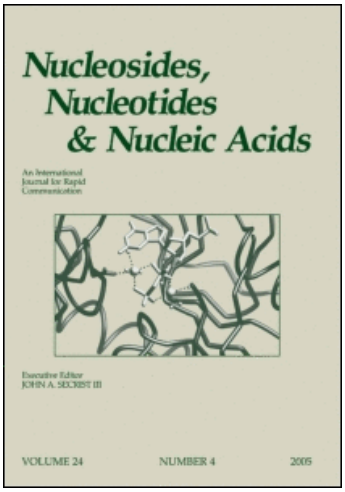

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SYNTHESIS OF UNSATURATED ANALOGUES OF 9-[2-(PHOSPHONO-METHOXY)PROPYL]GUANINE AS ANTIVIRAL AGENTS

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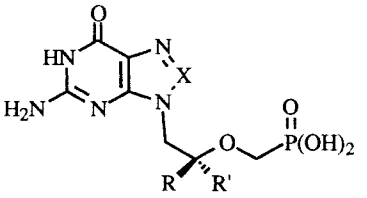
Abstract: The synthesis of 9-[2-(phosphonomethoxy)allyl]guanine (**1**) and 9-[2-(phosphonomethoxy)allyl]-8-aza-guanine (**2**), two new unsaturated acyclic phosphonate nucleosides analogues of the anti-HIV agents PMPG and 8-aza-PMPG, is described. Compounds **1** and **2** were evaluated for activity against human immunodeficiency virus (HIV-1 and HIV-2) and herpes simplex virus (HSV-1 and HSV-2).

Introduction

Acyclic nucleosides phosphonates have emerged as potent antiviral agents; in fact, several (phosphonomethoxy)alkyl derivatives of purines and pyrimidines possess high activity against DNA viruses and retroviruses. After penetration into the cell through an endocytosis-like process, these acyclo nucleotide analogues are converted into diphosphoryl derivatives and interact with the viral polymerases as either competitive inhibitors or alternative substrates.[1]

The 2-(phosphonomethoxy)ethyl-derivatives of adenine (PMEA), 2-monoaminopurine (PMEMAP), 2,6-diaminopurine (PMEDAP) and guanine (PMEG) proved active against a wide range of retroviruses and DNA viruses.[2,3] Since PMEG is cytotoxic at doses that are only sligthly higher than those effective, attempts have been made to modify its structure so as to provide new antiviral agents with higher selectivity index. As result, the (*R*)-2'-methyl derivative of PMEG [(*R*)-PMPG] has emerged as a good anti-HIV agent, with cytotoxicity lower than that shown by the parent compound.[4]

Recently we found that the substitution of the CH group with nitrogen at 8-position of the purine system of PMEG (8-aza-PMEG) and (*R*)-PMPG [(*R*)-8-aza-PMPG] resulted in a reduction of cytotoxicity.[5,6] Furthermore, we ascertained that both (*R*)-PMPG and (*R*)-8-aza-PMPG were effective in inhibiting HIV-1 multiplication in long-term cultures. On the contrary, the treatment with PMEG, PMEA and dideoxynucleosides gave rise in all cases to viral breakthrough within 12 days from the infection.[7] Therefore, considering that the efficacy in inhibiting HIV-1 multiplication in long-term cultures gives to (*R*)-PMPG and (*R*)-8-aza-PMPG a remarkable advantage over PMEG, we synthesized their unsaturated analogues (**1** and **2**) in which the 2'-methyl was replaced by a methylene group. In fact, the introduction of a double bond functionality in the sugar moiety of nucleosides, or in the side chain of acyclic nucleotide analogues, has led in some cases to compounds active as antitumor or anti-HIV agents.[8]

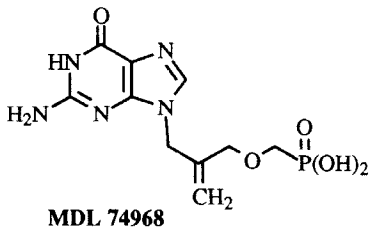


PMEG R, R' = H, X = CH

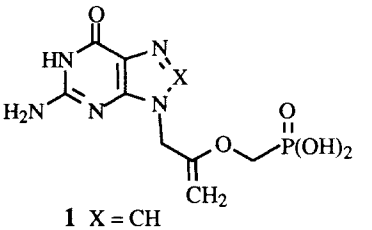
8-aza-PMEG R, R' = H, X = N

(*R*)-PMPG R = CH_3, R' = H, X = CH

(*R*)-8-aza-PMPG R = CH_3, R' = H, X = N



MDL 74968



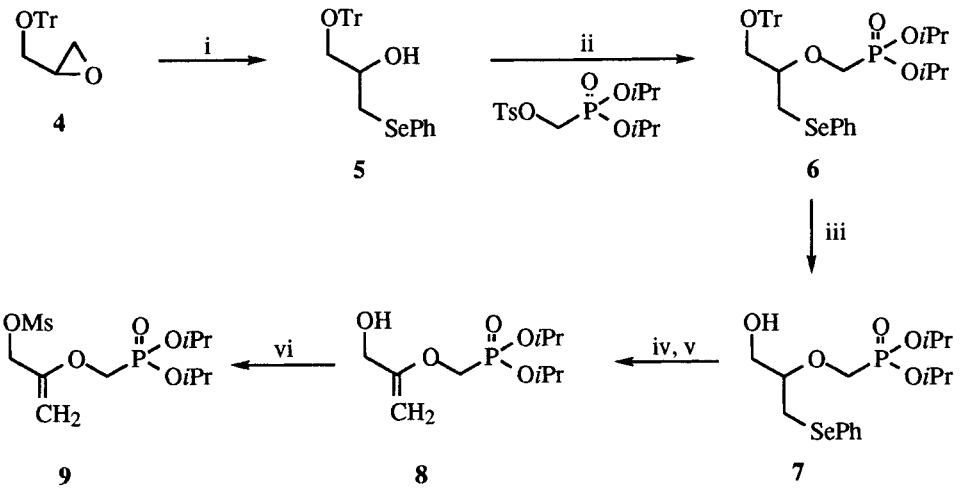
1 X = CH

2 X = N

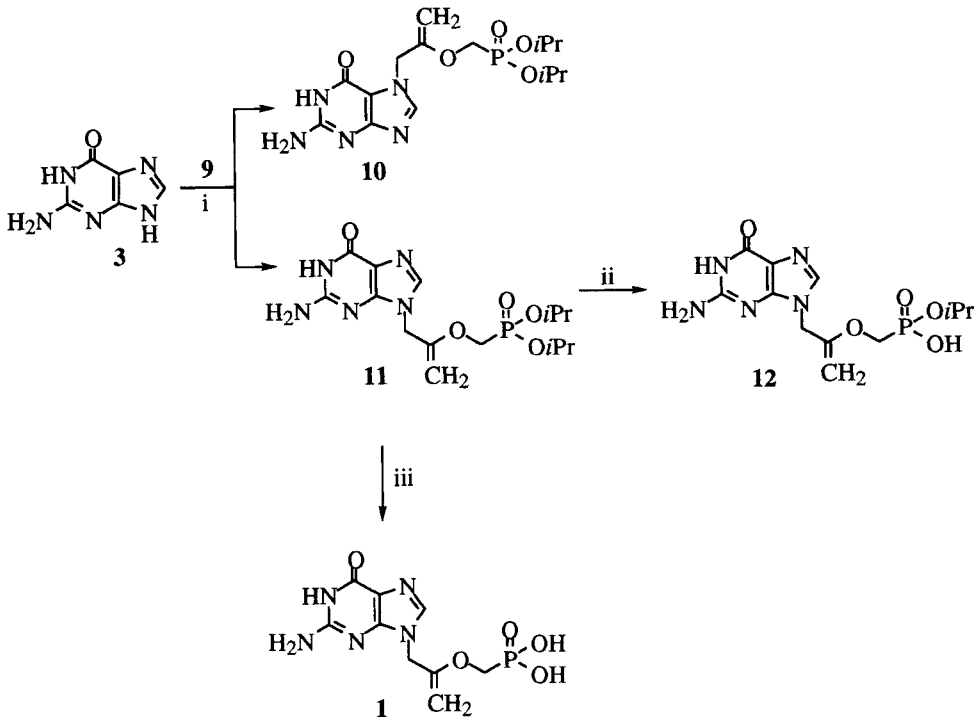
While our work was in progress, Casara *et al.* reported the synthesis of the 9-[2-methylene-3-(phosphonomethoxy)propyl]guanine (**MDL 74968**) a homologue of compound **1**, which inhibited HIV-1 replication *in vitro* with a potency comparable to that of PMEA but with a much lower cytotoxicity.[9]

Results and Discussion

The synthesis of phosphonate **1** was achieved by coupling of the side-chain derivative **9** with guanine (**3**) as shown in Scheme 2. The alkenyl phosphonate moiety of the acyclic substituent was synthesized according to the route shown in Scheme 1. The reaction of (±)-triphenylmethoxymethyloxirane (**4**) with the phenylselenenyl anion afforded the (±)-



Scheme 1 i: PhSe-SePh, $NaBH_4$, EtOH; ii: NaH, THF; iii: TsOH, MeOH; iv: $NaIO_4/NaHCO_3$; v: $NH(iPr)_2$; vi: MsCl.



Scheme 2 i: Cs_2CO_3, DMSO; ii: LiOH; iii: TMSBr, MeCN, 2,4,6-collidine, NaOH.

1-triphenylmethoxy-3-phenylselenenyl-propan-2-ol (**5**), which was converted into (±)-2-[(diisopropylphosphono)methoxy]-3-phenyselenenyl-1-(triphenylmethoxy)propane (**6**) by reaction with diisopropyl-(tosyloxy)-methyl-phosphonate. Selective hydrolysis of **6** with *p*-toluensulphonic acid gave the intermediate **7** which, after oxidation with sodium periodate followed by thermal elimination in benzene in the presence of diisopropylamine, was converted into vinyl-ether **8**. Conversion of **8** to mesylate **9** was obtained by treatment with mesyl chloride.

Compound **9** was coupled to guanine in DMSO in the presence of cesium carbonate to give the N^7- and N^9-substituted phosphonate nucleotides **10** and **11** (Scheme 2). Determination of the position of the acyclic substituent at the heterocyclic bases was deduced by comparison of their UV and ^{13}C-NMR spectral data with those of similar N^7- and N^9-alkylated guanine derivatives.[2,5,10,11]

Because of the instability of enol ether functionality in acidic medium we first attempted to hydrolyze the phosphonate **11** with lithium hydroxide in DMF at 40 °C. The mono-isopropyl ester **12** was obtained, which was not further hydrolyzable. Sequential cleavage of the ester **11** was effected by treatment with bromotrimethylsilane in acetonitrile in the presence of 2,4,6-collidine, followed by treatment with sodium hydroxide to provide the target compound **1** as disodium salt.

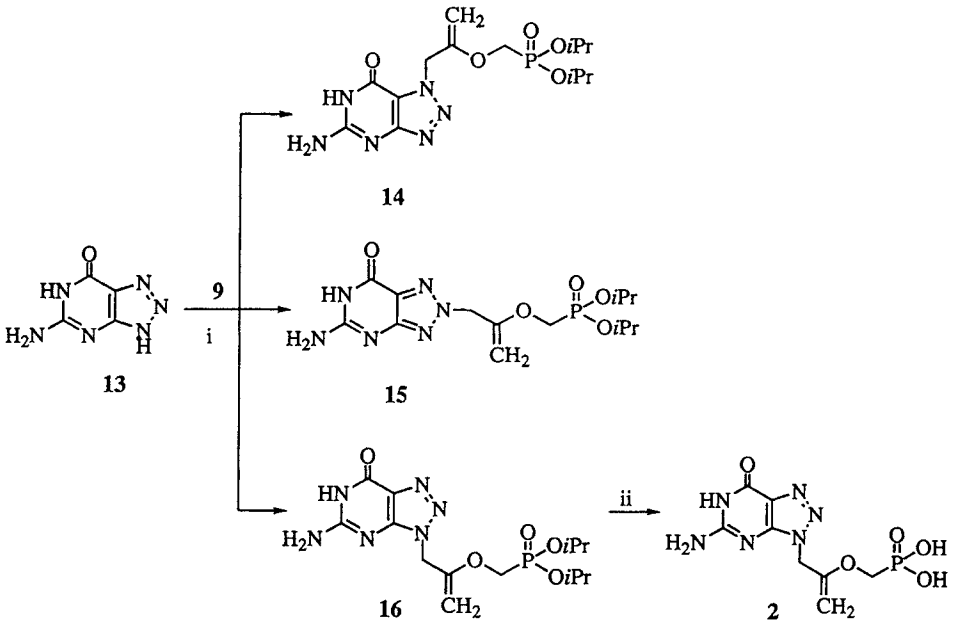
The 8-aza-analogue of **1** (compound **2**) was obtained in similar way by reaction of **9** with 8-aza-guanine (**13**) (Scheme 3). The alkylation reaction resulted in the formation of the N^7-, N^8-, and N^9-substituted isomers (**14**, **15**, and **16**) in 30 % yield.

The regioisomeric ratio $N^9/N^8/N^7$ (estimated by HPLC) was close to 2.5:1.1:0.3. The N^8-isomer **15** was separated from the mixture of N^9- and N^7-isomers **14** and **16** by flash chromatography. The alkylation position of **15** was established by the similarity of the UV spectrum with those reported for N^8-alkylated derivatives of 8-azaguanine.[5,6,11] The presence of N^7- and N^9-alkylated isomers in the mixture was deduced on the basis of the ^{1}H-NMR spectrum in DMSO-d_6 which shows two singlets for the NH_2 protons at δ 6.48 and 6.92 and two singlets for the NCH_2 protons at δ 5.22 and 4.91 of **14** and **16** respectively. Furthermore, the ^{13}C-NMR spectrum shows two signals at δ 113.9 and 124.1 which we have assigned to C(5) of **14** and C(5) of **16** respectively.[2,6]

These isomers were separated by preparative HPLC using a PrePak Cartridge Waters (25x100 mm, Porasil 15-20 μm) column with chloroform-ethanol (7:3).

Deesterification of **16** with bromotrimethylsilane in acetonitrile in the presence of 2,4,6-collidine, followed by treatment with sodium hydroxide, provided compound **2** as disodium salt.

Compounds **1**, **2**, and **12** were tested against HIV-1 (III_B strain), HIV-2 (ROD strain), HSV-1 and HSV-2 as previously reported.[6] While PMEG, (*R*)-PMPG and their



Scheme 3 i: Cs_2CO_3, DMSO; ii: TMSBr, MeCN, 2,4,6-collidine, NaOH.

8-aza-derivatives confirmed active as anti-HIV agents, the unsaturated phosphonates **1**, **2** and the isopropyl ester **12** proved inactive in protecting cell cultures against the HIV-1- and HIV-2-induced cytopathogenicity (Table 1). Like (*R*)-PMPG and (*R*)-8-aza-PMPG, these compounds were less cytotoxic than PMEG and 8-aza-PMEG. None of the compounds was found active against HSV-1 and HSV-2.

Since acyclic nucleotide phosphonates most likely exert their antiviral effect following conversion to the corresponding diphosphate derivatives and inhibition of viral polymerases,[1] the inactivity of phosphonates **1** and **2** might be due to one or more of the following reasons: difficulty to enter the cells, inefficient phosphorylation by guanylate kinase and other phosphorylating enzymes or poor affinity of the diphosphate derivatives for viral polymerases.

SAR studies carried out in order to investigate the effects of substitution at the 2'-position of PMEG, have demonstrated that active anti-HIV agents could be obtained by substituting the methyl group in PMPG with azidomethyl-, halomethyl-, ethyl- or vinyl-groups, whereas larger substituents substantially decrease the anti-HIV activity, indicating a limited steric tolerance at this position of the PMEG side chain.[12] The finding that the unsaturated phosphonates **1** and **2** are inactive may be explained by the lack of a sp^3

Table 1. Cytotoxicity and anti-HIV activity of unsaturated analogues of (*R*)-PMPG and (*R*)-8-aza-PMPG.

Compd	[a]CC_{50}	[b]EC_{50} HIV-1	[c]SI	[b]EC_{50} HIV-2	[c]SI
PMEG	2.4	0.19	12.6	0.2	12
8-aza-PMEG	69	15	4.6	6.2	11
(*R*)-PMPG	> 200	4.5	> 44	4.5	> 44
(*R*)-8-aza-PMPG	> 200	12	> 17	12.5	> 17
1	> 200	> 200	_	> 200	_
2	120	> 120	_	> 120	_
12	> 200	> 200	_	> 200	_

[a]Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%. [b]Compound concentration (μM) required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1 and HIV-2. [c]CC_{50}/EC_{50} ratio.

hybridized carbon atom at the 2'-position, which enables the substituent groups to adopt the right configuration and to assume a conformation suitable for interacting with the target enzyme(s).

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a Büchi apparatus and are uncorrected. Elemental analyses were determined on a Carlo Erba Model 1106 analyzer. UV spectra were recorded with an HP 8452 A diode array spectrophotometer driven by an Olivetti M 24. Thin layer chromatography (TLC) was performed on silica gel 60 F-254 plates and RP-18 F-254 S (Merck); silica gel 60 Merck (70-230, and 230-400 mesh) for column chromatography was used. Preparative HPLC was performed with a Waters 600 chromatograph. Nuclear magnetic resonance ^{1}H, ^{13}C and ^{31}P spectra were determined at 300, 75 and 121 MHz respectively, with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O.

(±)-1-Triphenylmethoxy-3-phenylselenyl-propan-2-ol (5). To stirred solution of diphenyldiselenide anion[13] (42.1 mmol) in absolute EtOH cooled to -30 °C, (±)-triphenylmethoxymethyloxirane[14] (**4**) (31.9 g, 100.8 mmol) was added portionwise. After 30 min. of stirring at -20 °C, the reaction mixture was evaporated to dryness and the residue was extracted with CH_2Cl_2 (2 x 100 mL). The organic phase was washed with

water (2 x 80 mL), dried (Na_2SO_4) and evaporated to dryness. The oily residue was chromatographed on a flash silica gel column using cyclohexane-EtOAc (99:1). The appropriate homogeneous fractions were collected and evaporated *in vacuo* to give **5** (33.6 g, 84.4%). TLC (hexane-EtOAc, 85:15): Rf = 0.6. ^1H NMR ($CDCl_3$): δ 2.58 (d, J = 4.5 Hz, 1H, OH); 3.1 (m, 2H); 3.22 (m, 2H); 3.92 (m, 1H); 7.20-7.47 (m, 20H). *Anal.* Calcd. for $C_{28}H_{26}O_2Se$: C 71.03, H 5.54. Found: C 71.15, H 5.42.

(±)-2-[(Diisopropylphosphono)methoxy]-3-phenylselenyl-1-(triphenylmethoxy)propane (6). To a stirred solution of **5** (32.35 g, 68.32 mmol) in anhydrous THF (160 mL) at 0 °C, under a nitrogen atmosphere, NaH (80% in oil) (2.46 g, 82.85 mmol) was added portionwise and the mixture was refluxed for 5 h. The resulting mixture was cooled at 0 °C and then a solution of diisopropyl-(*p*-tosyloxy)methanephosphonate[15] (28.68 g, 81.85 mmol) in anhydrous THF (60 mL) was added via a cannula. The mixture was stirred at 0 °C for 30 min. and then at room temperature for 14 h. The insoluble material was filtered through a pad of Celite and washed with CH_2Cl_2. The solvent was removed to dryness and the residue was chromatographed on a flash silica gel column using C_6H_6-EtOAc (97:3) as eluent to give **6** as yellow oil (18 g, 40.4%). TLC (ciclohexane-EtOAc, 70:30): Rf = 0.2. ^1H NMR ($CDCl_3$): δ 1.3 (m, 12H, 4xPOCHC*H*$_3$); 3.08 (d, J = 2.7 Hz, 1H, H-3'); 3.15 (d, J = 2.5 Hz, 1H, H-3'); 3.28 (d, J = 2.0 Hz, 1H, H-1'); 3.32 (d, J = 1.3 Hz, 1H, H-1'); 3.7 (m, 1H, H-2'); 3.78 (d, J = 2.9 Hz, 1H, CH_2P); 3.84 (d, J = 2.8 Hz, 1H, CH_2P); 4.75 (m, 2H, 2xPOCH); 7.25 (m, 15H, Ph_3C); 7.3, 7.45 (2m, 5H, C_6H_5Se). *Anal.* Calcd. for $C_{35}H_{41}O_5PSe$: C 64.51, H 6.34. Found: C 64.44, H 6.42.

(±)-2-[(Diisopropylphosphono)methoxy]-3-phenylselenyl-propan-1-ol (7). To a stirred solution of **6** (17 g, 26.08 mmol) in CH_3OH (100 mL), *p*-toluensulphonic acid (6.52 g, 34.27 mmol) was added and the mixture was heated at reflux for 3 h. After evaporation of the solvent, the residue was washed with $H_2O/CHCl_3$, and the organic phase was dried (Na_2SO_4), filtered and evaporated to dryness. The oily residue was purified by flash silica gel column using $CHCl_3$ as eluent to give **7** as yellow oil (6.4 g, 60.1%). TLC ($CHCl_3$-MeOH, 98:2): Rf = 0.69. ^1H NMR ($CDCl_3$): δ 1.35 (m, 12H, 4xPOCHC*H*$_3$); 2.92 (qq, 1H, H-3'); 3.05 (qq, 1H, H-3'); 3.55-3.85 (m, 4H, H-1', H-2', OH); 3.95 (d, J = 5.3 Hz, 1H, CH_2P); 4.05 (d, J = 4.6 Hz, 1H, CH_2P); 4.72 (m, 2H, 2xPOCH); 7.2, 7.5 (2m, 5H, C_6H_5Se). *Anal.* Calcd. for $C_{16}H_{27}O_5PSe$: C 46.95, H 6.65. Found: C 46.87, H 6.72.

2-[(Diisopropylphosphono)methoxy]prop-2-en-1-ol (8). To a solution of **7** (6.4 g, 15.64 mmol) in 280 mL of MeOH/H_2O (6:1), $NaIO_4$ (5 g, 23.44 mmol) and

$NaHCO_3$ (1.44 g, 17.19 mmol) were added and the reaction mixture was stirred at room temperature for 1 h. The suspension was filtered and the solution was concentrated in vacuo. The residue was taken up in CH_2Cl_2 and washed with H_2O. Evaporation of organic layer yields the crude selenoxide which was dried in vacuo.

The selenoxide was refluxing in benzene in the presence of diisopropylamine (17.33 g, 171.24 mmol) for 48 h. The mixture was evaporated to dryness, and the residue was chromatographed on a flash silica gel column using $CHCl_3$ to give **8** as a yellow oil (2.5 g, 63%). TLC ($CHCl_3$-CH_3OH, 96:4): Rf = 0.59. ^1H NMR ($CDCl_3$): δ 1.30 (m, 12H, 4xPOCHC*H*$_3$); 3.2 (pseudo-t, 1H, OH); 3.93 (d, J = 10.2 Hz, 2H, CH_2P); 4.03 (s, 2H, H-1'); 4.07 (d, J =3.9 Hz, 1H, H-3'); 4.3 (d, J = 3.0 Hz, 1H, H-3'); 4.8 (m, 2H, 2xPOCH). *Anal.* Calcd. for $C_{10}H_{21}O_5P$: C 47.62, H 8.39. Found: C 47.81, H 8.32.

2-[(Diisopropylphosphono)methoxy]-1-(methylsulfonyloxy)prop-2-ene (9). To a stirred solution of **8** (2.4 g, 9.51 mmol) in anhydrous CH_2Cl_2 (30 mL) and methanesulfonyl chloride (1.29 g, 11.4 mmol) at 0 °C under a nitrogen atmosphere, was added slowly triethylamine (1.92 g, 18.9 mmol). The resulting mixture was stirred for 10 min, filtered and the solution was concentrated *in vacuo*. Water (60 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3 x 60 mL). The organic layers combined were dried (Na_2SO_4) and filtered. After evaporation to dryness, the residue was chromatographed on a flash silica gel column using $CHCl_3$ to provide **9** as a yellow oil (2.4 g, 75%). TLC ($CHCl_3$-CH_3OH, 96:4): Rf = 0.83. ^1H NMR ($CDCl_3$): δ 1.35 (m, 12H, 4xPOCHC*H*$_3$); 3.04 (s, 3H, SO_3CH_3); 3.8 (d, J = 10.3 Hz, 2H, CH_2P); 4.3 (d, J = 3.5 Hz, 1H, H-3'); 4.45 (d, J = 3.5 Hz, 1H, H-3'); 4.63 (s, 2H, H-1'); 4.75 (m, 2H, 2xPOCH). *Anal.* Calcd. for $C_{11}H_{23}O_7PS$: C 40.0, H 7.02. Found: C 39.96, H 7.13.

7-[2-(Diisopropylphosphono)methoxy-allyl]guanine (10), and 9-[2-(Diisopropylphosphono)methoxy-allyl]guanine (11). A mixture of guanine (**3**) (0.5 g, 3.31 mmol), cesium carbonate (1.4 g, 4.3 mmol) and **9** (1.1 g, 3.31 mmol) in anhydrous DMSO (12 mL) was stirred under a nitrogen atmosphere at 90 °C for 1 h. The reaction mixture was filtered, and the solvent was evaporated *in vacuo*. The residue was chromatographed on a silica gel column with $CHCl_3$-MeOH-NH_4OH (95:4.7:0.3) to give **10** as a white solid (190 mg, 15%). M.p. 210-212 °C; TLC ($CHCl_3$-MeOH-NH_4OH, 90:9:1): Rf = 0.58. ^1H NMR (Me_2SO-d_6): δ 1.25 (m, 12H, 4xPOCHC*H*$_3$); 3.97 (d, J = 9.7 Hz, 2H, CH_2P); 4.12, 4.33 (2br s, 2H, H-3'); 4.6 (m, 2H, 2xPOCH); 4.95 (br s, 2H, H-1'); 6.35 (br s, 2H, NH_2); 7.8 (s, 1H, H-8); 11.35 (br s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): δ 17.5. ^{13}C NMR (Me_2SO-d_6): 158.0; 157.8; 154.8; 153.2; 143.6; 108.5; 86.1; 71.2; 71.0; 62.0 (d, J = 166.9 Hz); 48.2; 24.20; 24.15; 23.04; 23.98. *Anal.* Calcd. for $C_{15}H_{24}N_5O_5P$: C 46.75, H 6.28, N 18.17. Found: C 46.71, H 6.33, N 18.09.

Further elution of the same column provided **11** as a white solid (220 mg, 18.4%). M.p. 173-175 °C; TLC ($CHCl_3$-CH_3OH-NH_4OH, 90:9:1): Rf = 0.46. ^1H NMR (Me_2SO-d_6): δ 1.25 (m, 12H, 4xPOCHCH_3); 4.0 (d, J = 9.8 Hz, 2H, CH_2P); 4.06 (d, J = 2.8 Hz, 1H, H-3'); 4.35 (d, J = 2.9 Hz, 1H, H-3'); 4.6 (s, m, 4H, 2xPOCH, H-1'); 6.45 (br s, 2H, NH_2); 7.62 (s, 1H, H-8); 10.58 (br s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): δ 17.6. ^{13}C NMR (Me_2SO-d_6): 157.6; 157.4; 153.6; 151.7; 137.9; 116.5; 86.0; 71.4; 71.3; 62.0 (d, J = 167.5 Hz); 45.0; 24.15; 24.09; 23.98; 23.93. *Anal.* Calcd. for $C_{15}H_{24}N_5O_5P$: C 46.75, H 6.28, N 18.17. Found: C 46.88, H 6.16, N 18.24.

9-[2-(Isopropylphosphono)methoxy-allyl]guanine lithium salt (12). To compound **11** (170 mg, 0.363 mmol) in 10 ml of DMF was added lithium hydroxide (304 mg, 7.2 mmol). The reaction mixture was stirred under nitrogen at room temperature for 14 h. The solvent was evaporated in vacuo and the residue was chromatographed on reverse phase silica gel column (C18) eluting with CH_3CN-H_2O (80:20). The eluate was taken to dryness and the residue was crystallized from ethanol to give 49 mg of **12** (33.3%). M.p. >300 °C; ^1H NMR (Me_2SO-d_6): δ 1.25 (2 d, 6H, 2xPOCHCH_3); 3.50 (d, J = 1.6 Hz, 1H, H-3'); 3.60 (d, J = 9.6 Hz, 2H, CH_2P); 4.13 (d, J = 1.6 Hz, 1H, H-3'); 4.38 (m, 1H, POCH); 4.50 (s, 2H, H-1'); 7.30 (br s, 2H, NH_2); 7.70 (s, 1H, H-8); 11.30 (br s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): δ 8.2. *Anal.* Calcd. for $C_{12}H_{17}LiN_5O_5P$: C 41.27, H 4.91, N 20.05. Found: C 41.05, H 4.63, N 19.95.

9-[2-(Phosphonomethoxy)allyl]guanine disodium salt (1). To compound **11** (200 mg, 0.52 mmol) in anhydrous acetonitrile (30 mL), were added 2,4,6-collidine (126 mg, 1.02 mmol) and bromotrimethylsilane (694 mg, 5.18 mmol) under nitrogen atmosphere. The mixture was stirred at room temperature for 24 h and then evaporated *in vacuo* to afford a residue which was treated with an aqueous solution (20 ml) of sodium hydroxide (160 mg, 4 mmol). The resulting mixture was estracted with chloroform and the aqueous phase was lyophilized. The crude product was purified by reverse-phase HPLC on a silica gel column (C-18) eluting with CH_3CN-H_2O (80:20) to furnish the pure sodium salt of **1** as a white solid (45%). UV (water, pH 12) λ_{max} 256, and 266 nm. ^{31}P NMR (D_2O): δ 12.9. ^1H NMR (D_2O): δ 3.60 (d, J = 9.5 Hz, 2H, CH_2P); 3.72 (d, J = 1.6 Hz, 1H, H-3'); 4.09 (d, J = 1.6 Hz, 1H, H-3'); 4.52 (s, 2H, H-1'); 7.70 (s, 1H, H-8). *Anal.* Calc. for $C_9H_{10}N_5Na_2O_5P$: C 31.32, H 2.92, N 20.29. Trov. C 30.98, H 3.15, N 19.94.

7-[2-(Diisopropylphosphono)methoxy-allyl]-8-aza-guanine (14), 8-[2-(Diisopropylphosphono)methoxy-allyl]-8-aza-guanine (15), and 9-[2-(Diisopropylphosphono)methoxy-allyl]-8-aza-guanine (16). A mixture of 8-

aza-guanine (**13**) (1 g, 6.56 mmol), cesium carbonate (2.78 g, 8.52 mmol) and **9** (2.2 g, 6.62 mmol) in anhydrous DMSO (20 mL) was stirred under a nitrogen atmosphere at 90 °C for 1 h. The reaction mixture was cooled at room temperature, filtered, and the solvent was evaporated *in vacuo*. The residue was adsorbed from methanolic solution on silica gel. The sorbent was applied onto a silica gel column and eluted with $CHCl_3$-MeOH-NH_4OH (95:4.9:0.1). The fasted eluted minor fractions were discarded. The N^8-isomer (**15**) was then eluted to give 160 mg (8.3% yield) of a white solid. M.p. 191-193 °C; TLC ($CHCl_3$-MeOH, 85:15): Rf = 0.48. UV (ethanol) λ_{max} 240, and 292 nm. ^1H NMR (Me_2SO-d_6): δ 1.15 (m, 12H, 4xPOCHCH_3); 4.00 (d, J = 9.9 Hz, 2H, CH_2P); 4.40 (d, J = 2.93 Hz, 1H, H-3'); 4.47 (d, J = 3.90 Hz, 1H, H-3'); 4.55 (m, 2H, 2xPOCH); 5.13 (s, 2H, H-1'); 6.55 (br s, 2H, NH_2); 10.95 (br s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): δ 17.2. ^{13}C NMR (Me_2SO-d_6): 159.85; 155.9; 155.7; 154.2; 127.0; 88.4; 71.15; 71.03; 61.4 (d, J = 162 Hz); 58.4; 23.95; 23.87; 23.74; 23.64. *Anal.* Calcd. for $C_{14}H_{23}N_6O_5P$: C 43.52, H 6.00, N 21.75. Found: C 43.28, H 6.33, N 21.56.

Further elution of the same column provided 240 mg of a mixture N^7-, and N^9-isomers in the ratio 1:7.5 (estimated by HPLC). These isomers were separated by preparative HPLC using a PrePak Cartridge Waters (25x100 mm, Porasil 15-20 μm) and eluting with chloroform-ethanol (70:30). The fast eluted compound was N^7-isomer (**14**) as a foam (2.55%). UV (ethanol) λ_{max} 239, and 297 nm. ^1H NMR (Me_2SO-d_6): δ 1.19 (m, 12H, 4xPOCHCH_3); 3.96 (d, J = 9.9 Hz, 2H, CH_2P); 4.27 (d, J = 2.92 Hz, 1H, H-3'); 4.40 (d, J = 2.90 Hz, 1H, H-3'); 4.53 (m, 2H, 2xPOCH); 5.22 (s, 2H, H-1'); 6.48 (s, 2H, NH_2); 11.00 (br s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): δ 17.08. *Anal.* Calcd. for $C_{14}H_{23}N_6O_5P$: C 43.52, H 6.00, N 21.75. Found: C 43.33, H 6.25, N 21.53.

Further elution of the column gave the N^9-isomer (**16**) as a foam (19.13%): UV (ethanol) λ_{max} 254, and 264 (sh) nm. ^1H NMR (Me_2SO-d_6): δ 1.17 (m, 12H, 4xPOCHCH_3); 3.97 (d, J = 9.9 Hz, 2H, CH_2P); 4.13 (d, J = 2.9 Hz, 1H, H-3'); 4.40 (d, J = 2.9 Hz, 1H, H-3'); 4.53 (m, 2H, 2xPOCH); 4.91 (s, 2H, H-1'); 6.92 (br s, 2H, NH_2); 10.95 (br s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): δ 17.22. *Anal.* Calcd. for $C_{14}H_{23}N_6O_5P$: C 43.52, H 6.00, N 21.75. Found: C 43.37, H 6.14, N 21.50.

9-[2-(Phosphonomethoxy)allyl]-8-aza-guanine disodium salt (2). To compound **16** (200 mg, 0.52 mmol) in anhydrous acetonitrile (30 mL), were added 2,4,6-collidine (126 mg, 1.02 mmol) and bromotrimethylsilane (694 mg, 5.18 mmol) under nitrogen atmosphere. The mixture was stirred at room temperature for 24 h, filtered and evaporated *in vacuo* to afford a residue which was treated with an aqueous solution (20 ml) of sodium hydroxide (160 mg, 4 mmol). The resulting mixture was extracted with chloroform and the aqueous phase was lyophilized. The crude product was purified by

reverse-phase HPLC on a silica gel column (C-18) eluting with CH_3CN-H_2O (80:20) to furnish the pure sodium salt of **2** as white solid (53%). UV (water, pH 12) λ_{max} 252, and 278 nm. ^{31}P NMR (D_2O): δ 12.48. ^1H NMR (D_2O): δ 3.57 (d, J = 9.6 Hz, 2H, CH_2P); 3.79 (d, J = 1.6 Hz, 1H, H-3'); 4.14 (d, J = 1.6 Hz, 1H, H-3'); 4.83 (s, 2H, H-1'). *Anal.* Calc. for $C_8H_9N_6Na_2O_5P$: C 27.76, H 2.62, N 20.29. Trov. C 27.58, H 2.95, N 20.43.

Inhibition of HIV-1-, and HIV-2-induced Cytopathogenicity in MT-4 Cells. Activity of compounds against the HIV-1 and HIV-2 multiplication in acutely infected cells was based on the inhibition of the virus-induced cytopathogenicity (CPE) in MT-4 cells. Briefly, 50 μL of culture medium (RPMI 10% FCS) containing 1 x 10^4 MT-4 cells were added to each well of flat bottomed microtiter trays containing 50 μL of medium with or without various concentrations of the test compounds (dissolved in DMSO at 100 mg/ml and then diluted in culture medium). Twenty μL of HIV-1 or HIV-2 suspensions containing 100 $CCID_{50}$ were then added (m.o.i. = 0.01). After a 4-day incubation at 37 °C (8 days for HIV-2), the number of viable MT-4 cells was determined by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method.[15] Cytotoxicity of compounds was evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected MT-4 cells, as monitored by the MTT method. PMEG, 8-aza-PMEG, (*R*)-PMPG, and (*R*)-8-aza-PMPG were used as reference compounds.

Anti-HSV Assay. The anti-HSV activity of the compounds was evaluated by plaque reduction test (PRT) and was refered as the concentration of compound required to reduce the number of plaques by 50%.

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