyl)-9-methyl-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (12a). A mixture of **10a** (103.8 mg, 0.4 mmol) in methanolic NH_3 (40 mL) was heated at 120 °C

for 20 h in a sealed tube. The solution was cooled to 0 °C, and the precipitate was collected by filtration and washed with cold MeOH (5 mL) to obtain pure **12a**: 45.3 mg (46%); mp 289–291 °C; R_f 0.18 ($CHCl_3$MeOH = 3/1); UV λ_{max} nm (log ϵ) (H_2O) 281 (4.23); (pH 1) 291 (4.38); (pH 11) 280 (4.20); IR (KBr) 3374, 3318, 3170, 1666, 1623, 1518, 1061, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.98–6.76 (brs, 1 H, NH_2), 5.23 (s, 2 H, NCH_2O), 4.88 (t, 2 H, J = 5.6 Hz, OH), 3.40–3.23 (m, 4 H, CH_2O) 2.78 (s, 2 H, CH_2), 1.81 (s, 3 H, CH_3). Anal. Calcd for $C_{10}H_{15}N_3O_4$ (241.24): C, 49.78; H, 6.27; N, 17.42. Found: C, 49.82; H, 6.38; N, 17.46.

8-Amino-2,2-bis(hydroxymethyl)-1*H*,2*H*,4*H*-pyrimido-[1,6-*c*][1,3]oxazin-6-one (12b). Compound **12b** was prepared in a manner similar to that used for the preparation of compound **12a**. Reagent: **10b** (0.52 g, 2.15 mmol) in saturated methanolic NH_3 (100 mL). Crystallization from EtOH/H_2O: yield 313.8 mg (64%); mp 268–269 °C; R_f 0.24 ($CHCl_3$/MeOH = 2/1); IR (KBr) 3500, 3300, 3180, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.06 (s, 1 H, NH_2), 6.93 (s, 1 H, NH_2), 5.60 (s, 1 H, H-9), 5.21 (s, 2 H, NCH_2O), 4.91 (t, 2 H, J = 5.6 Hz, OH), 3.35 (d, 2 H, J = 5.6 Hz, CH_2O), 3.24 (d, 2 H, J = 5.6 Hz, CH_2O), 2.73 (s, 2 H, CH_2). Anal. Calcd for $C_9H_{13}N_3O_4$ (227.21): C, 47.57 H, 5.77; N, 18.49. Found: C, 47.36; H, 5.66; N, 18.24.

8-Amino-2,2-bis[(benzyloxy)methyl]-1*H*,2*H*,4*H*-pyrimido[1,6-*c*][1,3]oxazin-6-one (11b). Compound **11b** was prepared in a manner similar to that used for the preparation of compound **12b**. Reagent: **7b** (0.99 g, 2.34 mmol) in methanolic NH_3 (50 mL). Reaction time: 2 days. Crystallization with AcOEt–MeOH: yield 631 mg (66%); mp 168–169 °C; R_f 0.33 ($CHCl_3$/MeOH = 9/1); IR (KBr) 3360, 3128, 3086, 2861, 1673, 1631, 1469, 1082, 780, 730, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.31–7.28 (m, 10 H, Ph), 5.49 (s, 1 H, H-9), 5.45 (s, 2 H, NCH_2O), 4.51 (s, 4 H, CH_2Ph), 3.51 (d, 2 H, J = 9.8 Hz, CH_2O), 3.41 (d, 2 H, J = 9.8 Hz, CH_2O), 2.85 (s, 2 H, CH_2). Anal. Calcd for $C_{23}H_{25}N_3O_4$ (407.45): C, 67.79; H, 6.18; N, 10.31. Found: C, 67.63; H, 6.36; N, 10.30.

In Vitro Antiviral Evaluation. (a) Cells and Viruses. KB cells, an established human cell line derived from an epidermoid oral carcinoma, were routinely grown in minimal essential medium (MEM) with Hank salts [MEM(H)] supplemented with 5% fetal bovine serum. Diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[16] CEM-SS cells (a continuous human T-cell line) and $H9III_B$ cells (a continuous human T-cell line persistently infected with HIV-1) were grown in RPMI-1640 medium supplemented with 20% (v/v) fetal bovine serum, 100 unit/mL penicillin, 100 μg/mL streptomycin, and 2 mM glutamine as detailed elsewhere.[17] 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 Corp. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[16] HIV-1 (strain $HTLV\text{-}III_B$) was propogated in $H9III_B$ cells as previously described.[17] Infectious HIV-1 recovered from cell supernatants was clarified by centrifugation (400 × g, 5 min), filtered through a 0.45-μm Millipore filter, stored at −80 °C and assayed by syncytial plaque count.

(b) Assays for Antiviral Activity. HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above 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. Protocols for the HCMV yield reduction assay have been described previously.[18] HSV-1 was assayed using an enzyme immunoassay described by Prichard and Shipman.[19] HIV was assayed by syncytial plaque assay in CEM-SS cells as previously described by us.[17] The assay was used to measure infectious virus production in supernatant and cell lysate fractions from HIV infected and treated cells and to evaluate compounds for anti-HIV activity by inhibition of syncytial plaque count. The data were analyzed as described below for HIV syncytial plaque formation (plaque forming units, PFU).

(c) Cytotoxicity Assay. Two basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells during replication of HCMV 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 35- and 60-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration. Compounds also were evaluated for cytotoxicity in CEM-SS cells by seeding at a density of 10 000 cells per well in growth medium using a 96-well flat-bottom plate. Serial 5-fold dilutions of compounds were prepared in growth medium and added to the wells as a second overlay. After 48 h of incubation at 37 °C, the cells were pulsed labeled with [^{3}H]dThd (1 μCi per well; 20 Ci/mmol) for 6 h and the cells were harvested to measure total DNA synthesis.

(d) Data Analysis. Dose–response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against $\log_{10}$ 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.

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