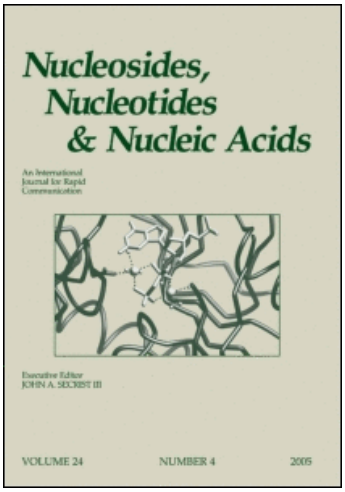

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-SUBSTITUTED ANTI-CONSTRAINED ACYCLIC ANALOGS OF CYTIDINE AND URIDINE

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-SUBSTITUTED ANTI-CONSTRAINED ACYCLIC ANALOGS OF CYTIDINE AND URIDINE

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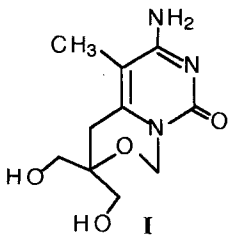
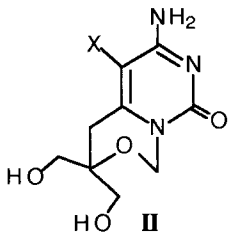
Abstract. A number of 5-substituted constrained acyclic analogs of cytidine and uridine have been prepared in which the glycosyl torsion angle is constrained in the anti conformation. Compounds **2a-c**, **3a-c**, **4**, **5** and **6** were tested for activity against HCMV and HSV-1. Compounds **2a** and **2b** showed moderate activity against HCMV. Compound **2c** exhibited a weak inhibitory activity against HSV-1. Compounds **2a**, **3a**, **4**, **5**, **6**, **8**, and **9** were screened for their anti-HIV or antitumor activity. None of them were active against HIV. However, compound **9** showed a 50% inhibition on MDA-MB-231/ATTC breast cancer cell growth at a concentration of 0.15 μM.

INTRODUCTION

Nucleoside mimicry has been a fruitful area in the search for the therapeutic agents,[1-6] notably for anti-cancer and anti-viral purposes. Much of this mimicry has been involved in the modification of the heterocyclic bases, carbohydrate moiety, and *syn-anti* glycosyl conformation of the common natural nucleosides. Since the discovery of the human immunodeficiency virus (HIV) as the etiological agent of AIDS,[7-10] increasing efforts have been devoted to the synthesis and biological evaluation of compounds with potential anti-HIV activity.[4, 11]

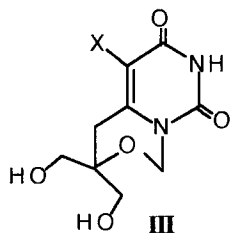


I



II

X = Cl, Br, I



III

X = Cl, Br, I, -CH=COOEt
-CH=COOH, -CH=CHBr

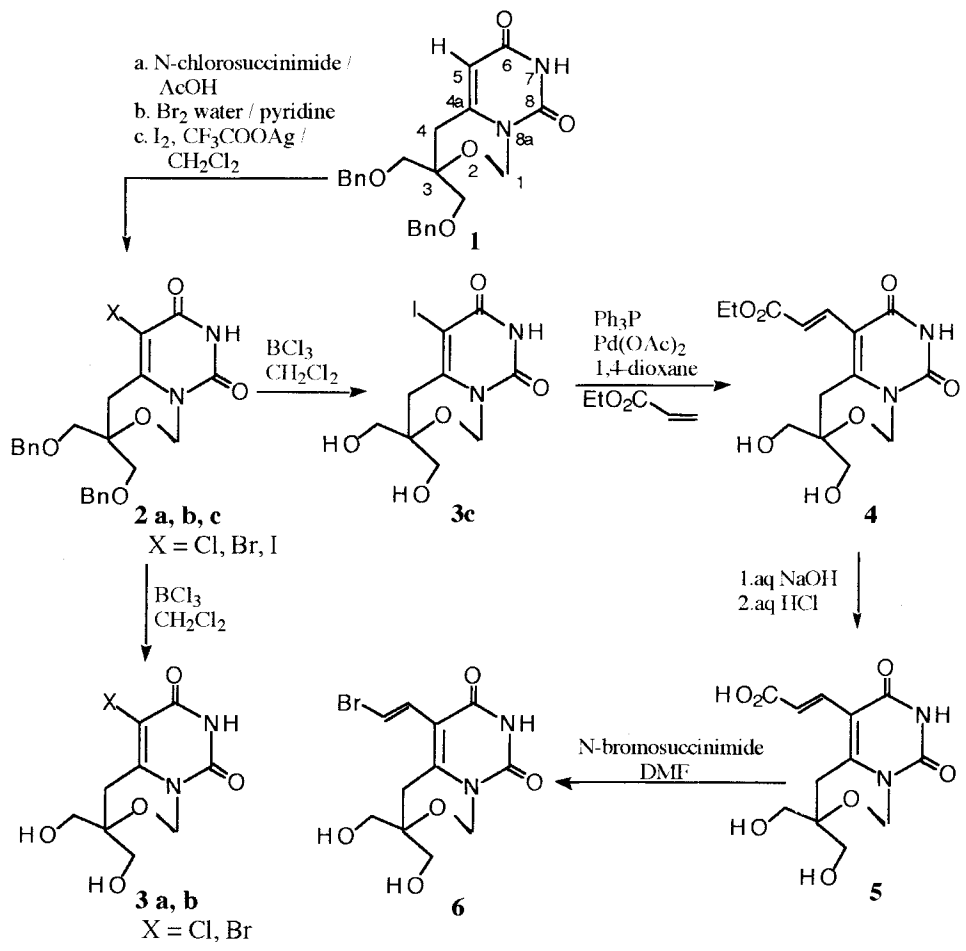
Recently, we reported a series of *anti*-conformationally restricted pyrimidine acyclic nucleosides and found 6-amino-3,3-bis(hydroxymethyl)-5-methyl-(1H, 2H, 4H, 7H)-pyrimido[1,6-*c*][1,3]oxazin-8-one (**I**),[12] a structural mimic of *anti*-constrained acyclic 2'-deoxycytidine, showed a slight inhibitory activity against HIV. In order to expand the structure-activity relationship and to improve anti-HIV activity, we began the preparation of 5-substituted analogs of constrained acyclic cytidine and uridine. In this paper, synthesis of these 5-substituted analogs (**II** and **III**) of constrained acyclic cytidine, and uridine, and their anti-viral and antineoplastic actvity is described.

RESULTS AND DISCUSSION

Chemical

The synthesis (scheme 1) of 5-substituted analogs of constrained acyclic uridine was started with **1** prepared by the methodology previously described.[12] Halogenation of **1** with N-chlorosuccinimide in the presence of acetic acid, bromine water in pyridine, and iodine in the presence of silver trifluoroacetate in dichloromethane, respectively, afforded the corresponding 5-halo derivatives **2a-c**. Deblocking the protected benzyl group of the 5-halo derivatives **2a-c** with boron trichloride in dichloromethane at -55 °C furnished **3a-c**. Reaction of 5-iodo derivative **2c** with palladium acetate, ethylacrylate, and triphenylphosphine in the presence of triethylamine in dioxane under reflux failed to produce the corresponding 5-ethoxycarbonylvinyl derivative. However, treatment of **3c** (the deblocked product of **2c**) with the same reagents produced the 5-ethoxycarbonylvinyl derivative **4** successfully. Hydrolysis of the ester group of **4** under basic condition gave the corresponding 5-carboxy-vinyl derivative **5** which was converted to the 5-bromovinyl derivative **6** by using N-bromosuccinimide.

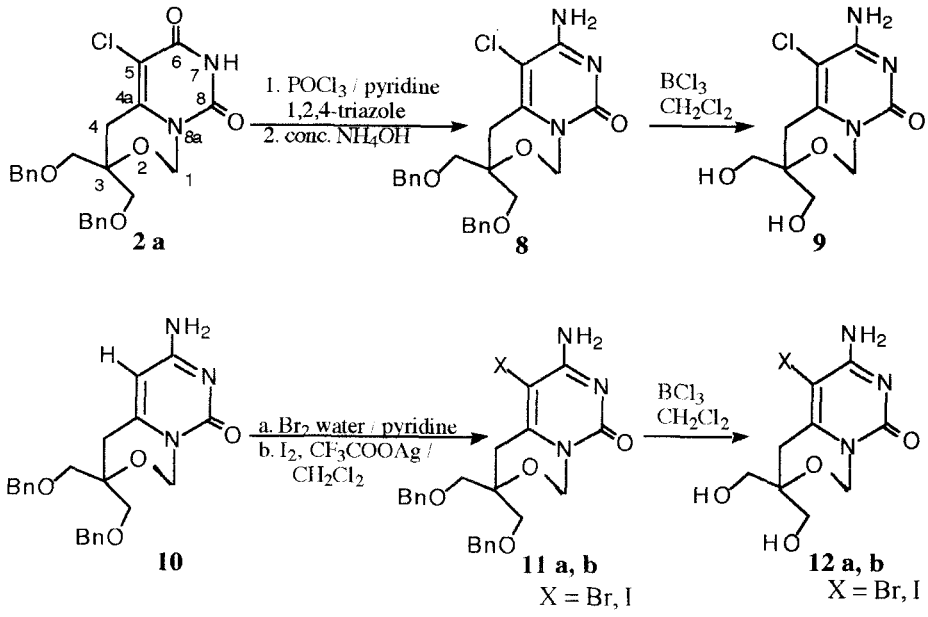
The 5-bromo and 5-iodo analogs of constrained acyclic cytidine were synthesized directly from rigid acyclic cytidine **10**[12] (scheme 2). Bromination or iodination of **10** with bromine water in pyridine, or iodine and silver trifluoroacetate in dichloromethane afforded the corresponding 5-bromo and 5-iodo derivatives **11a,b**, respectively. Deblocking the protected benzyl group of **11a,b** with boron trichloride in dichloromethane at -55 °C furnished **12a,b**. However the 5-chloro analog of constrained acyclic cytidine was synthesized from constrained acyclic uridine **2a**.[12] Treatment of **2a** with phosphoryl chloride and 1,2,4-triazole in pyridine at room temperature produced the 4-triazolyl derivative. Without isolation, the 4-triazolyl derivative was reacted with aqueous ammonia to give the constrained acyclic cytidine analog **8**, which was subsequently treated with boron trichloride in dichloromethane at -55 °C to produce **9**.



Reaction Scheme 1

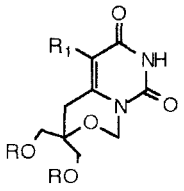
Biological

Compounds **2a-c**, **3a-c**, **4-6**, **10**, and the deprotected analog of **10** were tested for their *in vitro* inhibitory effects on the replication of two DNA viruses (i.e., human cytomegalovirus, and herpes simplex virus type 1), and the results for active compounds are presented in Table 1. Only uracil analogues with 5-halo substituent (*i.e.* **2a**, **2b**, and **2c**) showed anti-herpetic activity. Compounds **2a** and **2b** were weakly active against HCMV with IC_{50} of 36 μM and 29 μM, respectively and inactive against HSV-1. Compound **2c** showed inhibitory activity against HSV-1 with an IC_{50} of 35 μM. The anti-HCMV or anti-HSV-1 activity of **2a**, **2b** and **2c** was surprising because these molecules contain benzyl



Reaction Scheme 2

Table 1. Antiviral Activity and Cytotoxicity of 5-Substituted Analogs of Anti Constrained Acyclic Cytidine and Uridine



			50% inhibitory concentration (μM)			
			antiviral activity[a]			
			HCMV	HSV-1[b]	cytotoxicity[c]	
compd	R1	R	plaque	ELISA	visual[d]	growth
2a	Cl	$PhCH_2$	36[d]	>100[e]	>100	60
2b	Br	$PhCH_2$	29[d]	>100	32	60
2c	I	$PhCH_2$	>100[d]	35	>100	55
foscarnet[f]			39 + 26			
ganciclovir[g]			7.4 + 6.5	3.5 + 2.1	>100	>100

[a] Plaque assay was performed in duplicate as described in the text. [b] The plaque assay was used to determine the activity of DHPG against HSV-1; all other compounds were assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as describe in the text in quadruplicate assays. [d] Average derived from two experiments for each parameter. [e] >100 indicates IC_{50} not reached at the noted (highest) concentration tested. [f] Average + standard deviation from 15 experiments. [g] Average + standard deviation from 108, and 3 experiments, respectively.

groups instead of free hydroxyl groups at the acyclic sugar moiety. No anti-herpetic activity was observed at concentrations up to 100 μM with compounds **3a-c**, **4**, **5** and **6** which contained the free hydroxyl groups at the acyclic sugar portions. However, **2a**, **2b** and **2c** caused cytotoxicity in uninfected KB cells at concentrations near those required for antiviral activity.

Compounds **2a**, **3a**, **4**, **5**, **6**, **8**, and **9** were selected by National Cancer Institute (NCI), USA and screened for their *in vitro* antiviral or antitumor activity against human immunodeficiency virus (HIV) or 60 human tumor cell lines derived from nine clinically isolated cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate, breast). None of the tested compounds were active against HIV at the highest concentration tested (100 μM). Of the compounds tested, compound **9** was able to inhibit the growth of MDA-MB-231/ATTC breast cancer cells by 50% at concentration of 0.15 μM and the GI_{50} of **9** for renal cancer (786-0, and SN12C) was 36 μM (Table 2). Compound **2a** was relatively weakly active against three renal cancer cell lines (i.e., 786-0, CAKI-1, and SN12C) with a GI_{50} of 12, 17, and 11 μM, respectively. Compound **2a** also showed good activity against Melanoma (LOXIMVI) and breast cancer (MDA-MB-231/ATTC) at a GI_{50} concentration of 1.0 and 4.0 μM, respectively. Unfortunately, **2a** exhibited a very close concentration between the LC_{50} (cytotoxicity or net cell killing) and the GI_{50} on 786-0 and SN12C renal cancer cell lines.

EXPERIMENTAL SECTION

General Chemical Procedures

Melting Points were taken on a BUCHI 530 apparatus and are uncorrected. The silica gel used for chromatography was silica gel 60 70-230 mesh (E. Merck, Darmstadt, West Germany), TLC was performed on prescored DC-Alufolien Kieselgel $60F_{254}$ (E. Merk, Darmstart). 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 are reported as v/v. ^1H NMR spectra were obtained at 300 MHz. Where necessary, deuterium exchange, and homonuclear decoupling experiments were used to obtained proton shift assignments. IR spectra were recorded on a Perkin-Elmer 938G spectrophotometer. UV spectra were obtained on a Shimadzu UV-160 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 Perkin-Elmer 2400 Elemental Analyzer.

3,3-Bis[(benzyloxy)methyl]-5-chloro-1H, 2H, 4H, 7H-pyrimido [1,6*c*][1,3]oxazine-6,8-dione (2a) A mixture of compound **1** (4.05 g, 10 mmol) and N-chlorosuccinimide (2 g, 15 mmol) in glacial acetic acid (50 mL) was heated at 60 °C with

Table 2. Results of Antitumor Evaluations[a] of 5-Substituted Analogs of Anti Constrained Acyclic Cytidine and Uridine in the NCI In Vitro Primary Screen

	2a			**9**		
	Concentration in μM for					
Cancer Cell Line	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Melanoma						
(LOX IMV1)	1.0	7.6	44	>100	>100	>100
Renal Cancer						
(786-0)	12	26	58	36	>100	>100
(CAKI-1)	17	49	>100	36	>100	>100
(SN12C)	11	23	51	>100	>100	>100
Breast Cancer						
(MDA-MB-231/ATTC)	4.0	31	>100	0.15	>100	>100

[a] Compounds were tested in quadruplicate at five different concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} M). Calculations were based upon the averaged values from all available tests for each compound; standard errors averaged less than 10-15% of the respective means. GI_{50}: 50% growth inhibition. TGI: total growth inhibition. LC_{50}: 50% lethal concentration, the cytotoxicity parameter.

stirring for 4 h. The solution was evaporated in *vacuo* to dryness. The residue was coevaporated with methanol (3 x 10 mL). The solvent was removed. A small amount of methanol was added to the residue to produce precipitate product, 3.49 g (78%). Mp 118-119 °C; Rf: 0.27 ($CHCl_3$/MeOH, 9/1); UVλ_{max}nm(log ε): MeOH 279 (3.97). IR (KBr): 3144, 3017, 2829, 1700, 1609, 1455, 1084, 995, 741, 695 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.83(s, 1 H, NH); 7.36-7.24(m, 10 H, Ph); 5.40(s, 2 H, NCH_2O); 4.51(s, 4 H, $PhCH_2$); 3.47(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.20(s, 2 H, H_2-4). Anal. Calcd. for $C_{23}H_{23}ClN_2O_5$ (442.88): C, 62.37; H, 5.23; N, 6.33. Found: C, 61.97; H, 5.30; N, 6.30.

3,3-Bis[(benzyloxy)methyl]-5-bromo-1H, 2H, 4H, 7H-pyrimido-[1,6-*c*][1,3]oxazine-6,8-dione (2b) Compound **1** (0.41 g, 1 mmol) was dissolved in pyridine (10 mL). Bromine water (10 mL, 1.56 mmol) was added and stirred at room temperature for 3 days. The solvent was removed. The residue was chromatographed on silica gel (30 g, 2.5 x 10 cm) with chloroform / ethyl acetate (9 / 1). The desired fractions were collected and the solvent was removed. A small amount of methanol was added to produce precipitate product. Crystallization with methanol afforded the pure product, 0.15 g, (30%). Mp 118-119 °C; Rf: 0.21 ($CHCl_3$/MeOH, 9/1); UVλ_{max}nm(log ε): MeOH 280

(3.98); IR (KBr): 3425, 3005, 1685, 1659, 1062, 1437, 1411, 1070, 735 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.45(s, 1 H, NH); 7.36-7.26(m, 10 H, Ph); 5.41(s, 2 H, NCH_2O); 4.51(s, 4 H, $PhCH_2$); 3.47(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.25(s, 2 H, H_2-4). Anal. Calcd. for $C_{23}H_{23}BrN_2O_5$ (487.34): C, 56.68; H, 4.76; N, 5.75. Found: C, 56.39; H, 4.47; N, 5.41.

3,3-Bis[(benzyloxy)methyl]-5-iodo-1H, 2H, 4H, 7H-pyrimido[1,6-c][1,3]oxazine-6,8-dione (2c) To a stirred mixture of compound **1** (11.4 g, 28 mmol) and silver trifluoroacetate (6.64 g, 30 mmol) in dichloromethan (350 mL) was added dropwise a solution of iodine (9.95 g, 40 mmol) in 300 mL of dichloromethane at 0 °C (ice bath). The mixture was stirred at room temperature for 1 h. The suspension was filtered and washed with dichloromethane. The combined filtrate was washed successively with saturated sodium carbonate solution and water. The solution was dried over sodium sulfate. The solvent was removed under reduced pressure. The solid residue was crystalized with ethyl acetate to give the pure product 10.89 g (72%). Mp 91-92 °C; Rf: 0.14 ($CHCl_3$/AcOEt, 9/1); UVλ_{max}nm(log ε): MeOH 286 (3.95); IR (KBr): 3402, 3003, 2844, 1700, 1637, 1436, 1083, 744 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.54(s, 1 H, NH); 7.36-7.24(m, 10 H, Ph); 5.42(s, 2 H, NCH_2O); 4.52(s, 4 H, $PhCH_2$); 3.47(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.30(s, 2 H, H_2-4). Anal. Calcd. for $C_{23}H_{23}IN_2O_5$ (534.34): C, 51.69; H, 4.34; N, 5.24. Found: C, 51.79; H, 4.32; N, 5.17.

3,3-Bis(hydroxymethyl)-5-chloro-1H, 2H, 4H, 7H-pyrimido[1,6-c][1,3]oxazine-6,8-dione (3a) A mixture of **2a** (0.89 g, 2 mmol) in methylene chloride (12 mL) was cooled to 0 °C. Boron trichloride (1 M in methylene chloride, 6 mL, 6 mmol) was added via syringe and under nitrogen gas. The mixture was stirred in ice-bath for 3 hr. A solvent mixture of methanol / methylene chloride (1 / 1, 30 mL) was added and the cooling bath was removed. The solution was neutralized with saturated sodium bicarbonate solution to pH 7 and stirred for 30 min. The solvent was removed. The residue was crystallized with methanol to give pure product, 0.21 g (40%). Mp 230 °C; Rf: 0.32 ($CHCl_3$/MeOH, 9/1); UVλ_{max}nm(log ε): MeOH 279 (3.96); IR (KBr): 3406, 1615, 1423, 1069, 652. cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.73(s, 1 H, NH); 5.24(s, 2 H, NCH_2O); 4.95(t, 2 H, J = 6 Hz, OH); 3.35(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 2.99(s, 2 H, H_2-4). Anal. Calcd. for $C_9H_{11}ClN_2O_5$ (262.65): C, 41.15; H, 4.22; N, 10.67. Found: C, 41.26; H, 4.19; N, 10.42.

3,3-Bis(hydroxymethyl)-5-bromo-1H, 2H, 4H, 7H-pyrimido[1,6-c][1,3]oxazine-6,8-dione (3b) A mixture of **2b** (0.51 g, 1.05 mmol) in methylene chloride (10 mL) was cooled to 0 °C. Boron trichloride (1 M in methylene chloride, 3.2 mL, 3.2 mmol) was added via syringe and under nitrogen gas. The mixture was stirred in ice-bath for 3 hr. A solvent mixture of methanol / methylene chloride (1 / 1, 20 mL) was added

and the cooling bath was removed. The mixture was passed through a filter and the filtrate was concentrated. The residue was crystallized with methanol to give pure product, 0.14 g (44%). Mp 238-239 °C; R_f: 0.20 ($CHCl_3$/MeOH, 9/1); UVλ_{max}nm(log ε): MeOH 280 (3.99); IR (KBr): 3341, 2991, 2823, 1683, 1603, 1081, 1043 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 11.70(s, 1 H, NH); 5.24(s, 2 H, NCH_2O); 4.95(t, 2 H, J = 6 Hz, OH); 3.35(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.02(s, 2 H, H_2-4). Anal. Calcd. for $C_9H_{11}BrN_2O_5$ (307.11): C, 35.20; H, 3.61; N, 9.12. Found: C, 35.42; H, 3.58; N, 8.95.

3,3-Bis(hydroxymethyl)-5-iodo-1H, 2H, 4H, 7H-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (3c) A mixture of **2c** (1.6 g, 3 mmol) in methylene chloride (16 mL) was cooled to 0 °C. Boron trichloride (1 M in methylene chloride, 9 mL, 9 mmol) was added via syringe and under argon gas. The mixture was stirred at 0 °C for 3 h. A solution of methanol / methylene chloride (1/1, 30 mL) was added, and the cooling bath was removed. The solution was neutralized with sodium bicarbonate (powder) to pH 7 and stirred for an additional 30 min. The mixture was passed through a filter and the filtrate was concentrated under reduced pressure. The residue was crystallized with methanol to give the product, 0.839 g (79%). Mp 227 °C; R_f: 0.20 ($CHCl_3$/MeOH, 9/1); UVλ_{max}nm(log ε): MeOH 284 (3.98); IR (KBr): 3497, 3311, 1669, 1627, 1590, 1414, 1051 cm^{-1}; ^{1}H NMR (DMSO-d_6):δ 11.59(s, 1 H, NH); 5.24(s, 2 H, NCH_2O); 4.93(t, 2 H, J = 6 Hz, OH); 3.33(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.06(s, 2 H, H_2-4). Anal. Calcd. for $C_9H_{11}IN_2O_5$ (354.1): C, 30.53; H, 3.13; N, 7.91. Found: C, 30.27; H, 3.26; N, 7.45.

3,3-Bis(hydroxymethyl)-5-(E)-ethoxycarbonylvinyl-1H, 2H, 4H, 7H-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (4) To a solution of triethylamine (1.2 g, 12 mmol) in dioxane (20 mL) was added triphenylphosphine (0.2 g, 0.76 mmol) and palladium acetate (80 mg, 0.36 mmol), and the mixture was heated under reflux to give a dark solution. The heating source was removed. When the temperature droped below reflux temperature, compound **3c** (2.76 g, 7.8 mmol) and ethyl acrylate (2.4 g, 24 mmol) were added seperately. The mixture was heated under reflux for 4h and filtered through celite. The filtrate was concentrated under reduced pressure. After addition of a small amount of ethyl acetate to the residue, the solid product formed from oily residue. The crude product was crystalized from methanol from give the pure compound **4** (375 mg). The mother solution was coevaporated with silica gel (10 g) which was placed on the top of a column (60 g of silica gel, 4 x 12 cm). The column was eluted with chloroform / methanol (20 / 1). The desired fractions (R_f = 0.36; $CHCl_3$/MeOH, 9/1) were collected and concentrated *in vacuo* to give the product, 812 mg. Total yield was 1.19 g (46%). Mp 218-220 °C; UVλ_{max}nm(log ε): MeOH 300 (4.12); IR (KBr): 3440, 1689, 1294, 1188, 1147, 1062 cm^{-1}; ^{1}H NMR (DMSO-d_6):δ 11.6(s, 1 H, NH); 7.46, 7.01(d, 1 H each, J = 15.5 Hz,

CH=CH); 5.32(s, 2 H, NCH_2O); 4.98(t, 2 H, J = 5.4 Hz, OH); 4.14(q, 2 H, J = 7.0 Hz, $MeCH_2O$); 3.35(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.09(s, 2 H, H_2-4); 1.22(t, 3 H, J = 7.0 Hz, $MeCH_2O$). Anal. Calcd. for $C_{14}H_{18}N_2O_7$ (326.3): C, 51.53; H, 5.56; N, 8.59. Found: C, 51.24; H, 5.23; N, 8.32.

3,3-Bis(hydroxymethyl)-5-(E)-carboxyvinyl-1H, 2H, 4H, 7H-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (5) Compound **4** (286 mg, 0.88 mmol) was stirred with 2 M sodium hydroxide solution (5 mL) for 1 h. The solution was cooled to 0 °C and neutralized with conc HCl to pH 1. The mixture was filtered and washed with water. The precipitate was crystallized from methanol to give the pure product 230 mg (88%). Mp 263-265 °C; Rf: 0.26 [$CHCl_3$/MeOH + 5 drops of AcOH, (6/1)]; UVλ_{max}nm (log ε): MeOH 300 (4.15); IR (KBr): 3503, 3243, 1715, 1609, 1471, 1286, 1044, 797 cm^{-1}; ^{1}H NMR (DMSO-d_6):δ 12.12(s, 1 H, COOH); 11.57(s, 1 H, NH); 7.40, 6.95(d, 1 H each, J = 15.5 Hz, CH=CH); 5.31(s, 2 H, NCH_2O); 4.97(t, 2 H, J = 5.4 Hz, OH); 3.36(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.08(s, 2 H, H_2-4). Anal. Calcd. for $C_{12}H_{14}N_2O_7$ (298.25): C, 48.32; H, 4.73; N, 9.39. Found: C, 48.10; H, 4.68; N, 9.26.

3,3-Bis(hydroxymethyl)-5-(E)-bromovinyl-1H, 2H, 4H, 7H-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (6) Compound **5** (386 mg, 1.29 mmol) in DMF (15 mL) was stirred at room temperature for 10 min and sodium bicarbonate (325 mg, 3.87 mmol) was added. The mixture was stirred at room temperature for 20 min and a solution of N-bromosuccinimide (300 mg, 1.68 mmol) in DMF (8 mL) was added dropwise. The mixture was stirred overnight and filtered. The filtrate was evaporated in *vacuo* to dryness. The residue was chromatographed on 50 g of silica gel (14 x 3.5 cm) with chloroform / methanol (20 / 1). The desired fractions were collected and concentrated to give the product 200 mg (47%). Mp 179-180 °C; Rf: 0.38 ($CHCl_3$/MeOH, 9/1); UVλ_{max}nm (log ε): MeOH 293 (4.03); IR (KBr): 3391, 3263, 2959, 1543, 1515, 1429, 1347, 1022, 979 cm^{-1}; ^{1}H NMR (DMSO-d_6):δ 11.49(s, 1 H, NH); 7.33, 6.92(d, 1 H each, J = 13.3 Hz, CH=CH); 5.28(s, 2 H, NCH_2O); 4.93(t, 2 H, J = 5.4 Hz, OH); 3.32(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 2.99(s, 2 H, H_2-4). Anal. Calcd. for $C_{11}H_{13}BrN_2O_5$ (333.14): C, 39.66; H, 3.93; N, 8.41. Found: C, 39.32; H, 3.81; N, 8.11.

6-Amino-3,3-bis[(benzyloxy)methyl]-5-chloro-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-8-one (8) Phosphoryl chloride (750 μL, 8 mmol) was added to a solution of triazole (2.2 g, 32 mmol) in dry pyridine (70 mL). The mixture was cooled to °C, compound **2a** (1.77 g, 4 mmol) was added, and the mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure to give a residue which was then partitioned between dichloromethane and saturated sodium hydrogen carbonate, and the organic layer was dried over anhydrous sodium sulfate and evaporated to

give a residue. The residue was dissolved in dioxane (20 mL), concentrated aqueous ammonia solution (5 mL) was added, and the mixture was kept at room temperature in a sealed vessel overnight. The solvent was evaporated and the residue was crystalized in a solvent mixture (chloroform / methanol) to give the product **8**, 2.28 g (67%). Mp 257-258 °C; Rf: 0.34 ($CHCl_3$/MeOH, 95/5); ^{1}H NMR (DMSO-d_6):δ 7.73, 7.14(s, 1 H each , NH_2); 7.27(m, 10 H Ph); 5.26(s, 2 H, NCH_2O); 4.50(s, 4 H, $PhCH_2$); 3.50(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O); 3.05(s, 2 H, H_2-4). ^{13}C NMR (DMSO-d6):δ 28, 69, 73.02, 73.19, 77, 97, 127.57, 127.80, 129, 138, 149, 153, 162; MS *m/z* 441 (M^+). Anal. Calcd. for $C_{23}H_{24}ClN_3O_4 \cdot 1.25H_2O$(464.29): C, 59.50; H, 5.74; N, 9.05. Found: C, 59.37; H, 5.53; N, 9.30.

6-Amino-3,3-bis(hydroxymethyl)-5-chloro-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-8-one (9) A mixture of **8** (1.33 g, 3 mmol) in methylene chloride (30 mL) was cooled to 0 °C. Boron trichloride (1 M in methylene chloride, 9 mL, 9 mmol) was added *via* syringe and under nitrogen gas. The mixture was stirred at 0 °C for 3 h. A solution of methylene chloride / methanol (1 / 1, 40 mL) was added, and the cooling bath was removed. The solution was neutralized with methanolic ammonia. The solvent was removed under reduced pressure and the residue was crystallized with methanol to give the product, 0.34 g (43%). Rf: 0.24 ($CHCl_3$/MeOH, 4/1); ^{1}H NMR (DMSO-d_6):δ 8.66, 8.73(s, 1 H each, NH_2); 5.48(s, 2 H, NCH_2O); 5.14(t, 2 H, OH); 3.40(m, 4 H, CH_2O); 3.10(s, 2 H, H_2-4). Anal. Calcd. for $C_9H_{12}ClN_3O_4$ (261.66): C, 41.31; H, 4.62; N, 16.06. Found: C, 41,01; H, 4.31; N, 15.97.

6-Amino-3,3-bis[(benzyloxy)methyl]-5-bromo-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-8-one (11a) Compound **10** (0.8 g, 2 mmol) was dissolved in pyridine (120 mL). Bromine water (3 w/v %, 20 mL) was added and stirred at room temperature for 4 h. The solvent was removed under reduced pressure. The residue was crystallized from methanol to give the product 0.59 g, (60%). Mp 175-176 °C; Rf: 0.28 ($CHCl_3$/MeOH, 95/5); ^{1}H NMR ($CDCl_3$): δ 7.26-7.35(m, 10 H, Ph); 5.48(s, 2 H, NCH_2O); 4.53(s, 4 H, $PhCH_2$); 3.55, 3.46(d, 2 H each, J=10 Hz, CH_2O); 3.12(s, 2 H, H_2-4); MS *m/z* 486 (M^+); Anal. Calcd. for $C_{23}H_{24}Br \cdot N_3O_4H_2O$ (504.34): C, 54.77; H, 5.20; N, 8.33. Found: C, 55.14; H, 5.23; N, 8.33.

6-Amino-3,3-bis[(benzyloxy)methyl]-5-iodo-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-8-one (11b) To a stirred mixture of compound **10** (1.02 g, 2.5 mmol) and silver trifluoroacetate (553 mg, 2.5 mmol) in dichloromethan (32 mL) was added dropwise a solution of iodine (425 g, 3.33 mmol) in 14 mL of dichloromethane at 0 °C (ice bath). The mixture was stirred at room temperature for 2 h. The mixture was filtered and the filtrate was dried under reduced pressure. The residue was chromatographed with a solvent mixture of chloroform / methanol (97 / 3) to give the

product 516 mg (40%). Mp 170-171 °C; Rf: 0.1 ($CHCl_3$/AcOEt, 97/3); ^1H NMR ($CDCl_3$): δ 7.37-7.29(m, 10 H, Ph); 5.49(s, 2 H, NCH_2O); 4.54(s, 4 H, $PhCH_2$); 3.50(dd, 4 H, J_1=10 Hz, J_2=10 Hz, CH_2O) ; 3.17(s, 2 H, H_2-4). MS *m/z* 533 (M^+); Anal. Calcd. for $C_{23}H_{24}I\ N_3O_4$(533.34): C, 51.79; H, 4.54; N, 7.87. Found: C, 51.59; H, 4.33; N, 7.50.

6-Amino-3,3-bis(hydroxymethyl)-5-bromo-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-8-one (12a) A mixture of **11a** (1.5 g, 3.08 mmol) in methylene chloride (31 mL) was cooled to 0 °C. Boron trichloride (1 M in methylene chloride, 9.9 mL, 9.9 mmol) was added via syringe and under nitrogen gas. The mixture was stirred at 0 °C for 3h. A solution of methanol / methylene chloride (1:1, 20 mL) was added, and the cooling bath was removed. The solution was neutralized with methanolic ammonia. The solvent was removed under reduced pressure and the residue was crystallized with methanol to give the product 0.45 g (48%). Mp 228-229 °C; Rf: 0.31 ($CHCl_3$/MeOH, 5/1); ^1H NMR (DMSO-d_6): δ 7.65, 6.87(s, 1H each, NH_2); 5.23(s, 2 H, NCH_2O); 4.99(t, 2 H, J = 6 Hz, OH); 3.30-3.44(m, 4 H, CH_2O); 2.93(s, 2 H, H_2-4); MS *m/z* 305.6 (M^+); Anal. Calcd. for $C_9H_{12}BrN_3O_4$ (306.09): C, 35.51; H, 3.95; N, 13.73. Found: C, 35.27; H, 3.49; N, 13.57.

6-Amino-3,3-bis(hydroxymethyl)-5-iodo-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-8-one (12b) A mixture of **11b** (0.5 g, 0.94 mmol) in methylene chloride (6 mL) was cooled to 0 °C. Boron trichloride (1 M in methylene chloride, 3 mL, 3 mmol) was added via syringe and under nitrogen gas. The mixture was stirred at 0 °C for 3h. A solution of methanol / methylene chloride (1:1, 10 mL) was added, and the cooling bath was removed. The solution was neutralized with methanolic ammonia. The solvent was removed under reduced pressure and the residue was crystallized with methanol to give the product 0.23 g (68%). Mp 210-211 °C; Rf: 0.25 ($CHCl_3$/MeOH, 4/1); ^1H NMR (DMSO-d_6): δ 7.65, 6.51(s, 1H each, NH_2); 5.25(s, 2 H, NCH_2O); 4.93(t, 2 H, J = 6 Hz, OH); 3.36(m, 4 H, CH_2O); 2.97(s, 2 H, H_2-4); MS *m/z* 353 (M^+); Anal. Calcd. for $C_9H_{12}IN_3O_4$ (353.12): C, 30.61; H, 3.43; N, 11.90. Found: C, 30.94; H, 3.65; N, 11.56.

***In Vitro* Anti-HCMV or Anti-HSV-1 Evaluation.**

(a) Cells and Viruses. Diploid human foreskin fibroblasts (HFF cells) were grown in minimal essential medium (MEM) with Earle's salts [MEM(H)] supplemented with 10% fetal bovine serum. BSC-1 (African green monkey kidney) cells were grown 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 KOS strain of

HSV-1 was provided by Dr. S. K. Weller of University of Connecticut. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[13] The HTLV-IIIB strain of HIV-1 was propagated in the human T-lymphocyte cell line, H9 as detailed elswwhere.[14] The virus inoculum consisted of supernatant fluids from H9-IIIB 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) Cytotoxicity Assays. Two basic tests for cellular cytotoxicity 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 KB cells was determined colorimetrically using a staining assay as described previously.[16]

(d) Data Analysis. Dose-response relationships were constructed by linearly regressing 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, and 2-acetylpyridine thiosemicarbazone for KB cytotoxicity) 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.

***In vitro* Anti-HIV or Antineoplastic Assay.** Compounds were tested as described[17-19] in the NCI *in vitro* HIV or human tumor cell line screen. Data calculations and analyses were performed as described.[17-19]

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