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Design, Synthesis, and Antiviral Activity of Novel Phosphoramidates

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Novel phosphoramidates **8a-b** possessing structural features similar to nucleoside phosphonates HPMPA, PMEA, PMEG, and oxathiolane nucleoside BCH-189 were synthesized by chemical methods. The designed molecules exhibited significant antiviral activities. We hypothesized that, as a masked membrane-soluble form of the potential bioactive compounds **7a-b**, phosphoramidates **8a-b** may act as a proteinase substrate. Then, the potential drug **7a-b** will be liberated inside the infected cells. The triphosphate anabolites of each could act as competitive substrates for the HIV reverse transcriptase and terminate DNA synthesis after being incorporated into the growing DNA strand. Phosphoramidates **8a-b** were synthesized as racemates to allow access to both enantiomers.

Keywords: Nucleosides, Nucleotides, Phosphoramidates, Antivirals, Anti-HIV, Oxathiolane

INTRODUCTION

The development of novel selective antiviral agents for the treatment of hepatitis B and AIDS remains an important objective in medicine. Nucleoside and nucleotide analogs continue to provide a rich source of antiviral agents that can suppress or eliminate viral infections [1-6]. Most of these nucleoside analogs are 2',3'-dideoxynucleosides [7-9], including oxathiolane nucleosides (i.e., (2'*RS*,5'*RS*)-1-[2-(hydroxymethyl)oxathiolane-5-yl]cytosine (1)) [10a]. The dideoxynucleosides are not active in their native form. After membrane penetration, intracellular conversion of the nucleoside analogs to their 5'-triphosphates by host cell kinases is a prerequisite for the expression of the biological activity [7,11b,11c].

The general mode of action of these anabolites is the inhibition of reverse-transcriptase of the human immunodeficiency virus (HIV) [11]. They can also incorporate into the growing DNA chain, resulting in DNA chain termination [12]. In spite of great promise for the inhibition of viral replication, their full therapeutic efficacy is limited by several shortcomings. One problem is the limited ability of some dideoxynucleosides to undergo biotransformation catalyzed by host cell kinases into the triphosphate due to structural differences as compared to natural nucleosides [13]. Consequently, the rate and extent to which the nucleoside analogs are converted to their bioactive triphosphates may be as important as the affinity of these compounds for the target enzyme [14]. Monocytes and macrophages, which harbor high virus amounts, have very low levels of nucleoside kinases. Thus, the presence and activity of intracellular enzymes necessary for the phosphorylation of nucleosides are highly dependent on the host species, the cell type, and the stage in the cell cycle.

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The rate-limiting step in the activation process of certain dideoxynucleosides (i.e., d4T) is the first phosphorylation by the host cell thymidine kinase [14]. The eventual conversion to the ultimate bioactive triphosphates, as performed by thymidylate kinase and nucleoside diphosphate kinase, is not rate-limiting. Consequently, the direct application of the 2',3'-dideoxynucleoside monophosphates would bypass the metabolization-limiting thymidine kinase (TK-bypass) and could thus, in principle, improve the therapeutic potential of the drug. Phosphonates of purines have been utilized as excellent antiviral agents. These include (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (HPMPA), 9-[2-phosphonomethoxy)ethyl]-adenine (PMEA), and 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) [15].

3'-Heteroatom-substituted dideoxynucleosides, in which the 3'-CH_2 group is replaced by a sulfur, selenium, or oxygen atom (i.e., BCH-189) [10,16], have proven valuable and potent antiviral agents. These compounds exhibit potent activity toward HBV and HIV and are the most promising candidates for hepatitis B chemotherapy [6]. We synthesized nucleoside analogs **5a-b** and their respective nucleotides **7a-b**, which possess structural features similar to oxathiolane nucleosides (i.e., BCH-189).

A well-known problem in antiviral therapy is the bioavailability of nucleoside monophosphate analogs [1,14]. The ability of a drug to penetrate cell membrane and exhibit biological activity is correlated to its lipophilicity. Consequently, lipophilic prodrugs are expected to display superior antiviral activity. On the other hand, McGuigan *et al*. [17] suggested that HIV-aspartate proteinase [18] may recognize phosphoamidate derivatives of certain nucleosides and thus can specifically hydrolyze these membrane-soluble prodrugs. The resulting bioactive nucleotides would then be liberated inside the infected cells and act as potent inhibitors of viral proliferation. Accordingly, we synthesized phosphoramidates **8a-b** to explore this possible avenue towards combating viral infections.

Because naturally occurring nucleosides are in the β-D configuration, most nucleoside analogs designed for the treatment of cancer and viral diseases have also been synthesized in this stereochemical form. The discovery that L-enantiomer of BCH-189 had more potent anti-HBV and anti-HIV activity and less cytotoxicity than its D-enantiomer was the first indication that L-nucleoside analogs could have therapeutic potential [16]. As such, both enantiomers of the designed compounds **8a-b** were synthesized and evaluated for biological activity.

EXPERIMENTAL

General

For anhydrous reactions, glassware was dried overnight in an oven at 120 ºC and cooled in a desiccator over anhydrous $CaSO_4$. Reagents purchased from Sigma (St. Louis, USA) or Fluka (Switzerland). Solvents were reagent grade and purchased from Merck (Germany) and used as received.

Melting points were obtained with a Buchi 510 melting point apparatus. ^{1}H NMR and/or ^{13}C NMR spectra were obtained on a Varian XL-300 (300 MHZ) spectrometer. Mass spectra were carried out on a Finnigan MAT95XP mass spectrometer (Thermo Scientific).

Purification on silica gel refers to gravity column chromatography on Merck silica gel 60 (particle size 230-400 Mesh). Analytical TLC was performed on precoated plates purchased from Merck (Silica gel 60 F_{254}). Compounds were visualized by use of UV light.

Synthetic Procedures

The compounds **2a-8a** and **2b-8b** were synthesized based on the following procedures.

1-Chloro-3-mercapto-propan-2-ol (2a). Epichlorohydrine (**1a**, 18.5 g, 0.2 mol), and thioacetic acid (15.2 g, 0.2 mol) were placed in a 50 ml two-neck round bottom flack equipped with stirrer and reflux condenser. The reaction mixture was stirrer at 60ºC for 12 h. The reaction mixture then was cooled to room temperature. Distillation under vacuum gave (25.3 g, 0.15 mol, 75% yield) of α-acetylthiochlorohydrin. b.p. = 100-105 ºC/$_{5\text{-}6\ mm}$. α-Acetylthiochlorohydrin (25.3 g, 0.15 mol) in 50 ml MeOH containing 1% HCl were stirrered for 6 h at 60ºC. The reaction mixture then was cooled to room temperature. MeOH removed and the residue was purified by distillation under vacuum to give (13.7 g, 0.108 mol, 72% yield) of **2a**. b.p. = 60-65 ºC/$_{4\ mm}$. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.90-3.85 (m, 1H, CH); 3.69-3.64 (dd, J = 5.4 Hz, J = 5.2 Hz, 2H, CH_2Cl); 2.77-2.71 (dddd, J = 3.4 Hz, J = 3.9 Hz, J = 3.8 Hz, J = 3.6 Hz, 2H, CH_2S); 2.32

(br, 1H, OH); 1.50-1.44 (t, J = 8.9 Hz, 1H, SH).

(S,R) *cis*- and (S,R) *trans*-Benzoic acid 5-chloromethyl [1,3]oxathiolan-2-ylmethyl ester (3a). A solution of benzoyloxyacetaldehyde (2.46 g, 15 mmol), 1-chloro-3-mercapto-propan 2-ol (2.28 g, 18 mmol), of *p*-TsOH (0.1 g) in benzene (60 ml) was stirred and refluxed until the starting aldehyde has been completely used (8 h). The reaction mixture was then cooled to room temperature and washed once with 100 ml aq. Na_2CO_3 solution (2%) and twice with 50 ml of water. The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated in vacuum, and the crude product was purified by column chromatography on silica gel, using hexane-EA (20:1) to give (2.65 g, 9.75 mmol, 65% yield) of **3a** as a colorless liquid, diasteromer ratio (*cis:trans* = 2:1). Separation of diastereoisomers to respective *cis* and *trans* were done using column chromatography on silica gel (hexane-EA 50:1 as eluant) to give (1.77 g, 6.5 mmol) of (S,R) *cis*-benzoic acid 5-chloromethyl[1,3]oxathiolan-2-ylmethyl ester. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.06-8.03 (m, 2H, C_6H_5); 7.58-7.40 (m, 3H, C_6H_5); 5.50-5.46 (t, J = 5.0 Hz, 1H, H-2); 4.50-4.48 (d, J = 5.2 Hz, 2H, CH_2OBz); 4.35- 4.27 (m, 1H, H-5); 3.74-3.60 (dddd, J = 5.0 Hz, J = 4.9 Hz, J = 6.7 Hz, J = 6.9 Hz, 2H, CH_2Cl); 3.20-2.92 (ddddd, J = 5.0 Hz, J = 5.0 Hz, J = 8.5 Hz, J = 8.4 Hz, 2H, CH_2S)), and (0.88 g, 3.2 mmol) of (S,R) *trans*-benzoic acid 5-chloromethyl[1,3] oxathiolan-2-yl methyl ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.06-8.02 (m, 2H, C_6H_5); 7.58-7.40 (m, 3H, C_6H_5); 5.56-5.52 (dd, J = 4.0 Hz, J = 3.6 Hz, 1H, H-2); 4.61-4.53 (m, 1H, H-5); 4.53-4.27 (dddd, J = 7.6 Hz, J = 7.5 Hz, J = 4.1 Hz, J = 3.8 Hz, 2H, CH_2OBz); 3.63-3.58 (dd, J = 2.1 Hz, J = 2.2 Hz, 2H, CH_2Cl); 3.24-3.00 (dddd, J = 5.5 Hz, J = 5.5 Hz, J = 6.1 Hz, J = 6.0 Hz, 2H, CH_2S).

(S,R) *cis*- and (S,R) *trans*-Benzoic acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-ylmethyl ester (4a). To a stirred mixture of cytosine (266.4 mg, 2.4 mmol) and K_2CO_3 (1.38 g, 10 mmol) in DMF (20 ml) was added dropwise a solution of **3a** (545 mg, 2 mmol) in 4 ml DMF under N_2 over period of 15 min at 60 ºC. The resulting mixture was stirred under N_2 for 12 h at 100 ºC. The reaction mixture was then cooled to room temperature, and was filtered from insoluble inorganic salts, washed with DMF, and then DMF was evaporated in vacuum. The oily residue was extracted with $CHCl_3$, and concentrated in vacuum. The crude residue was purified by column on silica gel using CH_2Cl_2-MeOH (10:1) to give (347 mg, 1 mmol, 50% yield) alkylated product **4a** as white crystals. For (S,R) *cis*-Benzoic acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-ylmethyl ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.00-7.94 (m, 2H, C_6H_5); 7.56-7.37 (m, 3H, C_6H_5); 7.29-7.26 (d, J = 7.2 Hz, 1H, =CH-N); 5.69-5.67 (d, J = 7.2 Hz, =CH-); 5.38-5.35 (dd, J = 4.2 Hz, J = 4.2 Hz, 1H, H-2); 4.53-3.72 (m, 5H, H-5, CH_2OBz, CH_2N); 3.12-3.07 (dd, J = 4.8 Hz, J = 4.9 Hz, 1H, CH_2S), 2.77-2.70 (t, J = 9.8 Hz, 1H, CH_2S). For (S,R) *trans*-benzoic acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-ylmethyl ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.99-7.94 (m, 2H, C_6H_5); 7.56-7.37 (m, 3H, C_6H_5); 7.24-7.22 (d, J = 7.2 Hz, 1H, =CH-N); 5.74-5.71 (d, J = 7.3 Hz, =CH-); 5.49-5.45 (dd, J = 3.9 Hz, J = 3.7 Hz, 1H, H-2); 4.62-4.53 (m, 1H, H-5); 4.53-4.11 (m, 4H, CH_2OBz, CH_2N); 3.17-2.75 (dddd, J = 5.1 Hz, J = 5.0 Hz, J = 6.0 Hz, J = 5.9 Hz, 2H, CH_2S).

(S,R) *cis*- and (S,R) *trans*-2-Hydroxymethyl 5-(cytosin-1^1-ylmethyl) 1,3-oxathiolane (5a). To a stirred solution of **4a** (347 mg, 1 mmol) in 5 ml MeOH was added a solution of NaOH (200 mg) in 15 ml MeOH. The resulting mixture was stirred for 1 h at room temperature. To the reaction mixture was added 20 ml H_2O, and extracted with $CHCl_3$ + MeOH (2:1, 100 ml), and then organic phase was dried over $MgSO_4$. The filtrate was concentrated in vacuum. Crude product was purified by column on silica gel, using $CHCl_3$-MeOH (5:1) to give (206.5mg, 0.85 mmol, 85% yield) of **5a** as white crystals. For (S,R) *cis*-2-hydroxymethyl 5-(cytosin-1^1-ylmethyl) 1,3-oxathiolane: m.p.: 185-187 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) = 7.53-7.51 (d, J = 7.2 Hz, 1H, =CH-N); 7.11-7.02 (br, 2H, NH_2); 5.65-5.62 (d, J = 7.1 Hz, 1H, =CH-); 5.11 (br, 1H, OH); 5.08-5.04 (t, J = 5.4 Hz, 1H, H-2); 4.24-4.13 (m, 1H, H-5); 4.01-3.75 (dd, J = 3.7 Hz, J = 3.7 Hz, J = 7.0 Hz, J = 7.0 Hz, 2H, CH_2N); 3.65-3.42 (m, 2H, CH_2OH); 3.04-2.62 (dd, J = 5.3 Hz, J = 5.3 Hz, 1H, CH_2S) 2.77-2.70 (t, J = 9.9 Hz, 1H, CH_2S); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) = 166.22; 156.12; 146.80; 93.39; 85.78; 79.75; 64.84; 50.36; 33.50. HRMS-FAB: calcd. for $C_9H_{13}O_3N_3S$ $(M+H)^+$, 244.0756; found: 244, 0754. For (S,R) *trans*-2-hydroxymethyl 5-(cytosin-1^1-ylmethyl) 1,3-oxathiolane: m.p.: 173-175 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 7.52-7.50 (d, J = 7.2 Hz, 1H, =CH-N); 7.08-7.00 (br, 2H, NH_2); 5.64-5.62 (d,

J = 7.0 Hz, 1H, =CH-); 5.24-5.20 (dd, *J* = 5.0 Hz, *J* = 5.1 Hz, 1H, H-2); 5.09 (br, 1H, OH); 4.54-4.46 (m, 1H, H-5); 3.89-3.73 (dddd, *J* = 4.2 Hz, *J* = 4.1 Hz, *J* = 7.6 Hz, *J* = 7.6 Hz, 2H, CH_2N); 3.55- 3.29 (m, 2H, CH_2OH); 3.07-2.72 (dddd, *J* = 5.8 Hz, *J* = 5.9 Hz, *J* = 5.7 Hz, *J* = 5.8 Hz, 2H, CH_2S). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) = 166.03; 155.90; 146.61; 93.23; 81.49; 64.30; 50.68; 33.35. HRMS-FAB: calcd. for $C_9H_{13}O_3N_3S$ $(M+H)^+$, 244.0756; found: 244.0755.

(S,R) *cis*- and (S,R) *trans*-Phosphoric acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-yl methyl ester diphenyl ester (6a). To a solution of **5a** (121.5 mg, 0.5 mmol) and 1 ml of NMI in 5 ml CH_2Cl_2, was added a solution of diphenylchlorophosphate (134.5 mg, 0.5 mmol) in 5 ml CH_2Cl_2 with vigorous stirring at -30 ºC. The reaction mixture then warmed-up to room temperature and was stirred for 6 h. Methanol (1 ml) was added, and the solvents removed under vacuum. The residue was dissolved in 30 ml chloroform and washed with 50 ml Na_2CO_3 solution (2%) and twice with 50 ml of water. The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated in vacuum, and the crude product was purified by column chromatography on silica gel using CH_2Cl_2-MeOH (10:1) to give (164.0 mg, 0,32 mmol, 64% yield) of **6a** as colorless crystals. For (S,R) *cis*-phosphoric acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-yl methyl ester diphenyl ester: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.33-6.95 (m, 11 H, C_6H_5 + =CH-N); 5.64-5.62 (d, *J* = 7.0 Hz, 1H, =CH-); 5.26-5.24 (t, *J* = 4.7 Hz, 1H, H-2); 4.38-4.17 (m, 4H, H-4, CH_2N, CH_2OP); 3.64-3.58 (dd, *J* = 7.3 Hz, *J* = 7.2 Hz, 1H), 3.06-3.01 (dd, *J* = 4.5 Hz, *J* = 4.5 Hz, 1H, CH_2OS), 2.67-2.60 (t, *J* = 10.0 Hz, 1H, CH_2S). For (S,R) trans-phosphoric acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-ylmethyl ester diphenyl ester: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.30-6.99 (m, 11H, C_6H_5 + =CH-N); 5.76-5.73 (d, *J* = 7.1 Hz, 1H, =CH-); 5.26-5.24 (t, *J* = 3.9 Hz, 1H, H-2); 4.46-3.85 (m, 5H, H-4, CH_2N, CH_2OP); 3.10-3.72 (dddd, *J* = 5.5 Hz, *J* = 5.4 Hz, *J* = 5.8 Hz, *J* = 5.7 Hz, 2H, CH_2OS).

(S,R) *cis*-Phosphoric acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-yl methyl ester phenyl ester (7a). To a mixture of **6a** (119 mg, 0.25 mmol) in 5 ml water was added a solution of (315 mg, 1 mmol) of hydrated $Ba(OH)_2$ in 5 ml water, and the reaction mixture was shaken for a few minutes until the reaction mixture had become almost clear. The reaction mixture was neutralized to pH = 4 with dilute sulfuric acid at 0 ºC. $BaSO_4$ removed by centrifugation. The water solution was evaporated to dryness in vacuum, and residue was purified by column chromatography on silica gel, using $CHCl_3$-MeOH (3:1) to give (90 mg, 0.225 mmol, 90% yield) of **7a**. For (S,R) *cis*-phosphoric acid 5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-yl methyl ester phenyl ester: ^1H NMR (300 MHz, Methanol d_4) δ (ppm) = 7.36-7.34 (d, 1H, *J* = 7.3 Hz, =CH-N); 7.14-6.88 (m, 5H, C_6H_5); 5.68-5.65 (d, 1H, *J* = 6.9 Hz, =CH-); 5.13-5.09 (t, 1H, *J* = 4.8 Hz, H-2); 4.15-3.88 (m, 4H, H-5, 1H, CH_2N, CH_2O); 3.70-3.63 (dd, 1H, *J* = 7.4 Hz, *J* = 7.55 Hz, CH_2N), 2.95-2.89 (dd, 1H, *J* = 4.9 Hz, *J* = 4.86 Hz, CH_2S), 2.63-2.56 (t, 1H, *J* = 9.8 Hz, CH_2S). HRMS-FAB: calcd. for $C_{15}H_{18}O_6N_3PS$ $(M+H)^+$, 399.0761; found: 399.0761.

Synthesis of (S,R) *cis*-2^{11}-{[5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-ylmethoxy]-phenoxy-phosphorylamino}-propanic acid methyl ester (8a). To a solution of **5a** (121.5 mg, 0.5 mmol) and 1 ml of NMI in 5 ml CH_2Cl_2, was slowly added a solution of phenylmethoxy-L-alaninylchlorophosphate (139 mg, 0.5 mmol) in 5 ml CH_2Cl_2 with vigorous stirring at -30 ºC. The reaction mixture was then warmed-up to room temperature and stirred for 6 h. Methanol (1 ml) was added, and the solvents removed under vacuum. The residue was dissolved in 30 ml chloroform and washed with 50 ml aq. Na_2CO_3 solution (2%) and twice with 50 ml of water. The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated in vacuum, and the crude product was purified by column chromatography, on silica gel, using CH_2Cl_2-MeOH (10:1) to give **8a** (121 mg, 0.25 mmol) in 50% yield as a colorless glass (diasteromeric ratio (1:1)). For (S,R) *cis*-2^1-{[5-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]oxathiolan-2-ylmethoxy]-phenoxyphosphorylamino}propanic acid methyl ester: ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.31-7.10 (m, 6H, C_6H_5 + =CH-N); 5.75-5.71 (d, *J* = 7.1 Hz, 1H, =CH-); 5.43-5.38 (dd, *J* = 3.9 Hz, *J* = 3.4 Hz, 1H, H-2); 4.55-4.45 (m, 1H, H-5); 4.11-3.82 (m, 5H, CH, CH_2N, CH_2OP); 3.69 (s, 3H, OMe); 3.12-2.72 (dd, t, *J* = 5.0 Hz, *J* = 51 Hz, *J* = 8.5 Hz, 2H, CH_2OS); 1.36-1.32 (dd, *J* = 6.3 Hz, *J* = 5.6 Hz, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 174.01; 165.53; 156.30; 150.52; 146.52; 129.63; 124.91; 120.13; 94.90; 82.85; 80.09;

68.14; 52.47; 50.19; 34.03; 29.65; 20.71. HRMS-FAB: calcd. for $C_{19}H_{25}O_7N_4PS$ $(M+H)^+$, 485.1260; found: 485.1250.

(S,R) *cis*- and (S,R) *trans*-2-Benzoyloxymethyl-4-bromomethyl-1,3-dioxalane (3b). Benzyloxyacetaldehyde (2.46 g, 15 mmol), 3-bromo 1,2-propanediol (**2b**, 2.79 g, 18 mmol), and *p*-TsOH (0.1 g) were dissolved in 60 ml of benzene in a round bottomed flask equipped with a stirrer and Dean-Stark. The mixture was stirred and refluxed until the starting aldehyde has been completely used (8 h). The reaction mixture was then cooled to room temperature and washed once with 100 ml aq. Na_2CO_3 solution (2%) and twice with 50 ml of water. The orqanic layer was separated and dried over $MgSO_4$. The solvent was evaporated in vacuum, and the crude product was purified by column chromatography on silica gel using hexane-EA (20:1), to give (2.93 g, 9.75 mmol, 65% yield) of 2-benzoyloxymethyl-4-bromomethyl-1,3-dioxalane as a colorless liquid (diastereoisomeric ratio *cis:trans* = 2:1). Separation of *cis* and *trans* isomers were achieved by silica gel column chromatography using hexane-EA (40:1) to give *cis*-**3b** (1.95 g, 6.5 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.06-8.03 (m, 2H, C_6H_5); 7.58-7.40 (m, 3H, C_6H_5); 5.29-5.27 (t, *J* = 3.6 Hz, 1H, H-2); 4.43-4.40 (d, 2H, *J* = 3.2 Hz, CH_2OBz); 4.39- 4.34 (m, 1H, H-4); 4.07-3.99 (dd, *J* = 5.7 Hz, *J* = 4.8 Hz, 2H, CH_2O); 3.43-3.26 (dddd, *J* = 4.7 Hz, *J* = 4.6 Hz, *J* = 8.3 Hz, *J* = 8.2 Hz, 2H, CH_2Br), and *trans*-**3b** (0.97 g, 3.2 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.06-8.02 (m, 2H, C_6H_5); 7.58- 7.40 (m, 3H, C_6H_5), 5.45-5.43 (t, *J* = 4.1 Hz, 1H, H-2); 4.47- 4.42 (m, 1H, H-4); 4.35- 4.34 (d, *J* = 4.1 Hz ,2H, CH_2OBz); 4.27-3.82 (dddd, *J* = 6.2 Hz, *J* = 6.1 Hz, *J* = 5.7 Hz, *J* = 5.6 Hz, 2H, CH_2O); 3.50-3.32 (dddd, *J* = 4.4 Hz, *J* = 4.7 Hz, *J* = 7.9 Hz, *J* = 7.7 Hz, 2H, CH_2Br).

(S,R) *cis*- and (S,R) *trans*-Benzoic acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-ylmethyl ester (4b). To a stirred solution of cytosine (266.4 mg, 2.4 mmol) and K_2CO_3 (1.38 g, 10 mmol) in DMF (20 ml) was added dropwise a solution of *cis*- or *trans*-2-benzoyloxy-methyl-4-bromomethyl-1,3-dioxalane (**3b**, 602 mg, 2 mmol) in 4 ml DMF under N_2 over a period of 15 min at 60 ºC. The resulting mixture was stirred under N_2 for 12 h at 60 ºC until the starting ketal has been used. The reaction mixture was then cooled to room temperature, and was filtered from insoluble inorganic salts, washed with DMF. Then DMF was evaporated in vacuum. The oily residue was extracted with $CHCl_3$ and concentrated in vacuum. The crude residue was purified by column on a silica gel using CH_2Cl_2-MeOH (10:1) to give (430.3 mg, 1.3 mmol, 65% yield) of *cis*- or *trans*-alkylated products **4b** as white crystals. For (S,R) *cis*-benzoic acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3] dioxolan-2-ylmethyl ester. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.03-8.00 (m, 2H, C_6H_5); 7.56-7.41 (m, 3H, C_6H_5); 7.30-7.21 (d, *J* = 7.1 Hz, 1H, =CH-N); 5.63-5.61 (d, *J* = 6.9 Hz ,1H, =CH-); 5.22-5.20 (t, *J* = 3.4 Hz, 1H, H-2); 4.46-4.42 (m, 1H, H-4); 4.41-4.39 (d, *J* = 3.4 Hz, 2H, CH_2OBz); 4.20-4.02 (dddd, *J* = 2.4 Hz, *J* = 2.6 Hz, *J* = 6.7 Hz, *J* = 6.9 Hz, 2H, CH_2N); 3.82-3.61 (dddd, *J* = 5.9 Hz, *J* = 5.8 Hz, *J* = 7.0 Hz, *J* = 7.2 Hz, 2H, CH_2O). For (S,R) *trans*-Benzoic acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-ylmethyl, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.03-7.99 (m, 2H, C_6H_5); 7.58-7.40 (m, 3H, C_6H_5); 7.39-7.37 (d, *J* = 6.9 Hz, 1H, =CH-N); 5.79-5.76 (d, *J* = 7.0 Hz, =CH-); 5.37-5.34 (t, *J* = 3.8 Hz, 1H, H-2); 4.53-4.50 (m, 1H, H-4); 4.36-4.10 (m, 4H, CH_2OBz, CH_2N); 3.79-3.68 (dddd, *J* = 6.8 Hz, *J* = 4.4 Hz, *J* = 3.8 Hz, *J* = 6.3 Hz, 2H, CH_2O).

(S,R) *cis*- and (S,R) *trans*-2-Hydroxymethyl 4-(cytosin-1^1-yl methyl)-[1,3]dioxalane (5b). To a stirred solution of **4b** (331 mg, 1 mmol) in 5 ml MeOH was added a solution of 200 mg of NaOH in 15 ml MeOH. The resulting mixture was stirred for 1 h at room temperature. To the reaction mixture was added 20 ml H_2O and then extracted with a mixture of $CHCl_3$ and MeOH. Organic phase was dried over $MgSO_4$, filtered, and the solution was concentrated in vacuum. Crude product was purified by column on a silica gel using $CHCl_3$-MeOH (5:1) to give (193 mg, 0.85 mmol, 85% yield) of **5b**. For (S,R) *cis*-2-hydroxymethyl 4-(cytosin-1^1-yl methyl)-1,3-dioxalane: m.p.: 188-190 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 7.54-7.52 (d, *J* = 7.2 Hz, 1H, =CH-N); 7.05-6.86 (br, 2H, NH_2); 5.63-5.51 (d, *J* = 7.2 Hz, 1H, =CH-); 4.92-4.89 (t, *J* = 5.7 Hz, 1H, H-2); 4.82 (br, 1H, OH); 4.26-4.24 (m, 1H, H-4); 3.87-3.60 (dddd, *J* = 4.6 Hz, *J* = 4.9 Hz, *J* = 7.5 Hz, *J* = 7.5 Hz, 4H, CH_2O, CH_2N); 3.41-3.15 (br, 2H, CH_2OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) = 166.99; 156.01; 146.86; 104.35; 92.97; 73.76; 66.83; 61.96; 50.81. HRMS-FAB: calcd. for $C_9H_{13}O_4N_3$ $(M+H)^+$, 228.0984; found: 228.0984. For (S,R) *trans*-2-hydroxymethyl-4-(cytosin-1^1-yl methyl)-1,3-dioxalane: m.p.: 158-159 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 7.54-7.53 (d, *J* = 7.2 Hz, 1H, =CH-N);

7.08-6.98 (br, 2H, NH_2); 5.65-5.64 (d, *J* = 7.0 Hz, 1H, =CH-); 4.99-4.97 (t, *J* = 3.9 Hz, 1H, H-2); 4.86 (t, *J* = 6.0 Hz, 1H, OH); 4.32-4.26 (m, 1H, H-4); 4.03- 3.82 (dddd, *J* = 6.6 Hz, *J* = 6.8 Hz, *J* = 3.4 Hz, *J* = 3.2 Hz, 2H, CH_2N); 3.68-3.53 (dddd, *J* = 7.7 Hz, *J* = 7.7 Hz, *J* = 6.1 Hz, *J* = 6.0 Hz, 2H, CH_2O); 3.35-3.31 (d, *J* = 3.9 Hz, 2H, CH_2OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) = 166.03; 155.92; 146.71; 103.59; 93.11; 73.65; 66.81; 62.26; 50.81. HRMS-FAB: calcd. for $C_9H_{13}O_4N_3(M+H)^+$, 228.0984; found: 228.0988.

(S,R) *cis*- and (S,R) *trans*-Phosphoric acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-yl methyl ester diphenyl ester (6b). To a solution of **5b** (113.5 mg, 0.5 mmol) and 1 ml of NMI in 5 ml CH_2Cl_2 was slowly added a solution of (134.5 mg, 0.5 mmol) of diphenyl-chlorophosphate in 5 ml CH_2Cl_2 with vigorous stirring at -30 ºC. The reaction mixture was then warmed-up to room temperature and stirred for 6 h. Methanol (1 ml) was added and the solvents were removed under vacuum. The residue was dissolved in 30 ml chloroform and washed with 50 ml aq. Na_2CO_3 solution (2%) and twice with 50 ml of water. The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated in vacuum and the crude product was purified by column chromatography on a silica gel using CH_2Cl_2-MeOH (10:1) to give (161.0 mg, 0.35 mmol, 70% yield) of **6b**. For (S,R) *cis*-phosphoric acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-ylmethyl ester diphenyl ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.30-6.81 (m, 11H, C_6H_5 + =CH-N) ; 5.67-5.64 (d, *J* = 7.2 Hz, 1H, =CH-); 5.03 (br, 1H, H-2); 4.38-3.88 (m, 5H, H-4, CH_2N, CH_2O); 3.65-3.32 (dddd, *J* = 5.7 Hz, *J* = 5.6 Hz, *J* = 7.9 Hz, *J* = 7.9 Hz, 2H, CH_2OP). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 160.01; 156.84; 150.23; 146.23; 129.77; 129.74; 125.49; 120.18; 119.88; 101.59; 94.96; 74.76; 67.92; 67.49; 51.27. For (S,R) *trans*-phosphoric acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-ylmethyl ester diphenyl ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.30-6.99 (br, 11H, C_6H_5 + =CH-N); 5.78-5.76 (d, *J* = 7.2 Hz, 1H, =CH-); 5.16-5.14 (t, *J* = 3.2 Hz, 1H, H-2); 4.38-3.87 (m, 5H, H-4, CH_2N, CH_2O); 3.66-3.52 (dddd, *J* = 4.8 Hz, *J* = 4.9 Hz, *J* = 6.8 Hz, *J* = 6.9 Hz, 2H, CH_2OP).

(S,R) *cis*- and (S,R) *trans*-Phosphoric acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-yl methyl ester phenyl ester (7b). To a mixture of **6b** (115 mg, 0.25 mmol) in 5 ml water was added a solution of (315 mg, 1 mmol) of hydrated $Ba(OH)_2$ in 5 ml water. The reaction mixture was shaken for a few minutes until the reaction mixture had become almost clear. The reaction mixture was neutralized to pH = 4 with dilute sulfuric acid at 0 ºC. $BaSO_4$ removed by centrifugation. The water solution was evaporated to dryness in vacuum, and residue was purified by column chromatography on silica gel using $CHCl_3$-MeOH (3:1) to give (86 mg, 0.223 mmol, 90% yield) of **7b**. For (S,R) *cis*-phosphoric acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-yl methyl ester phenyl ester: ^{1}H NMR (300 MHz, Methanol d_4) δ (ppm) = 7.53-7.50 (d, *J* = 7.3 Hz, 1H, =CH-N); 7.24-6.97 (m, 5H, C_6H_5); 5.75-5.73 (d, *J* = 7.2 Hz, 1H, =CH-); 5.02 (br, 1H, H-2); 4.45-4.35 (m, 1H, H-4); 4.03-3.56 (m, 6H, CH_2N, CH_2O, CH_2OP). HRMS-FAB: calcd. for $C_{15}H_{18}O_7N_3P$ $(M+H)^+$, 384.0961; found: 384.0964. For (S,R) *trans*-phosphoric acid 4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-yl methyl ester phenyl ester: ^{1}H NMR (300 MHz, Methanol d_4) δ (ppm) = 7.42-7.40 (d, *J* = 7.3 Hz, 1H, =CH-N); 7.24-6.97 (m, 5H, C_6H_5); 5.80-5.77 (d, *J* = 7.1 Hz, 1H, =CH-); 5.16 (t, *J* = 3.5 Hz, 1H, H-2); 4.45-4.38 (m, 1H, H-4); 4.02-3.58 (m, 6H, CH_2N, CH_2O, CH_2OP). HRMS-FAB: calcd. for $C_{15}H_{18}O_7N_3P$ $(M+H)^+$, 384.0961; found: 384.0961.

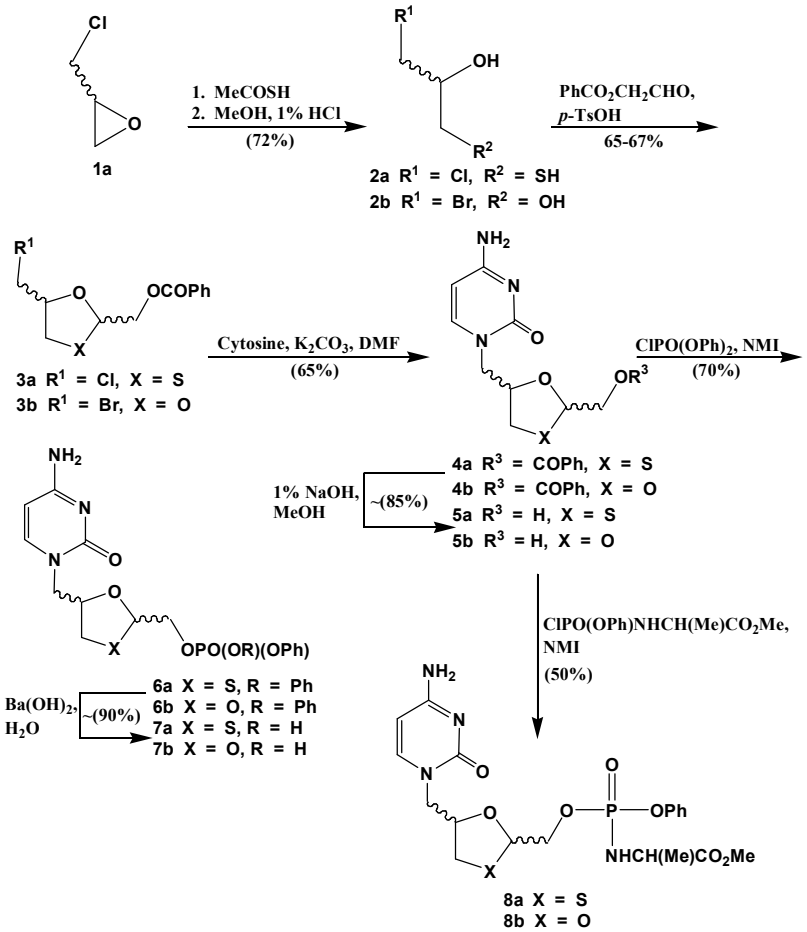
(S,R) *cis*- and (S,R) *trans*-2^{11}-{[4-(4^1-Amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-ylmethoxy]-phenoxyphosphorylamino}-propanic acid methyl ester (8b). To a solution of **5b** (113.5 mg, 0.5 mmol) and 1 ml of NMI in 5 ml CH_2Cl_2 was slowly added a solution of phenylmethoxy-L-alaninylchlorophosphate (139 mg, 0.5 mmol) in 5 ml CH_2Cl_2 with vigorous stirring at -30 ºC. The reaction mixture then warmed-up to room temperature and was stirred for 6h . Methanol (1ml) was added , and the solvents were removed under vacuum. The residue was dissolved in 30 ml chloroform and washed with 50 ml aq. Na_2CO_3 solution (2%) and twice with 50 ml of water. The organic layer was separated and dried over $MgSO_4$. The solvent was evaporated in vacuum and the crude product was purified by column chromatography on silica gel using CH_2Cl_2-MeOH (10:1) to give (117 mg, 0.25 mmol, 50% yield) of **8b**. For (S,R) *cis*-2^1-{[4-(4^1-amino-2^1-oxo-2H-pyrimidin-1^1-ylmethyl)-[1,3]dioxolan-2-ylmethoxy]-phenoxyphosphoryl-amino}-propanic acid methyl ester:

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.31-7.10 (m, 6H, C_6H_5 + =CH-N); 5.75-5.71 (d, 1H, =CH-); 5.35-5.31 (dd, 1H, H-2); 4.58-4.49 (m, 1H, H-4); 4.15-3.75 (m, 5H, CH, CH_2N, CH_2O); 3.71 (s, 3H, OMe); 3.61-3.30 (dd, 2H, CH_2OP); 1.35-1.31 (dd, 3H, Me). ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm) = 174.01; 165.85; 156.30; 150.58; 146.52; 129.61; 124.91; 120.16; 94.90; 82.85; 80.09; 67.92; 62.82; 50.66; 50.20; 50.11; 20.72. HRMS-FAB: calcd. for $C_{19}H_{25}O_8N_4P$ $(M+H)^+$, 469.1440; found: 485.1456.

RESULTS

Synthesis of Oxathiolane and Dioxalane Nucleoside Analogues 5a-b, Nucleotide Analogues 7a-b, and Phosphoramidates 8a-b (Scheme 1)

Epichlorohydrin (**1a**) was reacted with HSCOMe at 60 °C to afford the corresponding thioester, which upon treatment with 1% HCl/MeOH gave thiol **2a** in 72% overall yield. Then 1-Chloro-3-mercapto-propan-2-ol (**2a**) and 3-bromo-1,2-propandiol (**2b**) were converted to the corresponding 5-chloromethyl[1,3]oxathiolane **3a** (65%) and 4-bromomethyl [1,3]dioxalane **3b** (67%) by use of benzoyloxyacetaldehyde [19] and *p*-TsOH in benzene. Addition of cytosine to **3a** or **3b** at 60 °C in the presence of K_2CO_3 in DMF afforded the alkylated products **4a** (50%) and **4b** (65%), respectively. Removal of benzoyl group from **4a** and **4b** by 1% NaOH in MeOH gave the corresponding alcohol **5a** (85%) and **5b** (84%). Reaction of **5a** or **5b** with diphenylchlorophosphate in the presence of *N*-methyl imidazole (NMI) in CH_2Cl_2 at -30 ºC



Scheme 1

afforded the corresponding diphenylphosphates **6a** (70%) and **6b** (68%), respectively. Hydrolysis of **6a** or **6b** with $Ba(OH)_2$ in H_2O gave the respective phenylphosphates **7a** and **7b** in about 90% yield. On the other hand, individual treatments of **5a** and **5b** with phenylmethoxy-L-alaninyl chlorophosphate in the presence of NMI in CH_2Cl_2 afforded phosphoramidate **8a** and **8b** in about 50% yield [20,21].

Antiviral Activity

Compounds **5a**, **5b**, **7a**, **7b**, **8a**, and **8b** were tested for their inhibition of cytopathogenicity of human immunodeficiency virus (HIV-1 IIIB) in MT4 cells in a cell-protection assay. AZT was used as the reference compound. Compounds **7a-b** and **8a-b** demonstrated the ability to protect MT4 cells from HIV, which exhibits a cytopathic effect (Table 1) [22-24,29].

Table 1. Inhibitory Effects of Nucleoside and Nucleotide Analogues on the Cytopathogenicity of HIV-1 in MT4 Cells and Cellular Toxicity [29]

	IC_{50} (ug ml^{-1})[a]	
Compound	HIV-1(IIIB)	MT4 Cell[b]
AZT	0.04	58.32
5a	1.26	98.32
5b	1.12	112.50
7a	0.52	168.75
7b	0.43	186.98
8a	0.31	243.70
8b	0.24	285.68

[a]Inhibitory concentration (IC_{50}) represent the average of duplicate determinations. [b]Concentration of the compound required to reduce the number of viable uninfected cells by 50%.

DISCUSSION

The acyclic nucleoside phosphonates HPMPA, PMEA, and PMEG are potent inhibitors of DNA viruses and/or retroviruses [15g]. The phosphonomethyl ether functionality present in HPMPA, PMEA, and PMEG is chemically and metabolically stable, which may be responsible for their intrinsic *in vivo* antiviral activity [25]. The ethereal oxygen atom serves to enhance the acidity of the phosphonate due to the inductive electron-withdrawing effect of the oxygen atom, thus bringing the second pK_a of the phosphonate derivatives closer to that of a phosphate ester [26]. Consequently, respective rate of the formation of their biologically active diphosphate anabolites is similar to that of the natural nucleoside monophosphates. In addition to this isoelectronic nature, the ethereal oxygen in PMEG has been demonstrated to play a critical role for the enzymatic phosphorylation and thus for antiviral activity [26].

[(Phosphonomethoxy)alkyl]purine/pyrimidine derivatives are expected to be able to bypass the first enzymatic phosphorylation. Thus, HPMPA, PMEA, and PMEG have been shown to be active against thymidine kinase-deficient strain of herpes viruses [27]. As such, we designed nucleotide phosphonates **7a-b** (analog of BCH-189 monophosphate), to explore this interesting mode of action, especially against thymidine kinase-deficient viruses.

As mentioned previously, it has been shown that, in some cases, the L-enantiomer may be more active than the D-enantiomer [28]. This interesting fact prompted us to design new chemical structures **5a**, **5b**, **7a**, **7b**, **8a**, and **8b** possessing structural similarity to (2'*RS*,5'*RS*)-1-[2-(hydroxymethyl) oxathiolane-5-yl]cytosine (BCH-189). These compounds were synthesized as their respective racemates to allow access to both enantiomers.

A drug is useless if it is unable to penetrate the cell membrane of the infected cell. The ability of a drug to penetrate a membrane is correlated to its lipophilicity [1]. Consequently, we designed phosphoramidates **8a** and **8b** as lipophilic prodrugs, which displayed superior antiviral activities. A viral proteinase (i.e., HIV-aspartate proteinase) [17] may recognize and thus hydrolyze phosphoramidates **8a-b** to the corresponding potential drugs **7a-b** inside the infected cells. Then, they may be converted sequentially to the respective active triphosphates which should act as alternate substrates for the HIV reverse transcriptase to terminate DNA synthesis after being incorporated into the growing DNA strand.

CONCLUSIONS

New nucleoside and nucleotide analogs having an

oxathiolane or dioxalane ring were synthesized by chemical methods. These compounds include nucleoside analogues **5a-b**, phosphates **7a-b**, and phosphoramidates **8a-b**. These molecules exhibited significant anti-HIV activity *in vitro*.

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