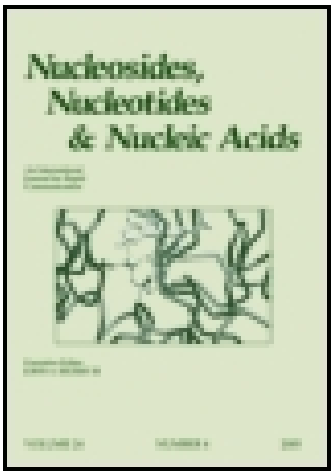

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Synthesis of 1-Hydroxy-10-methyl-pyrimido [1, 6-C][1, 3]oxazine and the Oxazepine Derivative, Structural Mimicry of Anti-Constrained Acyclic Thymidine

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SYNTHESIS OF 1-HYDROXY-10-METHYL-PYRIMIDO [1,6-*C*][1,3]OXAZINE AND THE OXAZEPINE DERIVATIVE, STRUCTURAL MIMICRY OF ANTI-CONSTRAINED ACYCLIC THYMIDINE

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Abstract

A number of pyrimido[1,6-*c*][1,3]oxazine and -oxazepine derivatives, mimicry analogs of anti-constrained acyclic thymidine, have been prepared via treatment of lithiated 5,6-dimethyl-2,4-dimethoxypyrimidine with benzylchloromethyl ether or oxiran to furnish 2,4-dimethoxy-6-(1-benzyloxyethyl)-5-methylpyrimidine (**2**) and 2,4-dimethoxy-6-(1-hydroxypropyl)-5-methylpyrimidine (**8**), respectively. Debenzylation of **2** afforded 2,4-dimethoxy-6-(1-hydroxyethyl)-5-methylpyrimidine (**3**). Chloromethylation of **3** and **8** with paraformaldehyde and gaseous hydrogen chloride produced reactive chloromethyl ether intermediates which were converted to the cyclized products 9-methyl-(1H, 2H, 4H, 7H)-pyrimido[1,6-*c*][1,3]-oxazine (**5**) and -oxazepine (**9**)-6,8-dione, respectively. By using selenium dioxide, allylic oxidation of **5** and **9** afforded the target compounds, a racemic mixture of (±)1-hydroxy-9-methyl-(1H, 2H, 4H, 7H)-pyrimido[1, 6-*c*][1, 3]-oxazine (**6**) and -oxazepine (**10**)-6, 8-dione, respectively. Compounds **5**, **6**, **7**, **9**, and **10** were evaluated for activity against human immunodeficiency virus (HIV), herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). All of these compounds were inactive.

Introduction

Human immunodeficiency virus-1 reverse transcriptase (HIV-1 RT) only being found in the virus-invaded host cell plays a central role in the proliferation of the virus and has been an ideal target for the design of drugs to combat acquired immune deficiency syndrome (AIDS).[1-6] Since the discovery of AZT as a potent inhibitor of HIV, a number of nucleoside analogs such as DDI, DDC and D4T have also been found to be potent inhibitors of HIV.[7-9] For the inhibition, these compounds must be phosphorylated by cellular kinases to their triphosphates which may act as either competitive inhibitors of the natural substrate for RT or growing chain terminators when

incorporated into nascent DNA since they do not possess the 3'-hydroxy group necessary for chain elongation. The high flexibility of the nucleoside conformation is well documented.[10,11] The orientation of the heterocyclic base about the glycosidic bond in nucleosides is an important conformational parameter, and has been clearly delineated in a variety of enzymatic reactions.[12] Recently, 8-amino-2,2-bis(hydroxymethyl)-9-methyl-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-6-one (Figure 1), an anti conformationally restricted acyclic pyrimidine nucleoside, was synthesized and found to have a slight inhibitory activity (IC_{50} = 36 μM) against HIV.[13] This result prompted us to synthesize more conformationally restricted acyclic pyrimidine nucleosides which mimic different glycosyl torsion angles of nucleoside analogs. In this article we describe the synthesis and the conformational features of these newly designed anti restricted acyclic pyrimidine nucleosides.

Results and Discussion

The 2,4-dimethoxypyrimidine bases **1** was synthesized from 5,6-dimethyluracil by chlorination with phosphorus oxychloride and subsequent substitution of the 2- and 4-chloro group with sodium methoxide in methanol. Treatment of **1** with lithium diisopropylamide or n-butyl lithium in tetrahydrofuran at -55 °C (Scheme 1) afforded the lithio intermediates which was reacted, without isolation, with benzylchloromethyl ether to furnish 2,4-dimethoxy-5-methyl-6-(1-benzyloxyethyl)pyrimidine (**2**). Debenzylation of **2** was accomplished by using hydrogen in the presence of 20% palladium on carbon at 50 psi for 14 hours to give 2,4-dimethoxy-5-methyl-6-(1-hydroxyethyl)pyrimidine (**3**). Chloromethylation of **3** with paraformaldehyde and gaseous hydrogen chloride at 0 °C produced reactive chloromethyl ether intermediates. Without isolation, these intermediates was converted to a mixture of cyclized products, 8-methoxy-9-methyl-(1H, 2H, 4H)-pyrimido[1,6-*c*][1,3]oxazine-6-one (**4**), and the 6, 8-dione derivative (**5**). Compound **4** could be converted to **5** at room temperature in methanolic hydrogen chloride solution. To generate a hydroxyl group at the C6 α-methylene of the pyrimidine ring, selenium dioxide was used. However, it has been reported[14,15] that the alcohol derivative, an allylic oxidation product by selenium dioxide, may further oxidize to the carbonyl level. This oxidation may be avoided by a careful choice of the reaction solvent and reaction conditions. Fortunetly, using pyridine as a solvent, compound **5** reacted with selenium dioxide at reflux temperature only a racemic alcohol product (±)-hydroxy-9-methyl-(1H, 2H, 4H, 7H)-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (**6**) was obtained in a yield of 42%. The existence of a hydroxyl group rather than a ketone was established by the presence of an exchangeable proton (D_2O, ^1H nmr: δ, 6.17-6.20 ppm) and a chemical conversion of **6** to the acetate derivative (**7**) with acetic anhydride in the

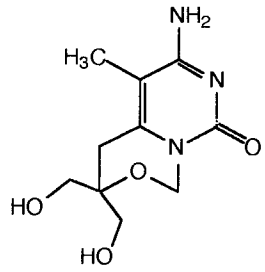
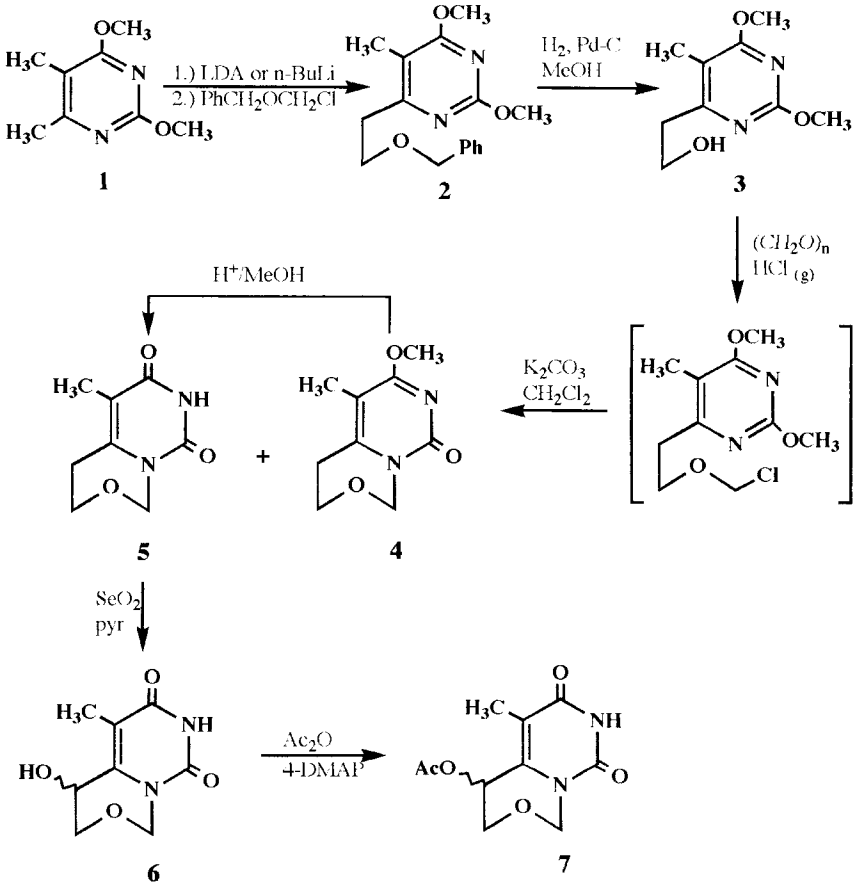
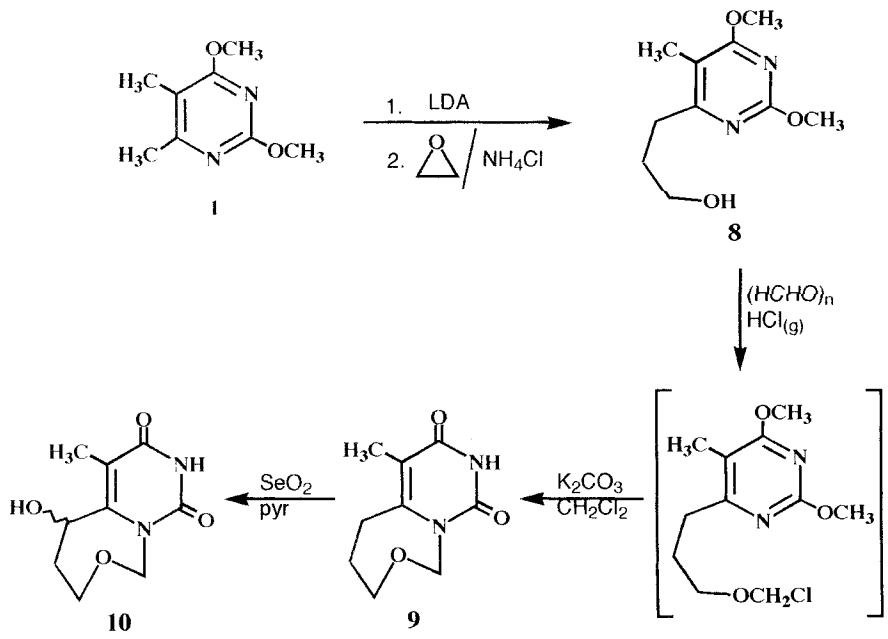


Figure 1. The chemical structure of 8-amino-2,2-bis(hydroxymethyl)-9-methyl-1H,2H,4H-pyrimido[1,6-c][1,3]oxazine-6-one



Scheme 1



Scheme 2

presence of 4-dimethylaminopyridine in pyridine. For the preparation (Scheme 2) of another target compound bearing a different tortion angle, a 7-membered oxazepine ring fused with a pyrimidine base was electected to be synthesized. Oxirane (ethylene oxide), an useful two-carbon homologation reagent was chosen to react with lithiated 2,4-dimethoxy-5,6-dimethylpyrimidine to prepare 2,4-dimethoxy-6-(1-hydroxypropyl)-5-methylpyrimidine (**8**). Chloromethylation of compound **8** with paraformadehyde and dried gaseous hydrogen chloride at 0 °C furnished a very active intermediate which was readily converted to 10-methyl-(1H, 2H, 3H, 5H, 8H)-pyrimido[1,6-*c*][1,3]oxazepine-7,9-dione (**9**) using potassium carbonate in methylene chloride. The yield of compound **9** was very low, only 8%. However, if the reaction was carried out in the presence of a catalytic amount of *tris*(3,6-dioxaheptyl)amine (TDA-1), a phase transfer reagent, the yield raised to 25%. Treatment of **9** with selenium dioxide in pyridine at reflux produced a racemic mixture of (±)1-hydroxy-10-methyl-(1H, 2H, 3H, 5H, 8H)-pyrimido[1,6-*c*][1,3]- oxazepine-7,9-dione (**10**). No evidence of a carbonyl product was observed.

In order to realize the 3D structural similarity of **6** or **10** to thymidine, a theoretical calculation[16-18] on (R)-**6** and (R)-**10** was carried out. Figure 2 shows the

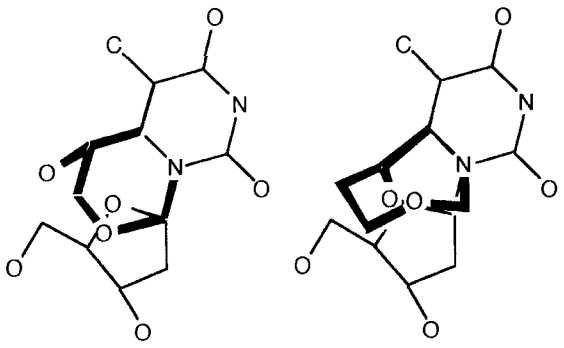


Figure 2. The PM3 optimized conformation of **6** and **10** (bold line). Superposition of **6** or **10** to thymidine. Only the non-hydrogen atoms are shown in the molecules.

superposition of PM3 optimized structure of (R)-**6** or (R)-**10** to thymidine. In the optimized structure of (R)-**6** or (R)-**10**, the pyrimidine ring is a planar system, but the oxazine and oxazepine rings are not planar. In (R)-**6** molecule, the oxygen atom in oxazine ring (corresponding to the O4' of ordinary nucleosides) projects towards the pyrimidine plane, while in (R)-**10** molecule, the oxygen atom in oxazepine ring projects behind the pyrimidine plane. The mimic glycosidic torsion in **6** or **10** is -19° or 66°, respectively, while a 39° is found in the crystal structure of thymidine. Superposition of (R)-**6** or (R)-**10** to thymidine reveal a distance deviation of 4.53 Å and 3.75 Å respectively between the mimic 5'-hydroxy group of (R)-**6** or (R)-**10** and the the 5'-hydroxy group of thymidine.

Target compounds **5**, **6**, **7**, **9**, and **10** were evaluated for activity against human immunodeficiency virus (HIV) and two herpes viruses, herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). All of these compounds were inactive against HIV, HSV-1 or HCMV at the highest concentration tested (100 μM). The lack of antiviral activity in these compounds may be attributed to the improper spatial position of the 5'-hydroxy group mimicry or a secondary hydroxy group instead of primary hydroxy group usually found in ordinary nucleosides.

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, USA).

Compounds were visualized by illuminating under UV light (254 nm). Evaporations were carried out at < 50 °C using a rotary evaporator at reduced presdure (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 obtained 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 hrs unless otherwise specified. Elemental analyses were obtained from M-H-W Laboratories, Phoenix, AZ.

2,4-Dimethoxy-5-methyl-6-(1-benzyloxyethyl)pyrimidine (2). Lithium diisopropylamide (1.5 M in n-hexane, 37 mL, 55 mmol) was added dropwise to a solution of 2,4-dimethoxy-5,6-dimethylpyrimidine[19] (8.41 g, 50 mmol) in tetrahydrofuran (200 ml) at -70 °C. The temperature was warmed to -55 °C and the solution was stirred for 30 min. Benzylchloromethyl 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 chloroform and water, the organic layer was separated and dried over sodium sulfate. 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/ethyl acetate (6/1). The fractions (R_f = 0.24, n-hexane/ethyl acetate = 5/1) containing product were collected and evaporated to give 13.19 g (90%) of **2** as an oily product; IR (neat): 2957, 2864, 1582, 1450, 1370, 1211, 1118, 1085 cm^{-1}. ^{1}H nmr (DMSO-d6): 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_2-6); 2.05(s, 3 H, CH_3). Anal. Calcd. for $C_{16}H_{20}N_2O_3$: C, 66.64; H, 6.99; N, 9.7. Found: C, 66.70; H, 7.02; N, 9.68.

2,4-Dimethoxy-5-methyl-6-(1-hydroxyethyl)pyrimidine (3). Compound **2** (3.16 g, 11 mmol) was treated with 20% palladium on carbon (0.5 g) in methanol (50 mL) at 50 psi of hydrogen for 20 h. The reaction mixture was passed through a Celite bed and the filtrate was concentrated under reduced pressure. The oily product was collected and evaporated to give 2.08 g (95%) of **3**. R_f: 0.47 (n-hexane / ethyl acetate/isopropanol = 6/1/1). IR (neat): 3668, 2957, 2871, 1583, 1457, 1370, 1211, 1125, 739, 699 cm^{-1}. ^{1}H NMR (DMSO-d6): δ 4.62(t, 1 H, J=5.6 Hz, OH); 3.87(s, 3 H, OCH_3); 3.82(s, 3 H, OCH_3); 3.69(q, 2 H, CH_2O); 2.75(t, 2 H, J=6.8 Hz, CH_2-6); 2.00(s, 3 H, CH_3). Anal. Calcd. for $C_9H_{14}N_2O_3$: C, 54.53; H, 7.12; N, 14.14. Found: C, 54.30; H, 7.17; N, 13.95.

8-Methoxy-9-methyl-(1H, 2H, 4H)-pyrimido[1,6-*c*][1,3]oxazine-6-one (4) and 9-Methyl-(1H, 2H, 4H, 7H)-pyrimido[1,6-*c*][1,3]oxazine-6,8-dione (5). To a mixture of **3** (4.78 g, 24.1 mmol), paraformaldehyde (2.0 g) in dichloromethane (16 mL) stirred vigorously in an ice bath under protection from moisture hydrogen chloride gas was passed rapidly through the solution for 5 minutes and then at slow rate for an additional 4 h. The flask containing the mixture was sealed tightly and allowed to stand below 0 °C for 42 h. The solution was then dried over calcium chloride and after filtration, the solvent was removed under reduced pressure. The oily residue was dissolved in dichloromethane (400 mL) and to this mixture was added finely ground potassium carbonate (0.8 g). The reaction mixture was stirred for 12 h at rt under under argon atmosphere. After filtration, the solvent was removed and the residue was coevaporated with methanol and silica gel. The residue was applied to a column (12 x 14 cm, 350 g of silica gel) and eluted with chloroform/ethyl acetate (9/1). The desired fractions (R_f = 0.2) were collected and concentrated *in vacuo* to give **4**, 392 mg (8%). Mp 197-198 °C; R_f: 0.2 (chloroform/ ethyl acetate = 9/1); UV λ_{max} nm (logε): methanol: 285(3.95); pH 1: 283(3.93); pH 11: , 283(3.99); IR (KBr): 2931, 1680, 1659, 1546, 1377, 1145, 780 cm^{-1}. ^1H nmr ($CDCl_3$): δ 5.31(s, 2H, OCH_2N); 3.99(t, 2H, J=6.2 Hz, CH_2O); 3.94(s, 3H, OCH_3); 2.79(t, 2H, H_2-1) ;1.87(s, 3H, CH_3). Anal. Calcd. for $C_9H_{12}N_2O_3$: C, 55.09; H, 6.17; N, 14.28. Found: C, 55.26; H, 6.14; N, 14.30.

The fractions (R_f = 0.5, chloroform/methanol = 9/1) were collected and concentrated *in vacuo* to give **5**, 632 mg (14%). Mp 221-222 °C; UV λ_{max} nm (logε): methanol: 274(4.11); pH 1: 275(4.11); pH 11: , 272(4.07); IR (KBr): 3142, 3079, 3001, 2875, 2826, 1694, 1644, 1490, 1427, 1172, 892 cm^{-1}. ^1H NMR (DMSO-d_6): d 11.28(s, 1 H, NH); 5.12(s, 2 H, OCH_2N); 3.94(t, 2 H, J=6.4 Hz, CH_2O); 2.78(t, 2 H, H_2-1) ;1.72 (s, 3 H, CH_3). Anal. Calcd. for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38; Found: C, 52.90; H, 5.69; N, 15.10.

Compound **4** (200 mg) could also be converted to **5** by treatment with concentrated HCl 2 ml) in methanol (10 ml).

(±) 1-Hydroxy-9-methyl-(1H, 2H, 4H, 7H)-pyrimido[1,6-*c*][1,3] oxazine-6,8-dione (6). A mixture of **5** (0.9 g, 4.9 mmo), selenium dioxide (1 g, 7.2 mmol) in pyridine (30 mL) was heated at reflux temperature for 6 h. The reaction mixture was passed through a Celite bed and the filtrate was concentrated *in vacuo* and then coevaporated with toluene. The residue was absorbed on a small amount silica gel, applied to a column (12 x 14 cm, 350 g of silica gel) and eluted with chloroform/methanol (95/5). The desired fractions (R_f = 0.27, chloroform/methanol = 9/1) were collected and evaporated to give 405.3 mg (42%) of **6**. Mp 190-191 °C;

IR(KBr): 3369, 3150, 3017, 2805, 1702, 1649, 1483, 1072, 792 cm^{-1}; ^1H NMR (DMSO-d6): δ 11.40(s, 1 H, NH); 5.94(d, 1 H, J=6.1 Hz, OH) 5.25(d, 1 H, J=8.8 Hz, OCH_2N), 5.00(d, 1 H, J=8.8 Hz, OCH_2N); 4.60(m, 1 H, H-1); 3.94(m, 2 H, CH_2O); 1.84 (s, 3 H, CH_3). Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.37; H, 5.08; N, 14.29.

(±)1-Acetoxy-9-methyl-(1H, 2H, 4H, 7H)-pyrimido[1,6-*c*][1,3]-oxazine-6,8-dione (7). A mixture of **6** (0.29 g, 1.5 mmol), 4-dimethylaminopyridine(30 mg), and acetic anhydride(10 ml) was stirred at room temperature for 14 h. A small amount of ice-water was added to the mixture. After filtration the solid product was collected and recrystallized with methanol to give 13.9 mg of a solid **7**. The filtrate was coevaporated with silica gel and the residue was applied to a column (3 x 10 cm, 15 g) and eluted with chloroform/methanol (95/5). The desired fractions (R_f: 0.26, chloroform/ethyl acetate = 1/2) were collected and evaporated to provide additional 132.4 mg (total 146.3 mg, 41%) of **7**. Mp 213-215 °C; IR(KBr): 3177, 3107, 3044, 2812, 1715, 1659, 1483, 1244 cm^{-1}; ^1H NMR (DMSO-d6): δ 11.59(s, 1 H, NH); 5.76(brs, 1 H, H-1); 5.38(d, 1 H, J=8.9 Hz, OCH_2N); 4.92(d, 1 H, J=8.9 Hz, OCH_2N); 4.12-3.98(m, 2 H, CH_2O); 2.09(s, 3 H, CH_3CO); 1.70 (s, 3 H, CH_3). Anal. Calcd. for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.86; H, 5.06; N, 11.86.

2,4-Dimethoxy-6-(1-hydroxypropyl)-5-methylpyrimidine (8). 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 addition of LDA (1.5 M in n-hexane, 10 mL, 15 mmol) to 2,4-dimethoxy-5,6-dimethyl-pyrimidine (1.68 g, 10 mmol) in THF. The mixture was allowed to warm to rt and then hydrolysed with saturated ammonium chloride solution. The solvent was removed *in vacuo*. The residue was partitioned between chloroform and water. The organic layer was washed with saturated sodium chloride solution and water and then dried over magnesium sulfate. The solvent was removed and the residue was chromatographed on silica gel (50 g, 3 x 20 cm column) with $CHCl_3$/AcOEt (9/1). The desired fractions (R_f = 0.31, $CHCl_3$/AcOEt = 1/1) were collected and evaporated to give an oil like product (1.21 g, 57%); IR (KBr): 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=7Hz, CH_2-6); 2.02 (s, 3 H, CH_3-5); 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.

10-Methyl-(1H, 2H, 3 H, 5H, 8H)-pyrimido[1,6-*c*][1,3]oxazepine -7,9-dione (9). A mixture of **8** (2.8 g, 13 mmol), paraformaldehyde (1.8 g) and dichloromethane (30 mL) was stirred vigorously in an ice bath under protection from moisture. Hydrogen chloride gas was passed through this mixture rapidly until a clear solution appeared and then at slow rate for 3 h. The solution was allowed to stand at 0 °C for 12 h and then dried over calcium chloride. After filtration, under an argon atmosphere, the solvent was removed. To the oily residue dissolved in dichloromethane (100 mL) was added finely ground potassium carbonate (0.9 g) and tris(3,6-dioxaheptyl)amine (TDA-1, 0.16 mL) was added. The reaction mixture was stirred for 3 h. under argon at room temperature and then passed through a filter with Celite bed. The solvent was removed. The residue was coevaporated with methanol and silica gel (2 g). This material was then applied to a column (3.2 x 20 cm, 30 g of silica gel) and eluted with $CHCl_3$/AcOEt = 9/1. The desired fractions (R_f = 0.36, CH_2Cl_2/MeOH = 20/1) were collected and evaporated to give the product (648 mg, 25%). Mp 246-248 °C; UV λ_{max} nm (logε): MeOH: 269(4.05); pH 1: 270(4.10); pH 11: 219(4.71); 270(4.08); IR (KBr): 3170, 3030, 1689, 1623, 1457, 1111, 1045 cm^{-1}. ^1H NMR (DMSO-d_6): δ 11.28(s, 1 H, NH); 5.39(s, 2 H, OCH_2N); 3.80(t, 2 H, J=5.1 Hz, CH_2O); 2.94(t, 2 H, J=5.3 Hz, CH_2-1); 1.84(s, 3 H, CH_3-10); 1.76(m, 2 H, C-CH_2-C). Anal. Calcd. for $C_9H_{12}N_2O_3$ (196.21): C, 55.09; H, 6.17; N, 14.28. Found: C, 55.19; H, 6.33; N, 14.04.

(±)1-Hydroxy-10-methyl-(1H, 2H, 3H, 5H, 8H)-pyrimido[1,6-*c*][1,3]oxazepine-7,9-dione (10). A mixture of **9** (0.648 g, 2.86 mmol), selenium dioxide (0.5 g, 4.5 mmol) and pyridine (10 mL) was heated at reflux temperature for 4 h. The reduced selenium dioxide was removed by filtration through a Celite bed filter. The filtrate was concentrated and then coevaporated with toluene *in vacuo*. The residue was absorbed on a small amount silical gel (1.5 g), then applied to a column (3.2 x 20 cm, 20 g of silica gel) and eluted with CH_2Cl_2/MeOH = 20/1. The desired fractions (R_f = 0.33, $CHCl_3$/AcOEt = 1/1) were collected and evaporated to give the product (0.44 g, 72%). Mp 222-224 °C; UV λ_{max} nm (logε): MeOH: 270(4.02); pH 1: 271(4.00); pH 11: 218(4.73); 271(4.00). IR (KBr): 3389, 3196, 3050, 1709, 1663, 1477, 1337, 1111, 978, 779, 726 cm^{-1}. ^1H-NMR (DMSO-d_6): δ 11.35(s ,1 H, NH); 6.13(brs, 1 H, OH); 5.87(d, 1 H, J=12.0 Hz, OCH_2N); 5.26(d, 1 H, J=12.0 Hz, OCH_2N); 5.22(brs, 1 H, CH-1); 3.93(t, 1 H, J=12 Hz, CH_2O); 3.77(q, 1 H, J=12 Hz, J=3 Hz, CH_2O); 1.98(s, 3 H, CH_3-10); 1.91-1.81(brs, 2 H, C-CH_2-C). Anal. Calcd. for $C_9H_{12}N_2O_4$ (212.21): C, 50.94; H, 5.70; N, 13.20. Found: C, 50.80; H, 5.70; N, 12.97.

***In Vitro* Antiviral Evaluation. (a) Cells and Viruses.** Diploid human foreskin fibroblasts (HFF cells) were grown in 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[20]. 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[20]. The HTLV-IIIB strain of HIV-1 was propagated in the human T-lymphocyte cell line, H9 as detailed elswwhere. 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[20] 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[21].

(C) Antiviral Assay for HIV. HIV was assayed using the XTT colorimetric technique as detailed previously[22-24]. 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 TCDI50/cell in MT-2 cells and approximately 0.12 TCID50/cell in CEM cells. Fresh medium was added to adjust the cell density to 1×10^5 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 supernants were discarded. Infected and uninfected cells were resuspended in an appropriate volumn of medium and added to plates in the amount of 100 μL/well to give a starting cell number of 1×10^4 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 plats. 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 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.[20] Cytotoxicity in CEM and MT2 cells was determined colormetrically using the XTT[22] assay as described above for HIV except uninfected cells were used.

(e) 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 containning positive controls (acyclovir for HSV-1, gancicclovir 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.

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REFERENCES

1. Hirsch, M. S. *J. Infec. Dis.* **1990**, *161*, 845-857.
2. Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911-1915.
3. Sandstrom, E. G.; Kaplan, J. C.; Byington, R. E.; Hirsch, M. S. *Lancet* **1985**, 1480-1481.
4. Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Gelder, J. V.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; Clercq, E. D.; Janssen, P. A. J. *Nature* **1990**, *343*, 470-474.
5. Nakane, H.; Ono, K. *Biochemistry* **1990**, *29*, 2841-2845.
6. Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C. K.; Eckner, K.; Hattox, S.; Adams, J.; Rosehthal, A. S.; Faanes, R.; Eckner, J.; Koup, R. A.; Sullivian, J. L. *Science* **1990**, *250*, 1411-1413.

7. Hao, Z.; Cooney, D. A.; Hartman, N. R.; Perno, C. F.; Fridland, A.; DeVico, A. L. S.; Broder, S.; John, D. G. *Mol. Pharmacol.* **1988**, *34*, 431-435.
8. Mansuri, M. M.; Starrett, Jr. J. E.; Ghazzouli, I.; Hitchcock, M. J. M.; Sterzycki, R. Z.; Brankovan, V. M.; Lin, T.-S.; August, E. M.; Prusoff, W. J.; Sommadossi, J. P.; Martin, J. C. *J Med .Chem.* **1989**, *32*, 461-466.
9. Hart, G. J.; Orr, D. C.; Penn, C. R.; Figueiredo, H. T.; Gray, N. M.; Boehme, R. E.; Cameron, J. M. *Antimicrob. Agents Chemother.* **1992**, *36*, 1688-1694.
10. Sundaralingam, M. In *Structure and Confromation of Nucleic Acid and Protein-Nucleic Acid Interactions*; Sundaralingam, M., Rao, S. T., Eds.; University Park Press: Baltimore, 1975, pp 487-536.
11. Saenger, W., Principles of Nucelic Acid Structure, Springer, New York, 1984.
12. Birbaum, G. I. and Shugar, D. Nucleic Acid Structure Part 3, In *Topics in Molecular and Structural Biology*, S. Neidle, Ed.; VCH Publishers: New York, 1987, pp 1-70.
13. Hsu, L.-Y; Wise, D. S.; Kucera L. S.; Drach, J. C.; Townsend, L. B. *J. Org. Chem.* , **1992**, *57*, 3354-3358.
14. Klein, R. S.;Fox, J. J. *J. Org. Chem.* , **1972**, *37*, 4381-4386.
15. Matsuda, A.; Tezuka, M.; Niizuma, K.; Sugiyama, E.; Ueda, T.*Tetrahedron* , **1978**, *34*, 2633-2637.
16. Calculation was carried out on a Silicon Graphics IRIS Indigo workstation. Total geometry optimization was determined by a semi-empirical quantum chemistry method PM3[17] in the MOPAC 6.0 package.[18] The main torsion angle (C6-N1-C1'-O4') was measured from the cis (eclipsed, 0°) position and were positive or negative for the right- or left-hand rotation, respectively.
17. (a) Stewart, J. J. P. *J. Comput. Chem.* **1989**, 10, 209-220. (b) Stewart, J. J. P. *J. Comput. Chem.* **1989**, 10, 221-231.
18. Stewart, J. J. P., Seiler, F. J. *MOPAC manual*, **1990**, 6th ed.; Research Laboratory, Air Force Academy: Boulder, CO.
19. Hull, R.; Lovell, B. J.; Openshaw, H. T.; Payman, L. C.; Todd, A. R. ?. *J. Chem. Soc.*, **1946**, 357-362.
20. Turk, S. R.; Shipman, Jr. C.; Nassiri, R.; Genzlinger, G.; Krawczyk, S. H.; Townsend, L. B.; Drach, J. C. *Antimicrob. Agents and Chemother.*, **1987**, *31*, 544-550.
21. Pirchard, M. N. and Shipman, Jr., C. *Antiviral Res.*, **1990**, *14*, 181-205.
22. Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader J.; Shoemaker, R. H.; Boyd, M. R. *J. Nat. Cancer Inst.*, **1989**, *81*, 577-586.

23. White, E. L.; Buckheit, R. W.; Jr., Ross, L. T.; Germany, J. M.; Andries, K.; Pauwels, R.; Janssen, P.A.J.; Shannon, W. M.; Chirigos, M. A. *Antiviral Res.*, **1991**, *16*, 257-266.
24. Swayze, E. E.; Shannon, W. M.; Buckheit, R. W.; Worting, L. L.; Drach, J. C.; Townsend, L. B. *Nucleosides & Nucleotides*, **1992**, *11*, 1507-1527.

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