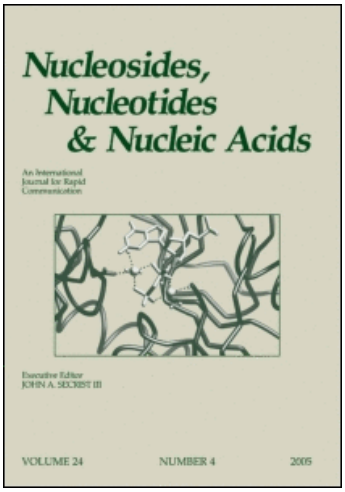

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8-Aza-analogues of PMEA and PMEG: Synthesis and *In Vitro* Anti-HIV Activity

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8-AZA-ANALOGUES OF PMEA AND PMEG: SYNTHESIS AND *IN VITRO* ANTI-HIV ACTIVITY.[1]

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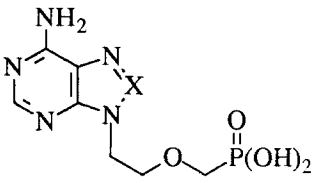
Abstract: 8-Aza-analogues of the potent antiviral nucleotide analogues 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) and 9-[2-(phosphonomethoxy)-ethyl]guanine (PMEG) were prepared and evaluated for activity against human immunodeficiency viruses. When compared to the parent compounds, 8-aza-PMEA (1) and -PMEG (2) were less cytotoxic for MT-4 cells, but also less potent against HIV-1 and HIV-2. A new synthesis of PMEG starting from guanine is also reported.

The discovery of the antiviral activity of (S)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine [(*S*)-HPMPA][2] has led to the synthesis of numerous phosphonomethoxyalkyl purine and pyrimidine derivatives, which have emerged as a new class of potent, broad-spectrum anti-DNA virus agents. Among the phosphonomethoxyalkyl purine analogues, the purine derivatives containing adenine (PMEA), 2-monoaminopurine (PMEMAP), 2,6-diaminopurine (PMEDAP) and guanine (PMEG) have been found active against HIV.[3] Following phosphorylation to diphosphorylphosphonates, they act as inhibitors of reverse transcriptase.[4] PMEG is one of the most potent, broad-spectrum antiviral agents reported to date; unfortunately, due to its high cytotoxicity, it is not very selective.[5]

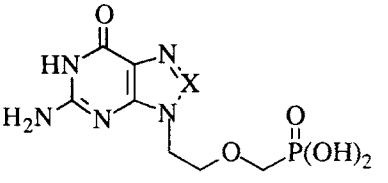
The distance between the base and the phosphonomethoxy group has been indicated as critical for the antiviral activity,[6] and systematic structure-activity studies have revealed loss of activity following alterations of the

base.[7] Nevertheless, in order to verify whether isosteric modifications of the purine moiety of PMEA and PMEG could afford compounds with both potent antiviral activity and low cytotoxicity, we substituted the CH group at 8-position in the purine ring with nitrogen. In this paper we report the synthesis and anti-HIV activity of 8-aza-PMEA (**1**) and 8-aza-PMEG (**2**) and a new facile synthesis of PMEG starting from guanine.



PMEA (X = CH)

8-Aza-PMEA (X = N, **1**)



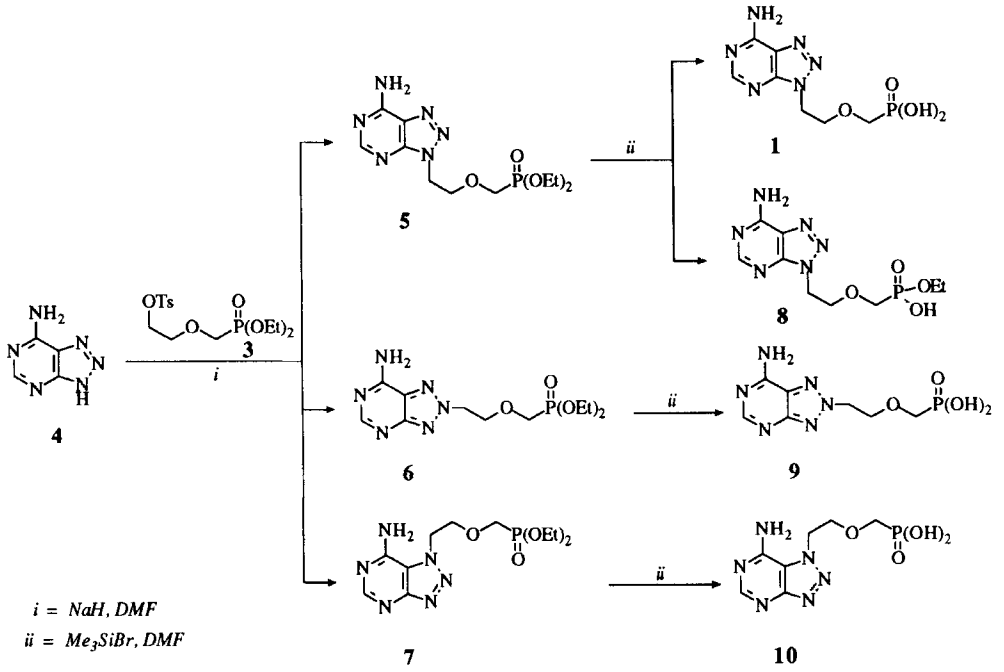
PMEG (X = CH)

8-Aza-PMEG (X = N, **2**)

CHEMISTRY

The synthesis of 8-aza-PMEA was achieved by coupling of the side-chain derivative **3**[8] with 7-amino-3*H*-triazolo[4,5-d]pyrimidine (8-azaadenine, **4**), followed by deprotection of the phosphonic moiety (Scheme I). The alkylation reaction, carried out by treatment of the sodium salt of **4** with tosylate **3** in DMF (method A), resulted in the formation of the N^9-substituted isomer **5** in a 34% yield, and of the isomers **6** (37%) and **7** (5%), which were separated by chromatography on silica gel. If the reaction was carried out in DMSO in the presence of cesium carbonate (method B), the three regioisomers were obtained in lower yields (N^9 16.5%, N^8 18% and N^7 2.5%). The alkylation position was established by the similarity of the UV spectrum of compound **5** with that reported for 9-alkylated derivatives of 8-azaadenine,[9] indicating that N^9 was the alkylation position. This was confirmed by the upfield shift of the C(4) [C(3a), if systematic numbering is used] signal in the ^{13}C-NMR spectrum of compound **5** (δ 149.1) as compared to that of **4** (δ 151.2).[10,11]

A downfield of C(4) of regioisomer **6** as compared to that of **5** immediately indicated that N^9 did not carry a substituent. As C(5) [C(7a), if systematic numbering is used] was unchanged, the position of alkylation was N^8; this was confirmed by the similarity of UV spectrum of **6** with that of 8-alkylated

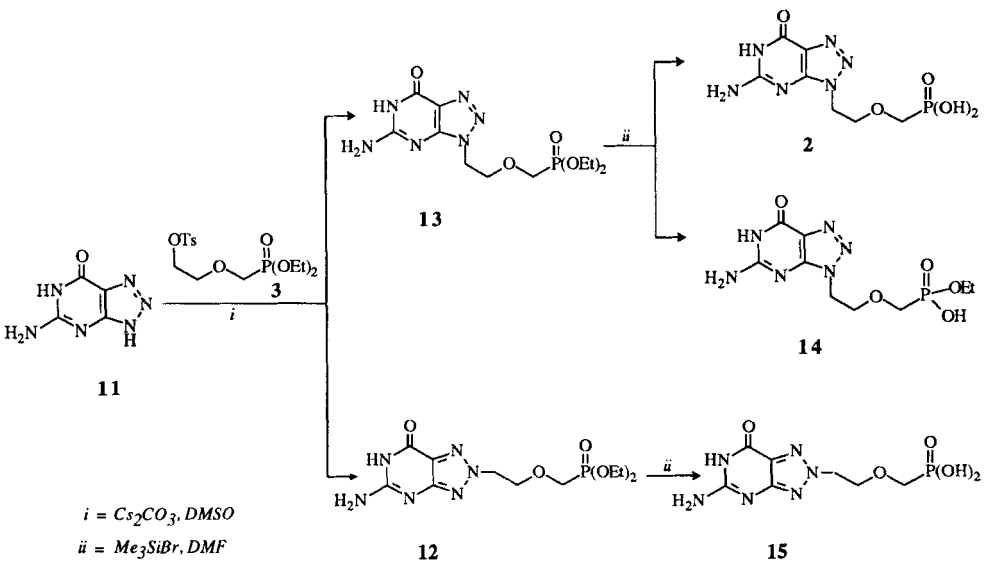


SCHEME I

derivatives of 8-azaadenine.[9] Since the chemical shift of C(5) of the isomer **7** was shifted upfield, when compared to the corresponding signal of the N^8 and N^9 isomers **5** and **6**, we concluded that **7** was the N^7-substituted isomer; this was confirmed by the UV spectrum which was similar to that reported for N^7-alkylated derivatives of 8-azaadenine.[9]

Sequential removal of the protecting groups of **5** with bromotrimethylsilane in DMF afforded 8-aza-PMEA (**1**) together with small amounts of its monoethylester (**8**). The phosphonate isomers **9** and **10** were prepared in similar way starting from **6** and **7**.

The synthesis of 8-aza-PMEG (**2**) was carried out as reported for 8-aza-PMEA starting from 8-azaguanine (**11**) and tosylate **3** in the presence of cesium carbonate in DMSO. A mixture of the N^8- and N^9-substituted regioisomers **12** and **13** in 22% and 30% yield, respectively, was obtained (Scheme II). The position of alkylation was deduced on the basis of the comparison of their UV and ^{13}C-NMR spectral data with those of N^8- and N^9-alkylated 8-azaguanine derivatives.[12] The structure of compound **13** was confirmed by the upfield



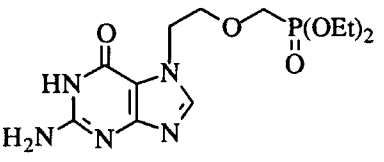
SCHEME II

shift of the C(3a) signal in the ^{13}C-NMR spectrum of compound **13** (δ 151.8) as compared to that of 8-azaguanine (δ 153.2).[11,13]

Conversion of compound **13** to 8-aza-PMEG (**2**) was achieved by deesterification with bromotrimethylsilane in DMF. A small amount of monoethylester **14** was also obtained. In similar way, the N^8-phosphonate isomer **15** was prepared starting from **12**.

A similar approach in only two steps starting from guanine and tosylate **3** was employed for the synthesis of PMEG, which we used as reference compound. Other authors have previously synthesized PMEG in three steps starting from 2-amino-6-chloropurine and **3** with an overall yield of 35%.[5] We found that the reaction of guanine with **3** in DMSO in the presence of cesium carbonate gave a 1:2 ratio of the alkylated products **16** and **17**, with the desired N^9 isomer **17** isolated as the major product in 41% yield. The structure assignment for **16** and **17** were based on ^{1}H and ^{13}C NMR spectroscopic data.[14]

Sequential removal of the protecting groups of **17** with bromotrimethylsilane in DMF afforded PMEG with an overall yield of 36%.



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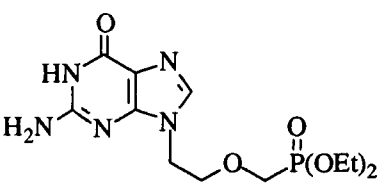

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BIOLOGICAL EVALUATION AND DISCUSSION SECTION

Test compounds were solubilized in DMSO at 100 mg/mL and then diluted in culture media.

The 8-aza analogues of PMEA and PMEG (compounds **1** and **2**), their monoethylesters **8**, **14** and N^8 and N^7 isomers **9**, **10**, **15** were evaluated *in vitro* for cytotoxicity and inhibitory effect on the multiplication of HIV-1 and HIV-2 in acutely infected cells. PMEA and PMEG were used as reference compounds.

The following cells were used: H9/IIIB cells, an H9 subline which is persistently infected with HIV-1; MT-4 and C8166, CD4+ T-cells.

T-cell lines were grown in RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS) and addition of 100 UI/mL penicillin G and 100 UI/mL streptomycin. Cell lines were grown at 37 °C in a CO_2 incubator and were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco).

The human immunodeficiency virus type 1 (HIV-1, HTLV/IIIB strain) used in anti-HIV assays was obtained from supernatants of H9/IIIB cells. HIV-2 (CBL 20 strain) was kindly provided by Prof. R. Weiss and the MRC AIDS Directed Program Reagent Project. HIV-2 stocks were obtained in MT-4 cells. Both HIV-1 and HIV-2 stocks were titrated in C8166 cells and stored at -80 °C until use. HIV-1 stocks had a titre of $2x10^5$ cell culture infective dose fifty ($CCID_{50}$/mL), whereas HIV-2 stocks had titres of $4.5x10^5$ $CCID_{50}$/mL.

Cytotoxicity of test compounds for MT-4 cells was evaluated in parallel with their anti-HIV-1 activity and was based on viability of mock-infected cells as determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method.[15]

Activity of the compounds against HIV multiplication in acutely infected cells was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 µL of growth medium containing $1x10^4$ MT-4 cells were added

TABLE 1. Comparative cytotoxicity and anti-HIV activity of 8-aza analogues of PMEA and PMEG.

Compound	CC_{50}[a]	EC_{50}[b] HIV-1	SI[c]	EC_{50}[b] HIV-2	SI[c]
PMEA	229	5.3	43	3.7	62
8-aza-PMEA (**1**)	>365	94	>3.9	91	>4
8	>331	>331	-	>331	-
9	>365	>365	-	>365	-
10	>365	>365	-	>365	-
PMEG	2.4	0.19	12.6	0.2	12
8-aza-PMEG (**2**)	69	15	4.6	6.2	11
14	>315	>315	-	>315	-
15	>315	>315	-	>315	-

[a]Compound dose (μM) required to reduce the viability of mock-infected MT-4 cells by 50% (4 days). [b]Compound dose (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.

to each well of flat-bottomed microtiter trays containing 50 μL of culture medium with or without various concentrations of test compounds. 20 μL of HIV suspensions were then added, so as to obtain a multiplicity of infection of 0.01. After a 4-day incubation for HIV-1 (8-days for HIV-2) at 37 °C, the number of viable MT-4 cells was determined by the MTT method.

The 8-aza analogues of PMEA and PMEG were active in protecting MT-4 cells from the virus-induced cytopathogenicity (Table 1), although their potency was somewhat lower than that of PMEA and PMEG.

Substitution of the CH group with nitrogen at 8-position of the purine ring system of both PMEA and PMEG resulted in a remarkable reduction of cytoxicity. However, the concomitant, strong decrease in anti-HIV potency resulted in a lowering of the selectivity index. The sole exception was 8-aza-PMEG, which maintained the same selectivity index of PMEG against HIV-2.

Monoethyl esters of 8-aza-PMEA and 8-aza-PMEG were found inactive against both HIV-1 and HIV-2.

The inactivity of regioisomers **9**, **10** and **15** confirmed that only PME-derivatives in which the phosphonate side chain is bonded at N^9 have affinity for both cellular kinases and HIV reverse transcriptase.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a Buchi 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 mesh) for column chromatography was used. Nuclear magnetic resonance ^1H, ^{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.

7-Amino-3-[2-(diethylphosphonomethoxy)ethyl]-3*H*-1,2,3-triazolo[4,5-d]pyrimidine (5). Method A) To a solution of 7-amino-3*H*-triazolo[4,5-d]-pyrimidine (**4**) (1 g, 7.34 mmol) in anhydrous DMF (50 mL) under a nitrogen atmosphere, sodium hydride (60% in mineral oil, 557 mg, 14.0 mmol) was added and the mixture was stirred at 80 °C for 1 h. A solution of **3** (2.68 g, 7.34 mmol) in anhydrous DMF (30 mL) was added and the resulting reaction mixture was heated at same temperature for 13 h. The solvent was removed under reduced pressure and the residue was treated with boiled $CHCl_3$ (3 x 80 mL). After filtration of insoluble material, the filtrate was concentrated to give a residue which was chromatographed on silica gel using $CHCl_3$-MeOH (92:8) to provide **5** as a colorless solid (0.78 mg, 34 %). M.p. 115-117 °C; TLC ($CHCl_3$-MeOH 90:10): R_f 0.40. UV (pH 12) λ_{max} 278 nm (ε 11000). ^1H NMR (Me_2SO-d_6): δ 1.10 (t, J = 7.0 Hz, 6H, CH_3); 3.83 (d, J = 8.4 Hz, 2H, P-CH_2); 3.80-3.95 (m, 4H, CH_2CH_3); 4.08 (t, J = 5.1 Hz, 2H, O-CH_2); 4.75 (t, J = 5.2 Hz, 2H, N-CH_2); 8.10, 8.42 (2br s, 2H, NH_2); 8.30 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6): 22.1. ^{13}C NMR (Me_2SO-d_6): 156.6 (C-5); 156.2 (C-7); 149.1 (C-3a); 123.7 (C-7a); 69.7 (d, $^3J_{c,p}$ = 15.4 Hz, C-2'); 63.2 (d, $^1J_{c,p}$ = 153.0 Hz, OCH_2P); 61.6 (d, $^2J_{c,p}$ = 8.0 Hz, $POCH_2$); 45.7 (C-1'); 16.1 (d, $^3J_{c,p}$ = 7.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_{11}H_{19}N_6O_4P$: C 40.00; H 5.80; N 25.44. Found: C 40.11; H 5.85; N 25.40.

Method B) Compound **3** (2.69 g, 7.34 mmol) was added dropwise under nitrogen atmosphere to a suspension of **4** (1 g, 7.34 mmol) in DMSO (30 mL) and Cs_2CO_3 (3.0 g, 9.22 mmol) and the mixture was heated at 80 °C for 6 h. After evapo-

ration of DMSO, the residue was chromatographed on silica gel with $CHCl_3$-MeOH (92:8) to give compound **5** (0.21 g, 16.5%).

7-Amino-2-[2-(diethylphosphonomethoxy)ethyl]-2*H*-1,2,3-triazolo[4,5-d]pyrimidine (6). The same chromatography column that provided compound **5** (method A) was further eluted to give compound **6** as a colorless oil (840 mg, 37 %). TLC ($CHCl_3$-MeOH 90:10): R_f 0.31. UV (pH 12) λ_{max} 252 nm (ε 3700); 294 nm (ε 7200). ^1H NMR (Me_2SO-d_6): δ 1.10 (t, J = 7.0 Hz, 6H, CH_3); 3.85 (d, J = 8.2 Hz, 2H, P-CH_2); 3.82-3.97 (m, 4H, CH_2CH_3); 4.17 (t, J = 5.0 Hz, 2H, O-CH_2); 4.90 (t, J = 5.1 Hz, 2H, N-CH_2); 8.08, 8.27 (2br s, 2H, NH_2); 8.30 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6): 22.0. ^{13}C NMR (Me_2SO-d_6): 156.7 (C-5); 143.7 (C-3a); 125.4 (C-7a); 70.1 (d, $^3J_{C,P}$ = 16.0 Hz, C-2'); 63.8 (d, $^1J_{C,P}$ = 214.4 Hz, OCH_2P); 61.7 (d, $^2J_{C,P}$ = 9.0 Hz, $POCH_2$); 56.2 (C-1'); 16.1 (d, $^3J_{C,P}$ = 8.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_{11}H_{19}N_6O_4P$: C 40.00; H 5.80; N 25.44. Found: C 39.92; H 5.77; N 25.58.

7-Amino-1-[2-(diethylphosphonomethoxy)ethyl]-1*H*-1,2,3-triazolo[4,5-d]pyrimidine (7). Further elution of the same chromatography column that provided compounds **5** and **6**, gave **7** as a colorless solid (134 mg, 5 %). M.p. 123-125 °C; TLC ($CHCl_3$-MeOH 90:10): R_f 0.27. UV (pH 12) λ_{max} 288 nm (ε 7900). ^1H NMR (Me_2SO-d_6): δ 1.10 (t, J = 7.0 Hz, 6H, CH_3); 3.82 (d, J = 8.0 Hz, 2H, P-CH_2); 3.78-3.90 (m, 4H, CH_2CH_3); 3.95 (t, J = 4.7 Hz, 2H, O-CH_2); 5.10 (t, J = 4.9 Hz, 2H, N-CH_2); 7.75 (br s, 2H, NH_2); 8.32 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6): 16.3. ^{13}C NMR (Me_2SO-d_6): 160.3 (C-7); 154.3 (C-5); 151.6 (C-3a); 113.9 (C-7a); 71.1 (d, $^3J_{C,P}$ = 21.0 Hz, C-2'); 63.9 (d, $^1J_{C,P}$ = 321.0 Hz, OCH_2P); 61.6 (d, $^2J_{C,P}$ = 12.0 Hz, $POCH_2$); 49.8 (C-1'); 16.1 (d, $^3J_{C,P}$ = 11.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_{11}H_{19}N_6O_4P$: C 40.00; H 5.80; N 25.44. Found: C 39.95; H 5.83; N 25.38.

7-Amino-3-[2-(phosphonomethoxy)ethyl]-3*H*-1,2,3-triazolo[4,5-d]-pyrimidine (1) and 7-Amino-3-[2-(ethylphosphonomethoxy)-ethyl]-3*H*-1,2,3-triazolo[4,5-d]pyrimidine (8). A solution of **5** (0.7 g, 2.1 mmol) in anhydrous DMF (20 mL) at room temperature and under a nitrogen atmosphere was treated dropwise with bromotrimethylsilane (3.22 g, 21.0 mmol). The reaction mixture was stirred at 22 °C for 24 h. After evaporation, the oil residue was treated with H_2O (5 mL) and acetone (30 mL) and stirred for 1 h. The mixture was cooled at -20 °C for 14 h and the resulting precipitate was collected by filtration to give compound **1** as colorless solid (0.5 g, 87.3 %). M.p. 243-245 °C dec.; TLC (H_2O-CH_3CN 80:20): R_f 0.80. UV (pH 12) λ_{max} 278 nm (ε 8600). ^1H NMR (Me_2SO-d_6): δ 3.59 (d, J = 8.4 Hz, 2H, P-CH_2); 3.65 (s, 2H, OH); 4.08 (t, J = 5.5 Hz, 2H, O-CH_2); 4.72 (t, J = 5.5 Hz, 2H, N-CH_2); 8.10, 8.45 (2br s, 2H, NH_2); 8.30 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6): 17.4. ^{13}C NMR (Me_2SO-d_6): 156.5 (C-5);

156.2 (C-7); 149.3 (C-3a); 124.1 (C-7a); 69.7 (d, $^3J_{C,P}$ = 19.0 Hz, C-2'); 66.5 (d, $^1J_{C,P}$ = 318.0 Hz, O*C*H_2P); 46.2 (C-1'). Anal. Calcd. for $C_7H_{11}N_6O_4P$: C 30.67; H 4.04; N 30.65. Found: C 30.53; H 4.15; N 30.68.

Evaporation of the filtrate gave a residue which was chromatographed by preparative layer chromatography (RP-18 F254 S, Merck), using H_2O-acetonitrile (80:20). Compound **8** was obtained as white solid (25 mg, 5.4 %). M.p. 235-237 °C dec.; TLC (H_2O-CH_3CN 80:20): R_f 0.75. UV (pH 12) λ_{max} 276 nm (ε 10800). ^1H NMR (Me_2SO-d_6): δ 0.90 (t, J = 7.0 Hz, 3H, CH_3); 3.28 (d, J = 7.0 Hz, 2H, P-CH_2); 3.48 (m, 2H, *C*H_2CH_3); 3.95 (t, J = 5.0 Hz, 2H, O-CH_2); 4.15 (s, 1H, OH); 4.70 (t, J = 5.1 Hz, 2H, N-CH_2); 8.12, 8.45 (2br s, 2H, NH_2); 8.30 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6): 12.3. ^{13}C NMR (Me_2SO-d_6): 157.0 (C-5); 156.6 (C-7); 149.5 (C-3a); 124.2 (C-7a); 69.4 (d, $^3J_{C,P}$ = 18.0 Hz, C-2'); 67.2 (d, $^1J_{C,P}$ = 212.0 Hz, O*C*H_2P); 59.7 (d, $^2J_{C,P}$ = 8.0 Hz, POCH_2); 46.5 (C-1'); 17.4 (d, $^3J_{C,P}$ = 8 Hz, POCH$_2$*C*H_3). Anal. Calcd. for $C_9H_{15}N_6O_4P$: C 35.75; H 5.00; N 27.81. Found: C 35.70; H 5.08; N 27.79.

If the reaction mixture was stirred at 28 °C for 3 h, only compound **1** was obtained.

7-Amino-2-[2-(phosphonomethoxy)ethyl]-2*H*-1,2,3-triazolo[4,5-d]-pyrimidine (9). Compound **9** was prepared from **6** (0.8 g, 2.42 mmol) as described for **1**, but in anhydrous MeCN for 4 h. After evaporation and treatment of the residual oil with H_2O-acetone for 1 h, the precipitate was filtered to give **9** which as a white solid (0.54 g, 82.7 %). M.p. 265-267 °C dec.; TLC (H_2O-CH_3CN 80:20): R_f 0.83. UV (pH 12) λ_{max} 254 nm (ε 5000); 292 nm (ε 11100). ^1H NMR (Me_2SO-d_6): δ 3.58 (d, J = 8.4 Hz, 2H, P-CH_2); 3.80 (s, 2H, OH); 4.18 (t, J = 5.2 Hz, 2H, O-CH_2); 4.84 (t, J = 5.2 Hz, 2H, N-CH_2); 8.05, 8.28 (2br s, 2H, NH_2); 8.30 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6): 17.3. ^{13}C NMR (Me_2SO-d_6): 158.0 (C-7); 156.0 (C-5); 146.8 (C-3a); 125.7 (C-7a); 70.1 (d, $^3J_{C,P}$ = 18.0 Hz, C-2'); 67.3 (d, $^1J_{C,P}$ = 318.0 Hz, O*C*H_2P); 56.6 (C-1'). Anal. Calcd. for $C_7H_{11}N_6O_4P$: C 30.67; H 4.04; N 30.65. Found: C 30.71; H 4.18; N 30.55.

7-Amino-1-[2-(phosphonomethoxy)ethyl]-1*H*-1,2,3-triazolo[4,5-d]-pyrimidine (10). Compound **10** was prepared from **7** (120 mg, 0.36 mmol) as described for **9** to give a white solid (75 mg, 75.7 %). M.p. 250-252 °C dec.; TLC (H_2O-CH_3CN 80:20): R_f 0.86. UV (pH 12) λ_{max} 288 nm (ε 7600). ^1H NMR (Me_2SO-d_6): δ 3.37 (d, J = 8.2 Hz, 2H, P-CH_2); 3.80 (s, 2H, OH); 4.18 (t, J = 4.9 Hz, 2H, O-CH_2); 4.84 (t, J = 5.1 Hz, 2H, N-CH_2); 8.05, 8.28 (2br s, 2H, NH_2); 8.30 (s, 1H, H-2). ^{31}P NMR (Me_2SO-d_6): 12.5. ^{13}C NMR (Me_2SO-d_6): 160.5 (C-7); 154.6 (C-5); 152.3 (C-3a); 114.7 (C-7a); 71.2 (d, $^3J_{C,P}$ = 23.0 Hz, C-2'); 65.9 (d, $^1J_{C,P}$ = 313.0 Hz, O*C*H_2P); 51.3 (C-1'). Anal. Calcd. for $C_7H_{11}N_6O_4P$: C 30.67; H 4.04; N 30.65. Found: C 30.58; H 4.09; N 30.70.

5-Amino-2-[2-(diethylphosphonomethoxy)ethyl]-2*H*-1,2,3-triazolo[4,5-d]pyrimidin-7-one (12) and 5-Amino-3-[2-(diethylphosphonomethoxy)ethyl]-3*H*-1,2,3-triazolo[4,5-d]pyrimidin-7-one (13). To a mixture of 8-azaguanine (**11**) (1 g, 6.57 mmol) in DMSO (30 mL) and Cs_2CO_3 (2.7 g, 8.3 mmol) compound **3** (2.4 g, 6.57 mmol) was added dropwise under N_2. The mixture was heated at 80 °C for 4 h. After evaporation of the DMSO, the residue was chromatographed on silica gel with $CHCl_3$-MeOH (92:8) to give, as a first fraction, compound **12** as a colorless solid (0.5 g, 22 %). M.p. 203-205 °C; TLC ($CHCl_3$-MeOH 85:15): R_f 0.51. UV (pH 12) λ_{max} 280 nm (ε 7300). ^1H NMR (Me_2SO-d_6): δ 1.15 (t, J = 7.0 Hz, 6H, CH_3); 3.85 (d, J = 8.0 Hz, 2H, P-CH_2); 3.94 (m, 4H, CH_2CH_3); 4.05 (t, J = 5.4 Hz, 2H, O-CH_2); 4.67 (t, J = 5.0 Hz, 2H, N-CH_2); 6.58 (br s, 2H, NH_2); 10.98 (s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): 21.5. ^{13}C NMR (Me_2SO-d_6): 160.0 (C-7); 156.8 (C-5); 154.5 (C-3a); 126.8 (C-7a); 70.4 (d, $^3J_{c,p}$ = 16.0 Hz, C-2'); 64.2 (d, $^1J_{c,p}$ = 214.0 Hz, OCH_2P); 62.2 (d, $^2J_{c,p}$ = 8.0 Hz, $POCH_2$); 55.6 (C-1'); 16.6 (d, $^3J_{c,p}$ = 7.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_{11}H_{19}N_6O_5P$: C 38.15; H 5.53; N 24.27. Found: C 38.22; H 5.63; N 24.18.

Further elution gave **13** (0.68 g, 30 %) as a colorless solid. M.p. 128-130 °C; TLC ($CHCl_3$-MeOH 85:15): R_f 0.39. UV (pH 12) λ_{max} 252 nm (ε 4300); 296 nm (ε 6100). ^1H NMR (Me_2SO-d_6): δ 1.18 (t, J = 7.1 Hz, 6H, CH_3); 3.82 (d, J = 8.3 Hz, 2H, P-CH_2); 3.88 (q, J = 7.1, 14.7 Hz, 4H, CH_2CH_3); 3.90 (t, J = 4.9 Hz, 2H, O-CH_2); 4.45 (t, J = 5.4 Hz, 2H, N-CH_2); 6.50 (br s, 2H, NH_2); 11.02 (s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): 21.4. ^{13}C NMR (Me_2SO-d_6): 156.2 (C-7); 155.9 (C-5); 152.0 (C-3a); 124.5 (C-7a); 70.2 (d, $^3J_{c,p}$ = 15.0 Hz, C-2'); 64.3 (d, $^1J_{c,p}$ = 214.0 Hz, OCH_2P); 62.1 (d, $^2J_{c,p}$ = 8.0 Hz, $POCH_2$); 45.6 (C-1'); 16.6 (d, $^3J_{c,p}$ = 8.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_{11}H_{19}N_6O_5P$: C 38.15; H 5.53; N 24.27. Found: C 38.10; H 5.48; N 24.39.

5-Amino-3-[2-(phosphonomethoxy)ethyl]-3*H*-1,2,3-triazolo[4,5-d]pyrimidin-7-one (2) and 5-Amino-3-[2-(ethylphosphonomethoxy)ethyl]-3*H*-1,2,3-triazolo[4,5-d]pyrimidin-7-one (14). Compound **2** was prepared from **13** (0.65 g, 1.87 mmol) by the same procedure used for **1** (22 °C, 48 h). After evaporation, the oily residue was treated with H_2O (5 mL) and acetone (30 mL) and stirred for 1 h. The mixture was cooled at -20 °C overnight and the resulting precipitate was collected by filtration to give compound **2** as a white solid (0.45 g, 78.5 %). M.p. 236-238 °C dec.; TLC (H_2O): R_f 0.81. UV (pH 12) λ_{max} 278 nm (ε 9100). ^1H NMR (Me_2SO-d_6): δ 3.56 (d, J = 8.3 Hz, 2H, P-CH_2); 3.98 (t, J = 5.6 Hz, 2H, O-CH_2); 4.48, 4.75 (t, J = 5.5 Hz, 2H, N-CH_2); 4.70, 6.50 (2s, 2H, OH); 6.95 (br s, 2H, NH_2); 10.98 (s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): 15.4. ^{13}C NMR (Me_2SO-d_6): 156.1 (C-7); 155.8 (C-5); 151.8 (C-3a); 124.6 (C-7a); 69.8 (d, $^3J_{c,p}$ = 13.0 Hz, C-2'); 66.7 (d, $^1J_{c,p}$

= 212.0 Hz, OCH_2P); 45.6 (C-1'). Anal. Calcd. for $C_7H_{11}N_6O_5P$: C 28.97; H 3.82; N 28.96. Found: C 28.80; H 3.78; N 28.89.

The filtrate from above was evaporated and chromatographed as described for **8** to obtained **14** as a white solid (18 mg, 3.9 %). M.p. 265-267 °C dec. TLC (H_2O): R_f 0.67. UV (pH 12) λ_{max} 278 nm (ε 8900). ^1H NMR[16] (Me_2SO-d_6): δ 1.00 (t, J = 7.1 Hz, 3H, CH_3); 3.50 (d, J = 8.6 Hz, 2H, P-CH_2); 3.70 (m, 2H, CH_2CH_3); 3.95 (m, 2H, O-CH_2); 4.43, 4.52 (2t, J = 6.7, 5.5 Hz, 2H, N-CH_2); 6.60 (s, 1H, OH); 6.58, 7.00, (2br s, 2H, NH_2); 11.08, 11.39 (2br s, 1H, NH, OH-7). ^{31}P NMR[16] (Me_2SO-d_6): 11.2, 11.8. ^{13}C NMR[16] (Me_2SO-d_6): 161.4 (C-7); 156.1 (C-5); 154.1 (C-3a); 124.6 (C-7a); 70.0 (d, $^3J_{c,p}$ = 13.0 Hz, C-2'); 67.2 (d, $^1J_{c,p}$ = 229.0 Hz, OCH_2P); 59.9 (d, $^2J_{c,p}$ = 7.0 Hz, $POCH_2$); 45.9 (C-1'); 17.1 (d, $^3J_{c,p}$ = 8.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_9H_{15}N_6O_5P$: C 33.97; H 4.75; N 26.41. Found: C 33.88; H 4.80; N 26.50.

5-Amino-2-[2-(phosphonomethoxy)ethyl]-2*H*-1,2,3-triazolo[4,5-d]pyrimidin-7-one (15). Compound **15** was obtained from **12** (0.5 g, 1.44 mmol, reaction time 5 h), as a colorless solid (0.3 g, 73.2 %), as described for **2**. M.p. 260-262 °C dec.; TLC (H_2O): R_f 0.76. UV (pH 12) λ_{max} 248 nm (ε 7500); 298 nm (ε 9000). ^1H NMR (Me_2SO-d_6): δ 3.52 (d, J = 8.2 Hz, 2H, P-CH_2); 3.92 (s, 2H, OH); 4.04 (t, J = 5.2 Hz, 2H, O-CH_2); 4.63 (t, J = 5.0 Hz, 2H, N-CH_2); 6.50 (br s, 2H, NH_2); 10.98 (s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): 16.8. ^{13}C NMR (Me_2SO-d_6): 159.8 (C-7); 156.9 (C-5); 154.3 (C-3a); 126.8 (C-7a); 70.1 (d, $^3J_{c,p}$ = 12.1 Hz, C-2'); 66.9 (d, $^1J_{c,p}$ = 208.0 Hz, OCH_2P); 55.8 (C-1'). Anal. Calcd for $C_7H_{11}N_6O_5P$: C 28.97; H 3.82; N 28.96. Found: C 28.99; H 3.70; N 29.03.

5-Amino-1-[2-(diethylphosphonomethoxy)ethyl]-1*H*-imidazo[4,5-d]pyrimidin-7-one (16) and 5-Amino-3-[2-(diethylphosphonomethoxy)ethyl]-3*H*-imidazo[4,5-d]pyrimidin-7-one (17). Compounds **16** and **17** were prepared from guanine (1 g, 6.61 mmol) as described for **12** and **13**. Chromatographic separation on a silica gel column eluting with $CHCl_3$-MeOH-NH_4OH (80:18:2), provided **16** as a white solid (0.45 g, 20 %). M.p. 208-210 °C; TLC ($CHCl_3$-MeOH-NH_4OH 80:19:1): R_f 0.61. ^1H NMR (Me_2SO-d_6): δ 1.18 (t, J = 7.0 Hz, 6H, CH_3); 3.80 (d, J = 8.2 Hz, 2H, P-CH_2); 3.92 (apparent quintet, J = 7.0 Hz, 4H, CH_2CH_3); 4.19 (t, J = 6.5 Hz, 2H, O-CH_2); 4.34 (t, J = 4.8 Hz, 2H, N-CH_2); 6.20 (br s, 2H, NH_2); 7.83 (s, 1H, H-2); 10.88 (s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): 21.8. ^{13}C NMR (Me_2SO-d_6): 160.0 (C-7); 155.0 (C-5); 153.0 (C-3a); 144.1 (C-8); 104.3 (C-7a); 71.5 (d, $^3J_{c,p}$ = 16.0 Hz, C-2'); 64.2 (d, $^1J_{c,p}$ = 205.0 Hz, OCH_2P); 62.2 (d, $^2J_{c,p}$ = 8.0 Hz, $POCH_2$); 45.9 (C-1'); 16.6 (d, $^3J_{c,p}$ = 7.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_{12}H_{20}N_5O_5P$: C 41.74; H 5.84; N 20.28. Found: C 41.82; H 5.73; N 20.31.

From the same column, further elution provided **17** as a colorless solid (0.94 g, 41 %). M.p. 120-122 °C; TLC ($CHCl_3$-MeOH-NH_4OH 80:19:1): R_f 0.49. ^1H NMR (Me_2SO-d_6): δ 1.20 (t, J = 7.0 Hz, 6H, CH_3); 3.82 (t, J = 5.1 Hz, 2H, O-CH_2); 3.85 (d, J = 8.4 Hz, 2H, P-CH_2); 3.97 (apparent quintet, J = 7.0 Hz, 4H, CH_2CH_3); 4.12 (t, J = 5.1 Hz, 2H, N-CH_2); 6.50 (br s, 2H, NH_2); 7.65 (s, 1H, H-2); 10.57 (s, 1H, NH). ^{31}P NMR (Me_2SO-d_6): 21.7. ^{13}C NMR (Me_2SO-d_6): 157.2 (C-7); 154.0 (C-5); 151.6 (C-3a); 144.2 (C-8); 116.8 (C-7a); 70.8 (d, $^3J_{c,p}$ = 15.0 Hz, C-2'); 64.3 (d, $^1J_{c,p}$ = 215.0 Hz, OCH_2P); 62.1 (d, $^2J_{c,p}$ = 8.0 Hz, $POCH_2$); 42.6 (C-1'); 16.6 (d, $^3J_{c,p}$ = 7.0 Hz, $POCH_2CH_3$). Anal. Calcd. for $C_{12}H_{20}N_5O_5P$: C 41.74; H 5.84; N 20.28. Found: C 41.80; H 5.80; N 20.19.

5-Amino-3-[2-(phosphonomethoxy)ethyl]-3*H*-imidazo[4,5-d]pyrimidin-7-one (PMEG). This compound was prepared from **17** (0.80 g, 0.23 mmol) by the same procedure used for **2** (22 °C, 8 h). After treatment with H_2O-acetone, the resulting precipitate was collected by filtration to provide 0.59 g (88 %) of PMEG. ^1H and ^{13}C NMR spectra (Me_2SO-d_6) were similar to those reported in the literature.[5]

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