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Synthesis and Conformation of Novel 3'-Branched Threosyl-5'-Deoxyphosphonic Acid Nucleoside Analogues

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The discovery that threosyl phosphonate nucleoside (PMDTA, EC_{50} = 2.53 μM) is a potent anti-HIV agent has led to the synthesis and biological evaluation of 5'-deoxy versions of threosyl phosphonate nucleosides. In the present study, (*E*)-3'-phosphonoalkenyl and 3'-phosphonoalkyl nucleoside analogues **13**, **16**, **20** and **23** were synthesized from acetol and tested for anti-HIV activity and cytotoxicity. The adenine analogue **16** was found to exhibit moderate *in vitro* anti-HIV-1 activity (EC_{50} = 22.2 μM).

Key Words : Antiviral agent, Threosyl nucleoside phosphonic acid, Conformation analysis

Introduction

Phosphorus-modified nucleoside analogues, bearing a phosphonate group in their sugar moiety, have shown potent antiviral activity.[1] Since antiviral activity is often associated with nucleoside analogues bearing a phosphonomethoxy group in the sugar moiety, comparatively little attention has been paid to the properties and scopes of other phosphonate functions in relationship to biological activity.

On the other hand, considerable attention has been paid to unusual nucleosides since modified nucleosides were reported to be promising anti-human immunodeficiency virus (HIV) and anti-hepatitis B virus (HBV) agents. Of these compounds, threose nucleosides,[2] such as, PMDTA (**1**) and PMDTT (**2**), have been previously synthesized (Figure 1) because they can be assembled from natural precursors.[3] Furthermore, it has been demonstrated that threose nucleic acids (TNA) form duplexes with DNA and RNA that are thermally stable, in an analogous manner to natural nucleic acid association. The triphosphates of threose nucleosides are substrates of several polymerases, and can be enzymatically incorporated into DNA.[4] Actually, these nucleosides are accepted as substitutes for ribonucleosides in the catalytic site of hammerhead ribozyme, although subsequently, the catalytic efficiency of the ribozyme is significantly reduced.[5] The phosphonoalkoxy group of the proposed threose nucleoside phosphonates is bound at the 3'-position, which brings the phosphorus atom and the nucleobase closer together than in previously synthesized nucleoside phosphonates, where the phosphonate group is bound to the primary hydroxyl group of the nucleoside.

In the literature, nucleoside phosphonates have been prepared from several 5'-phosphate isosteres. As shown in Figure 1, compound **3**[6] is a simple 5'-deoxynucleoside phosphonate, in which the 5'-oxygen of a nucleoside phosphate is replaced by a methylene (Figure 1). More recently, we synthesized the novel threosyl 5'-deoxynucleoside adenine phosphonate **4**.[7] All phosphonates mimic the overall shape and geometry of nucleoside monophosphates.

Phosphorylation by kinases and the incorporation into nucleic acid (eventually leading to chain termination) is considered as important mechanism underlying the antiviral activities of nucleosides. In fact, lack of antiviral activity by a nucleoside phosphonate is generally attributed to poor substrate properties for cellular and viral kinases. On the other hand, the potent antiviral activities of phosphonylated alkylated nucleobases are ascribed to their intracellular phosphorylation to diphosphates and to refractory incorporation of the modified nucleosides in nucleic acids.[8] Furthermore, the enzymatic incorporation of phosphonate nucleosides into nucleic acids is almost irreversible, which is not the case for regular nucleotides.

Phosphonates have certain advantages over their phosphate counterparts because they are metabolically stable due to the lack of susceptibility of the phosphorus-carbon bond to hydrolytic cleavage.[9] Moreover, the spatial location of the carbon atom, namely the β-position from the phosphorus atom in the nucleoside analogue, has been demonstrated to play a critical role in antiviral activity.[10] The antiviral activities conferred by these atoms may be due to the

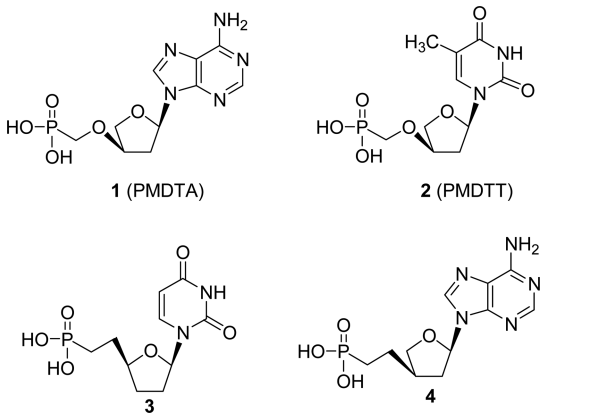
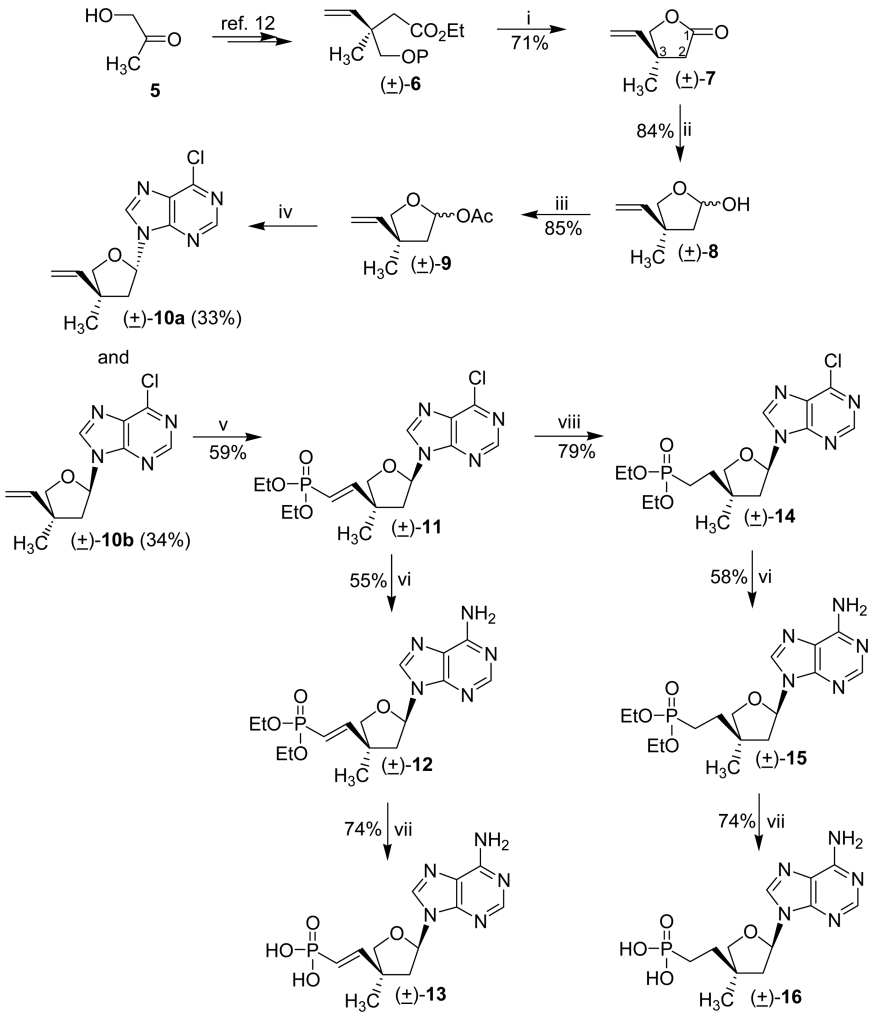


Figure 1. Structures of some threosyl phosphonic acid nucleosides as potent antiviral agents.



Scheme 1. Synthesis of threosyl-4'-methyl-5'-deoxyphosphonate adenine analogue. Reagents: i) TBAF, THF; ii) DIBALH, toluene; iii) Ac_2O, pyridine; iv) silylated 6-chloropurine, TMSOTf, DCE; v) Vinyldiethylphosphonate, Grubbs cat.(II) CH_2Cl_2; vi) NH_3, MeOH, 60 °C; vii) TMSBr, 2,6-lutidine, CH_3CN; viii) Pd/C, cyclohexene, MeOH.

increased binding capacity of the phosphonate analogues for target enzymes.[11]

Encouraged by these findings that threosyl nucleoside analogues and 5'-deoxynucleoside phosphonates have excellent biological activities, we explored the antiviral activities conferred by removing the 5'-oxygen or replacing this oxygen with carbon moieties, which resulted in 5'-phosphonate derivatives, and we evaluated their activities against various viruses.

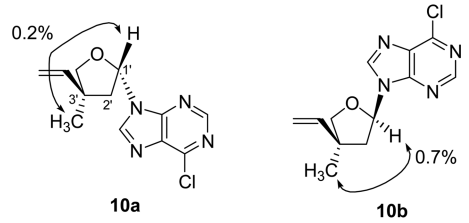
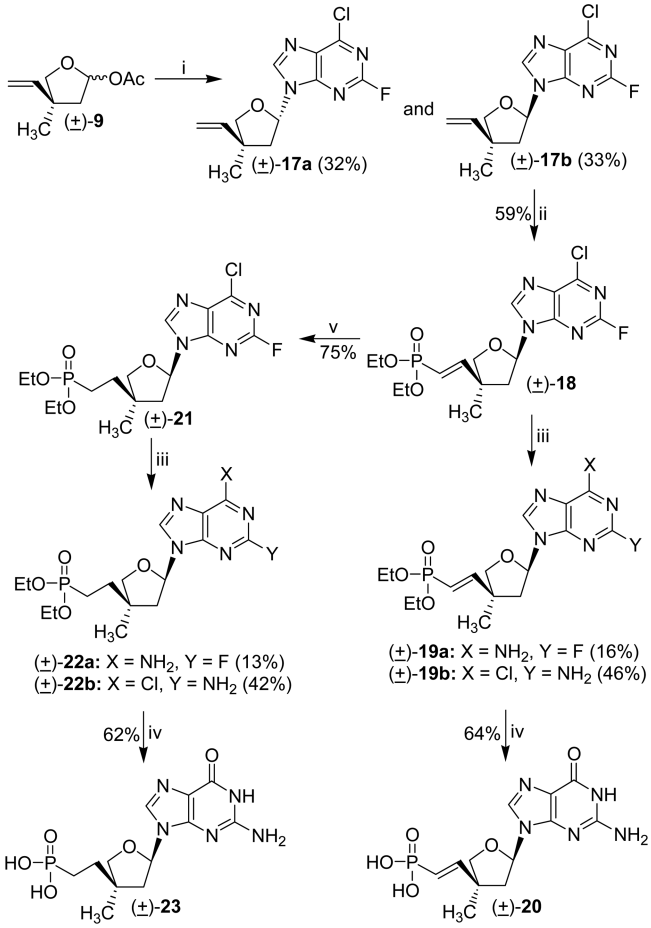


Figure 2. NOE differences between the proximal hydrogens of **10a** and **10b**.

Results and Discussion

As shown in Scheme 1, target compounds were prepared from acetol *via* an acyclic synthesis route.[12] The lactone functional group of **7** was prepared *via* desilylation and cyclization from **6**, and **7** was subsequently reduced using DIBALH in toluene at –78 °C to give lactol **8**, which was acetylated in pyridine to furnish the key intermediate **9** (a glycosyl donor) (Scheme 1). The synthesis of adenine nucleoside was carried out by condensation between **9** and silylated 6-chloropurine using TMSOTf as a catalyst in DCE to give the protected 6-chloropurine derivatives **10a** and **10b**, respectively. A complete NOE study allowed the unambiguous determination of their respective stereochemistries (Figure 2). For compound **10b**, strong NOE (0.7%) of H-1' ↔ CH-3', showing 1',3'-*cis* relationships, was observed. According to this result, the 3'-vinyl group and the 1'-purine base of **10b** were located on the β face. On the other hand, for **10a**, weak NOE (0.2%), such as, H-1' ↔ CH-3', were assigned to the 1',3'-*trans* relationship.

Cross-metathesis[13] of **10b** with diethylphosphonate using 2nd generation Grubbs catalyst[14] gave the vinylidene phos-



Scheme 2. Synthesis of threosyl-4'-methyl-5'-deoxyphosphonate guanine analogue. Reagents: i) silylated 2-fluoro-6-chloropurine, TMSOTf, DCE; ii) vinyldiethylphosphonate, Grubbs cat.(II) CH_2Cl_2; iii) NH_3, DME, rt; iv) (a) TMSBr, 2,6-lutidine, CH_3CN; (b) NaOMe, $HSCH_2CH_2OH$, MeOH; v) Pd/C, cyclohexene, MeOH.

phonate nucleoside analogue **11**, the chlorine group of which was then converted to amine using methanolic ammonia at 60 °C to give the corresponding adenosine phosphonate derivative **12**. Hydrolysis of the diethyl phosphonate functional groups of **12** with bromotrimethylsilane in CH_3CN in the presence of 2,6-lutidine then gave the adenosine phosphonic acid derivative **13**.[15] The vinylidene phosphonate of **13** was then saturated under transfer catalytic hydrogenation conditions to give the ethyl phosphonate nucleoside analogue **14**. Adenine analogue **16** was prepared using reaction conditions (ammonolysis and hydrolysis) similar to those described to prepare **13**.

To synthesize guanine analogues, 2-fluoro-6-chloropurine[16] was condensed with glycosyl donor using conditions similar to those used for the condensation of 6-chloropurine. Vorbruggen coupling[17] of the acetate **9** with 2-fluoro-6-chloropurine provided the analogues **17a** (32%) and **17b** (33%). Cross-metathesis of **17b** and diethylvinylphosphonate then produced **18** at a yield of 59%.

Bubbling ammonia into compound **18** provided the two separable analogues 2-fluoro-6-aminopurine[18] **19a** (16%) and 2-amino-6-chloropurine **19b** (46%). Fluorine atom acts as a good leaving group than chlorine atom in nucleophilic aromatic substitution. The 2-amino-6-chloropurine derivative **19b** was treated with TMSBr to provide phosphonic acid, and then treated with sodium methoxide and 2-mercaptoethanol in methanol to give the desired guanine vinylidene phosphonic acid **20** (Scheme 2).[19] The guanine phosphonate **23** was synthesized from **18** by transfer catalytic hydrogenation and by ammonolysis and hydrolysis using conditions similar to those described for the synthesis of **20**.

Table 1. The antiviral activities of the synthesized compounds

Compound	HIV-1		cytotoxicity IC_{50} (μM)		
	EC_{50} (μM)	EC_{90} (μM)	PBM	CEM	Vero
13	50.6	90	>100	>100	>100
16	**22.2**	80	42.4	30.4	>100
20	70	95	>100	>100	>100
23	85	95	>100	>100	>100
PMEA	5.4	ND	>100	50.3	>100
PMDTA	2.6	ND	>100	>100	>100
AZT	0.16	ND	>100	14.7	51.2

ND: Not Determined. PMEA: 9-[2-(Phosphonomethoxy)ethyl]adenine. PMDTA: Phosphonomethoxy-2-deoxy-threosyladenine. AZT: Azidothymidine. EC_{50} (μM): EC_{50} values are for 50% inhibition of virus production as indicated by supernatant RT levels. EC_{90} (μM): EC_{90} values are for 90% inhibition of virus production as indicated by supernatant RT levels. IC_{50} (μM): IC_{50} values indicates 50% inhibition of cell growth.

The antiviral activity of phosphonate nucleosides is largely due to their intracellular conversions to diphosphates, their subsequent incorporation into the viral genome, and chain termination.[20] The synthesized compounds **13**, **16**, **20**, and **23** were tested against HIV-1 and for cytotoxicity using AZT and PMEA as positive controls; results are summarized in Table 1. Anti-HIV activity was determined in human peripheral blood mononuclear (PBM) cells infected with HIV-1 strain *LAI*. In particular, the adenine analogue **16** show moderate antiviral activity against HIV-1, indicating that this virus might allow the sugar moiety for diphosphorylation or some affinity of its diphosphate toward viral polymerases. PBM cells (1×10^5 cell/mL) were infected with HIV-1 at a multiplicity of infection (MOI) of 0.02 and cultured in the

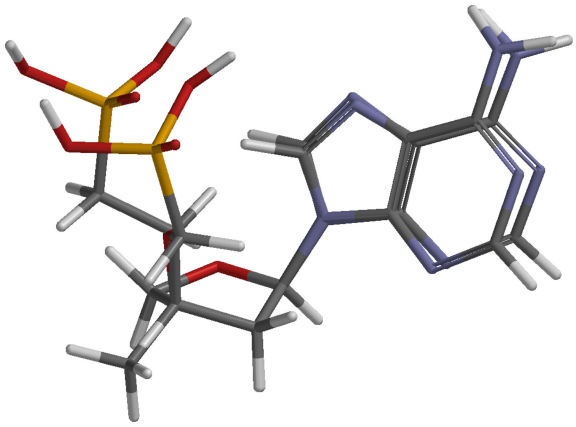


Figure 3. Superimpose model of PMDTA and **16**.

presence of various concentrations of the test compounds. After 4 days of incubation at 37 °C, numbers of viable cells were determined using the 3-(4,5-di-methylthiazole-2-yl)-2,5-diphenyltetrazolium bromide method. The cytotoxicities of the compounds were evaluated in parallel with their antiviral activities, which were assessed based on the viabilities of mock-infected cells.[21]

Conclusion

In summary, based on the known potent anti-HIV activities of threosyl 5'-norcarbocyclic nucleoside analogues, we designed and successfully synthesized novel 5'-deoxyphosphonate nucleoside analogues starting from acetol. The previously synthesized adenine **4** exhibited better cell-based activity than 4'-methyl branched adenine phosphonic acid **16**, which suggests that the methyl substituent at the 4'-position is possibly responsible for the apparent lack of activity of **16**. Superimposed modeling of PMDTA and **16** highlighted differences in adenine bases and phosphonic acid moieties (Figure 3).[22]

Experimental Section

Melting points were determined on a Mel-temp II laboratory device and were not corrected. NMR spectra were recorded on a JEOL 300 Fourier transform spectrometer (JEOL, Tokyo, Japan); chemical shifts are reported in parts per million (δ) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets). UV spectra were obtained using a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). MS spectra were collected in electrospray ionization (ESI) mode. The elemental analyses were performed using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were carried out under a nitrogen atmosphere unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

(±)-3-Methyl-3-vinyl-dihydrofuran-1-one (7). To a solution of **6** (1.2 g, 4.19 mmol) in THF (10 mL), TBAF (5.03 mL, 1.0 M solution in THF) was added at 0 °C. The mixture was stirred overnight at rt and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/EtOAc, 10:1) to give **7** (375 mg, 71%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.73-5.67 (m, 1H), 5.05-4.98 (m, 2H), 4.30 (d, J = 6.4 Hz, 1H), 4.21 (d, J = 6.5 Hz, 1H), 2.31 (d, J = 7.0 Hz, 1H), 2.23 (d, J = 7.0 Hz, 1H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.6, 148.8, 109.1, 84.2, 50.2, 31.7, 27.1; MS *m/z* 127 $(M+H)^+$.

(±)-3-Methyl-3-vinyl-tetrahydrofuran-1-ol (8). To a cooled (−78 °C), stirred solution of lactone **7** (320 mg, 2.53 mmol) in dry toluene (12 mL) was added dropwise a 1.0 M solution of diisobutylaluminium hydride (DIBALH) (3.0 mL, 3.0 mmol). The reaction was stirred for 20 min at −78 °C, methanol (3.0 mL) was added dropwise, and the mix was diluted with ethyl acetate. The reaction mixture was then warmed to room temperature and stirred for 2 h, and the precipitate that formed was removed by filtration through a pad of Celite and washed with ethyl acetate. Filtrate and washings were concentrated *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give **8** (272 mg, 84%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.76-5.65 (m, 1H), 5.50-5.43 (m, 2H), 5.02-4.99 (m, 2H), 3.73-3.68 (m, 2H), 2.01 (m, 2H), 1.25 (s, 3H).

(±)-Acetic acid 3-methyl-3-vinyl-tetrahydrofuran-1-yl ester (9). To a solution of compound **8** (151 mg, 1.18 mmol) in anhydrous pyridine (8 mL), Ac_2O (0.177 g, 1.75 mmol) was slowly added, and the mixture was then stirred overnight under nitrogen. The pyridine was then evaporated under reduced pressure and co-evaporated with toluene, and the residue so obtained was diluted with H_2O (50 mL), extracted with EtOAc (60 mL), dried over $MgSO_4$, and filtered. The filtrate was then concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give compound **9** (170 mg, 85%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.25-6.20 (m, 1H), 5.71-5.67 (m, 1H), 5.04-4.95 (m, 2H), 3.73-3.70 (m, 2H), 2.06-2.00 (m, 2H), 2.03 (s, 3H), 1.24 (s, 3H).

(*rel*)-(1'*R*,3'*R*)-9-(3'-Methyl-3'-vinyl-tetrahydrofuran-1'-yl) 6-chloropurine (10a) and (*rel*)-(1'*S*,3'*R*)-9-(3'-methyl-3'-vinyl-tetrahydrofuran-1'-yl) 6-chloropurine (10b). 6-Chloropurine (158 mg, 1.027 mmol), anhydrous HMDS (8 mL), and a catalytic amount of ammonium sulfate (12 mg) were refluxed to provide a clear solution, and the solvent was then distilled under anhydrous conditions. The residue so obtained was dissolved in anhydrous 1,2-dichloroethane (8 mL), and a solution of **9** (102 mg, 0.6 mmol) in dry DCE (10 mL) and TMSOTf (228 mg, 1.027 mmol) was added and stirred for 8 h at rt. The reaction mixture was then quenched with 2.5 mL of saturated $NaHCO_3$ and stirred for 1 h, and the resulting solid was filtered through a Celite pad. The filtrate was extracted with CH_2Cl_2 twice, and combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/hexane, 4:1) to give compounds **10a** (52 mg, 33%) and **10b** (54 mg, 34%). Data for **10a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.74 (s, 1H), 8.31 (s, 1H), 5.96 (t, J = 5.2 Hz, 1H), 5.72 (m, 1H), 5.04-4.95 (m, 2H), 3.72 (d, J = 5.8 Hz, 1H), 3.61 (d, J = 5.9 Hz, 1H), 2.28 (d, J = 6.2 Hz, 1H), 2.21 (d, J = 6.2 Hz, 1H), 1.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.7, 151.4, 151.1, 144.7, 142.4, 132.6, 109.7, 84.4, 76.2, 43.5, 34.3, 21.7; Anal. Calc. for $C_{12}H_{13}ClN_4O$: C, 54.45; H, 4.95; N, 21.17. Found: C, 54.42; H, 4.96; N, 21.15; MS *m/z* 265 $(M+H)^+$. Data for **10b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (s, 1H), 8.27 (s, 1H), 5.94 (dd, J = 5.8, 2.0 Hz, 1H), 5.73-5.70 (m, 1H), 5.02-4.96 (m, 2H), 3.71 (d, J = 6.0 Hz, 1H), 3.64 (d, J = 6.0 Hz, 1H), 2.29

(d, J = 6.2 Hz, 1H), 2.22 (d, J = 6.1 Hz, 1H), 1.23 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.5, 151.3, 151.0, 144.3, 142.9, 132.3, 108.7, 83.6, 74.8, 43.5, 34.8, 21.7; Anal. Calc. for $C_{12}H_{13}ClN_4O$: C, 54.45; H, 4.95; N, 21.17. Found: C, 54.46; H, 4.93; N, 21.18; MS *m/z* 265 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-Methyl-3'-vinyl-tetrahydrofuran-1'-yl) 6-chloropurine} phosphonate (11). To a solution of 6-chloropurine derivative **10b** (218 mg, 0.824 mmol) and diethyl vinylphosphonate (676 mg, 4.12 mmol) in CH_2Cl_2 (10 mL), 2nd-generation Grubbs catalyst (34.98 mg, 0.0412 mmol) was added. The reaction mixture was refluxed for 20 h under dry argon and concentrated under reduced pressure. The residue so obtained was purified by silica gel column chromatography (EtOAc/*n*-hexane/MeOH, 3:1:0.03) to give **11** (194 mg, 59%) as a form: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.74 (s, 1H), 8.31 (s, 1H), 6.57 (dd, J = 16.4, 20.5 Hz, 1H), 6.06 (dd, J = 16.5, 19.8. Hz, 1H), 5.97 (dd, J = 5.8, 1.8 Hz, 1H), 4.15-4.10 (m, 4H), 3.73 (d, J = 6.4 Hz, 1H), 3.66 (d, J = 6.5 Hz, 1H), 2.28 (d, J = 7.2 Hz, 1H), 2.22 (d, J = 7.2 Hz, 1H), 1.21-1.31 (m, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.7, 151.4, 153.2, 149.9, 144.6, 133.1, 115.3, 84.2, 76.1, 63.6, 63.1, 43.6, 35.5, 21.3, 14.4; Anal. Calc. for $C_{16}H_{22}ClN_4O_4P$ (+1.0 MeOH): C, 47.17; H, 6.05; N, 12.94; Found: C, 47.21; H, 6.02; N, 12.91; MS *m/z* 401 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-methyl-3'-vinyl-tetrahydrofuran-1'-yl) adenine}phosphonate (12). A solution of **11** (213 mg, 0.533 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at 60 ºC in a steel bomb, and volatiles were evaporated. The residue obtained was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:8) to give **12** (112 mg, 55%) as a white solid: mp 174-176 ºC; UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.31 (s, 1H), 8.10 (s, 1H), 6.61 (dd, J = 20.4, 17.0 Hz, 1H), 6.15 (dd, J = 18.9, 17.1 Hz, 1H), 5.96 (dd, J = 6.4, 1.8 Hz, 1H), 4.15-4.07 (m, 4H), 3.73 (d, J = 6.4 Hz, 1H), 3.65 (d, J = 6.3 Hz, 1H), 2.26-2.14 (m, 6H), 1.24-1.19 (m, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.4, 152.5, 149.4, 148.3, 140.5, 119.5, 116.2, 84.6, 75.4, 62.4, 61.6, 42.7, 35.2, 21.5, 14.5; Anal. Calc. for $C_{16}H_{24}N_5O_4P$ (+0.5 MeOH): C, 49.87; H, 6.59; N, 17.62; Found: C, 49.85; H, 6.61; N, 17.59; MS *m/z* 382 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-9-(3'-Methyl-3'-vinyl-tetrahydrofuran-1'-yl) adenine}phosphonic acid (13). To a solution of the phosphonate **12** (153 mg, 0.403 mmol) in anhydrous CH_3CN (10 mL) and 2,6-lutidine (0.938 mL, 8.06 mmol) was added trimethylsilyl bromide (0.616 mg, 4.03 mmol). The mixture was heated overnight at 70 ºC under nitrogen and then concentrated *in vacuo*. The residue obtained was partitioned between CH_2Cl_2 (100 mL) and purified water (100 mL), and the aqueous layer was washed with CH_2Cl_2 (2 × 70 mL) and then freeze-dried to give phosphonic acid **13** (97 mg, 74%) as a yellowish foam: UV (H_2O) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.34 (s, 1H), 8.14 (s, 1H), 6.61 (dd, J = 20.4, 17.0 Hz, 1H), 6.15 (dd, J = 18.9, 17.1 Hz, 1H), 5.95 (dd, J = 6.4, 1.8 Hz, 1H), 3.75 (d, J = 6.2 Hz, 1H), 3.67 (d, J = 6.3 Hz, 1H), 2.24-2.12 (m, 2H), 1.25 (m, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.3, 152.3, 149.4, 148.7, 139.3, 118.9, 115.2, 84.6, 75.7, 43.5, 35.3, 19.8; Anal. Calc. for $C_{12}H_{16}N_5O_4P$ (+2.0 H_2O): C, 39.89; H, 5.58; N, 19.38; Found: C, 39.92; H, 5.60; N, 19.41; MS *m/z* 326 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-methyl-3'-ethyltetrahydrofuran-1'-yl) 6-chloropurine} phosphonate (14). A solution of vinyl phosphonate nucleoside analogue **11** (320 mg, 0.798 mmol) in methanol (15 mL) was added to 10% Pd/C (10 mg) in cyclohexene (5 mL) under Ar. The reaction mixture was refluxed for 25 h, and then filtered through a pad of Celite, evaporated, and purified by silica gel column chromatography using methanol and methylene chloride (10:1) as eluant to give the ethyl phosphonate analogue **14** (254 mg, 79%) as a white solid: mp 162-164 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.78 (s, 1H), 8.35 (s, 1H), 5.96 (dd, J = 5.6, 1.8 Hz, 1H), 4.18-4.12 (m, 4H), 3.71 (d, J = 6.4 Hz, 1H), 3.62 (d, J = 6.3 Hz, 1H), 2.28-2.12 (m, 6H), 1.72-1.63 (m, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.8, 151.5, 150.4, 143.8, 135.2, 85.6, 76.1, 63.3, 62.3, 43.5, 32.1, 27.7, 21.2, 19.4, 14.0; Anal. Calc. for $C_{16}H_{24}ClN_4O_4P$: C, 47.31; H, 6.26; N, 13.38; Found: C, 47.27; H, 6.24; N, 13.40; MS *m/z* 403 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-methyl-3'-ethyltetrahydrofuran-1'-yl) adenine} phosphonate (15). The adenine derivative **15** was prepared from the 6-chloropurine analogue **14** using an ammonolysis procedure similar to that described for **12**: yield 58%; mp 167-169 ºC; UV (MeOH) λ_{max} 262.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.28 (s, 1H), 8.07 (s, 1H), 5.94 (dd, J = 6.2, 1.8 Hz, 1H), 4.12-4.06 (m, 4H), 3.72 (d, J = 6.4 Hz, 1H), 3.63 (d, J = 6.3 Hz, 1H), 2.26-2.14 (m, 6H), 1.24-1.19 (m, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.3, 152.4, 149.3, 140.6, 120.1, 84.4, 75.3, 62.5, 61.5, 42.6, 32.3, 28.6, 21.5, 18.4, 14.5; Anal. Calc. for $C_{16}H_{26}N_5O_4P$ (+1.0 MeOH): C, 49.15; H, 7.28; N, 16.86; Found: C, 49.12; H, 7.30; N, 16.84; MS *m/z* 384 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-{9-(3'-Methyl-3'-ethyl-tetrahydrofuran-1'-yl) adenine} phosphonic acid (16). The phosphonic acid **16** was synthesized from **15** using by hydrolysis in a manner similar to that described for **13**: yield 74%, UV (H_2O) λ_{max} 262.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.33 (s, 1H), 8.14 (s, 1H), 5.93 (dd, J = 6.3, 1.8 Hz, 1H), 3.72 (d, J = 6.2 Hz, 1H), 3.66 (d, J = 6.2 Hz, 1H), 2.23-2.12 (m, 6H), 1.26 (m, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.5, 152.2, 149.1, 138.5, 119.8, 83.7, 74.6, 42.7, 32.2, 28.4, 20.7, 18.8; Anal. Calc. for $C_{12}H_{18}N_5O_4P$ (+1.0 H_2O): C, 41.74; H, 5.84; N, 20.28; Found: C, 41.71; H, 5.82; N, 20.30; MS *m/z* 328 $(M+H)^+$.

(*rel*)-(1'*R*,3'*S*)-3'-Methyl-3'-vinyl-tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine (17a) and (*rel*)-(1'*R*,3'*R*)-3'-methyl-3'-vinyl-tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine (17b). Coupling of **9** with 2-fluoro-6-chloropurine by condensation in a manner similar to that described for **10** yielded **17a** and **17b**. Data for **17a**: yield 32%; UV (MeOH) λ_{max} 269.0 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.47 (s, 1H), 5.98 (t, J = 5.5 Hz, 1H), 5.73 (m, 1H), 5.06-4.94 (m, 2H), 3.74 (d, J = 6.8 Hz, 1H), 3.53 (d, J = 6.7 Hz, 1H), 2.28 (dd, J

= 10.2, 8.2 Hz, 1H), 2.20 (dd, *J* = 10.2, 6.8 Hz, 1H), 1.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.9, 152.4, 149.3, 147.9, 144.5, 128.6, 110.1, 84.2, 75.7, 43.3, 34.2, 20.7; Anal. Calc. for $C_{12}H_{12}ClFN_4O$: C, 50.98; H, 4.28; N, 19.82; Found: C, 51.02; H, 4.30; N, 19.80; MS *m/z* 283 $(M+H)^+$. Data for **17b**: yield 33%; UV (MeOH) λ_{max} 268.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.48 (s, 1H), 5.97-5.90 (dd, *J* = 6.2, 2.8 Hz, 1H), 5.74-5.69 (m, 1H), 3.73 (d, *J* = 6.6 Hz, 1H), 3.68 (d, *J* = 6.7 Hz, 1H), 1H), 2.31 (dd, *J* = 6.8, 10.4 Hz, 1H), 2.23 (dd, *J* = 8.8, 10.3 Hz, 1H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.0, 152.7, 149.6, 143.7, 136.5, 129.1, 109.8, 84.4, 76.2, 42.9, 33.8, 20.3; Anal. Calc. for $C_{12}H_{12}ClFN_4O$: C, 50.98; H, 4.28; N, 19.82; Found: C, 50.95; H, 4.27; N, 19.81; MS *m/z* 283 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-methyl-3'-vinyltetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine} phosphonate (18). The phosphonate nucleoside analogue **18** was prepared from **17b** by cross-metathesis as described for **11**: yield 59%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.50 (s, 1H), 6.63 (dd, *J* = 16.9, 19.7 Hz, 1H), 6.16 (dd, *J* = 17.1, 19.7 Hz, 1H), 5.99 (dd, *J* = 1.6, 6.0 Hz, 1H), 4.16-4.08 (m, 4H), 3.74 (d, *J* = 7.0 Hz, 1H), 3.63 (d, *J* = 6.9 Hz, 1H), 2.29 (d, *J* = 6.8, 10.4 Hz, 1H), 2.20 (dd, *J* = 8.4, 10.4 Hz, 1H), 1.32 (m, 6H), 1.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.1, 153.5, 150.5, 145.7, 129.1, 115.7, 110.2, 84.6, 76.3, 63.2, 62.4, 43.5, 35.3, 21.6, 20.1, 14.3; Anal. Calc. for $C_{16}H_{21}ClFN_4O_4P$ (+0.5 MeOH): C, 45.58; H, 5.33; N, 12.88; Found: C, 45.61; H, 5.31; N, 12.90; MS *m/z* 419 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-methyl-3'-vinyl-tetrahydrofuran-1'-yl) 2-fluoro-6-aminopurine} phosphonate (19a) and (*rel*)-(1'*R*,3'*R*)-diethyl {9-(3'-methyl-3'-vinyl-tetrahydrofuran-1'-yl) 2-amino-6-chloropurine} phosphonate (19b). Dry ammonia was bubbled into a stirred solution of **18** (390 mg, 0.96 mmol) in DME (18.4 mL) at room temperature overnight. Salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:8) to give **19a** (41 mg, 16%) and **19b** (170 mg, 46%). Data for **19a**: UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.22 (s, 1H), 7.74 (br s, NH_2, 2H), 6.66 (dd, *J* = 21.1, 17.2 Hz, 1H), 6.13 (dd, *J* = 20.5, 17.2 Hz, 1H), 5.94 (dd, *J* = 2.0, 6.0. Hz, 1H), 4.15-4.05 (m, 4H), 3.73 (d, *J* = 6.8 Hz, 1H), 3.64 (d, *J* = 6.7 Hz, 1H), 2.32 (dd, *J* = 8.0, 10.6 Hz, 1H), 2.23 (dd, *J* = 6.4, 10.6 Hz, 1H), 1.26-1.20 (m, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.3, 152.5, 149.3, 147.6, 143.3, 125.3, 116.7, 84.7, 75.6, 63.1, 62.8, 62.1, 43.8, 35.4, 21.1, 14.5, 13.9; Anal. Calc. for $C_{16}H_{23}FN_5O_4P$ (+0.5 MeOH): C, 47.70; H, 6.06; N, 16.86; Found: C, 47.73; H, 6.07; N, 16.84; MS *m/z* 400 $(M+H)^+$. Data for **19b**: UV (MeOH) λ_{max} 309.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.15 (s, 1H), 7.69 (br s, NH_2, 2H), 6.60 (dd, *J* = 20.9, 17.3 Hz, 1H), 6.12 (dd, *J* = 21.2, 17.2 Hz, 1H), 5.92 (dd, *J* = 1.8, 6.5. Hz, 1H), 4.15-4.06 (m, 4H), 3.75 (d, *J* = 6.9 Hz, 1H), 3.62 (d, *J* = 6.8 Hz, 1H), 2.31 (dd, *J* = 6.4, 10.6 Hz, 1H), 2.20 (dd, *J* = 8.2, 10.6 Hz, 1H), 1.27-1.20 (m, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.7, 154.6, 151.5, 149.3, 143.5, 125.8, 116.2, 84.5, 75.4, 63.0, 62.3, 61.7, 43.4, 35.7, 21.5, 15.6; Anal. Calc. for $C_{16}H_{23}ClN_5O_4P$ (+1.0 MeOH): C, 45.59; H, 6.07; N, 15.63; Found: C, 45.63; H, 6.05; N, 15.60; MS *m/z* 416 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-9-{(3'-Methyl-3'-vinyl-tetrahydrofuran-1'-yl) guanine} phosphonic acid (20). To a solution of **19b** (65.7 mg, 0.158 mmol) dry CH_3CN (12 mL) was added trimethylsilyl bromide (0.0364 mL, 2.76 mmol) at room temperature. This mixture was stirred for 36 h, solvent was removed by coevaporation three times using methanol. The residue was dissolved in MeOH (6.0 mL) and 2-mercaptoethanol (43.2 mL, 0.633 mmol) and NaOMe (33.6 mg, 0.633 mmol) was added. The mixture was then refluxed for 12 h under N_2, cooled, neutralized with glacial AcOH, and evaporated to dryness under vacuum. The residue was purified by chromatography on a column of reversed-phase C18 silica gel using water as eluant to give **20** (34.5 mg, 64%) as a yellowish form. UV (H_2O) λ_{max} 254.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.7 (br s, NH, 1H), 8.11 (s, 1H), 7.02 (br s, NH_2, 2H), 6.65 (dd, *J* = 20.4, 17.6 Hz, 1H), 6.14 (dd, *J* = 19.3, 17.6 Hz, 1H), 5.92 (dd, *J* = 2.4, 6.6. Hz, 1H), 3.75 (d, *J* = 6.8 Hz, 1H), 3.59 (d, *J* = 6.7 Hz, 1H), 2.33 (dd, *J* = 6.8, 10.8 Hz, 1H), 2.22 (dd, *J* = 8.4, 10.8 Hz, 1H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.7, 154.1, 152.6, 148.9, 137.2, 120.5, 112.7, 86.3, 76.4, 62.5, 61.9, 43.6, 36.0, 34.6, 20.5, 15.1; Anal. Calc. for $C_{12}H_{16}N_5O_5P$ (+2.0 H_2O): C, 38.20; H, 5.34; N, 18.56; Found: C, 38.23; H, 5.32; N, 18.55; MS *m/z* 342 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-methyl-3'-ethyl tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine} phosphonate (21). Compound **21** was synthesized from **18** by catalytic hydrogenation as described for **16**: yield 75%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.64 (s, 1H), 5.94 (dd, *J* = 1.8, 6.6 Hz, 1H), 4.15-4.03 (m, 4H), 3.73 (d, *J* = 6.8 Hz, 1H), 3.60 (d, *J* = 6.7 Hz, 1H), 2.31 (dd, *J* = 6.4, 10.7 Hz, 1H), 2.13 (m, 3H), 1.73 (m, 2H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.3, 152.5, 147.7, 142.8, 124.5, 83.6, 75.5, 63.4, 62.8, 61.7, 44.1, 32.7, 28.4, 20.5, 18.6, 14.3; Anal. Calc. for $C_{16}H_{23}ClFN_4O_4P$ (+0.5 MeOH): C, 45.37; H, 5.77; N, 12.83; Found: C, 45.34; H, 5.79; N, 12.82; MS *m/z* 421 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-Diethyl {9-(3'-methyl-3'-ethyl-tetrahydrofuran-1'-yl) 2-fluoro-6-aminopurine} phosphonate (22a) and (*rel*)-(1'*R*,3'*R*)-diethyl {9-(3'-methyl-3'-ethyl-tetrahydrofuran-1'-yl) 2-amino-6-chloropurine} phosphonate (22b). Ammonolysis of **21** was performed as described for **15**: Data for **22a**: yield 13%; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.19 (s, 1H), 7.76 (br s, NH_2, 2H), 5.94 (dd, *J* = 2.2, 6.2. Hz, 1H), 4.14-4.10 (m, 4H), 3.74 (d, *J* = 6.8 Hz, 1H), 3.62 (d, *J* = 6.8 Hz, 1H), 2.30 (dd, *J* = 6.6, 10.4 Hz, 1H), 2.14-2.09 (m, 3H), 1.69 (m, 2H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.0, 152.5, 147.2, 143.8, 125.6, 84.2, 75.7, 62.4, 61.7, 43.8, 33.0, 27.6, 20.6, 18.9, 14.3; Anal. Calc. for $C_{16}H_{25}FN_5O_4P$ (+1.0 MeOH): C, 47.11; H, 6.74; N, 16.16; Found: C, 47.08; H, 6.72; N, 16.15; MS *m/z* 402 $(M+H)^+$. Data for **22b**: yield 42%; UV (MeOH) λ_{max} 309.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.15 (s, 1H), 7.68 (br s, NH_2, 2H), 5.96 (dd, *J* = 2.4, 6.4, Hz, 1H), 4.14-4.09 (m, 4H), 3.73 (d, *J* = 6.8 Hz, 1H), 3.59 (d, *J* =

6.8 Hz, 1H), 2.18-2.10 (m, 4H), 1.72 (m, 2H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.8, 153.8, 151.5, 143.7, 125.7, 85.0, 74.6, 62.7, 62.0, 61.5, 44.1, 33.2, 27.5, 21.2, 18.9, 16.2, 15.1; Anal. Calc. for $C_{16}H_{25}ClN_5O_4P$ (+1.0 MeOH): C, 45.38; H, 6.49; N, 15.57; Found: C, 45.42; H, 6.51; N, 15.55; MS *m/z* 418 $(M+H)^+$.

(*rel*)-(1'*R*,3'*R*)-9-{(3'-Methyl-3'-ethyl-tetrahydrofuran-1'-yl) guanine} phosphonic acid (23). Nucleoside phosphonic acid **23** was prepared from **22b** by hydrolysis as described for **20**: yield 62%; UV (H_2O) λ_{max} 253.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.9 (br s, NH, 1H), 8.07 (s, 1H), 6.98 (br s, NH_2, 2H), 5.95 (dd, J = 2.3, 6.6. Hz, 1H), 3.72 (d, J = 6.7 Hz, 1H), 3.62 (d, J = 6.8 Hz, 1H), 2.34 (dd, J = 6.4, 10.2 Hz, 1H), 2.18-2.10 (m, 3H), 1.72 (m, 2H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.7, 154.8, 152.4, 136.2, 118.5, 75.8, 73.1, 45.3, 33.1, 28.5, 22.4, 20.3; Anal. Calc. for $C_{12}H_{18}N_5O_5P$ (+2.0 H_2O): C, 37.99; H, 5.84; N, 18.46; Found: C, 38.05; H, 5.81; N, 18.49; MS *m/z* 344 $(M+H)^+$.

References

1. (a) Holy, A. *Curr. Pharm. Des.* **2003**, *9*, 2567. (b) De Clercq, E.; Holy, A. *Nat. Rev. Drug Discovery* **2005**, *4*, 928.
2. Vina, D.; Wu, T.; Renders, M.; Laflamme, G.; Herdewijn, P. *Tetrahedron* **2007**, *63*, 2634.
3. Schöning, K.; Scholz, P.; Guntha, S.; Wu, X.; Krishnamurthy, R.; Eschenmoser, A. *Science* **2000**, *5495*, 1347.
4. (a) Kempeneers, V.; Vastmans, K.; Rozenski, J.; Herdewijn, P. *Nucleic Acids Res*. **2003**, *31*, 6221. (b) Chaput, J. C.; Szostak, J. W. *J. Am. Chem. Soc*. **2003**, *125*, 9274.
5. Kempeneers, V.; Froeyen, M.; Vastmans, K.; Herdewijn, P. *Chem. Biodivers* **2004**, *1*, 112.
6. (a) Secrist, J. A. 3rd; Riggs, R. M.; Comber, R. N.; Montgomery, J. A. *Nucleosides, Nucleotides* **1992**, *11*, 947. (b) Montgomery, J. A.; Thomas, H. J.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem*. **1979**, *22*, 109.
7. Liu, J. L.; Kim, E. A.; Hong, J. H. *Nucleosides, Nucleotides & Nucleic Acids* **2012**, *31*, 411.
8. Wang, W.; Jin, H.; Fuselli, N.; Mansour, T. S. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 2567.
9. Wu, T.; Froeyen, M.; Kempeneers, V.; Pannecouque, C.; Wang, J.; Busson, R.; De Clercq, E.; Herdewijn, P. *J. Am. Chem. Soc*. **2005**, *127*, 5056.
10. Koh, Y. H.; Shim, J. H.; Wu, J. Z.; Zhong, W.; Hong, Z.; Girardet, J. L. *J. Med. Chem.* **2005**, *48*, 2867.
11. Kim, C. U.; Luh, B. Y.; Misco, P. F.; Bronson, J. J.; Hitchcock, M. J.; Ghazzouli, I.; Martin, J. C. *J. Med. Chem*. **1990**, *33*, 1207.
12. Hong, J. H. *Arch. Pharm. Res*. **2007**, *30*, 131.
13. (a) Kumamoto, H.; Topalis, D.; Broggi, J.; Pradere, U.; Roy, V.; Berteina-Raboin, S.; Nolan, S. P.; Deville-Bonne, D.; Andrei, G.; Snoeck, R.; Garin, D.; Crance, J.-M.; Agrofoglio, L. A. *Tetrahedron* **2008**, *64*, 3517. (b) Montagu, A.; Pradere, U.; Roy, V.; Nolan, S. P.; Agrofoglio, L. A. *Tetrahedron* **2011**, *67*, 5317. (c) Huang, Q.; Herdewijn, P. *J. Org. Chem*. **2011**, *76*, 3742.
14. Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett*. **1999**, *1*, 953.
15. Hocková, D.; Holý, A.; Masojídková, M.; Keough, D. T.; De Jersey, J.; Guddat, L. W. *Bioorg. Med. Chem*. **2009**, *17*, 6218.
16. Robins, M. J.; Uznanski, B. *Can. J. Chem*. **1981**, *59*, 2608.
17. Vorbruggen, H.; Ruh-Pohlenz, C. New York, *Handbook of Nucleoside Synthesis*; 2001.
18. Montgomery, J.; Hewson, K. *J. Med. Chem*. **1969**, *12*, 498.
19. Tong, G. L.; Ryan, K. J.; Lee, W. W.; Acton, E. M.; Goodman, L. *J. Org. Chem*. **1967**, *32*, 859.
20. Holy, A.; Votruba, I.; Merta, A.; Cerny, J.; Vesely, J.; Vlach, J.; Sediva, K.; Rosenberg, I.; Otmar, M.; Hrebabecky, H.; Travniekb, M.; Vonkac, V.; Snoeck, R.; De Clercq, E. *Antiviral Res*. **1990**, *13*, 295.
21. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309.
22. All geometries were optimized with the framework of the density functional theory (DFT), with Spartan modeling software. The B3LYP functional with 6-31G* basis set was employed.