Synthesis and Antiviral Activity of 8-Aza Analogs of Chiral [2-(Phosphonomethoxy)propyl]guanines

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Received April 25, 1995®

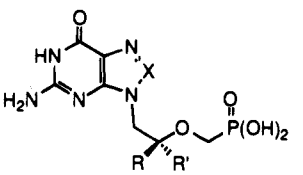
(*R*)- And (*S*)-8-aza-9-[2-(phosphonomethoxy)propyl]guanine [(*R*)- and (*S*)-8-aza-PMPG] were synthesized and tested *in vitro* for anti-human immunodeficiency virus (HIV) activity. The synthesis of the above compounds and of (*R*)-9-[2-(phosphonomethoxy)propyl]guanine [(*R*)-PMPG] was carried out through the alkylation of 8-azaguanine or guanine with (*R*)- and (*S*)-2-*O*-[(diisopropylphosphono)methyl]-1-*O*-(tolylsulfonyl)-1,2-propanediol followed by deprotection of the phosphonic moiety. A different, even more convenient synthesis of (*R*)-8-aza-PMPG starting from 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone and (*R*)-[2-[(diisopropylphosphono)-methoxy]propyl]amine is also reported. Both (*R*)-8-aza-PMPG and (*R*)-PMPG demonstrated anti-HIV activity in the MTT assay with EC_{50} values of 12 and 4.5 μM, respectively. The corresponding *S* enantiomers were found to be less potent. When evaluated in combination with AZT, ddI, or DABO 603, (*R*)-8-aza-PMPG gave additive, additive, and synergistic anti-HIV-1 effects, respectively.

Introduction

Acyclic nucleoside phosphonates have received considerable attention after the demonstration of the broad antiviral activity of (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine [(*S*)-HPMPA].[1] Since then, numerous (phosphonomethoxy)alkyl derivatives of purines and pyrimidines have been shown to possess antiviral activity,[2,3] reinforcing the concept that the phosphonomethoxy group is a metabolically stable bioisosteric replacement for the phosphate. Among them, (phosphonomethoxy)-ethyl derivatives of adenine (PMEA), 2-aminopurine (PMEMAP), and 2,6-diaminopurine (PMEDAP) emerged as specific inhibitors of retroviruses,[4,5] whereas the (phosphonomethoxy)ethyl derivative of guanine (PMEG) turned out to be one of the most potent anti-herpes virus and antiretrovirus agents reported so far.[6] Since PMEG is cytotoxic at concentrations that are only slightly higher than effective ones, a number of attempts have been made to modify its structure so as to provide new antiviral agents with a higher selectivity index. From these studies, the (*R*)-2′-methyl isomer of PMEG [(*R*)-PMPG][7] has emerged as a good anti-HIV agent *in vitro*, being 5-fold less potent than the parent compound but at least 30-fold less cytotoxic. Moreover, we have recently reported that the substitution of the CH group with nitrogen at position 8 of the purine ring of PMEG results in a remarkable reduction of cytotoxicity.[8]

In order to verify whether a similar isosteric modification in the purine moiety of both (*R*)- and (*S*)-PMPG could afford compounds endowed with favorable selectivity indexes, we synthesized the 8-aza derivatives of the latter compounds. As a matter of fact, the synthesis of (*R*)- and (*S*)-8-aza-PMPG has been reported by Holy et al.[9] However, as far as (*R*)-8-aza-PMPG and its 8-isomer were concerned, no biological data have been provided.

In this paper we present new procedures for the synthesis of the *R* and *S* isomers of PMPG and 8-aza-PMPG, which are more convenient than those reported by Yu et al.[7] and Holy et al.,[9] and reported on their *in vitro* cytotoxicity and anti-HIV activity. We also tested the anti-HIV activity of (*R*)-8-aza-PMPG in combination with nucleoside analogs, such as AZT and ddI, and with a nonnucleoside reverse transcriptase inhibitor, DABO 603,[10] and the anti-herpes activity of the new phosphonates.



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 (**1**)
(*S*)-8-aza-PMPG R = H, R′ = CH_3, X = N (**2**)

Chemistry

The synthesis of the *R* and *S* isomers of 8-aza-PMPG (**1**, **2**) reported by Holy et al.[9] has been performed in three steps starting from 8-azaguanine with an overall yield of 10.6%. In a recent paper[8] we described the synthesis of 8-aza-PMEG in only two steps by reaction of 8-azaguanine with [[di-(2-ethyl)phosphonyl]methoxy]-[(1-tolylsulfonyl)oxy]ethane in DMSO in the presence of cesium carbonate with an overall yield of 23.5%. This finding prompted us to apply this synthetic method also to the *R* and *S* isomers of 8-aza-PMPG.

The reaction of 8-azaguanine (**3**) with (*R*)-2-*O*-[(diisopropylphosphono)methyl]-1-*O*-(methylsulfonyl)-1,2-propanediol (**4**) (synthesized as described by Yu et al.;[7] optical purity exceeding 99% as determined by HPLC

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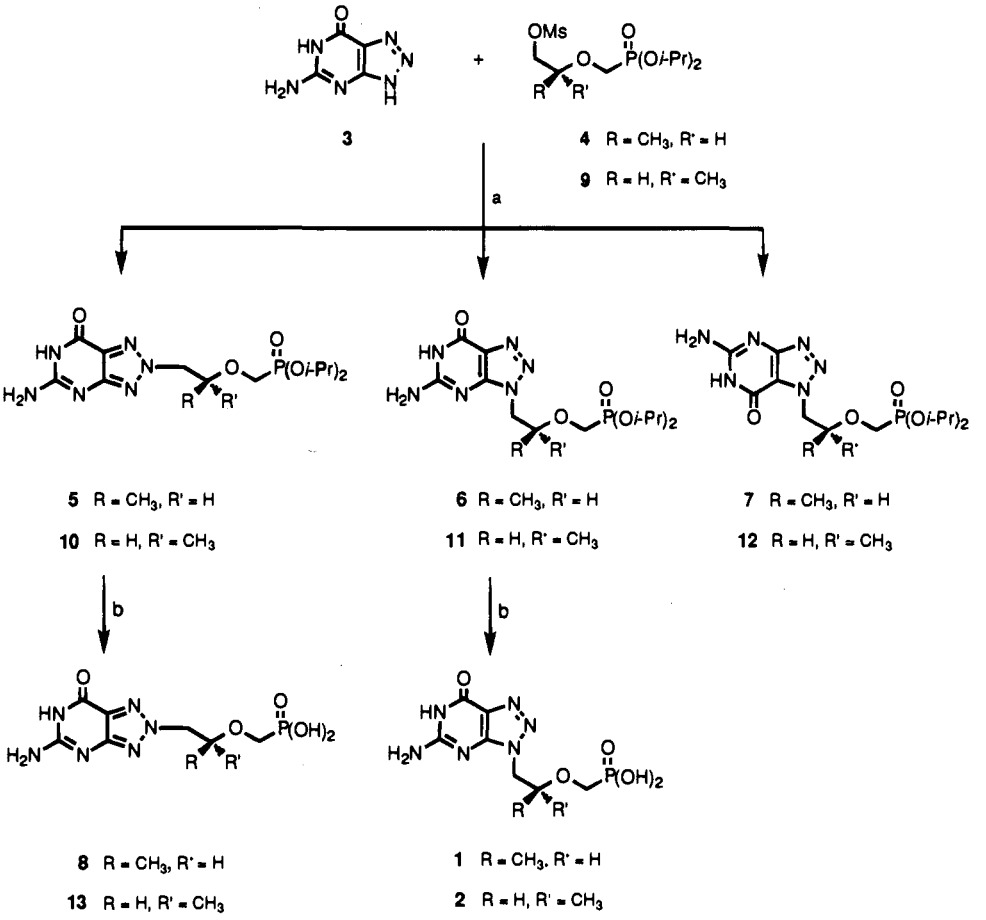
® Abstract published in *Advance ACS Abstracts*, September 1, 1995.

0022-2623/95/1838-4007$09.00/0

Scheme 1[a]



[a] (a) Cs_2CO_3, DMSO; (b) TMSBr, CH_3CN.

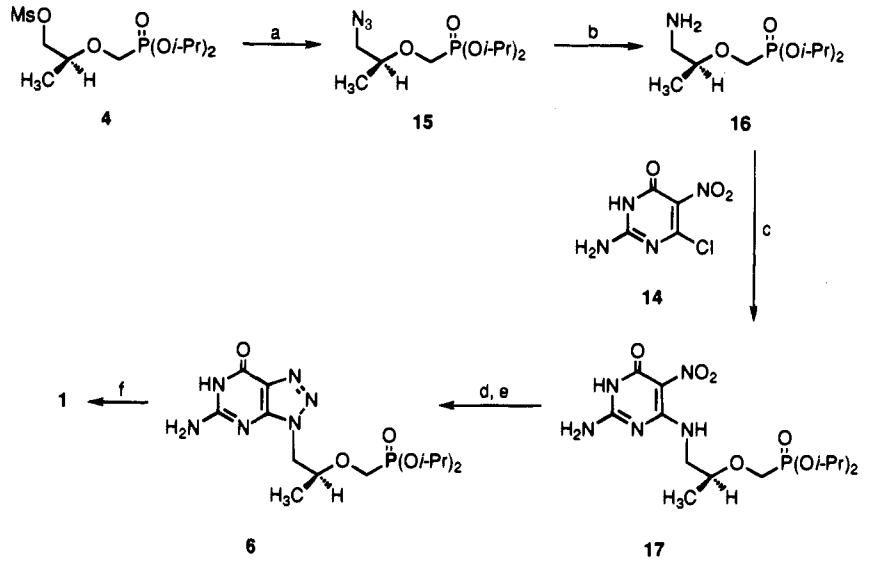
on a Chiracel OA column) in DMSO resulted in a mixture of 7-, 8-, and 9-alkylated isomers (**5**–**7**) in 57% overall yield. The regioisomer ratio $N^9/N^8/N^7$ was close to 2:1.5:1. The 8-isomer **5** was separated from the mixture of 9- and 7-isomers **6** and **7** by flash chromatography. The position of alkylation was deduced on the basis of the comparison of the UV spectral data of **5** as compared with those of N^8-alkylated 8-azaguanine derivatives.[8,11,12] 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 NH proton at δ 10.98 and 11.22; furthermore, the ^{13}C-NMR spectrum shows two signals at δ 113.9 and 124.3 which we have assigned respectively to C(7a) of **7** and C(7a) of **6**.[8,11,12] The possibility of two tautomeric forms was excluded because the chemical shifts of these signals remained unmodified until 120 °C. We attempted to separate these isomers utilizing a variety of chromatographic technics but with no success. So, we decided of utilizing this mixture in the next step. Deesterification with bromotrimethylsilane in MeCN gave a mixture of (*R*)-8-aza-PMPG (**1**) and its 7-substituted isomer (overall yield 90%). After two crystallizations from acetone–water, **1** was obtained as a pure compound (51%). In a similar way the phosphonate **8** was obtained from N^8-alkylated derivative **5**. The structure of **1** was confirmed by its UV spectrum which was similar to that reported for 8-aza-PMEG.[8]

The (*S*)-8-aza-PMPG (**2**) was synthesized as reported above starting from **3** and (*S*)-2-*O*-[(diisopropylphosphono)methyl]-1-*O*-(methylsulfonyl)-1,2-propanediol (**9**) (Scheme 1). The mixture of N^9- and N^7-alkylated isomers **11** and **12** was hydrolyzed with bromotrimethylsilane in MeCN; from the mixture of phosphonates, **2** was obtained as a pure compound after repeated crystallizations from acetone–water. In a similar way, the N^8-alkylated derivative **10** was converted into phosphonate **13**.

We have also developed an additional, more convenient procedure for the synthesis of (*R*)-8-aza-PMPG (**1**) (Scheme 2). Reaction of 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (**14**) with (*R*)-2-[(diisopropylphosphono)-methoxy]propylamine[13] (**16**) [prepared by reaction of **4** with sodium azide followed by catalytic hydrogenation (Pd/C) of the azido derivative **15**] gave the dialkyl phosphonate **17**. Compound **17** was hydrogenated in the presence of Raney nickel in methanol to give the amino derivative which was immediately cyclized to **6** in the presence of nitrous acid (91.7% yield). This compound was converted into **1** with bromotrimethylsilane in MeCN.

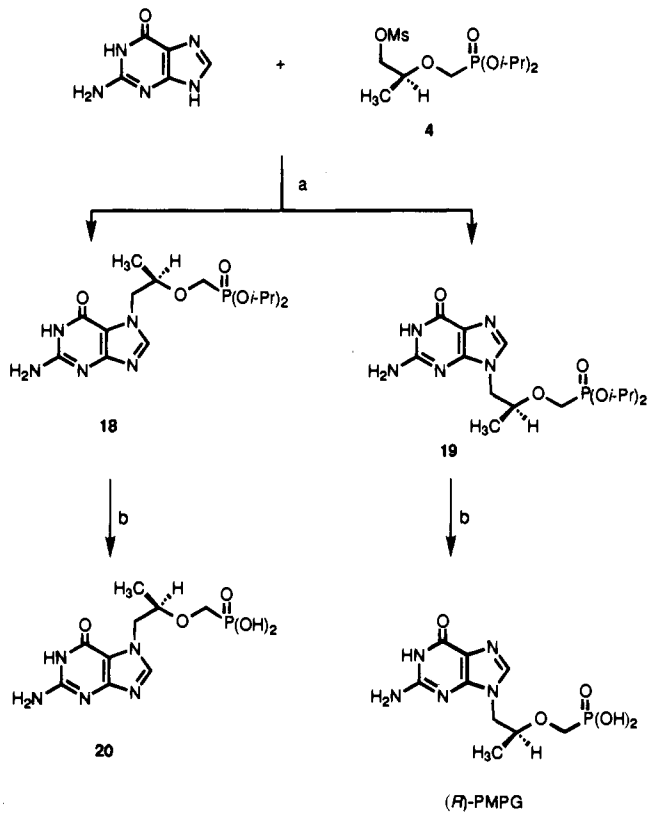
An approach similar to that used for the synthesis of **1** reported in Scheme 1 was employed for the synthesis of (*R*)-PMPG, which we used as a reference compound. Other authors have previously synthesized this compound starting from 2-amino-6-chloropurine and **4**, with an overall yield of 37%.[7] On the other hand, Holy et al.[9] have described the synthesis of (*R*)-PMPG starting from 9-(*R*)-(2-hydroxypropyl)-N^2-benzoylguanine [prepared in three steps from 2-amino-6-chloropurine and

Scheme 2[a]



[a] (a) NaN_3, DMF; (b) H_2/Pd/C; (c) TEA, DMF; (d) H_2/Raney Ni; (e) $NaNO_2$/AcOH/H_2O; (f) TMSBr, CH_3CN.

Scheme 3[a]



[a] (a) Cs_2CO_3, DMSO; (b) TMSBr, CH_3CN.

(*R*)-2-*O*-tetrahydropyranyl-1-*O*-(*p*-tolylsulfonyl)propane-1,2-diol] and di(2-propyl)[[(*p*-tolylsulfonyl)oxy]methyl]-phosphonate. We found that the reaction of guanine with **4** in DMSO in the presence of cesium carbonate gave a 1:2 ratio of the alkylated products **18** and **19**, with the desired N^9 isomer **19** being isolated as the major product in 44% yield (Scheme 3). The structure assignment for **18** and **19** was based on ^1H- and ^{13}C-NMR spectroscopic data.[14]

Sequential removal of the protecting groups of **19** with bromotrimethylsilane in MeCN afforded (*R*)-PMPG, with an overall yield of 42%. In a similar way, the N^7-substituted isomer **20** was obtained starting from **18**.

Table 1. Cytotoxicity and Anti-HIV Activity of 8-Aza Analogues of (*R*)- and (*S*)-PMPG

compd	CC_{50}[a]	EC_{50} (HIV-1)[b]	SI[c]	EC_{50} (HIV-2)[b]	SI[c]
(*R*)-8-aza-PMPG (1)	>400	12	>33	12.5	>32
8	>400	53	>7.5	73	>5.5
(*S*)-8-aza-PMPG (**2**)	>400	93	>4.3	200	2
13	>400	>400		>400	
(*R*)-PMPG	>400	4.5	>89	4.5	>89
(*S*)-PMPG	>48	19	2.5	30	1.6
PMEG	2.4	0.19	12.6	0.2	12
8-aza-PMEG	69	15	4.6	6.2	11
AZT	>20	0.01	>2000	0.04	>500

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50% (4 days). [b] Compound concentration (μM) required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1 (4 days) and HIV-2 (8 days). [c] CC_{50}/EC_{50} ratio.

Results and Discussion

The 8-aza analogs of (*R*)- and (*S*)-PMPG (both N^9 and N^8 isomers **1**, **2**, **8**, and **13**) were evaluated *in vitro* for inhibitory activity against HIV-1 and HIV-2 in acutely infected MT-4 cells. The cytotoxicity of compounds was evaluated in parallel with their anti-HIV activity. (*R*)- and (*S*)-PMPG, 8-aza-PMEG, and PMEG were used as reference compounds, together with AZT.

(*R*)-8-aza-PMPG (**1**) was effective in protecting MT-4 cells from the cytopathogenicity induced by both HIV-1 and HIV-2 (Table 1). The antiviral potency of (*R*)-8-aza-PMPG (EC_{50} = 12 μM) was 8-fold higher than that of its *S* enantiomer **2**, 3-fold lower than that of (*R*)-PMPG, and equivalent to those of (*S*)-PMPG and 8-aza-PMEG. Surprisingly, the N^8 isomer of (*R*)-8-aza-PMPG (compound **8**) showed selective, although not very potent, activity against both HIV-1 and HIV-2. The N^8 isomer of (*S*)-8-aza-PMPG (compound **13**) was devoid of antiviral activity.

When the CC_{50} values of PMEG, 8-aza-PMEG, (*R*)- and (*S*)-PMPG, and their 8-aza counterparts are compared, it can be concluded that the substitution of the CH group with nitrogen at position 8 of the purine ring leads to a loss of cytotoxicity. However, contrary to what happens when PMEG is converted to its 8-aza counterpart, the conversion of (*R*)-PMPG into (*R*)-8-aza-

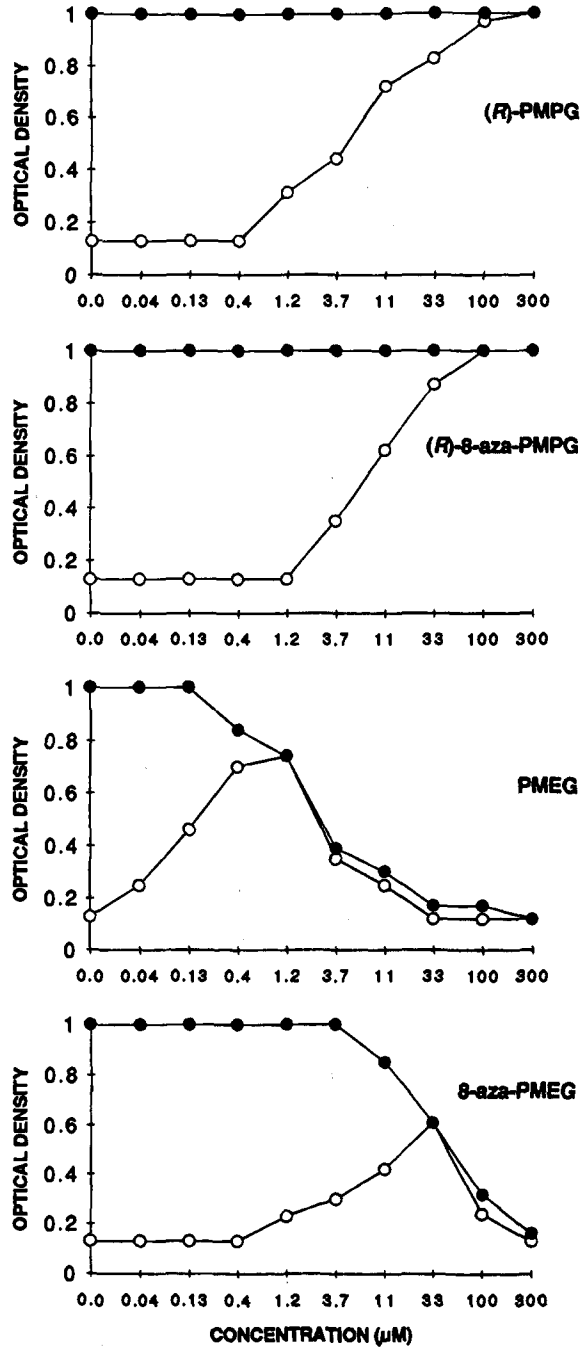


Figure 1. Anti-HIV activity of (*R*)-PMPG, (*R*)-8-aza-PMPG, PMEG, and 8-aza-PMEG. Toxicity (●), uninfected MT-4 cells. Anti-HIV activity (○), infected MT-4 cells. The optical density values are directly proportional to the number of the viable cells, as determined by the MTT assay.

PMPG causes only a very small loss of anti-HIV potency [compare EC_{50}s of PMEG and 8-aza-PMEG and of (*R*)-PMPG and (*R*)-8-aza-PMPG]. As expected, AZT was confirmed as a potent and selective inhibitor of both HIV-1 and HIV-2.

Dose–response curves (Figure 1), obtained by plotting cytotoxicity and anti-HIV-1 activity values over a range of concentrations, illustrate more clearly the improvement in selectivity for the *R* enantiomers of PMPG and its 8-aza derivative over PMEG and 8-aza-PMEG. In fact, the former two compounds provide full protection from the virus-induced CPE up to very high doses, whereas PMEG and 8-aza-PMEG protect over a much narrower concentration range before becoming cytotoxic.

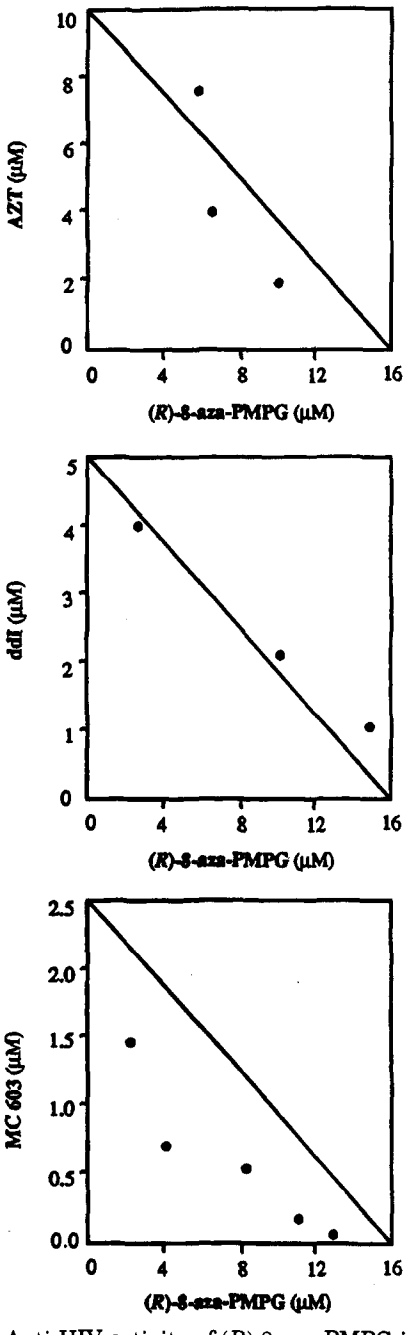


Figure 2. Anti-HIV activity of (*R*)-8-aza-PMPG in combination with nucleoside and nonnucleoside inhibitors of reverse transcriptase. EC_{50} values of the drugs, alone or in combination, are reported.

The cytotoxicity and anti-HIV-1 effects of combinations of (*R*)-8-aza-PMPG (used in the range **1–16** μM) with AZT (0.001–0.01 μM), ddI (0.1–5 μM), or DABO 603 (0.05–2.25 μM) were evaluated by the MTT assay[15] (Figure 2). None of the combinations was cytotoxic for mock-infected MT-4 cells, whereas the combinations of (*R*)-8-aza-PMPG with AZT, ddI, and DABO 603 resulted additive, additive, and synergistic effects, respectively, when evaluated by both isobologram (Figure 2) and FIC indices (data not shown).

When 8-aza analogs of (*R*)- and (*S*)-PMPG were evaluated in plaque reduction assays against HSV-1 and HSV-2 in Vero cells, only PMEG was found effective (EC_{50} = 0.5 and 2.0 μM, respectively), although not as potent as previously reported.[6]

Experimental Section

Melting points were determined on a Buchi apparatus and are uncorrected. Elemental analyses were determined on a EA 1108 CHNS-O (Fisons Instruments) analyzer. Infrared spectra were recorded on a Perkin Elmer instrument, model 1310. Ultraviolet spectra were recorded on an HP 8452 A diode array spectrophotometer driven by an Olivetti M 24. Thin layer chromatography (TLC) was run on silica gel 60 F_{254} plates and RP-18 F_{254} S columns (Merck); silica gel 60 (Merck) (70–230 and 230–400 mesh) for column chromatography was used. Nuclear magnetic resonance (^1H, ^{13}C, ^{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.

(*R*)-5-Amino-2-[2-[(diisopropylphosphono)methoxy]propyl]-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (5), (*R*)-5-Amino-3-[2-[(diisopropylphosphono)methoxy]propyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (6), and (*R*)-5-Amino-1-[2-[(diisopropylphosphono)methoxy]propyl]-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (7). To a stirring mixture of 8-azaguanine (1 g, 6.57 mmol) in anhydrous DMSO (30 mL) and cesium carbonate (2.66 g, 8.18 mmol) under a nitrogen atmosphere was added (*R*)-2-*O*-[(diisopropylphosphono)methoxy]-1-*O*-(methylsulfonyl)-1,2-propanediol[7] (**4**) (2.18 g, 6.57 mmol). The mixture was stirred at 95 °C for 6 h and then at room temperature for 30 min and filtered. The filtrate was evaporated to dryness, and the residue was purified by flash chromatography on silica gel eluting with $CHCl_3$–MeOH (95:5) to give **5** as a yellow oil (0.5 g, 19%). TLC ($CHCl_3$–MeOH, 80:20): R_f 0.67. UV (pH 12): λ_{max} 280 nm (ϵ 7.320). ^1H NMR (Me_2SO-d_6): δ 1.11–1.17 (m, 12 H), 1.19 (d, J = 6.0 Hz, 3 H), 3.65 (dd, J = 9.3, 13.7 Hz, 1 H), 3.80 (dd, J = 9.3, 13.7 Hz, 1 H), 4.16 (m, 1 H), 4.50 (m, 4 H), 6.52 (s, 2 H), 10.98 (s, 1 H). Anal. ($C_{14}H_{25}N_6O_5P$) C, H, N.

Further elution of the same column provided a mixture of **6** and **7** as a yellow foam (0.98 g, 38%). It was impossible to differentiate **6** from **7** by TLC or HPLC.

(*R*)-5-Amino-3-[2-(phosphonomethoxy)propyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (1). Method A. To a mixture of **6** and **7** (0.9 g, 2.32 mmol) in anhydrous acetonitrile (40 mL) was added bromotrimethylsilane (3.55 g, 23.2 mmol) at room temperature under a nitrogen atmosphere. The reaction mixture was stirred at 28 °C for 24 h. After evaporation, the oil residue was treated with water–acetone (5:30 mL) and stirred for 30 min. The resulting mixture was cooled at −20 °C for 14 h to give a precipitate which was collected by filtration (90%). By repeated recrystallization with acetone–water was obtained 1 as a white solid (0.4 g, 51%). Mp: 252–254 °C dec. TLC (H_2O–CH_3CN, 80:20): R_f 0.83. UV (pH 12): λ_{max} 256 nm (ϵ 8300), 280 (ϵ 13 400). $[\alpha]^{20}_D$ −4.85° (*c* 0.62, HCl 0.1 M). ^{31}P NMR (Me_2SO-d_6): 16.3. ^1H NMR (Me_2SO-d_6): δ 1.15 (pseudo-t, 3 H), 3.49 (d, J = 9.1 Hz, 2 H), 4.10 (q, 1 H), 4.43–4.65 (m, 2 H), 6.50 (s, 2 H), 11.05 (s, 1 H). ^{13}C NMR (Me_2SO-d_6): δ 156.0, 155.8, 151.8, 124.3, 74.8 (d, $^3J_{cp}$ = 10.5 Hz), 65.5 (d, $^1J_{cp}$ = 160 Hz), 49.9, 17.9. Anal. ($C_8H_{13}N_6O_5P$) C, H, N.

Method B. Compound **6** (obtained from **17**) (0.6 g, 1.54 mmol) in anhydrous acetonitrile (30 mL) was hydrolyzed with bromotrimethylsilane as described above. The oil residue was treated with a mixture of water–acetone (4:20 mL) and stirred for 30 min at 28 °C. The mixture was cooled at −20 °C for 14 h, and the resulting precipitate was collected by filtration and washed with acetone to give 1 (0.47 g, 90%).

(*R*)-5-Amino-2[2-(phosphonomethoxy)propyl]-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (8). The title compound was prepared from **5** (0.48 g, 1.24 mmol), as described for 1, as a white solid (0.35 g, 90%) Mp: 286–288 °C dec. TLC (H_2O–CH_3CN, 80:20): R_f 0.80. UV (pH 12): λ_{max} 250 nm (ϵ 5000), 298 (ϵ 6500). $[\alpha]^{20}_D$ −23.5° (*c* 0.16, HCl 0.1 M). ^{31}P NMR (Me_2SO-d_6): 16.1. ^1H NMR (Me_2SO-d_6): δ 1.12 (pseudo-t, 3 H), 3.34 (d, J = 9.1 Hz, 2 H), 4.10 (m, 1 H), 4.46–4.52 (m, 2 H), 6.52 (s, 2 H), 10.98 (s, 1H). ^{13}C NMR (Me_2SO-d_6): δ 159.7, 156.7, 154.1, 126.7, 75.4 (d, $^3J_{cp}$ = 9.5 Hz), 65.1 (d, $^1J_{cp}$ = 159 Hz), 59.8, 17.7. Anal. ($C_8H_{13}N_6O_5P$) C, H, N.

(*S*)-5-Amino-2-[2-[(diisopropylphosphono)methoxy]propyl]-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (10), (*S*)-5-Amino-3-[2-[(diisopropylphosphono)methoxy]propyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (11), and (*S*)-5-Amino-1-[2-[(diisopropylphosphono)methoxy]propyl]-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (12). Compound **3** (1 g, 6.57 mmol) in anhydrous DMSO (30 mL) was reacted with cesium carbonate (2.14 g, 6.57 mmol) and (*S*)-2-*O*-[(diisopropylphosphono)methyl]-1-*O*-(methylsulfonyl)-1,2-propanediol[7] (**9**) (1.82 g, 5.47 mmol), as described for the *R* isomer (100 °C, 7 h). Chromatographic purification on a silica gel column with $CHCl_3$–MeOH–NH_4OH (90:0.9:0.1) yielded **10** as an oil (0.55 g, 20%). TLC ($CHCl_3$–MeOH, 80:20): R_f 0.46. UV (pH 12): λ_{max} 280 nm (ϵ 7.300). ^1H NMR (Me_2SO-d_6): δ 1.10–1.20 (m, 15 H), 3.60–3.98 (m, 2 H), 4.15 (dd, J = 6.0, 9.0 Hz, 1 H), 4.40–4.55 (m, 4 H), 6.50 (s, 2 H), 11.0 (s, 1 H). Anal. ($C_{14}H_{25}N_6O_5P$) C, H, N.

Further elution of the same column provided a mixture of 11 and **12** as a yellow foam (0.93 g, 36%). It was impossible to differentiate **11** from **12** by TLC or HPLC.

(*S*)-5-Amino-3-[2-(phosphonomethoxy)propyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (2). The title compound **2** was prepared from the mixture of **11** and **12** (0.9 g, 2.32 mmol) as described for 1 (0.43 g, 55%). Mp: 250–252 °C dec. TLC (H_2O–CH_3CN, 80:20): R_f 0.77. UV (pH 12): λ_{max} 252 nm (ϵ 5900), 278 (ϵ 8900). $[\alpha]^{20}_D$ +4.82° (*c* 0.44, HCl 0.1 M). ^{31}P NMR (Me_2SO-d_6): 16.3. ^1H NMR (Me_2SO-d_6): δ 1.10 (d, J = 5.8 Hz), 3.40 (d, J = 9.3 Hz, 2 H), 4.05 (m, 1H), 4.40–4.60 (m, 2H), 6.50 (s, 2 H), 11.25 (s, 1 H). ^{13}C NMR (Me_2SO-d_6): δ 155.9, 155.6, 151.8, 124.3, 74.9 (d, $^3J_{cp}$ = 9.1 Hz), 65.3 (d, $^1J_{cp}$ = 159.5 Hz), 49.9, 17.9. Anal. ($C_8H_{13}N_6O_5P$) C, H, N.

(*S*)-5-Amino-2-[2-(phosphonomethoxy)propyl]-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (13). The title compound was prepared from **10** by the same procedure used for 1 in 88% yield, as a white solid. Mp: 280–282 °C dec. TLC (H_2O–CH_3CN, 80:20): R_f 0.80. UV (pH 12): λ_{max} 250 nm (ϵ 4100), 298 (ϵ 6500). $[\alpha]^{20}_D$ +23.4° (*c* 0.18, HCl 0.1 M). ^1H NMR (Me_2SO-d_6): δ 1.18 (d, J = 6.1 Hz), 3.55 (d, J = 9.1 Hz, 2 H), 4.18 (dd, J = 7.4, 10.2 Hz, 1 H), 4.47–4.62 (m, 2 H); 6.58 (s, 2 H), 11.00 (s, 1 H). ^{13}C NMR (Me_2SO-d_6): δ 159.7, 156.7, 154.1, 126.7, 75.5 (d, $^3J_{cp}$ = 10.7 Hz), 64.8 (d, $^1J_{cp}$ = 160 Hz), 59.8, 17.7. Anal. ($C_8H_{13}N_6O_5P$) C, H, N.

(*R*)-1-Azido-2-[(diisopropylphosphono)methoxy]propane (15). A stirred mixture of **4** (5 g, 15.04 mmol) in anhydrous DMF (100 mL) and sodium azide (9 g, 138.4 mmol) was heated to 80 °C for 12 h under a nitrogen atmosphere. At this time the reaction was complete by TLC. The resulting mixture was allowed to cool to room temperature and then concentrated in vacuo. The residue was diluted with 50 mL of H_2O and extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to give **15** as an oil (3.6 g, 84%). TLC ($CHCl_3$–MeOH, 95:5): R_f 0.76. IR (cm^{-1}): 2100 (azide). ^1H NMR ($CDCl_3$): δ 1.18 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 12 H), 3.22 (d, J = 5.7 Hz, 2 H), 3.65 (d, J = 5.7 Hz, 2 H), 3.75 (m, 1 H), 4.73 (m, 2 H). Anal. ($C_{10}H_{22}N_3O_4P$) C, H, N.

(*R*)-[2-[(Diisopropylphosphono)methoxy]propyl]-amine (16). The title compound was obtained by reaction of **15** (3 g, 10.74 mmol) in 50 mL of MeOH with palladium on carbon (10%, 0.50 g) under a hydrogen atmosphere at 40 psi for 1.5 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness to give **16** as a colorless oil (2.5 g, 92.3%). TLC ($CHCl_3$–MeOH, 80:20): R_f 0.2. ^1H NMR ($CDCl_3$): δ 1.02 (d, J = 6.2 Hz, 3H); 1.23 (d, J = 6.2 Hz, 12 H), 1.38 (br s, 2 H), 2.62 (m, 2 H), 3.40 (m, 1 H), 3.55, 3.72 (2dd, J = 4.7, 9.5 Hz, 2 H), 4.58–4.70 (m, 2 H). Anal. ($C_{10}H_{24}NO_4P$) C, H, N.

(*R*)-2-Amino-5-nitro-6-[[2-[(diisopropylphosphono)-methoxy]propyl]amino]-3*H*-pyrimidin-4-one (17). A mixture of 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone[16] (**14**) (1 g, 4.79 mmol), **16** (1.22 g, 4.81 mmol), and triethylamine (1 mL, 7.2 mmol) in anhydrous DMF (20 mL) was stirred under a nitrogen atmosphere for 2 h at room temperature. The reaction mixture was filtered, and the filtrate was evaporated

to dryness. The residue was purified by flash chromatography on silica gel eluting with $CHCl_3$–MeOH (95:5) to give **17** as an oil which was crystallized from diethyl ether/petroleum ether (1.65 g, 84%, yellow crystals). Mp: 158–160 °C. TLC ($CHCl_3$–MeOH, 85:15): R_f 0.58. ^1H NMR ($CDCl_3$): δ 1.23 (d, J = 6.2 Hz, 3 H); 1.35 (m, 12 H), 3.48 (m, 1 H), 3.83 (m, 4 H), 4.77 (m, 2 H), 5.90, 8.31 (2 br s, 2 H), 9.82 (t, 1 H), 10.72 (br s, 1 H). Anal. ($C_{14}H_{26}N_5O_7P$) C, H, N.

(*R*)-5-Amino-3-[2-[(diispropylphosphono)methoxy]propyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (6). A mixture of **17** (0.8 g, 1.96 mmol) in 40 mL of anhydrous MeOH and Raney nickel (0.4 g) was reacted at a hydrogen pressure of 50 psi. After 1 h the reaction was complete by TLC. The stirred crude mixture was cooled at 0 °C; a mixture of acetic acid/deoxygenated H_2O (40:10) and a solution of sodium nitrite (0.55 g, 8.0 mmol) in 30 mL of deoxygenated H_2O were added. The reaction mixture was stirred at room temperature for 3 h, and filtered, and the filtrate was evaporated to dryness. Water (40 mL) and $CHCl_3$ (50 mL) were added to the residue. The aqueous layer was extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and filtered. The solvent was evaporated in vacuo to yield 0.7 g (91.7%) of **6** as a yellow foam. TLC ($CHCl_3$–MeOH, 80:20) R_f 0.64. ^1H NMR (Me_2SO-d_6): δ 1.11–1.26 (m, 15 H), 3.65 (dd, J = 9.3, 13.7 Hz, 1 H), 3.78 (dd, J = 9.3, 13.7 Hz, 1 H); 4.10 (q, 1 H), 4.31 (d, J = 5.9 Hz, 2 H), 4.49 (m, 2 H), 6.95 (s, 2 H); 10.98 (s, 1 H). ^{31}P NMR (Me_2SO-d_6): 21.8. ^{13}C NMR (Me_2SO-d_6): δ 155.1, 155.8, 151.9, 124.3, 75.3 (d, $^3J_{cp}$ = 15.0 Hz), 70.4 (t, $^2J_{cp}$ = 5.0 Hz), 62.8 (d, $^1J_{cp}$ = 160 Hz), 49.9, 23.9 (t, $^3J_{cp}$ = 4.0 Hz), 17.3. Anal. ($C_{14}H_{25}N_6O_5P$) C, H, N.

(*R*)-5-Amino-1-[2-[(diisopropylphosphono)methoxy]propyl]-1*H*-imidazo[4,5-*d*]pyrimidin-7-one (18) and (*R*)-5-Amino-3[2-[(diisopropylphosphono)methoxy]propyl]-3*H*-imidazo[4,5-*d*]pyrimidin-7-one (19). The title compounds were prepared from guanine (1 g, 6.62 mmol), cesium carbonate (2.15 g, 6.61 mmol) in anhydrous DMSO (20 mL), and **4** (1.83 g, 5.51 mmol) (80 °C for 6 h). The reaction was performed as described for **5**. Purification by chromatography on a silica gel column ($CHCl_3$-MeOH–NH_4OH, 90:9.5:0.5) gave **18** as white crystals (0.28 g, 14%). Mp: 219–222 °C. TLC ($CHCl_3$–MeOH, 85:15): R_f 0.35. ^1H NMR (Me_2SO-d_6): δ 1.08 (d, J = 6.2 Hz, 3H), 1.21 (m, 12 H), 3.75 (m, 2 H), 3.95 (q, 1 H), 4.27 (d, J = 5.8 Hz, 2 H), 4.43–4.68 (m, 2 H), 6.15 (s, 2 H), 7.85 (s, 1 H), 10.83 (s, 1 H). ^{31}P NMR (Me_2SO-d_6): 21.8. ^{13}C NMR (Me_2SO-d_6): δ 160.0, 155.0, 153.0, 144.1, 104.3. Anal. ($C_{15}H_{26}N_5O_5P$) C, H, N.

Further elution of the same column provided **19** as a white solid (0.91 g, 43%). Mp: 188–190 °C. TLC ($CHCl_3$–MeOH, 85:15): R_f 0.29. ^1H NMR (Me_2SO-d_6): δ 1.08 (d, J = 6.0 Hz, 3 H), 1.20 (m, 12 H), 3.65–3.92 (m, 3 H), 3.95–4.05 (pseudo-t, 2 H), 4.54 (m, 2 H), 6.42 (s, 2 H), 7.63 (s, 1 H), 10.55 (s, 1 H). ^{31}P NMR (Me_2SO-d_6): 21.7. ^{13}C NMR (Me_2SO-d_6): δ 157.2, 154.0, 151.6, 144.2, 116.8. Anal. ($C_{15}H_{26}N_5O_5P$) C, H, N.

(*R*)-5-Amino-3-[2-(phosphonomethoxy)propyl]-3*H*-imidazo[4,5-*d*]pyrimidin-7-one [(R)-PMPG]. The title compound was prepared from **19** (0.8 g, 1.29 mmol) as described for 1 and isolated as colorless crystals (0.59 g, 96%). ^1H- and ^{13}C-NMR spectra were similar to those reported in the literature.[7,9]

(*R*)-5-Amino-1-[2-(phosphonomethoxy)propyl]-1*H*-imidazo[4,5-*d*]pyrimidin-7-one (20). Compound **20** was prepared from **18** as a colorless solid (78%). Mp: 256–258 °C. TLC (H_2O–CH_3CN, 80:20): R_f 0.82. UV (pH 12): λ_{max} 242 nm (ϵ 4600), 280 (ϵ 5100). ^{31}P NMR (Me_2SO-d_6): 17.4. ^1H NMR (Me_2SO-d_6): δ 1.10 (d, J = 6.2 Hz, 3 H), 3.60–3.75 (m, 2 H), 3.98 (m, 1 H), 4.25–4.40 (m, 2 H), 7.20 (br s, 2 H), 8.90 (s, 1 H), 11.82 (br s, 1 H). Anal. ($C_9H_{14}N_5O_5P$) C, H, N.

Cells and Viruses. MT-4 and C8166 cells were grown at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% metal calf serum (FCS), 100 IU/mL penicillin G, and 100 μg/mL streptomycin. Vero cells (grown in D-MEM, FCS, 100 IU/mL penicillin G, and 100 μg/mL streptomycin) were used for anti-HSV assays. Cell cultures were checked periodically for the absence of mycoplasma contamination with a Myco Teck Kit (Gibco).

Human immunodeficiency viruses type 1 (HIV-1, III_B strain) and type 2 (HIV-2, ROD strain; kindly provided by Dr. L. Montagnier) were obtained from supernatants of persistently infected H9/III_B and CEM cells, respectively. HIV-1 and HIV-2 stock solutions had titers of 2.5 × 10^5 and 1.4 × 10^5 cell culture infectious dose fifty ($CCID_{50}$)/mL, respectively.

Herpes simplex type 1 (HSV-1, ATCC, VR 733) and herpes simplex type 2 (HSV-2, ATCC, VR 734) stocks were prepared in Vero cells and had titers of 2 × 10^8 and 8 × 10^7 PFU/mL, respectively.

HIV Titration. Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1:2, four replica wells/dilution) in 96-well plates. The infectious virus titer was determined by light microscope scoring of syncytia after 4 days of incubation, and the virus titers are expressed as $CCID_{50}$/mL according to the Reed and Muench method.[17]

Anti-HIV Assays. Compounds were dissolved in DMSO at 100 mg/mL and then diluted in culture medium. 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 × 10^4 MT-4 cells was added to each well of flat-bottomed microtiter trays containing 50 μL of medium with or without various concentrations of the test compounds. Twenty microliters of HIV-1 or HIV-2 suspensions containing 100 $CCID_{50}$ were then added (MOI = 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-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium 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.

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

Drug Combination Studies. Combinations of antiviral compounds were evaluated in MT-4 cells infected with HIV-1 at a MOI of 0.01. The effective concentration of compound that, alone or in combination, reduced the virus-induced cytopathogenicity of 50% (EC_{50}) was determined by the MTT method. A graphic method (isobologram) of evaluating combinations was utilized. The method consists of plotting on an arithmetic scale the concentrations of drugs that, alone or in various combinations, produce the same antiviral effect (i.e., 50% inhibition of CPE).

Acknowledgment. This investigation was supported by grants (92-0468 and 92-0462) from the Ministry of Health I.S.S. AIDS Project 1994, FATMA Project CNR (94.00597PF41), and Ministry of University and Scientific-Technological Research (60% funds).

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JM950311Y