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Synthesis of Isomeric Nucleoside Phosphonates: Cyclic Analogs of the Anti-HIV Active Compound, PMEA

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Abstract: Synthesis of cyclic analogs of the anti-HIV active acyclic nucleoside phosphonate, PMEA, is described. A key reaction in the synthesis was the stereoselective cyclization of an acyclic phosphonyl intermediate to a phosphonyltetrahydrofuran. Complete stereostructures of the target isomeric phosphonyl-nucleosides were established by COSY and NOESY NMR data and X-ray crystallographic analysis. Preliminary anti-HIV data in infected CEM-SS cells are mentioned.

Key words: isonucleosides, cyclic phosphonates, stereoselective synthesis, NMR data, anti-HIV activity

Introduction

Inhibition of the infectivity and cytopathicity of the human immunodeficiency virus (HIV) by 3′-deoxy-3′-azidothymidine (AZT) (Figure 1) furnished the first chemotherapeutic agent for the treatment of acquired immunodeficiency syndrome (AIDS).[1] Successes with AZT led to the development of 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxycytidine (ddC), 2′,3′-didehydro-2′,3′-dideoxythimydine (D4T), and 2′,3′-dideoxy-3′-thiacytidine (3TC) as anti-HIV agents.[2-7] The mechanism of action of all of these 2′,3′-dideoxynucleosides

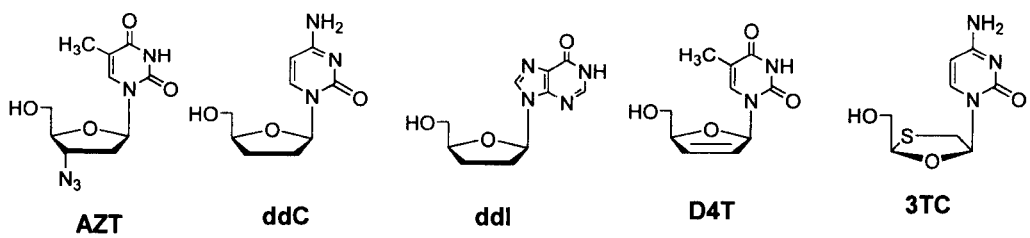

Figure 1. General structural representation of anti-HIV active normal dideoxynucleosides

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(ddNs) is similar, i.e., following intracellular phosphorylation to their 5'-triphosphate forms, they serve as chain terminators and/ or inhibitors in the viral reverse transcription reaction.[8-12] However, the toxicity and stability problems associated with these ddNs have led to the search for diverse structural types which are less toxic, more stable, but comparable in anti-HIV activity to the clinically used ddNs.

Another problem of the known anti-HIV ddNs is the inefficiency of the first intracellular phosphorylation step by nucleoside kinases. It has been suggested that the anti-HIV activity of ddNs may be critically dependent on their initial intracellular phosphorylation.[8,9] One way to overcome the difficulty of the first phosphorylation step is to work with prodrugs that deliver intracellularly the monophosphate forms.[13,14] Another approach to bypass the first phosphorylation step more completely is represented by a class of compounds called acyclic nucleoside phosphonates such as PMEA, PMPA, FPMPA (Figure 2), that, after intracellular conversion to their diphosphate forms, PMEApp, FPMPApp, and PMPApp,[15] serve as inhibitors/ chain terminators in the HIV RT reaction.[15-18] An advantage of these acyclic nucleoside phosphonates is that their antiviral activity spectra are not limited to retroviruses but also extend to herpesviruses.[18] A unique feature common to all acyclic nucleoside phosphonates is their prolonged antiviral action.

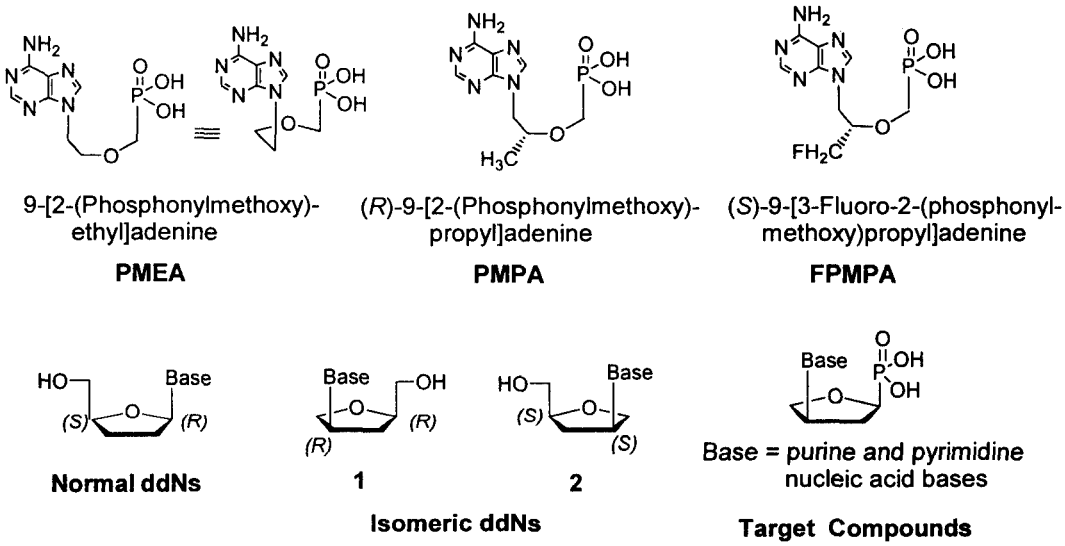


Figure 2. Examples of acyclic nucleoside phosphonates, isomeric dideoxynucleosides and target isomeric nucleoside phosphonates

Close examination of the structures of these acyclic nucleoside phosphonates suggests that these compounds could be classified as isomeric nucleotides. The structures of two types of isomeric dideoxynucleosides **1** and **2** in which the base has been translocated from the natural 1'-position to the isomeric

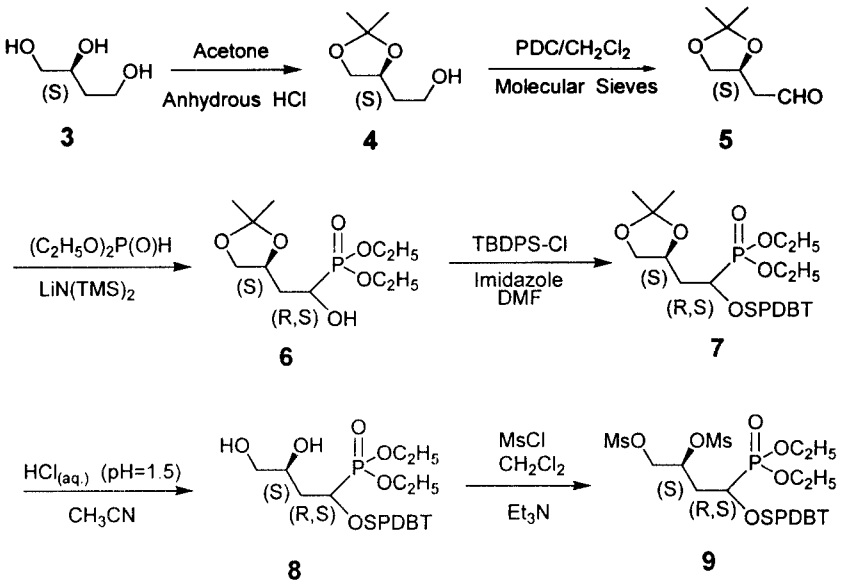
2'-position are shown in Figure 2.[19] Nair and coworkers have reported previously on the synthesis[20] and biological studies[21] of the isomeric dideoxynucleoside, 4(*S*)-(6-amino-9H-purin-9-yl)tetrahydro-2(*S*)-furan-methanol, [(*S,S*)-IsoddA]. This compound exhibits significant activity against HIV-1 and HIV-2 and is extremely stable with respect to nucleobase cleavage and enzymatic deamination.[21] Combination of the characteristics of the isomeric dideoxynucleosides with those of acyclic nucleoside phosphonates led us to the design of the target compounds shown in Figure 2 which retain the advantages of the circumvention of the requirement of the first phosphorylation step. While methylenephosphonate analogs of nucleoside 3'- and 5'-positions have been known for many years,[22-26] very few examples of direct attachment of a phosphono group to a furanose ring at the 1'-position have been described.[27] This paper reports on the synthesis and antiviral studies of isomeric nucleoside phosphonates that are cyclic analogs of PMEA.

Results and Discussion

*Development of Synthetic Approach to Cyclic Isomeric Phosphonylnucleosides (***14**)

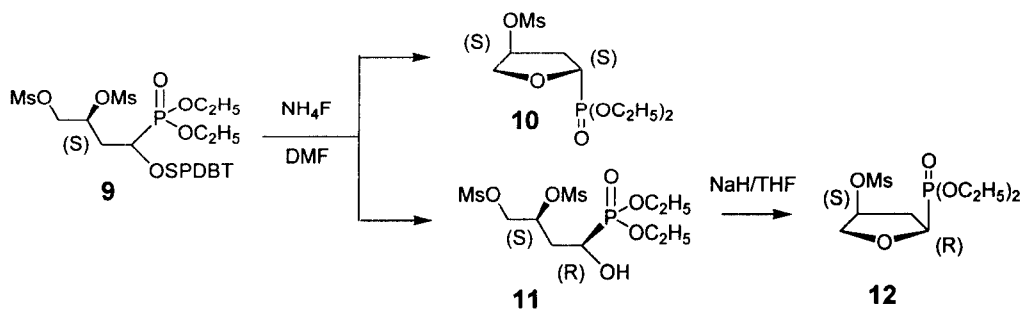
Synthesis of the target compounds was achieved by coupling of the desired nucleobases with an appropriate phosphono sugar. The chiral synthon, 1,(2*S*),4-butanetriol (**3**), which is commercially available, was chosen as the starting compound. It was protected to form the ketal **4** (71%) which was oxidized with PDC to provide the aldehyde **5**[28] in 83% yield (Scheme 1). Nucleophilic addition of a dialkyl phosphite anion to a carbonyl group was the methodology chosen for the introduction of the phosphonyl group.[29,30] Using this approach, the hydroxyphosphonate **6** was prepared in 58% yield by the addition of compound **5** with the diethylphosphite anion [prepared from diethylphosphite and lithium bis(trimethylsilyl)amide]. The hydroxyl group in **6** was needed for cyclization to produce the substituted tetrahydrofuran ring and the stereochemistry of this hydroxyl group is important in this reaction. However, the stereochemical course of the conversion of **5** to **6** produced a diastereoisomeric mixture based on the ^{13}C and ^{31}P NMR spectra of compound **6**. As the separation and differentiation of these two diastereomers were difficult at this stage, the mixture was carried forward to the next steps.

Selection of a protecting group with properties of stability in acid and appropriate deprotection conditions to mask the α-hydroxy group in phosphonate **6** was deemed important at this stage. The protecting group of choice was the *tert*-butyldiphenylsilyl (TBDPS) group. Treatment of **6** with TBDPS-Cl gave **7** in excellent yield (84%). When **7** was hydrolyzed in aqueous HCl, the diol **8** was produced (79%) without cleavage of the TBDPS protecting group. Mesylate was chosen as the leaving group for **8** because tosyl chloride was not reactive enough to generate the ditosylate in good yields. Instead, monotosylation was the major reaction. However, treatment of **8** in CH_2Cl_2 with mesyl chloride and triethylamine gave **9** in 89 % yield.



Scheme 1. Synthetic pathway to acyclic phosphonyl intermediate **9**

Deprotection of the TBDPS group in compound **9** with NH_4F in dry DMF (Scheme 2) was accompanied by spontaneous cyclization to afford the key intermediate **10** (*S,S* isomer) as the major product (51%). Cyclization of the other deprotection product from **9**, the (*R,S*)-isomer **11,** was markedly slower and it was isolated from the reaction mixture in 35 % yield. Treatment of **11** in anhydrous THF with sodium hydride furnished **12** (*R,S*-isomer) in 79 % yield. It was possible to determine the stereochemistry of these compounds at this stage. One



Scheme 2. Cyclization of **9** to key precursor **10** and its diastereoisomer **12**

would expect that cyclization would favor the diastereoisomer with the less hindered (*S,S*)-stereochemistry because this would place the mesyl and diethyl phosphonyl groups in a *trans*-relationship. NMR studies

(discussed below) confirmed the proposed structures. The reaction conditions also provided some information related to stereochemistry. For example, when the reaction of **9** with NH_4F in DMF was heated to 70 °C, the ratio of diastereomers **10 : 12** became 4 : 1 and with higher overall yield.

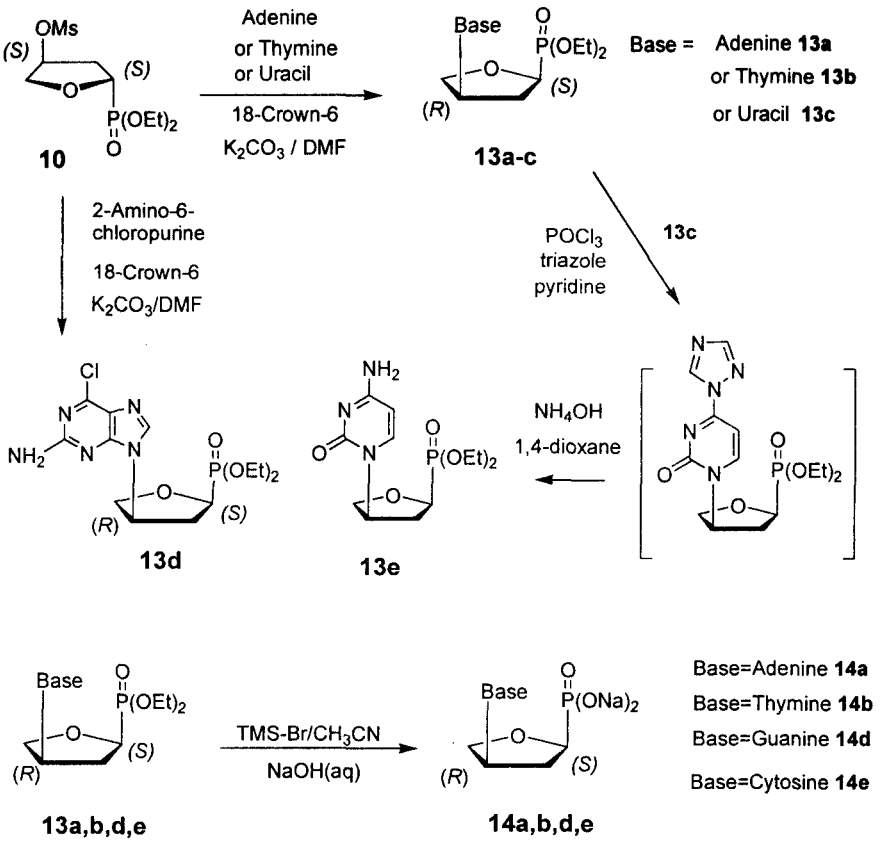
The protected target isodideoxynucleoside phosphonates, **13a**, **13b** and **13c**, were synthesized by direct coupling of adenine, thymine and uracil with mesylate **10** (18-crown-6, K_2CO_3, Δ). The yields varied from 27% to 61%. The isoguanosine derivative (**13d**) was prepared in 48% yield by coupling of 2-amino-6-chloropurine with mesylate **10** (Scheme 3). Isocytidine phosphonate analog, **13e,** was synthesized by converting isouridine (**13c**) to the corresponding 4-triazole intermediate followed by ammonolysis of this intermediate (Scheme 3). Conversion of the ethyl phosphonate esters to their corresponding sodium salts or free acids (**14a**), (**14b**), (**14d**), and (**14e**) was necessary for biological evaluation. This was accomplished in good yields (59-81%) by reaction with bromotrimethylsilane (TMS-Br) followed by treatment with NaOH (Scheme 3). Chemical shift change of carbon-6 in the ^{13}C NMR spectra of **13d** and **14d** from 153 to 181 ppm, together with UV data, confirmed that the 6-chloro group was converted to the 6-oxo (carbonyl) group under above reaction conditions.

Differentiation of "O-Alkylation" and "N-Alkylation" Products Arising From Direct Coupling Reactions

While coupling of adenine or 2-amino-6-chloropurine with the key intermediate **10** affords, in each case, one major product in good yields, the results of the same reaction with uracil and thymine turned out to be more complicated and gave lower yields. This is because "O-alkylation" competes with "N-alkylation" in the latter couplings. Differentiation of the "N-alkylated" product from the "O-alkylated" isomer was accomplished previously by Nair and coworkers using selective INEPT techniques.[20] In the case of the compounds of this paper, differentiation could be achieved by using ^{13}C NMR and DEPT techniques because of sufficient chemical shift differences between the carbon directly attached to nitrogen versus the carbon bonded to oxygen. Thus, in coupling product **13c**, the carbon of the "sugar" moiety linked to 1-N-uracil appeared at δ 53.5 ppm, while the same carbon attached to oxygen was, as expected, much further downfield (δ 70.4 ppm). These assignments were supported by the observed chemical shift for related N-bonded carbons (for **13b** at 53.8 ppm and for **13e** at 53.9 ppm).

Separation of Diastereomers and Determination of Stereochemistry

So far, in every case of dideoxynucleosides active against HIV-RT, the hydroxymethyl group and the nucleic acid base had a *cis*-relationship. Thus, it was important for the relative stereochemistry of our target molecules (**14**) to be established. Purification of final products **14** was accomplished by using reversed-phase HPLC which gave essentially pure diastereoismeric products as evidenced from their ^{13}C and ^{31}P NMR data. For example, in compound **13a**, the chemical shifts of the hydrogens on the tetrahydrofuran ring were first



Scheme 3. Final steps in the synthesis of isomeric phosphonyl nucleosides

determined by COSY experiments which aided the relative stereochemical assignments by NOE studies (Figure 3). The NMR data established the cis-configuration of the final products and suggested that the adenine ring was in a preferred anti conformation. NMR techniques were used, not only to confirm the assigned relative stereochemistry of **14d**, but also to establish that epimerization did not occur when **13d** was treated with sodium hydroxide to obtain **14d** (Figure 3).

The configuration and conformation of the target structures were further confirmed by single crystal X-ray diffraction analysis (Figure 4). For example, for compound **14a,** the crystal structure data established the absolute configuration, the preferred glycosidic bond conformation, and the puckering of the sugar moiety.

In summary, methodologies have been developed for the synthesis of cyclic analogs of the anti-HIV compound, PMEA. A key reaction in the synthesis was the stereoselective cyclization of an acyclic phosphonyl intermediate to a phosphonyltetrahydrofuran. Configurations and conformations of the target

isomeric phosphonylnucleosides were established by COSY and NOESY NMR data and single crystal X-ray crystallographic analysis. Preliminary antiviral evaluation suggested that these compounds did not show significant anti-HIV activity *in vitro* in infected CEM-SS cells. Further biological studies are in progress.

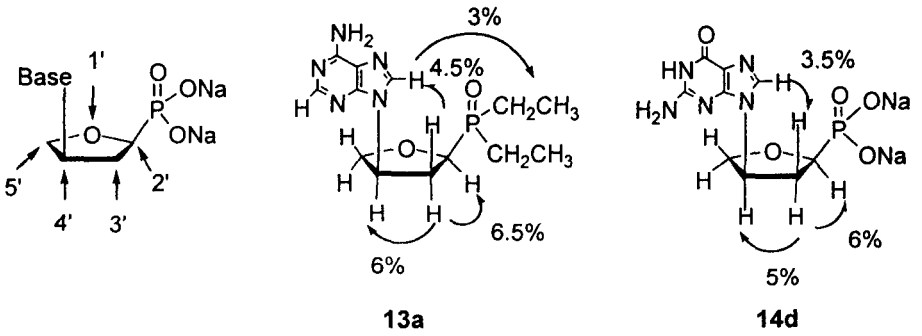


Figure 3. NOESY NMR correlations of compounds **13a** and **14d**

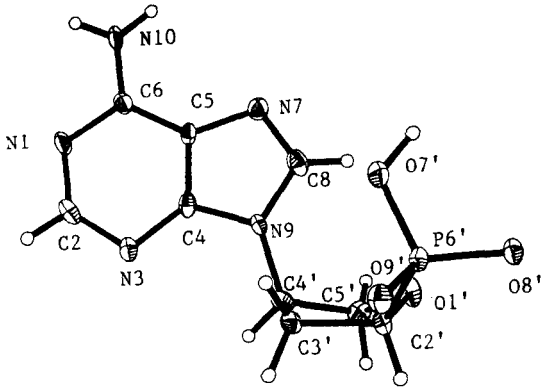


Figure 4. ORTEP plot of single-crystal X-ray structure of compound **14a**

Experimental

NMR spectra were recorded on a Brüker Model AC-300 spectrometer. Chemical shifts (δ, ppm) are relative to TMS (^{1}H and ^{13}C) or H_3PO_4 (^{31}P). High resolution ESI mass spectra were determined on a Micromass, Inc. Autospec instrument. UV spectra were recorded on a Gilford Response spectrophotometer. Optical rotations were measured on a Perkin Elmer Model 141 Polarimeter at 25 °C. Elemental analyses were determined at Desert Analytics, Tucson, AZ. Preparative layer chromatography used plates prepared with E.

Merck PF_{254} silica gel. Flash chromatography was carried out on columns packed with 240-400 mesh silica gel. HPLC separations were done on a Waters automated 600E system using a Delta-Pak C_{18} column.

Coupling Reaction: Procedure A. Typically, 10 mmol of key intermediate **10,** 20 mmol of desired nucleic bases, 15 mmol of 18-crown-6, and 15 mmol of potassium carbonate were dissolved in 50 mL of dried DMF. The reaction mixture was heated with stirring at 70 °C for 18 h. DMF was removed in *vacuo and* the residue was extracted with 3 x 50 mL of chloroform. The inorganic salts were filtered and washed with chloroform (3 x 10mL). Concentration of the combined organic layers gave a residue which was purified by flash silica gel chromatography (0-10 % methanol/ chloroform).

Deprotection of Phosphonate Esters: Procedure B. The diethyl phosphonate ester (10 mmol) was dissolved in 80 mL of acetonitrile and 10.0 equiv (13.1 mL) of bromotrimethylsilane was slowly added to it at 0 °C. The reaction mixture was warmed to room temperature and set aside in a stoppered flask for 48 h and then concentrated. The residue was stirred with acetonitrile (3 x 30 mL) and concentrated to afford a pale yellow solid which was washed with ethyl acetate (3 x 20 mL) and dried. The residue was dissolved in 50 mL of water and neutralized with 1 M NaOH. The crude product was purified by HPLC using a C-18 preparative column with an acetonitrile/ water gradient as the mobile phase.

1-Diethylphosphonyl-3,4-O-isopropylidene-1(*R,S*),3(*S*),4-butanetriol (6). To a solution of (*S*)-1,2,4-butanetriol (**3**) (25.00 g, 235.6 mmol) in 250 mL of anhydrous acetone at 0 °C, a catalytic amount of HCl gas was added. The reaction mixture was stirred at room temperature for 24 h and then quenched with solid sodium carbonate. Ethyl acetate (100 mL) was added and inorganic salts were filtered and washed with ethyl acetate. All organic solvents were combined and concentrated to give a pale yellow oil which was purified by flash chromatography (0-3 % methanol/chloroform) to afford the protected triol (**4**) as a colorless oil (24.12 g, 167.4 mmol, 71% yield). ^{1}H NMR ($CDCl_3$) δ: 4.27 (1H, quintet), 4.09 (1H, dd), 3.80 (1H, m), 3.59 (1H, t), 2.46 (1H, m), 1.96 (1H, bs, OH peak), 1.82 (2H, m), 1.42 (3H, s), 1.37 (3H, s).

Compound **4** (42.18 g, 292.7 mmol) was dissolved in 600 mL of dichloromethane followed by slow addition of pyridinium dichromate (132.1g, 351.2 mmol) and powdered 4A molecule sieves (66 g). The suspension was stirred vigorously overnight at room temperature. Hexanes/ethyl acetate (1:1, 300 mL) was added to the reaction mixture that was then stirred for 30 min. The black suspension was filtered through a short flash silica gel column to remove excess PDC and its reduced forms. The organic solvents were removed to give the crude aldehyde **5**[28] (34.77 g, 244.7 mmol) as a colorless oil in 83% yields. This aldehyde was used for the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ: 9.80 (1H, s), 4.52 (1H, m), 4.17 (1H, m),

3.57 (1H, m), 2.80 (1H, m), 1.40 (3H, s), 1.34 (3H, s). ^{13}C NMR ($CDCl_3$) δ: 199.9, 109.5, 70.7, 69.1, 47.8, 26.8, 25.4.

To a solution of diethyl phosphite (38.73 g, 280.67 mmol) in 80 mL of THF at -78 °C, was added 245.6 mL of 1.0 M lithium bis(trimethylsilyl)amide in THF. The solution was allowed to warm to room temperature and stirred for 45 min, and then cooled down to -20 °C. Crude aldehyde **5** (33.24 g, 233.89 mmol) in 200 mL of THF was transferred into the solution at this temperature. The reaction mixture was allowed to warm to room temperature slowly and stirred for 16 h and then quenched by slow addition of acetic acid (14.73 g, 245.58 mmol) in 50 mL of ether. It was filtered through celite which was washed with ethyl acetate. The organic solvents were concentrated to give a colorless oil which was purified by flash chromatography (0-5 % methanol/chloroform) to afford the title phosphonate **6** (37.89 g, 135.2 mmol, 58 % yield) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 4.77 (1H,bs, OH peak), 4.38 (1H, quintet) 4.20-4.08 (6H, m), 3.65 (1H, t), 2.12-1.94 (2H, m), 1.41-1.32 (12H, m). ^{13}C NMR (major form) ($CDCl_3$) δ: 108.7 and 108.3, 74.0 and 73.8, 69.0, 68.9, 65.5 and 65.2 (d, $^1J_{P\text{-}C}$ = 114 Hz), 62.5 and 62.4 (d, $^2J_{P\text{-}C}$ = 12 Hz), 62.3 (d, $^2J_{P\text{-}C}$= 12 Hz), 35.0 and 34.8, 26.7 and 26.5, 25.4 and 25.3, 16.2, 16.1. ^{31}P NMR ($CDCl_3$) δ: 24.3, 25.0. Anal. Calcd for $C_{11}H_{23}O_6P$: C, 46.61; H, 8.21. Found: C, 46.23; H, 8.13.

1-Diethylphosphonyl-1-O-*tert*-butyldiphenylsilyl-3,4-O-isopropylidene-1(*R,S*),3(*S*),4-butanetriol (7). Compound **6** (13.24 g, 47.26 mmol) and imidazole (4.82 g, 70.9 mmol) were dissolved in 35mL of DMF. *Tert*-butyldiphenylsilyl chloride (15.59 g, 56.7 mmol) was added and the reaction mixture was stirred overnight at room temperature and quenched by the addition methanol (5 mL). DMF was removed under high vacuum. The residue was taken up in ethyl acetate (100 mL). Imidazole hydrochloride salts were filtered and washed with ethyl acetate (3 x 40 mL). The combined ethyl acetate portions were concentrated and the crude oil was purified by flash chromatography (0-1 % methanol/ chloroform) to provide **7** (20.75 g, 40.0 mmol, 84 % yield). ^1H NMR ($CDCl_3$) δ: 7.82-7.69 (4H, m), 7.44-7.36 (6H, m), 4.15-3.88 (6H, m), 3.65 (1H, dd), 3.22 (1H, t), 2.04-1.94 (2H, m), 1.30-1.14 (12H,m), 1.08 (9H, s). ^{13}C NMR ($CDCl_3$) δ: 136.1 and 135.9, 133.0, 132.5 and 132.3, 129.7 and 129.6, 127 and 127.4, 108.5 and 108.4, 72.4 and 71.4 (d, $^2J_{P\text{-}C}$ = 12Hz), 69.2 and 69.1, 67.0 and 66.6 (d, $^1J_{P\text{-}C}$ = 118 Hz), 62.0 (d, $^2J_{P\text{-}C}$ = 11 Hz), 61.7 (d, $^2J_{P\text{-}C}$ = 11 Hz), 37.3 and 36.6, 26.8 and 25.4, 19.5 and 19.4, 16.2 (d, $^3J_{P\text{-}C}$ = 4 Hz), 16.1 (d, $^3J_{P\text{-}C}$ = 4 Hz). ^{31}P NMR ($CDCl_3$) δ: 24.6, 24.1. Anal. Calcd for $C_{22}H_{41}O_6PSi$: C, 62.28; H, 7.94. Found: C, 62.31; H,7.96.

1-Diethylphosphonyl-1-O-*tert*-butyldiphenylsilyl-1(*R,S*),3(*S*),4-butanetriol (8). Compound **7** (20.57 g, 39.7 mmol) was dissolved in 210 mL of CH_3CN and 30 mL of 0.6 % HCl (aq.) (pH = 1.5) was added. The mixture was stirred overnight at room temperature and neutralized with solid sodium carbonate. Anhydrous sodium

sulfate was added and the mixture was stirred for 10 min. The inorganic salts were filtered and the volume of filtrate was reduced and the solution was extracted with ethyl acetate (4 x 140 mL). The ethyl acetate layers were concentrated to provide a clear oil which was purified by flash chromatography (0-5 % methanol /chloroform) to give **8** (13.10 g, 27.4 mmol, 79 % yield). ^{1}H NMR ($CDCl_3$) δ: 7.74-7.76 (4H, m), 7.43-7.29 (6H, m), 4.38 (1H, m), 4.25-3.97 (6H, m), 3.32 (1H, m), 3.21 (1H, m), 2.97 (1H, m), 1.82-1.71 (2H, m), 1.28-1.23 (6H,m), 1.09 (9H, s). ^{13}C NMR (major form) ($CDCl_3$) δ: 136.1, 135.8, 132.8, 132.5, 132.3, 129.9, 129.7, 127. 6, 127.4, 67.8, 66.7 (d, $^{3}J_{P\text{-}C}$ = 4 Hz), 66.6 (d, $^{1}J_{P\text{-}C}$ = 124 Hz), 66.2, 65.5, 62.4 (d, $^{2}J_{P\text{-}C}$ = 12 Hz), 62.3 (d, $^{2}J_{P\text{-}C}$ = 12 Hz), 36.4 (d, $^{2}J_{P\text{-}C}$ = 8 Hz), 26.7, 19.2, 16.2 (d, $^{3}J_{P\text{-}C}$ = 4 Hz), 16.1 (d, $^{3}J_{P\text{-}C}$ = 4 Hz). ^{31}P NMR ($CDCl_3$) δ: 24.4. Anal. Calcd for: $C_{24}H_{37}O_6PSi$: C, 59.96; H, 7.78. Found : C, 59.94 ; H, 7.92.

1-Diethylphosphonyl-1-O-*tert*-butyldiphenylsilyl-3,4-bis-O-methanesulfonyl-1(*R*,*S*),3(*S*),4-butanetriol (9). To a solution of compound **8** (13.10 g, 27.4 mmol) in 300 mL of methylene chloride at -10 °C, was added triethyl- amine (11.06 g, 109.5 mmol) followed by slow addition of mesyl chloride (9.41g, 82.1 mmol). The reaction mixture was kept in a freezer for 24 h and then quenched by addition of 10 mL of methanol. All the solvents were removed and the residue was taken up by ethyl acetate (200 mL). The salts were filtered and washed with ethyl acetate (3 x 40 mL). The combined organic layers were concentrated to provide a clear oil which was purified by flash chromatography (0-2 % methanol/ chloroform) to give **9** (15.46 g, 24.37 mmol, 89 % yield). ^{1}H NMR ($CDCl_3$) δ: 7.74-7.76 (4H, m), 7.43-7.29 (6H, m), 4.37 (1H, m), 4.15-4.01 (6H, m), 3.09 (3H, s), 3.04 (3H, s), 2.96 (1H, m), 1.90-1.71 (2H, m), 1.26-1.20 (6H,m), 1.09 (9H, s). ^{13}C NMR ($CDCl_3$) δ: 136.1, 133.6, 133.0, 132.5, 132.3, 129.7, 129.6, 127. 6, 127.4, 108.4, 76.0 (d, $^{3}J_{P\text{-}C}$ = 4 Hz), 69.7 (d, $^{1}J_{P\text{-}C}$ = 124 Hz), 66.9, 62.4 (d, $^{2}J_{P\text{-}C}$ = 11 Hz), 62.2 (d, $^{2}J_{P\text{-}C}$ = 11 Hz), 37.9, 37.4, 34.9 (d, $^{2}J_{P\text{-}C}$= 8 Hz), 26.7, 19.2, 16.3, 16.2. ^{31}P NMR ($CDCl_3$) δ: 24.4, 24.3. Anal. Calcd for $C_{26}H_{41}O_{10}PS_2Si$: C, 49.01; H, 6.48. Found : C, 48.49; H, 6.40.

2(*S*)-Diethylphosphonyl-4(*S*)-O-methanesulfonyltetrahydrofuran (10). To a solution of **9** (5.50 g, 8.67 mmol) in 20 mL of dry DMF, NH_4F (1.28 g, 34.67 mmol) was added. The mixture was heated to 40 °C overnight. DMF was removed using a high vacuum pump and the residue was taken up in 100 mL of chloroform. The inorganic salts were filtered and washed with chloroform. All of the chloroform portions were combined and concentrated to provide an oil, which was purified by flash chromatography (0-2 % methanol/ chloroform) to provide **10** (1.34 g, 4.42 mmol, 51 % yield) as a colorless oil. The deprotected product **11** was also isolated (1.21 g, 3.03 mmol, 35 %). By dissolving in 20 mL of THF then adding sodium hydride (0.011g, 0.47 mmol) and stirring overnight, compound **11** (0.19g, 0.47 mmol) could be converted to compound **12** (0.11, 0.37 mmol, 79% yield after purification). ^{1}H NMR ($CDCl_3$) δ: 5.36 (1H,m), 4.31 (1H,m), 4.25-4.14 (6H, m),

3.21 (3H, s), 2.60-2.52 (2H, m), 1.39-1.35 (6H, 2 sets of triplet). Data for compound **10**: ^{1}H NMR ($CDCl_3$) δ: 5.39 (1H,m), 4.36 (1H, m), 4.24-4.11 (6H, m), 3.06 (3H, s), 2.57-2.44 (2H, m), 1.35 (6H, sextet). ^{13}C NMR ($CDCl_3$) δ: 80.2 (d, $^3J_{P-C}$ = 5 Hz), 73.94 (d, $^3J_{P-C}$ = 5 Hz), 73.8 (d, $^1J_{P-C}$ = 116 Hz), 63.0 (d, $^2J_{P-C}$ = 13 Hz), 62.4 (d, $^2J_{P-C}$ = 13 Hz), 38.6, 34.7, 16.4, 16.3. ^{31}P NMR ($CDCl_3$) δ: 24.8. Anal. Calcd for $C_9H_{19}O_7PS$: C, 35.76; H, 6.34. Found: C, 36.14; H, 6.72. Data for compound **11**: ^{1}H NMR ($CDCl_3$) δ: 4.39 (1H, m), 4.22 (2H, m), 4.08-4.01 (4H, 2 sets of quartet), 3.78 (1H, bs OH peak), 3.21 (1H, m), 1.97 (1H, m), 1.82 (1H, m), 1.26-1.20 (6H, 2 sets of triplet). ^{13}C NMR ($CDCl_3$) δ: 75.3 (d, $^3J_{P-C}$ = 4 Hz), 70.0 (d, $^1J_{P-C}$ = 124 Hz), 66.8, 62.4 (d, $^2J_{P-C}$ = 11 Hz), 62.2 (d, $^2J_{P-C}$ = 11 Hz), 37.9, 37.45, 35.0 (d, $^2J_{P-C}$ = 8 Hz), 16.3 16.2. ^{31}P NMR ($CDCl_3$) δ: 24.4. Anal. Calcd for $C_{10}H_{23}O_{10}PS_2$: C, 30.14; H, 5.88. Found: C, 30.08; H, 6.03.

4(*R*)-(6-Amino-9H-purin-9-yl)-2(*S*)-diethylphosphonyltetrahydrofuran (13a). Adenine was coupled with mesylate **10** following Procedure A to give **13a** as white crystals in 61% yields. UV (ethanol) λmax = 260 nm (ε= 14,600). $[\alpha]_D$ = -4.41° (c = 0.074 M in methanol). ^{1}H NMR ($CDCl_3$) δ: 8.35 (1H, s), 7.96 (1H, s), 6.77 (2H, s), 5.45 (1H,m), 4.56 (1H,t), 4.36-4.19 (6H, m), 2.87 (1H, m), 2.61 (1H, m), 1.35 (6H, sextet). ^{13}C NMR ($CDCl_3$) δ: 155.9, 152.9, 149.4, 138.0, 119.3, 73.22 (d, $^3J_{P-C}$ = 5 Hz), 72.55 (d, $^1J_{P-C}$ =114 Hz), 63.19 (d, $^2J_{P-C}$ = 6 Hz), 62.60 (d, $^2J_{P-C}$ = 6 Hz), 54.76 (d, $^3J_{P-C}$ = 5 Hz), 34.42, 16.56, 16.53. ^{31}P NMR ($CDCl_3$) δ: 24.4. Anal. Calcd for $C_{13}H_{22}N_5O_4P$: C, 45.44; H, 6.49; N, 20.40. Found: C, 45.39; H, 6.22; N, 20.05.

2(*S*)-Diethylphosphonyl-4(*R*)-[3,4-dihydro-2,4-dioxo-5-methyl-1(2H)-pyrimidinyl]tetrahydrofuran (13b). Thymine was coupled with **10** under the conditions of Procedure A to give the desired "N-alkylated" product **13b** in 27 % yields. The "O-alkylated" product (11%) was separated from the "N-alkylated" product on a preparative TLC plate. UV (ethanol) λ max = 270nm (ε = 9100). $[\alpha]_D$ = +3.41° (c = 0.20 M in methanol). ^{1}H NMR ($CDCl_3$) δ: 9.56 (1H, bs), 7.02 (1H, s), 4.48 (1H, m), 4.33-4.17 (6H, m), 2.52-2.36 (2H, m), 1.94 (3H, s), 1.35 (6H, sextet). ^{13}C NMR ($CDCl_3$) δ: 163.6, 151.1, 136.9, 112.0, 73.9 (d, $^3J_{P-C}$ = 13.5 Hz), 72.5 (d, $^1J_{P-C}$ = 175 Hz), 63.3 (d, $^2J_{P-C}$ = 6.7 Hz), 62.5 (d, $^2J_{P-C}$ = 6.7 Hz), 53.8 (d, $^3J_{P-C}$ = 8.5 Hz), 33.9, 16.5, 16.4, 12.5. ^{31}P NMR ($CDCl_3$) δ: 24.6. Anal. Calcd for $C_{13}H_{21}N_2O_6P$: C, 46.98; H, 6.39; N, 8.40. Found : C, 46.95; H, 6.39; N, 7.94.

2(*S*)-Diethylphosphonyl-4(*R*)-[4-amino-2-oxo-1(2H)-pyrimidinyl]tetrahydrofuran (13e). Uracil was coupled with **10** under the conditions of Procedure A to provide **13c** as colorless oil in 54 % yield (35% "N-alkylated" product and 19% "O-alkylated" product after preparative TLC separation. UV (ethanol) λ max = 266 nm (ε = 8000). ^{1}H NMR ($CDCl_3$) δ: 10.42 (1H, bs), 7.84 (1H, d), 5.79 (1H, d), 5.55 (1H,m), 4.30-4.02 (7H,

m), 2.67 (1H, m), 2.33 (1H, m), 1.35 (6H, sextet). ^{13}C NMR ($CDCl_3$) δ: ^{13}C NMR ($CDCl_3$) δ: 162.9, 150.5, 140.8, 102.8, 73.3 (d, $^3J_{P-C}$ = 6 Hz), 72.6 (d, $^1J_{P-C}$ = 114 Hz), 62.8 (d, $^2J_{P-C}$ = Hz), 62.4 (d, $^2J_{P-C}$ = 6 Hz), 53.5 (d, $^3J_{P-C}$ = 5 Hz), 33.2, 15.9, 15.8. ^{31}P NMR ($CDCl_3$) δ: 24.1. Anal. Calcd for $C_{12}H_{19}N_2O_6P$: C, 45.29; H, 6.02; N, 8.80. Found: C, 45.17; H, 6.09 ; N, 8.71. The uracil derivative, compound **13c** (50.00 mg, 0.157 mmol) was dissolved in 1 mL of dry pyridine. A solution of 44 μl (0.32 mmol) of $POCl_3$ and 32.5 mg (0.32 mmol) of triazole in 4 mL of anhydrous pyridine was added dropwise at 0 °C. The reaction mixture was warmed to room temperature and allowed to stir for 22 h to provide the triazole intermediate which was not isolated but was dissolved in 5 mL of dioxane. Concentrated NH_4OH solution (5 mL) was then added and the clear yellow solution was stirred overnight at room temperature. The solvents were removed and the residue was extracted with chloroform (3 x 10 mL). The chloroform portions were combined and concentrated to provide a yellow oil which was purified on a silica gel column to afford **13e** as a colorless oil in 34 % yields. UV (in $CHCl_3$) λ max = 276 nm (ε = 9,000). $[\alpha]_D$ = + 4.8° (c = 0.51 M in methanol). ^1H NMR ($CDCl_3$) δ: 7.91 (1H, d), 6.12 (1H, d), 5.85 (2H, s), 5.45 (1H,m), 4.28-4.10 (6H, m), 4.00 (1H, dd), 2.71 (1H, m), 2.38 (1H, m), 1.35 (6H, 2 sets of triplet). ^{13}C NMR ($CDCl_3$) δ: 164.7, 147.0, 142.9, 95.5, 74.6 (d, $^3J_{P-C}$ = 6 Hz), 72.9 (d, $^1J_{P-C}$ = 116 Hz), 62.8 (d, $^2J_{P-C}$ = 6 Hz), 62.4 (d, $^2J_{P-C}$ = 6 Hz), 53.9 (d, $^3J_{P-C}$ = 5 Hz), 33.2, 16.5 (d, $^3J_{P-C}$ = 5 Hz), 16.4 (d, $^3J_{P-C}$ = 5 Hz). ^{31}P NMR ($CDCl_3$) δ: 24.7. Anal. Calcd for $C_{12}H_{20}N_3O_5P$. H_2O: C, 42.93; H, 6.56; N, 11.50. Found: C, 43.19; H, 6.43; N, 11.38.

4(*R*)-(2-Amino-6-chloro-9H-purin-9-yl)-2(*S*)-diethylphosphonyltetrahydrofuran (13d). The 2-amino-6-chloropurine derivative (**13d**) was made by coupling 2-amino-6-chloropurine with compound **10** under the conditions of Procedure A to give a white solid in 48 % yield. $[\alpha]_D$ = -5.8° (c = 0.017 M in methanol). ^1H NMR ($CDCl_3$) δ: 7.87 (1H, s), 5.53 (2H, s), 5.25 (1H, m), 4.52 (1H, t), 4.46-4.19 (6H, m), 2.87 (1H, m), 2.51 (1H, m), 1.35 (6H, sextet). ^{13}C NMR ($CDCl_3$) δ: 159.1, 153.2, 151.3, 139.9, 124.9, 72.8 (d, $^3J_{P-C}$ = 4Hz), 72.4 (d, $^1J_{P-C}$ = 141 Hz), 63.2 (d, $^2J_{P-C}$ = 6 Hz), 62.5 (d, $^2J_{P-C}$ = 6 Hz), 54.6 (d, $^3J_{P-C}$ = 5 Hz), 34.0, 16.6 (d, $^3J_{P-C}$ = 4Hz), 16.5 (d, $^3J_{P-C}$ = 4Hz). ^{31}P NMR ($CDCl_3$) δ: 24.5. Anal. Calcd for $C_{13}H_{21}ClN_5O_4P$: C, 41.33; H, 5.66. Found : C, 41.64; H, 5.53.

Sodium [4(*R*)-(6-amino-9H-purin-9-yl)]tetrahydrofuran-2(*S*)-ylphosphonate (14a). Procedure B was followed for the deprotection of compound **13a** to yield **14a** (sodium salt) as white crystals in 68% yield after HPLC purification. The product **14a** came out from the C-18 column at 34 min with elution with a 0-100% acetonitrile/ water (1 h) gradient. UV (H_2O) λ max = 260 nm (ε = 13700). $[\alpha]_D$ = -32.0° (c = 0.058 M in water). ^1H NMR (D_2O) δ: 8.31(1H, s), 8.08(1H, s), 5.19 (1H, m), 4.13 (1H, m), 4.01 (2H, m), 2.85 (1H, m), 2.24 (1H,

m). ^{13}C NMR (D_2O) δ: 153.4, 149.5, 148.2, 141.1, 117.9, 74.9 (d, $^{1}J_{P-C}$ = 162 Hz), 73.1 (d, $^{3}J_{P-C}$= 12 Hz), 54.8 (d, $^{3}J_{P-C}$ = 8.5 Hz), 34.4. ^{31}P NMR (D_2O) δ: 17.9. HRMS (FAB): calcd for $C_9H_{11}N_5NaO_4P$: (M–Na)$^-$ 284.0549, found: 284.0564.

Sodium [4(*R*)-3,4-dihydro-2,4-dioxo-5-methyl-1(2H)-pyrimidinyl]tetrahydrofuran-2(*S*)-ylphosphonate (14b). The thymine phosphonate as its sodium salt, **14b**, was made from compound **13b** by using Procedure B (66% yield). UV (H_2O) λ max =271 nm. ^{1}H NMR (D_2O) δ: 7.65 (1H, s), 5,40 (1H, m), 4.14 (1H, m), 3.98 (1H, m), 3.84 (1H, m), 2.64 (1H, m), 2.39 (1H, m), 1.77 (3H, s). ^{13}C NMR (D_2O) δ: 167.5, 153.1, 140.2, 112.3, 75.8 (d, $^{1}J_{P-C}$ =1 65 Hz), 73.3 (d, $^{3}J_{P-C}$ = 11 Hz), 58.2 (d, $^{3}J_{P-C}$ = 3.5 Hz), 34.7 (d, $^{2}J_{P-C}$ = 16 Hz), 12.4. ^{31}P NMR (D_2O) δ: 16.9. HRMS (FAB): calcd for $C_9H_{13}N_2NaO_6P$: (M-Na)$^-$ 275.0433, found: 275.0424.

Sodium [4(*R*)-(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)]tetrahydrofuran-2(*S*)-ylphosphonate (14d). Procedure B was followed for the deprotection of the diethyl phosphonate ester of compound **13d** except at the point of neutralization with sodium hydroxide. The pH was adjusted to 14 instead of 7 and the aqueous solution was stirred for 14 h to hydrolyze the 2-amino-6-chloropurine to the corresponding guanine derivative. The above solution was neutralized with 1N HCl to pH = 7. The resulting pale precipitate was filtered and the filtrate was concentrated and purified on HPLC (81 % yield). UV (H_2O) λ max = 251 nm (ε =11,700). $[\alpha]_D$ = -22.8° (c = 0.060 M in water). ^{1}H NMR (D_2O) δ: 8.25 (1H, s), 5.02 (1H, m), 4.04 (1H, m), 3.93 (2H, m), 2.76 (1H, m), 2.22 (1H, m). ^{13}C NMR (D_2O) δ: 181.4, 158.9, 151.6, 142.6, 126.0, 76.6 (d, $^{1}J_{P-C}$ = 141Hz), 73.1 (d, $^{3}J_{P-C}$ = 12 Hz), 54.6 (d, $^{3}J_{P-C}$ = 8.5 Hz), 34.6. ^{31}P NMR (D_2O) δ: 16.4. HRMS (FAB) calcd for $C_9D_3H_9N_5NaO_5P$ (deuterated): (M-Na)$^-$ 304.0187, found: 304.0194.

Sodium [4(*R*)-(4-amino-2-oxo-1(2H)-pyrimidinyl)]tetrahydrofuran-2(*S*)-ylphosphonate (14e). Compound **14e**, the cytosine phosphonate sodium salt was made from **13e** by following general procedure B. The crude water solution was purified by reversed phase HPLC and eluted with 0 to 20% acetonitrile/water gradient/120 minutes. The product **14e** came out at 28 minute as a white solid after evaporation in 59% yields. UV (H_2O) λ max =2 76nm (ε = 9,600). ^{1}H NMR (D_2O) δ: 7.65 (1H, s), 5,40 (1H, m), 4.10-3.81 (3H, m), 2.64 (1H, m), 2.39 (1H, m), 1.77 (3H, s). ^{13}C NMR (D_2O) δ: 167.5, 153.1, 140.2, 112.3, 75.8 (d, $^{1}J_{P-C}$ = 165 Hz), 73.3 (d, $^{3}J_{P-C}$ = 11 Hz), 58.2 (d, $^{3}J_{P-C}$ = 3.5 Hz), 34.7 (d, $^{2}J_{P-C}$ = 16 Hz), 12.4. ^{31}P NMR (D_2O) δ: 17.1. HRMS (FAB) calcd for $C_8H_{11}N_3NaO_5P$ (M-Na)$^-$ 260.0436, found: 260.0425.

Single Crystal X-ray Structure Determination of Compound 14a. A colorless thin lath (0.54 x 0.29 x 0.11mm) was isolated from the sample and mounted with grease on the tip of a glass capillary epoxied to a brass pin. The crystal was placed in the cold stream with the long dimension (c-axis) approximately parallel to the diffractometer ϕ axis. Data were collected on an Enraf-Nonius CAD4 diffractometer (Mo K-α radiation, graphite monochromator) at 213 (2) K (N_2 cold gas stream) using θ-2θ scans. Intensity standards were measured at 2 h intervals. Net intensities were obtained by profile analysis of the 4295 data. Lorentz and polarization corrections were applied. No change in the intensity standards was detected. An empirical absorption correction based on three ψ scans measured at 10 degree intervals was applied. The transmittance range was 0.9150 – 0.9982, with an average value of 0.9538. Equivalent data were averaged yielding 2817 unique data [R-int = 0.080, 1487 > 4 *σ (F)]. Based on preliminary examination of the crystal, the space group P2 (1) /c was assigned. The systematically absent data (0k0, k = odd; h01, 1 = odd) were deleted. The computer programs from the MoLEN package were used for data reduction. The preliminary model of the structure was obtained using XS, a direct methods program. Least-squares refining of the model vs. the data was done with the XL computer program. Illustrations were made with the XP program and tables were made with the XCIF program. All are in the SHELXTL v5.0 package. Thermal ellipsoids are drawn at the 35% level unless otherwise noted. The crystal was split giving rise to systematically large values for low angle reflections (hk1, h=0,1,2,3,4; all k; 1= -3 – 3). These reflections were corrected using the HKL5 scheme in the XL program. All non-hydrogen atoms were refined with anisotropic thermal parameters. The adenine and furan hydrogen atoms were included with the riding model using program default values. The H07′ and water hydrogen atom coordinates were refined with Uiso H07′ = 1.5*Uiso (07′) and a single Uiso for all water H atoms. All water O-H distances were restrained to be the same as were all H-H distances within each water molecule. No other constraints or restraints were imposed.

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References

1. Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. USA,* **1985**, *82*, 7096-7100.
2. Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. USA,* **1986**, *83*, 1911-1915.

3. Yarchoan, R.; Mitsuya, H.; Thomas, R. V.; Pluda, J. M.; Hartman, N. R.; Perno, C-F.; Marczyk, K. S.; Allain, J-P.; Johns, D.; Broder, S. *Science* **1989**, *245*, 412-415.
4. Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990**, *249*, 1533-1544.
5. De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491-2517, and references therein.
6. Soudeyns, H.; Yao, X.-J.; Gao, Q,; Belleau, B.; Kraus, J.-L.; Nguyen-Ba, N.; Spira, B.; Wainberg, M. A. *Antimicrob. Agents Chemother.* **1991**, *35*, 1386-1390.
7. Schinazi, R. F.; Chu, C. K.; Peck, A.; McMillian, A.; Mathis, R.; Cannon, D.; Jeong, L-S.; Beach, J. W.: Choi, W-B.; Yeola, S.; Liotta, D. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 672-676.
8. Hao, Z.; Cooney, D. A.; Hartman, N. R.; Perno, C-F.; Fridland, A.; DeVico, A. L.; Sarngadharan, M. G.; Broder, S.; Johns, D. G. *Mol. Pharmacol.* **1988**, *34*, 431-435.
9. Cooney, D. A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johnson, M. A.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1987**, *36*, 1765-1768.
10. Huang, P.; Farquhar, D.; Plunkett, W. *J. Biol. Chem.* **1990**, *265*, 11914-11918.
11. Nair, V.; Sells, T. B. *Biochim. Biophys. Acta* **1992**, *1119*, 201-204.
12. Ho, H-T.; Hitchcock, M. J. M. *Antimicrob. Agents Chemother.* **1989**, *33*, 844-849.
13. Sastry, J. K.; Nehete, P. N.; Khan, S.; Nowak, B. J.; Plunkett, W.; Arlinghaus, R. B.; Farquhar, D. *Mol. Pharmacol.* **1992**, *41*, 441-445.
14. Puech, F.; Gosselin, G.; Lefebvre, I.; Pompon, A.; Aubertin, A-M.; Kirn, A.; Imbach, J-L. *Antiviral Res.* **1993**, *22*, 155-174.
15. Balzarini, J.; Holy, A.; Jindrich, J.; Dvorakova, H.; Hao, Z.; Snoeck, R.; Herdewijn, P.; Johns, D. G.; De Clercq, E. *Proc. Natl. Acad. Sci.* **1991**, *88*, 4961-4965.
16. Balzarini, J.; Holy, A.; Jindrich, J.; Naesens, L., Snoeck, R.; Schols, D.; De Clercq, E. *Antimicro. Agents Chemther.* **1993**, *37*, 332-338.
17. Balzarini, J.; Hao, Z.; Herdewijn, P.; Johns, D. G.; De Clercq, E. *Proc. Natl. Acad. Sci.* **1991**, *88*, 1499-1503.
18. De Clercq, E. *Clin. Microb. Rev.* **1997**, *10*, 674-693, and references therein
19. Nair, V.; Jahnke, T. S. *Antimicrob. Agents and Chemother.* **1995**, *39*, 1017-1029, and references therein.
20. Bolon, P. J.; Sells, T. B.; Nuesca, Z. M.; Purdy, D. F.; Nair, V. *Tetrahedron* **1994**, *50*, 7747-7764; Nair, V.; Nuesca, Z. M. *J. Am. Chem. Soc.* **1992**, *114*, 7951-7953.
21. Nair, V.; St. Clair, M.; Reardon, J. E.; Krasny, H. C.; Hazen, R. J.; Paff, M. T.; Boone, L. R.; Tisdale, M.; Najera, I.; Dornsife, R. E.; Everett, D. R.; Borroto-Esoda, K.; Yale, J. L.; Zimmerman, T. P.; Rideout, J. L. *Antimicrob. Agents and Chemother.* **1995**, *39*, 1993-1999.
22. Albrecht, H. P.; Jones, G. H.; Moffatt, J. G. *Tetrahedron* **1984**, *40*, 79-87.

23. Jones, G. H.; Moffatt, J. G., *J. Am. Chem. Soc.* **1968**, *90*, 5337-5342.

24. Montgomery, J. A.; Laseter, A. G.; Hewson, K. *J. Heterocycl. Chem.* **1974**, *11*, 211-219.

25. Secrist, J. A.; Riggs, R. M.; Comber, R. N.; Montgomery, J. A. *Nucleosides and Nucleotides*, **1992**, *11*, 947-954.

26. Serra, C.; Dewynter, G.; Montero, J-L.; Imbach, J-L. *Tetrahedron* **1994**, *50*, 8427-8444.

27. Kofoed, T.; Ismail, A. E.; Pederson, E. B.; Nielsen, C. *Societe Chimique de France Bulletin* **1997**, 59-65.

28. Meyers, A. I.; Lawson, J. P.; Walker, D. G.; Linderman, R. J. *J. Org. Chem.* **1986**, *51*, 5111-5123.

29. Ohler, E.; Zbiral, E. *Synthesis* **1991**, 357-359.

30. Evelyn, L.; Hall, L. D.; Lynn, L.; Steiner, P. R.; Stokes, D. H. *Carbohydrate Research* **1973**, *27*, 21-27.