6-[2-(Phosphonomethoxy)alkoxy]pyrimidines with Antiviral Activity

Antonín Holý,*,† Ivan Votruba,† Milena Masojídková,† Graciela Andrei,‡ Robert Snoeck,‡ Lieve Naesens,‡ Erik De Clercq,‡ and Jan Balzarini‡

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-166 10 Praha 6, Czech Republic, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received November 7, 2001

6-Hydroxypyrimidines substituted at positions 2 and 4 by hydrogen, methyl, amino, cyclopropylamino, dimethylamino, methylsulfanyl, or hydroxyl group afford by the reaction with diisopropyl 2-(chloroethoxy)methylphosphonate in the presence of NaH, Cs_2CO_3, or DBU a mixture of N^1- and O^6-[2-(diisopropylphosphorylmethoxy)ethyl] isomers which were converted to the free phosphonic acids by treatment with bromotrimethylsilane followed by hydrolysis. Analogously, 2,4-diamino-6-hydroxypyrimidine gave on reaction with [(*R*)- and (*S*)-2-(diisopropylphosphorylmethoxy)propyl] tosylate, followed by deprotection, the enantiomeric 6-[2-(phosphonomethoxy)propoxy]pyrimidines. 2,4-Diamino-6-sulfanylpyrimidine gave, on treatment with diisopropyl 2-(chloroethoxy)methylphosphonate in the presence of NaH and subsequent deprotection, 2,4-diamino-6-{[2-(phosphonomethoxy)ethyl]sulfanyl}pyrimidine. 2-Amino-4-hydroxy-6-[2-(phosphonomethoxy)ethyl]pyrimidine was obtained from the appropriate 2-amino-4-chloropyrimidine derivative by alkaline hydrolysis and ester cleavage. Direct alkylation of 2-amino-4,6-dihydroxypyrimidine afforded a mixture of 2-amino-4,6-bis[2-(phosphonomethoxy)ethyl]- and 2-amino-1,4-bis[2-(phosphonomethoxy)ethyl]pyrimidine. None of the N^1-[2-(phosphonomethoxy)ethyl] isomers exhibited any antiviral activity against DNA viruses or RNA viruses tested in vitro. On the contrary, the O^6-isomers, namely the compounds derived from 2,4-diamino-, 2-amino-4-hydroxy-, or 2-amino-4-[2-(phosphonomethoxy)ethoxy]-6-hydroxypyrimidine, inhibited the replication of herpes viruses [herpes simplex type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), and cytomegalovirus (CMV)] and retroviruses [Moloney sarcoma virus (MSV) and human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2)], their activity being most pronounced against the latter. The antiviral activity was lower if the oxygen at the position 6 was replaced by a sulfur atom, as in 2,4-diamino-6-[2-(phosphonomethoxy)ethylsulfanyl]pyrimidine. In analogy to N^9-[2-(phosphonomethoxy)propyl]-2,6-diaminopurine (PMPDAP), solely the (*R*)-2,4-diamino-6-[2-(phosphonomethoxy)propoxy]-pyrimidine exerted antiviral activity, whereas its (*S*)-enantiomer was essentially inactive.

Introduction

9-[2-(Phosphonomethoxy)alkyl]purines are attractive for their antiviral and cytostatic activity.[1] Among them, particularly 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir, **1**, Chart 1) is active against DNA viruses and retroviruses;[2] its bis(pivaloyloxymethyl) ester (adefovir dipivoxil)[3] is in the ultimate clinical phase for use in hepatitis B therapy.[4] Adefovir has pronounced cytostatic activity.[5] Another related adenine derivative, 9-(*R*)-[2-(phosphonomethoxy)propyl]adenine (PMPA, tenofovir, **2**), is a promising anti-HIV drug: its prodrug Viread has been approved for treatment of AIDS.[6] Also the 2,6-diaminopurine congeners of these two drugs demonstrated interesting biological properties: the (*R*)-9-[2-(phosphonomethoxy)propyl] derivative (*R*)-PMPDAP (**4**) belongs among the most active compounds against retroviruses in vitro[7] and the corresponding PME derivative PMEDAP (**3**) exhibits, in addition to inhibiting DNA viruses and retroviruses,[8] also selective antitumor properties.[9] In addition to adenine and 2,6-diaminopurine derivatives, their guanine counterparts PMEG (**5**) and (*R*)-PMPG (**6**) are potent antivirals[10] and exhibit powerful antitumor activity.[11] However, their potential use is substantially limited by their narrow safety margin.[12] The acyclic nucleoside phosphonates of the PME or PMP-type derived from all the above purine bases induce NO production in macrophages, stimulate in vitro secretion of cytokines,[13] and display antiarthritic activity.[14]

Thorough SAR studies in the series of acyclic nucleoside phosphonates[15] led to the conclusion that, except for an N∼C interchange at positions 3 and 8,[16] any substitution or other alteration of the purine base at the position 2 or 8 results in the loss of antiviral or cytostatic activity.[15] The influence of substitution of the amino group at position 6 (in adenine or 2,6-diaminopurine) is different. Generally, this group can be replaced by numerous mono- or dialkyl, cycloalkyl, alkenyl, or alkynyl functions while preserving antiviral[17] and/or cytostatic activity, particularly in the PMEDAP series.[18] Presumably, at least in some cases, this activity may be due to the intracellular deamination resulting in guanine derivatives.[19]

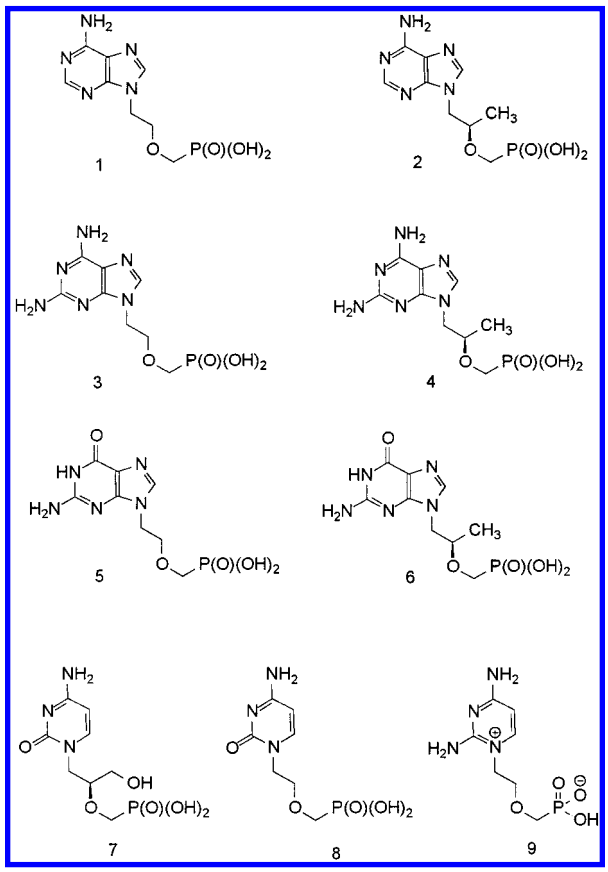
* Corresponding author: Dr. Antonín Holý, DSc., Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-166 10 Praha 6, Czech Republic. Phone: (4202)-20183333. Fax: (4202)-24310090. E-mail: holy@uochb.cas.cz.

† Academy of Sciences of the Czech Republic.

‡ Katholieke Universiteit Leuven.

10.1021/jm011095y CCC: $22.00
Published on Web 03/27/2002

Chart 1



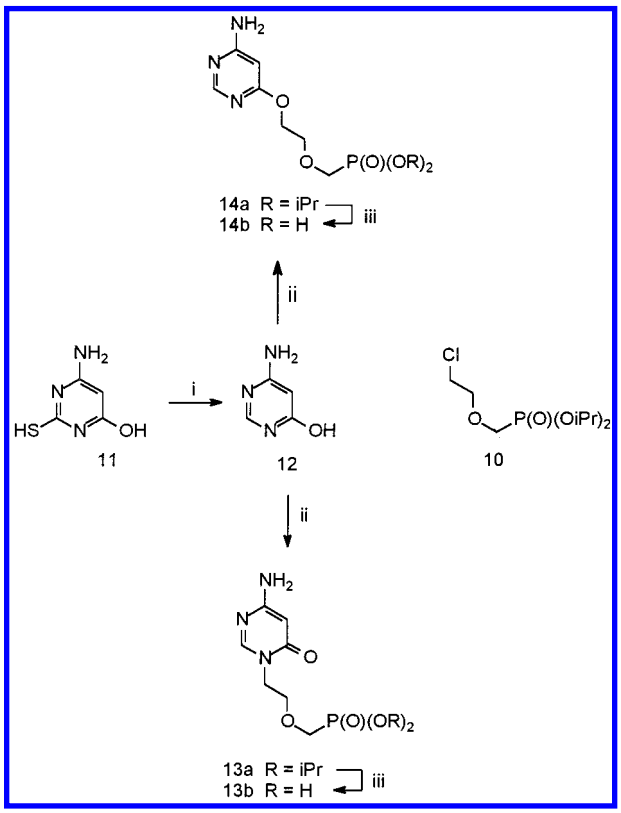
The pharmacophore of purine acyclic nucleoside phosphonates is characterized by the presence of amino groups at the pyrimidine part of the purine system. Replacement of these groups by aminomethyl or amidino functions, which are more strongly basic compared to their amino counterparts and cannot participate in the heteroaromatic π-electron system, resulted in completely inactive products both in the PME- and (*R*)-PMP-series.[20]

We have also investigated acyclic nucleoside phosphonates derived from pyrimidines bearing amino group(s). Only the cytosine derivative of the structurally related HPMP—[3-hydroxy-2-(phosphonomethoxy)propyl]—series [(S)-HPMPC, cidofovir, Vistide, **7**] showed antiviral[21] and antitumor activity,[22] while its 1-[2-(phosphonomethoxy)ethyl] (PMEC, **8**)[15] or (*R*)-1-[2-(phosphonomethoxy)propyl][23] congeners were essentially inactive in both aspects. N^1-[2-(phosphonomethoxy)ethyl] derivatives of 2-aminopyrimidine, 2,4-diaminopyrimidine (**9**), and their regioisomers[24] were devoid of antiviral or cytostatic activity. However, the critical feature of these compounds which distinguishes them from the above compounds might be their quaternary character. In an attempt to overcome this drawback, we synthesized and investigated the biological activity of the structurally related nonquaternary acyclic nucleoside phosphonates derived from pyrimidine bases bearing an hydroxyl group at position 6.

Chemistry

The major effort in this study focused on the synthesis of pyrimidine N^1-[2-(phosphonomethoxy)ethyl] (PME) derivatives. These syntheses were accomplished by

Scheme 1[a]

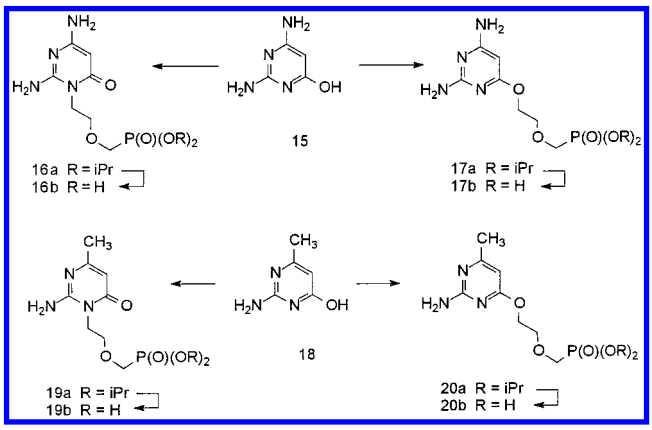


[a] (i) Ra-Ni/EtOH, reflux; (ii) **10**/Cs_2CO_3/DMF, 100 °C; (iii) (a) $BrSiMe_3$/acetonitrile; (b) H_2.

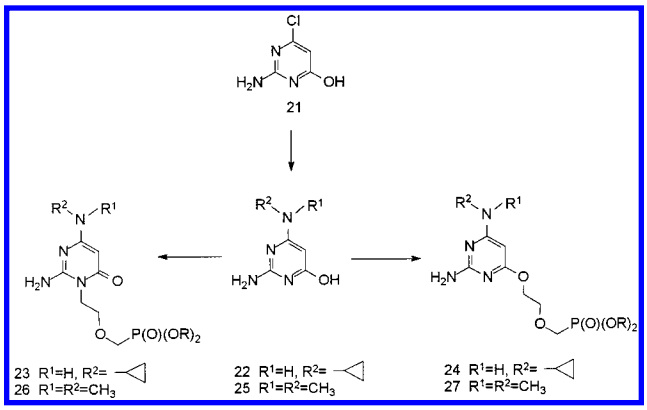
treatment of the appropriate 6-hydroxypyrimidine with diisopropyl 2-(chloroethoxy)methylphosphonate (**10**) in the presence of a suitable base [NaH, Cs_2CO_3, or DBU (1,8-diazabicyclo[5.4.0]undec-7-ene)] in dimethylformamide. The reaction course is illustrated by the reaction of compound **10** with the parent compound of the series, 4-amino-6-hydroxypyrimidine (**12**). The starting material was obtained by desulfurization of 4-amino-6-hydroxy-2-sulfanylpyrimidine (**11**). Treatment with the synthon **10** in the presence of Cs_2CO_3 afforded a mixture of two isomers: N^1-[2-(diisopropylphosphorylmethoxy)-ethyl]-4-aminopyrimidin-6-one (**13a**) and 4-amino-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (**14a**). The isomeric diesters gave, on treatment with bromotrimethylsilane followed by hydrolysis, the free phosphonic acids **13b** and **14b,** respectively (Scheme 1). Their structure was unequivocally assigned on the basis of ^{13}C NMR spectra. Similarly, 2,4-diamino-6-hydroxypyrimidine (**15**) gave N^1-[2-(diisopropylphosphorylmethoxy)ethyl]-2,4-diaminopyrimidin-6-one (**16a**) and 2,4-diamino-6-[2-(diisopropylphosphorylmethoxy)ethoxy]-pyrimidine (**17a**) and the free phosphonic acids **16b** and **17b**. In the same reaction sequence, 2-amino-4-hydroxy-6-methylpyrimidine (**18**) was transformed to the isomeric diesters **19a** and **20a** and free phosphonic acids **19b** and **20b**, respectively (Scheme 2).

With regard to the high biological activity of N^6-substituted 2,6-diaminopurine derivatives (vide supra), we have also examined the PME derivatives of 2-amino-4-cyclopropylamino-6-hydroxypyrimidine (**22**) and 2-amino-4-(dimethylamino)-6-hydroxypyrimidine (**25**). Starting pyrimidines were prepared by treatment of 2-amino-4-chloro-6-hydroxypyrimidine (**21**) with cyclopropylamine and dimethylamine, respectively. It should

Scheme 2



Scheme 3[a]



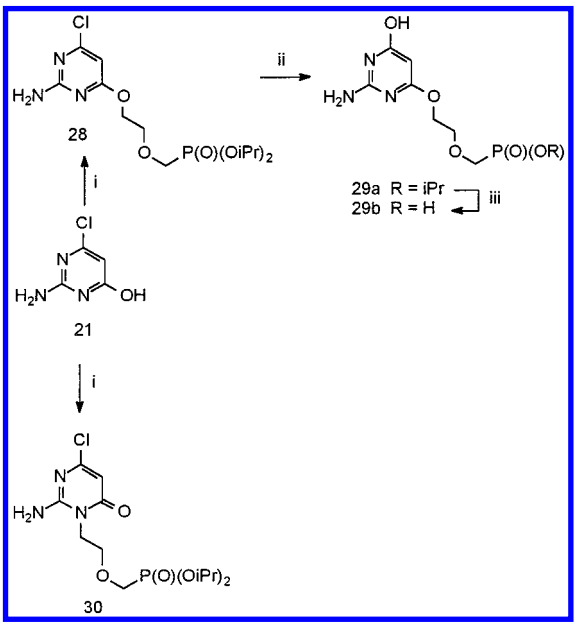
[a] In formulae **23**, **24**, **26**, and **27**, (a) R = ^{i}Pr, (b) R = H.

be noted that this reaction should not be performed in DMF: heating of compound **21** with cyclopropylamine in DMF in an autoclave gave the dimethylamino derivative **25** as the sole product, evidently due to the reaction with the solvent. Reaction of pyrimidines **22** and **25** with the synthon **10** under standard conditions gave diesters of N^1-isomers **23a** and **26a** and O^6-isomers **24a** and **27a.** Their deprotection gave N-phosphonates **23b** and **26b** and O-phosphonates **24b** and **27b**, respectively (Scheme 3).

2-Amino-4-chloro-6-hydroxypyrimidine (**21**) also gave, with the synthon **10** in the presence of a base, a mixture of diesters: N^1-isomer **30** and O^6-isomer **28**. The latter compound was transformed by alkaline hydrolysis in the presence of DABCO (1,4-diazabicyclo[2.2.2]octane)[25] to 2-amino-4-hydroxy-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (**29a**), which gave, after ester cleavage, the free phosphonate **29b** (Scheme 4). Compound **29a** is not formed by direct alkylation of 2-amino-4,6-dihydroxypyrimidine (**31**) with the synthon **10**. Disregarding the character of the base used, this reaction gave isomeric disubstituted products only: 2-amino-4,6-bis[2-(diisopropylphosphorylmethoxy)ethoxy]-pyrimidine (**32a**) and 2-amino-1-[2-(diisopropylphosphorylmethoxy)ethyl]-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (**33a)**. Bromotrimethylsilane-mediated cleavage of ester groups afforded the free phosphonic acids **32b** and **33b** (Scheme 5).

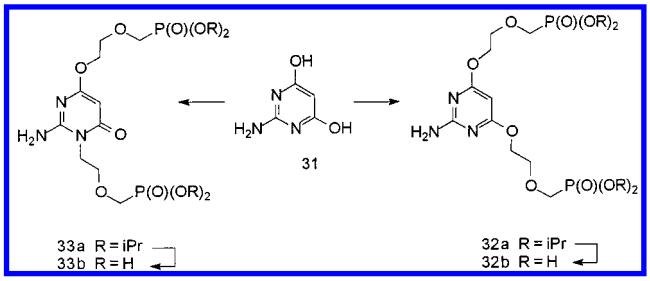
In contrast to 6-hydroxypyrimidines which give, due to −N=C−(OH)− ↔ −NH−C(=O)− tautomerism, by alkylation with the synthon **10** in the presence of a strong base always a mixture of N^1- and O^6-isomers, the

Scheme 4[a]



[a] **10**/base/DMF 100 °C; (ii) K_2CO_3/DABCO/H_2O; (iii) (a) $BrSiMe_3$, (b) H_2O.

Scheme 5

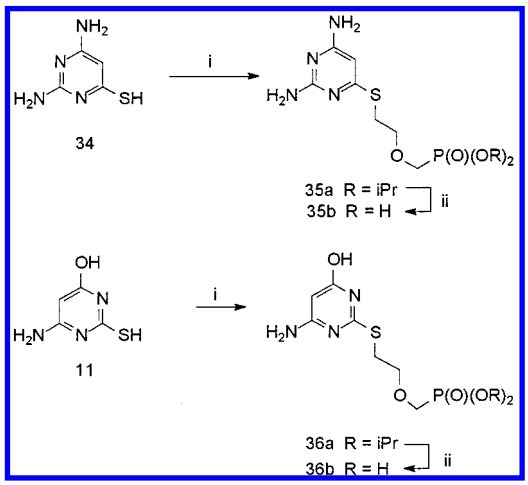


alkylation of sulfanylpyrimidines with the synthon **10** afforded the S-alkyl derivatives only. Thus, 2,4-diamino-6-sulfanylpyrimidine (**34**) gave diester **35a** and therefrom the free phosphonate **35b**. The preference of S-alkylation over the formation of N/O-isomers is clearly manifested by the reaction of 4-amino-6-hydroxy-2-sulfanylpyrimidine (**11**) with synthon **10** in the presence of 1 equiv of sodium hydride. The 2-[2-(diisopropylphosphorylmethoxy)ethyl]sulfanyl derivative **36a**, formed as the only reaction product, gave by ester cleavage the free phosphonate **36b** (Scheme 6).

The alkylation of the 6-hydroxypyrimidines is not limited to the ω-chloroalkyl derivatives (e.g., **10**). Reactions of 2,4-diamino-6-hydroxypyrimidine (**15**) with [(*R*)- or (*S*)-2-(diisopropylphosphoryl)methoxy]propyl tosylate [**(*R*)-37**, **(*S*)-37**] in the presence of cesium carbonate gave the enantiomeric 2,4-diamino-6-[2-(diisopropylphosphorylmethoxy)prop-1-oxy]pyrimidines [**(*R*)-38a**, **(*S*)-38a**] and the corresponding free phosphonates (analogues of PMP-compounds) [**(*R*)-38b**, **(*S*)-38b**] (Scheme 7). The formation of N^1-isomers was in these cases nearly nondetectable.

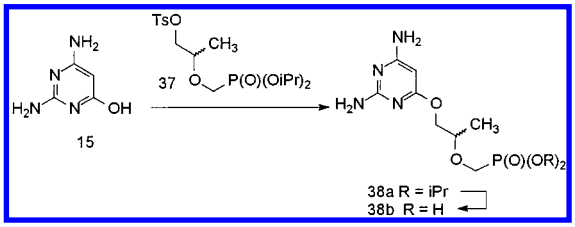
Another unequivocal reaction alternative which leads exclusively to the O^6-isomers makes use of the reactivity of the 6-chlorine atom at the pyrimidine ring (Scheme 8): reaction of 4-amino-6-chloro-2-methylsulfanylpyrimidine (**39**) with sodium alkoxide of diethyl 2-(hydroxyethoxy)methylphosphonate **40** generated in situ gave compound **41a** as the only product. Its deprotection

Scheme 6[a]

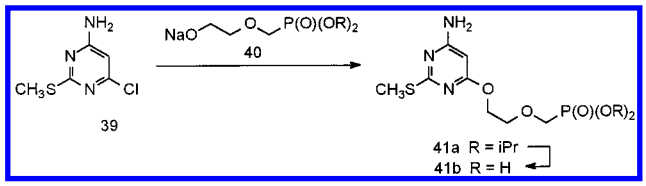


[a] (i) **10**, 1 equiv of NaH/DMF; (ii) (a) $BrSiMe_3/CH_3CN$; (b) H_2O.

Scheme 7



Scheme 8



afforded the free phosphonate **41b**. This alternative could be a method of choice for large-scale syntheses. Limiting factor will be the availability of the starting 6(4)-halogenopyrimidine derivatives.

The structure of N^1- and O^6-isomers of ANPs synthesized in this study could be derived from their ^{13}C NMR spectra. The N^1-isomers were characterized by the chemical shift of carbon atom C-1′ ($\delta \sim 40$ ppm) and by triplets of carbons C-2 [^3J(C-2′,H-1′) = 2.9] and C-6 [^3J(C-6,H-1′) = 3.9], and by doublets of carbons C-4 [^2J(C-4,H-5) = 4.9] and C-5 [^1J(C,H) = 164.2]. In the ^{13}C NMR spectra of O^6-isomers there is a low-field shift of C-1′ carbon ($\delta \sim 65$ ppm), which is due to the linkage to oxygen atom. We have also observed characteristic alkylation low-field shifts of pyrimidine base carbons C-6 and C-2 (ca. 15 and 3 ppm, respectively) and an absence of vicinal interaction of α-hydrogen atoms of the side chain with carbon C-6, which is typical for the N^1-isomers. The presence of the phosphonate function at the side chain is manifested by splitting of the appropriate hydrogens and carbons due to spin−spin interactions J(H,P) and J(C,P), respectively.

Biology

Among the 6-[2-(phosphonomethoxy)ethoxy] (PMEO) pyrimidine derivatives, several analogues showed a pronounced antiviral activity in cell culture. PMEO derivatives that carry an amino group at C-2 of the pyrimidine ring [i.e., **17b** and **29b**] emerged as the most active compounds. They were inhibitory to herpes simplex virus type 1 (HSV-1), HSV-2, and the thymidine kinase (TK)-deficient TK^-/HSV-1 strain at EC_{50} values ranking between 6.5 and 24 μg/mL (Table 1). The compounds were even more potent against two wild-type VZV and two TK-deficient VZV strains (EC_{50}: 0.6−2.5 μg/mL), but they were not active against cytomegalovirus (CMV) at subtoxic concentrations (data not shown). Compound **17b** was not active against adenovirus type 2 and type 3 infections in HEL cells.

Both **17b** and **29b** were exquisitely inhibitory to Moloney murine sarcoma virus (MSV) in C3H/3T3 cell cultures (EC_{50}: 0.04−0.08 μg/mL). Compound **17b** was also very effective (EC_{50}: 0.4−0.8 μg/mL) against HIV-1 and HIV-2 in CEM cell cultures. In contrast, **29b** was not inhibitory at 0.8 μg/mL, that is, at a compound concentration close to its toxicity threshold.

PMEO derivatives bearing an amino group solely in the C-4, but not in the C-2, position of the pyrimidine ring (i.e., compound **14b**) or that have the 4-NH_2 group replaced by dimethylamino (**27b**), cyclopropylamino (**24b**), or by methyl group (**20b**) or, wherein the 2-amino group is replaced by a 2-methylsulfanyl group (i.e., **41b**), were devoid of significant antiviral activity (Table 1). Replacement of the ether oxygen of compounds **17b** or **29b** by sulfur in 6-[2-(phosphonomethoxy)ethylsulfanyl] derivatives resulted in a marked (5- to 10-fold) decrease (**35b**) or complete loss (**36b**) of the antiviral activity. In contrast, the sulfanyl derivative **35b** gained some anti-adenovirus activity (EC_{50}: 74−78 μg/mL), comparable with PMEDAP (38−47 μg/mL) but inferior to (*S*)-HPMPA (EC_{50}: 0.21−0.24 μg/mL). Esterification of the phosphonate residue in **35b** by isopropyl groups (as in compound **35a**) annihilated the antiviral activity of the parent compound.

We have also synthesized the (*R*)- and (*S*)-6-[2-(phosphonomethoxy)propoxy] (PMPO) homologues of the most active PMEO derivative **17b**. Interestingly, the (*R*)-enantiomer of **38b** showed pronounced anti-herpes and anti-retroviral activity that was comparable to the activity of **17b**, whereas the (*S*)-derivative of **38b** was virtually devoid of antiviral activity. The residual activities of the (*S*)-enantiomer of **38b** noted for MSV and HIV-1 and HIV-2 may be due to contamination by traces (≤1%) of the (*R*)-enantiomer of **38b**. It should also be noted that the free pyrimidine bases of **14b**, **24b**, and **27b** (i.e., compounds **13b**, **22**, and **25**, respectively) were devoid of antiviral activity, except for a marginal activity against MSV.

None of the isomeric 1-[2-(phosphonomethoxy)ethyl] (PME) pyrimidin-6-one derivatives substituted at the C-2 and/or C-4 positions of the pyrimidine ring with 4-methyl (**19b**), 2,4-diamino (**16b**), 2-amino-4-dimethylamino (**26b**), or even 2-amino-4-[2-(phosphonomethoxy)-ethoxy] (**33b**) group showed appreciable antiviral activity with the exception of a poor but notable anti-MSV activity.

The N^1 and O^6-isomers were inactive in vitro against all RNA viruses tested [vesicular stomatitis virus (VSV), parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B-4 virus, or Punta Toro virus].

The majority of the compounds showed no appreciable cytostatic activity against E_6SM, HEL, and CEM cell

Table 1. Antiviral Activity of PMEO and PMPO Pyrimidine Derivatives in Cell Culture

	EC_{50} (μg/mL)									
formula	HSV-1 (KOS)	HSV-2 (G)	HSV-1 TK$^-$ (VMW 1837)	VZV (OKA)	VZV (YS)	VZV TK$^-$ (07/1)	VZV TK$^-$ (YS/R)	MSV	HIV-1 (III_B)	HIV-2 (ROD)
12	>400	>400	>400	>50	>50	>50	>50		>100	>100
13b	>400	>400	>400	>50	>50	>50	>50	138 ± 3	>100	>100
14a	>400	>400	>400	>50	>50	>50	>50		>20	>20
14b	>16	>16	>16	>50	>50	>50	>50	13 ± 7.6	>100	>100
16b	240	>400	>400	32	>50	>50	>50	2.92 ± 2.48	80.0 ± 28.3	25.0 ± 7.1
17b	6.5	24	9.6	1.2	1.1	2.5	1.6	0.04 ± 0.002	0.80 ± 0.17	0.43 ± 0.32
19b	>400	>400	>400	>50	>50	>50	>50	122 ± 3	>100	>100
20b	>400	>400	>400	>50	>50	>50	>50	89 ± 37.6	>100	>100
22	>400	>400	>400	>50	>50	>50	>50	133 ± 19	>100	>100
23b	>16	>16	>16	>50	>50	>50	>50		>100	>100
24b	240	>80	240	>20	>50	>50	>50	>40	>100	>100
25	>400	>400	>400	>50	>50	>50	>50	132 ± 5	>100	>100
26b	>80	>80	>80	>50	>50	>50	>50	105 ± 30	>100	>100
27b	>400	>400	>400	>50	>50	>50	>50	133 ± 11	>100	>100
29b	9.6	9.6	9.6	1.1	0.9		0.6	0.08	>0.8	>0.8
32b	240	>400	240	50	>50	>50	>50	43 ± 0.75	57 ± 38	80 ± 35
33b	>400	>400	>400	>50	>50	>50	>50	107 ± 10	>100	>100
35a	>400	>400	>400	>50	>50	>50	>50		47 ± 18	52 ± 14
35b	29	≥80	48	7.5	7	20	15	1.7	5.5 ± 2.1	3.0 ± 1.4
36b	240	>400	>400	>50	>50	>50	>50	72 ± 17	>100	>100
38b(*R*)	16	48	9.6	3.8	5.9	6.3	5.7	0.046 ± 0.012	1.9 ± 0.5	1.3 ± 0.4
38b(*S*)	>80	>80	>80	>50	>50	>50	>50	6.1 ± 1.4	51	33
41b	>80	>80	>80	>50	>50	>50	>50	>40	>100	>100
PMEA	7[b]	7[b]	7[b]	10[b,c]	10[b,c]	10[b,c]	10[b,c]	0.25[d]	1.8[d]	2.5[d]

[a] Fifty percent effective concentration, or compound concentration required to inhibit virus-induced cytopathicity by 50%. [b] Data taken from ref 33. [c] Data taken from ref 30; data are the average values for two wild-type (YS, OKA) and two TK$^-$ (07/1, YS/R) VZV strains. [d] Data taken from ref 27.

Table 2. In Vitro Cytotoxicity of Free Phosphonates

	% control			
formula	L-1210	L-929	HeLaS3	CCRF-CEM
13b	98	90	94	86
14b	81	84	86	95
16b	76	96	93	95
17b	62	61	89	63
19b	95	81	83	100
20b	99	85	87	95
24b	71	93	88	87
26b	90	99	92	98
27b	88	104	89	104
29b	50		96	61
32b	77	56	72	109
33b	80	77	72	101
35b	83	88	98	89
(*S*)-38b	80	94	92	89
41b	96	92	79	87

growth. The antivirally active compounds **17b**, **35b**, **38b** (*R*), and **29b** did not affect microscopically visible cell morphology at 50 (HEL) or 400 (E_6SM) μg/mL, and **17b**, **35b**, and **29b** were inhibitory to HEL cell proliferation at 25−50 μg/mL.

Detailed evaluation of cytostatic activity in vitro was performed in two mouse cell lines (L1210, L929) and two cell lines of human origin (HeLaS3, CCRF−CEM). The cells were grown in the presence of the tested compounds at a constant drug concentration (10 μM) and, after a 72 h incubation period, the cells were counted and the inhibition expressed relative to cell count in the control culture grown in the culture medium without compound (Table 2). The IC_{50} values of **17b** and **29b** were 12.4 μM and 11.2 μM (3.3 μg/mL and 3.0 μg/mL, respectively) for CCRF−CEM cells, 25 and 7.5 μM (6.5 μg/mL and 2.0 μg/mL, respectively) for L-1210 cells, and 25 and 2 μM (6.5 μg/mL and 5.3 μg/mL, respectively) for L929 cells. However, the IC_{50} values of **17b** and **29b** in CCRF−CEM cells are significantly (40−80×) higher compared to their EC_{50} values, e.g., against retroviruses (Table 1).

In conclusion, we report on a novel subclass of acyclic (pyrimidine) nucleoside phosphonates that are endowed with inhibitory activity against both DNA and retroviruses. From our studies, it could be concluded that the 6-[2-(phosphonomethoxy)ethoxy]pyrimidines must bear an (unsubstituted) amino group concomitantly on both C-2 and C-4, or an amino on C-2 and an OH group on C-4, to display antiviral activity. Alkyl ether derivatives are preferred over alkyl thioethers. The most active compounds of the 6-[2-(phosphonomethoxy)ethoxy] and 6-[2-(phosphonomethoxy)propoxy]pyrimidine series have an in vitro antiviral activity that is comparable to PMEA and (*R*)-PMPA and should be further investigated for their utility as novel antiviral agents.

Experimental Section

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. TLC was performed on Silufol UV254 plates (Kavalier Votice, Czech Republic) in chloroform−ethanol (4:1). Paper electrophoresis (20 V/cm, 1 h) was made on Whatman 3 MM paper in 0.05 M triethylammonium hydrogen carbonate pH 7.5.

NMR spectra were measured on an FT NMR spectrometer Varian UNITY 500 (^{1}H at 500 M and ^{13}C at 125.7 M frequency) in $CDCl_3$, dimethyl sulfoxide-d_6, or D_2O. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). UV spectra were measured on a Shimadzu type UV 1240 Mini spectrophotometer in aqueous solutions.

Materials. Bromotrimethylsilane, cesium carbonate, sodium hydride, and dimethylamine (30% solution in ethanol) were purchased from Fluka (Switzerland); DBU, DABCO, cyclopropylamine, and all pyrimidine derivatives were obtained from Sigma-Aldrich (Praha, Czech Republic). Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å).

Methods. Deionization of the Reaction Mixtures. The solution of reaction products in water (20−25 mL) was applied onto a column of Dowex 50 × 8 (H^+-form) (100 mL, if not stated otherwise), and the column was washed with water (20% aqueous methanol for phosphonate diesters) till the drop of the UV absorption (254 nm) and acid reaction of the eluate. Standard elution rate: 3 mL/min. Elution was continued with 2.5% ammonia (in water or 20% aqueous methanol, respectively), and the UV-absorbing eluate was collected and evaporated in vacuo.

Purification of the Phosphonates by Column Chromatography on Dowex 1 × 2. Unless stated otherwise, 100 mL columns of Dowex 1 × 2 (100−200 mesh, acetate form, prewashed with water) were used. The sample was dissolved in water (20−25 mL), alkalified with concentrated aqueous ammonia to pH 9−9.5, and applied onto the column. Elution with water (3 mL/min) was continued till the drop of the initial UV absorption (254 nm) of the eluate. The column was then eluted with the linear gradient of acetic acid (0−0.4 M acetic acid, 1 L each; 3 mL/min, fractions 30 mL).

Antiviral Activity Assays. The antiviral assays, other than HIV-1, were based on inhibition of virus-induced cytopathicity in either E_6SM (HSV-1, HSV-2, VV) or HEL (VZV, CMV, adenovirus type 2 and 3) cell cultures, following previously established procedure.[26] Briefly, confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1−2 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

Anti-Adenovirus Activity in HEL Cells (cf. ref 27). On day 0, confluent cultures of human embryonic lung (HEL) fibroblasts were infected with 100 $CCID_{50}$ of human adenovirus type 2 or type 3. Virus was removed after 2 h adsorption at 37 °C and replaced by serial dilutions of the compounds. On day 7−10 p.i., microscopy was performed to determine the cytopathic effect for calculation of the EC_{50} and the minimum cytotoxic concentration (MCC).

Inhibition of HIV-1-Induced Cytopathicity in CEM Cells. The methodology of the anti-HIV assays has been described previously.[28] Briefly, human CEM (~3×10^5 cells mL^{-1}) cells were infected with 100 $CCID_{50}$ HIV-1 ((III_B) or HIV-2 (ROD)/mL and seeded in 200 μL wells of a microtiter plate, containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, CEM giant cell formation was examined microscopically.

Inhibition of MSV-Induced Transformation of Murine C3H/3T3 Embryo Fibroblasts. The anti-MSV assay was performed as described previously.[28] Murine C3H/3T3 embryo fibroblast cells were seeded at 5×10^5 cells mL^{-1} into 1 cm^2 wells of a 48-well microplate. At 24 h later, the cell cultures were infected with 80 focus-forming units of MSV (prepared from tumors induced following intramuscular inoculation of 3-day-old NMRI mice with MSV, as described previously[29]) for 90−120 min at 37 °C. The medium was then replaced by 1 mL of fresh medium containing various concentrations of the test compounds. After 6 days, transformation of the cell culture was examined microscopically.

Cytostatic Activity Assays. Inhibition of the cell growth was estimated in mouse leukemia L1210 cells (ATCC CCL 219), CCRF-CEM T lymphoblastoid cells (ATCC CCL 119), murine L929 cells (ATCC CCL 1), and human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2) as described.[30]

4-Amino-6-hydroxypyrimidine (12). 4-Amino-6-hydroxy-2-sulfanylpyrimidine (**11**) (20 g, 0.14 mol) in boiling ethanol (300 mL) was treated under stirring with Raney-Ni until the starting material disappeared. The supension was filtered while hot, the precipitate washed with hot ethanol (300 mL), and the filtrate evaporated to dryness. The residue afforded on crystallization from ethanol (ether added to turbidity) 4-amino-6-hydroxypyrimidine (**12**), mp 272 °C. Yield, 10.0 g (64.4%). Anal. ($C_4H_5N_3O$) C, H, N. MS: 112 (MH^+). ^{1}H NMR (CD_3SOCD_3): 4.97 s, 1H (H-5); 6.42 brs, 2H (NH_2); 7.77 s, 1H (H-2); 11.41 brs, 1H (OH).

4-Amino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (14b) and 4-Amino-1-[2-(phosphonomethoxy)ethyl]-pyrimidin-6(1*H*)-one (13b). Compound **12** (3.6 g, 33.6 mmol) in DMF (70 mL) was treated with NaH (1.36 g, 34 mmol, 60% dispersion in paraffin oil) for 0.5 h under stirring, and diisopropyl 2-(chloroethoxy)methylphosphonate (**10**) (9.4 mL, 40.5 mmol) was added. The mixture was stirred for 8 h at 80 °C, filtered through Celite pad, and evaporated in vacuo. The residue in chloroform was purified on silica gel; elution with chloroform−ethanol (97.5:2.5) afforded 4-amino-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (**14a**), which was crystallized from ethyl acetate−petroleum ether. Yield, 3.0 g (26.8%); mp 112 °C. Anal. ($C_{13}H_{24}N_3O_5P$) C, H, N, P. ^{1}H NMR (CD_3SOCD_3): 1.22 d, 6 H and 1.23 d, 6 H, $J(CH_3,CH) = 6.1$ (4 × CH_3); 3.78 brt, 2 H, $J(2',1') = 4.5$ (H-2′); 3.78 d, $J(CH_2-P) = 8.4$ (CH_2-P); 4.31 brt, 2H, $J(1',2') = 4.5$ (H-1′); 4.59 m, 2 H (P−OCH); 5.67 s, 1 H (H-5); 6.62 bs, 2H (NH_2); 8.07 s, 1H (H-2). ^{13}C NMR (CD_3SOCD_3): 23.84 d, 2C, $J(CH_3,P) = 3.9$ (2 × CH_3); 23.99 d, 2C, $J(CH_3,P) = 2.9$ (2 × CH_3); 64.42 (C-1′); 64.99 d, $J(CH_2,P) = 165.0$ (CH_2-P); 70.35 d, 2C, $J(CH,P) = 5.9$ (2CHO); 70.88 d, $J(2',P) = 11.7$ (C-2′); 85.80 (C-5); 157.85 (C-2), 165.64 and 168.89 (C-4 and C-6).

This compound was treated with $BrSiMe_3$ (10 mL) in acetonitrile (70 mL) overnight at room temperature. After evaporation in vacuo, water (100 mL) was added to the residue, followed by concentrated aqueous ammonia to give an alkaline reaction, and the mixture was evaporated. The residue was deionized on a Dowex 50 × 8 column (100 mL) further purified by Dowex 1 × 2 (acetate form) column (150 mL) chromatography [elution with water followed by linear gradient of acetic acid (0−1 M, 1L each)]. The main UV-absorbing fraction was evaporated, the residue codistilled with water (3 × 50 mL) and crystallized from water. Yield, 1.8 g (80%) of 4-amino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (**14b**), mp 254 °C. Anal. ($C_7H_{12}N_3O_5P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): 3.71 d, 2H, $J(CH_2,P) = 8.5$ (CH_2P); 3.94 m, 2H (H-2′); 4.37m, 2H (H-1′); 5.95 d, 1H, $J(5,2) = 0.95$ (H-5); 8.10 d, 1H, $J(2,5) = 0.95$ (H-2). ^{13}C NMR (D_2O + NaOD): 69.11 (C-1′), 70.10 d, $J(CH_2,P) = 155.7$ (CH_2-P); 73.38 d, $J(2',P) = 11.0$ (C-2′); 89.29 (C-5); 160.16 (C-2); 167.99 (C-6); 172.02 (C-4).

Further elution of the crude reaction mixture on silica gel column with chloroform−ethanol (95:5) gave the oily 4-amino-1-[2-(diisopropylphosphorylmethoxy)ethyl]pyrimidin-6(1*H*)-one (**13a**), which was dried in vacuo [yield, 4.6 g (41.1%)]. This compound was treated with $BrSiMe_3$ (10 mL) in acetonitrile (70 mL) overnight at room temperature. After evaporation in vacuo, the residue was treated with water (100 mL). After 10 min, concentrated aqueous ammonia was added to give an alkaline reaction, and the mixture was evaporated. The residue was applied onto a column (100 mL) of Dowex 50 × 8 and eluted with water. The main UV-absorbing fraction was evaporated, and the residue was crystallized from 70% aqueous ethanol (ether added to turbidity). Yield, 2.8 g (91%) of 4-amino-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (**13b**), mp 233 °C. Anal. ($C_7H_{12}N_3O_5P$) C, H, N, P. ^{1}H NMR (CD_3SOCD_3): 3.56 d, 2H, $J(CH_2,P) = 8.8$ (CH_2P); 3.64 t, 2H, $J(2',1') = 4.9$ (H-2′); 3.90t, 2H, $J(1',2') = 4.9$ (H-1′); 5.06 s, 1 H (H-5); 6.45 brs, 2H (NH_2); 6.90 brs, 2H (P−OH); 7.98 s, 1H (H-2). ^{13}C NMR (CD_3SOCD_3): 44.44 (C-1′), 66.54 d, $J(CH_2,P) = 160.2$ (CH_2-P); 70.13 d, $J(2',P) = 11.7$ (C-2′); 84.78 (C-5); 152.35 (C-2), 161.05 and 163.74 (C-4 and C-6).

2,4-Diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (17b) and 2,4-Diamino-1-[2-(phosphonomethoxy)-ethyl]pyrimidin-6(1*H*)-one (16b). A mixture of 2,4-diamino-6-hydroxypyrimidine (**15**) (2.52 g, 20 mmol), cesium carbonate (3.25 g, 10 mmol) in dimethylformamide (40 mL) was stirred for 30 min at 80 °C, and diisopropyl 2-chloroethoxymethylphosphonate (**10**) (3.5 mL, 23.4 mmol) was added. The mixture was stirred for 16 h at 100 °C and filtered from salts. The filtrate was taken down in vacuo, and the residue

chromatographed on silica gel column (300 mL) with chloroform. The eluate gave a product which was crystallized from ethyl acetate−petroleum ether to afford 1.2 g (17.2%) of 2,4-diamino-6-[2-(diisopropylphosphorylmethoxy)ethoxy]-pyrimidine (**17a**), mp 159 °C. Anal. ($C_{13}H_{25}N_4O_5P$) C, H, N, P. MS: 349.3 (MH^+) (100), 265.1 ($MH^+ - 2 \times {}^i$Pr) (6); 139 (26); 127.1 (BaseH^+) (37). ^{1}H NMR (CD_3SOCD_3): 1.24 d, 6H and 1.24 d, 6 H, $J(CH_3,CH) = 6.2$ (4 × CH_3); 3.74 m, 2 H (H-2′); 3.78 d, 2H, $J(CH_2-P) = 8.2$ (CH_2−P); 4.22 m, 2H (H-1′); 4.59 dh, 2 H, $J(CH,P) = 8.2$, $J(CH,CH_3) = 6.2$ (2xCH); 5.02 s, 1 H (H-6); 5.85 bs, 2 H and 6.00 bs, 2H (2 × NH_2). ^{13}C NMR (CD_3SOCD_3): 23.85 d, 2C, $J(CH_3,P) = 4.6$ (2 × CH_3); 23.99 d, 2C, $J(CH_3,P) = 4.1$ (2 × CH_3); 63.65 (C-1′); 65.02 d, $J(CH_2-P) = 164.4$ (CH_2P); 70.32 d, 2C, $J(CH,P) = 6.0$ (2 × CH−O); 71.05 d, $J(2',P) = 11.9$ (C-2′); 76.35 (C-5); 163.01, 166.15 and 169.92 (C-2, C-4 and C-6).

This compound (1.0 g, 2.9 mmol) was treated with $BrSiMe_3$ (4 mL) in acetonitrile (40 mL) overnight. The solvents were stripped down in vacuo, the residue was codistilled with acetonitrile (2 × 25 mL), and water (50 mL) was added to the residue. The solution was alkalized with concentrated aqueous ammonia and evaporated in vacuo. The residue was deionized on a Dowex 50 × 8 column (100 mL) and the UV-absorbing fraction of the ammonia eluate was collected. It was taken down in vacuo, redissolved in water (20 mL), brought to pH 9−10 by concentrated aqueous ammonia, and applied onto a column (70 mL) Dowex 1 × 2 (acetate form) thoroughly prewashed with water. Elution with water gave (with retention) product which was crystallized from water to afford 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (**17b**) (0.60 g, 78.3%), mp 279 °C (water). E_{Up} 0.80. Anal. ($C_7H_{13}N_4O_5P$) C, H, N, P. UV spectrum [λ_{max} (ϵ_{max})] (pH 2): 276 (9100), (pH 7): 265 (7500). These data agree with the UV spectra published[31] for 2,4-diamino-6-methoxypyrimidine ((λ_{max} 263 and 275, respectively). ^{1}H NMR (CD_3SOCD_3, 40 °C): 3.58 d, 2 H, $J(CH_2,P) = 8.7$ (CH_2P); 3.74 t, 2 H, $J(2',1') = 4.9$ (H-2′); 4.23 t, 2 H, $J(1',2') = 4.9$ (H-1′); 5.07 s, 1 H (H-5); 5.86 bs, 2 H and 6.01 bs, 2 H (2 × NH_2). ^{1}H NMR (D_2O): 3.69 d, 2H, $J(CH_2,P) = 8.7$ (CH_2P); 3.91 m, 2H (H-2′); 4.30 m, 2 H (H-1′); 5.45 s, 1 H (H-5). ^{13}C NMR (D_2O): 69.30 (C-1′); 70.28 d, $J(CH_2-P) = 151.3$ (CH_2P); 73.35 d, $J(2',P) = 10.3$ (C-2′); 79.63 (C-5); 165.46, 169.35 and 171.08 (C-2, C-4 and C-6). Low-field position of C-1′-carbon signal in the O-isomers (diester **17a** and free phosphonate **17b**) (δ 63.65 and 69.30, respectively) indicates that the PME group is linked to the oxygen atom at C-6.

Further elution of the silica gel column with chloroform gave 1.8 g (26%) of amorphous 2,4-diamino-1-[2-(diisopropylphosphorylmethoxy)ethyl]pyrimidin-6(1*H*)-one (**16a**) (R_F 0.35, S1) which was dried in vacuo over P_2O_5. mp 196−197 °C. MS: 349.2 (MH^+) (100); 265.1($MH^+ - 2 \times {}^i$Pr) (35); 126 (BH^+) (26). ^{1}H NMR (CD_3SOCD_3): 1.23 d, 6 H and 1.21 d, 6 H, $J(CH_3,CH) = 6.3$ (4 × CH_3); 3.62 t, 2 H, $J(2',1') = 4.9$ (H-2′); 3.78 d, 2H, $J(CH_2-P) = 8.3$ (CH_2−P); 3.96 t, 2H, $J(1',2') = 4.9$ (H-1′); 4.57 m, 2 H (2 × CH); 4.59 s, 1 H (H-5); 6.55 bs, 2 H and 5.84 bs, 2H (2 × NH_2). ^{13}C NMR (CD_3SOCD_3): 23.94 d, 2C, $J(CH_3,P) = 4.9$ (2 × CH_3); 24.00 d, 2C, $J(CH_3,P) = 3.9$ (2 × CH_3); 39.39 (C-1′); 65.00 d, $J(CH_2-P) = 163.1$ (CH_2P); 70.22 d, $J(2',P) = 11.7$ (C-2′); 70.40 d, 2C, $J(CH,P) = 6.9$ (2 × CH−O); 76.66 (C-5); 155.71 (C-6); 162.22 (C-2); 163.38 (C-4).

The residue (5.17 mmol) was treated with $BrSiMe_3$ (6 mL) in acetonitrile (60 mL) overnight and worked up as described for the O-isomer. Purification of the desalted mixture on Dowex 1 column (elution with water) gave a product which was crystallized from water to afford 0.95 g (65%) of 2,4-diamino-1-[2-(phosphonomethoxy)ethyl]pyrimidin-(1*H*)-one (**16b**), mp 228 °C (water). E_{Up} 0.90. Anal. ($C_7H_{13}N_4O_5P\cdot H_2O$) C, H, N, P. UV spectrum [λ_{max} (ϵ_{max})] (pH 2): 264 (18000), (pH 7): 267 (12200). The λ_{max} value coincides with the value published[32] for 2,4-diamino-1-methylpyrimidin-6(1*H*)-one (268 nm). ^{1}H NMR (CD_3SOCD_3): 3.59 d, 2 H, $J(CH_2,P) = 8.4$ (CH_2P); 3.60 t, 2 H, $J(2',1') = 6.1$ (H-2′); 3.96 t, 2 H, $J(1',2') = 6.1$ (H-1′); 4.61 s, 1 H (H-5); 5.88 bs, 2 H and 6.61 bs, 2 H (2 × NH_2).^{1}H NMR (D_2O): 3.48 d, 2 H, $J(CH_2,P) = 8.4$ (CH_2P); 3.76 t, 2 H, $J(1',2') = 5.2$ (H-1′); 4.09 t, 2H, $J(2',1') = 5.2$ (H-2′); 5.06 s, 1 H (H-5). ^{13}C NMR (D_2O): 45.15 (C-1′); 70.41 d, $J(CH_2-P) = 154.7$ (CH_2P); 74.09 d, $J(2',P) = 11.8$ (C-2′); 81.05 (C-5); 159.73, 166.79 and 168.04 (C-2, C-4 and C-6). The upfield position of C-1′ (δ 45.15) indicates N-substitution. Two signals of NH_2 groups (δ 6.61 and 5.88) which are observed in ^{1}H NMR spectrum in DMSO exclude the substitution at the *exo*-positions 2-NH_2 and/or 4-NH_2 and are consistent with the expected substitution at N^1.

2-Amino-4-methyl-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (20b) and 2-Amino-4-methyl-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (19b). Diisopropyl 2-(chloroethoxy)methylphosphonate (**10**) (15 mL, 62.5 mmol) was added to a mixture of 2-amino-6-hydroxy-4-methylpyrimidine (**18**) (6.25 g, 50 mmol) and cesium carbonate (11.3 g, 25 mmol) in DMF (70 mL) which had been stirred at 100 °C for 1 h prior to addition. The reaction mixture was then stirred at 100 °C for 14 h, filtered while hot, and evaporated in vacuo. The residue gave, on extraction with chloroform and subsequent purification on silica gel column (250 mL), compound **20a**, which was crystallized from ethyl acetate−petroleum ether. Yield, 5.75 g (33,1%), mp 72−73 °C. Anal. ($C_{14}H_{26}N_3O_5P$) C, H, N, P. ^{1}H NMR ($CDCl_3$): 1.33 d, 6H, $J = 6.2$ and 1.34 d, 6H, $J = 6.2$ (4 × CH_3); 2.26 d, 3H, $J = 0.6$ (CH_3); 3.82 d, 2H, $J(H,P) = 8.2$ (P−CH_2); 3.90 m, 2H (2 × H-2′); 4.76 dh, 2H, $J(H,P) = 7.6$ and $J(H,H) = 6.2$ (2 × OCH (^{i}Pr)); 4.42 m, 2H (2 × H-1′); 4.90 b, 2H (NH_2); 5.95 q, 1H, $J = 0.6$ (H-5); ^{13}C NMR ($CDCl_3$): 23.61 (CH_3); 23.90 d, 2C, $J(C,P) = 4.9$ (2 × CH_3); 24.04 d, 2C, $J(C,P) = 3.9$ (2 × CH_3); 64.60 (C-1′); 65.99 d, $J(C,P) = 167.6$ (P−CH_2); 71.06 d, $J(C,P) = 6.8$ (OCH (^{i}Pr)); 71.17 d, $J(C,P) = 10.8$ (C-2′); 97.03 (C-5); 162.48, 168.27 and 170.36 (C-2, C-4 and C-6).

This compound (5.55 g, 16 mmol) was treated with $BrSiMe_3$ (5 mL) in acetonitrile (50 mL) at room temperature overnight, and the volatiles were evaporated in vacuo. The residue was dissolved in water (100 mL), and concentrated aqueous ammonia was added to an alkaline reaction. The mixture was deionized on a Dowex 50 × 8 column (100 mL) and further purified by Dowex 1 × 2 column (150 mL) chromatography [elution with water followed by linear gradient of acetic acid (0−0.4 M, 1 L each)]. The main UV-absorbing fraction was evaporated, and the residue was codistilled with water (3 × 50 mL) and crystallized from water. Yield, 3.67 g (87%) of 2-amino-4-methyl-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (**20b**), mp 245 °C. Anal. ($C_8H_{14}N_3O_5P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): 2.26 s, 3H (CH_3); 3.57 d, 2H, $J(H,P) = 8.5$ (P−CH_2); 3.92 m, 2H (2 × H-2′); 4.39 m, 2H (2 × H-1′); 6.13 s, 1H (H-5). ^{13}C NMR (D_2O + NaOD): 25.42 (CH_3); 68.85 (C-1′); 72.06 d, $J(C,P) = 149.4$ (P−CH_2); 73.09 d, $J(C,P) = 10.2$ (C-2′); 98.75 (C-5); 165.50, 172.80 and 173.63 (C-2, C-4 and C-6).

Further elution of the silica gel column followed by crystallization from ethyl acetate−petroleum ether gave 2-amino-4-methyl-1-[2-(diisopropylphosphorylmethoxy)ethyl]pyrimidin-6(1*H*)-one (**19a**) (4.2 g, 24%), mp 88 °C. Anal. ($C_{14}H_{26}N_3O_5P$) C, H, N, P. ^{1}H NMR ($CDCl_3$): 1.29 d, 6H, $J = 6.2$ and 1.32 d, 6H, $J = 6.2$ (4 × CH_3); 2.12 d, 3H, $J = 0.8$ (CH_3); 3.74 d, 2H, $J(H,P) = 8.6$ (P−CH_2); 3.90 m, 2H (2 × H-2′); 4.20 m, 2H (2 × H-1′); 4.72 dh, 2H, $J(H,P) = 7.6$ and $J(H,H) = 6.2$ (2 × OCH); 5.62 b, 2H (NH_2); 5.79 q, 1H, $J = 0.8$ (H-5). ^{13}C NMR ($CDCl_3$): 23.64 (CH_3); 23.97 d, 2C, $J(C,P) = 3.9$ and 23.91 d, 2C, $J(C,P) = 4.9$ (2 × CH_3); 43.06 (C-1′); 66.17 d, $J(C,P) = 168.1$ (P−CH_2); 71.27 d, $J(C,P) = 6.8$ (OCH); 72.88 d, $J(C,P) = 11.7$ (C-2′); 102.22 (C-5); 156.37, 162.93 and 164.33 (C-2, C-4 and C-6).

This product (4.0 g, 11.5 mmol) was treated analogously with $BrSiMe_3$ (5 mL) in acetonitrile (50 mL) to afford, after chromatography of the deionized reaction mixture on Dowex 1 × 2 and crystallization from water, 2-amino-4-methyl-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (**19b**). Yield, 2.3 g (76%), mp 283 °C. Anal. ($C_8H_{14}N_3O_5P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): 2.12 s, 3H (CH_3); 3.50 d, 2H, $J(H,P) = 8.7$ (P−CH_2); 3.82 t, 2H, $J = 5.3$ (2 × H-2′); 4.18 t, 2H, $J = 5.3$ (2 × H-1′); 5.77 s, 1H (H-5). ^{13}C NMR (D_2O + NaOD): 25.43 (CH_3); 45.60 (C-1′); 72.55 d, $J(C,P) = 142.0$ (P−CH_2); 73.17 (C-2′); 102.40 (C-5); 160.25, 168.32 and 169.48 (C-2, C-4 and C-6).

2-Amino-4-cyclopropylamino-6-hydroxypyrimidine (22). 2-Amino-4-chloro-6-hydroxypyrimidine monohydrate (**21**, 5.0 g, 30.5 mmol) was refluxed in ethanol (150 mL) with cyclopropylamine (15 mL) for 12 h. The mixture was evaporated in vacuo, codistilled with ethanol (3 × 50 mL), adsorbed from methanol on silica gel and applied onto a column of silica gel (200 mL) in chloroform. Elution with chloroform−ethanol gradient afforded a crystalline product which was filtered from ether and dried in vacuo to afford 2-amino-4-cyclopropylamino-6-hydroxypyrimidine (**22**), mp 229 °C. Yield, 3.0 g (59%). Anal. ($C_7H_{10}N_4O$) C, H, N. ^{1}H NMR (DMSO): 0.40m, 2H and 0.62, m, 2H (C−CH_2, N−CH); 2.30 m, 1H; 4.66 s, 1H (H-5); 6.08 brs, 2H (NH_2); 6.55 bs, 2H (NH); 9.73 brs, 1H (OH).

2-Amino-4-cyclopropylamino-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine (24b) and 2-Amino-4-cyclopropylamino-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (23b). The mixture of compound **22** (3.0 g, 18 mmol) and cesium carbonate (2.92 g, 9 mmol) in DMF (50 mL) was stirred at 100 °C for 1 h, and compound **10** (6 mL) was added. The reaction mixture was stirred at 100 °C for 24 h, filtered while hot, and evaporated in vacuo. The residue was extracted with hot chloroform (100 mL), filtered, concentrated in vacuo, and purified by chromatography on silica gel column (2 × 200 mL). Elution with chloroform gave 2-amino-4-cyclopropylamino-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (**24a**) as a thick oil (1.8 g, 25.8%). It was treated with $BrSiMe_3$ (5 mL) and acetonitrile (50 mL) overnight at room temperature. After evaporation in vacuo, the residue was treated with water (100 mL), alkalized with concentrated aqueous ammonia, and evaporated. The residue was deionized on a column (100 mL) of Dowex 50 × 8 and further purified by Dowex 1 × 2 column (150 mL) chromatography [elution with water followed by linear gradient of acetic acid (0−0.4 M, 1 L each)]. The main UV-absorbing fraction was evaporated, and the residue was codistilled with water (3 × 50 mL) and crystallized from water. Yield, 1.0 g (70.7%) of 2-amino-4-cyclopropylamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (**24b**), mp 244 °C. Anal. ($C_{10}H_{17}N_4O_5P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): 0.54 m, 2H and 0.80 m, 2H (C−CH_2); 2.53 m, 1H (N−CH); 3.57 d, 2H, J(CH_2,P) = 8.4 (CH_2P); 3.91 m, 2H (H-2′); 4.32 m, 2H (H-1′); 5.61 s, 1 H (H-5).

Further elution of the silica gel column with chloroform−ethanol gradient afforded (after crystallization from ethanol−ether) yellow 2-amino-4-cyclopropylamino-1-[2-(diisopropylphosphorylmethoxy)ethyl]pyrimidin-6(1*H*)-one (**23a**) (1.6 g, 23%), which was treated with $BrSiMe_3$ (5 mL) and acetonitrile (50 mL) overnight and worked up similarly. The product eluted with water during Dowex 50 chromatography. It was evaporated in vacuo and crystallized from water to yield 2-amino-4-cyclopropylamino-1-[2-(phosphonomethoxy)ethyl]pyrimidin-2-one (**23b**), not melting under 290 °C. Yield, 0.90 g (71.8%). Anal. ($C_{10}H_{17}N_4O_5P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): 0.54 m, 2H and 0.81 m, 2H (C−CH_2); 2.50 m,1H (N−CH); 3.59 d, 2H, J(CH_2,P) = 8.5 (CH_2−P); 3.95 t, 2H, J(2′,1′) = 4.9 (H-2′); 4.30 t, 2H, J(1′,2′) = 4.9 (H-1′); 5.54 s, 1H (H-5). ^{13}C NMR (D_2O + NaOD): 9.43 (2 × CH_2, cyclopropyl); 25.96 (N−CH); 69.14 (C-1′); 72.19 d, J(C,P) = 149.4 (P−CH_2); 73.31 d, J(C,P) = 10.3 (C-2′); 78.75 (C-5); 165.53, 170.07 and 173.71 (C-2, C-4 and C-6).

2-Amino-4-(dimethylamino)-6-hydroxypyrimidine (25). 2-Amino-4-chloro-6-hydroxypyrimidine monohydrate (5.0 g) was stirred with 30% dimethylamine in ethanol (180 mL) at 100 °C in an autoclave for 16 h. The crystalline product was filtered, washed with water, acetone, and ether, and dried in vacuo to afford 2-amino-4-(dimethylamino)-6-hydroxypyrimidine (**25**), not melting under 300 °C. Yield, 4.3 g (81%). Anal. ($C_6H_{10}N_4O$) C, H, N. ^{1}H NMR (CD_3SOCD_3): 2.89 s, 6H (N−CH_3); 4.51 s, 1H (H-5); 6.18 brs, 2H (NH_2); 9.75 brs, 1H (OH).

2-Amino-4-(dimethylamino)-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (27b) and 2-Amino-4-(dimethylamino)-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (26b). Compound **25** (4.0 g, 26 mmol) and cesium carbonate (4.22 g, 13 mmol) in DMF (60 mL) was stirred at 100 °C for 1 h, and diisopropyl 2-(chloroethoxy)methylphosphonate (**10**) (8 mL) was added. The mixture was stirred at 100 °C for 24 h, filtered while hot, and evaporated in vacuo. The residue was extracted with hot chloroform (100 mL), filtered, and purified by chromatography on silica gel column (200 mL). Elution with chloroform gave 2-amino-4-(dimethylamino)-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (**27a**) as a thick oil (4.6 g, 47%). It was treated with $BrSiMe_3$ (5 mL) and acetonitrile (50 mL) overnight at room temperature. After evaporation in vacuo, the residue was treated with water (100 mL), alkalized with concentrated aqueous ammonia, and the mixture was evaporated. The residue was deionized on Dowex 50 × 8 column (100 mL) and purified by Dowex 1 × 2 column (150 mL) chromatography [elution with water followed by linear gradient of acetic acid (0−0.4 M, 1 L each)]. The main UV-absorbing fraction was evaporated, and the residue was codistilled with water (3 × 50 mL) and crystallized from water. Yield, 1.1 g (31%) of 2-amino-4-(dimethylamino)-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (**27b**), mp 168 °C. Anal. ($C_9H_{17}N_4O_5P$) C, H, N, P. ^{1}H NMR (DMSO): 2.93 s, 6H (N(CH_3)$_2$); 3.58 d, 2H, J(H,P) = 8.8 (P−CH_2); 3.74 m, 2H (H-2′); 4.26 m, 2H (2 × H-1′); 5.20 s, 1H (H-5); 6.03 bs, 2H (NH_2). ^{13}C NMR (DMSO): 36.93, 2C (N(CH_3)$_2$); 64.28 (C-1′); 66.96 d, J(C,P) = 160.7 (P−CH_2); 70.89 d, J(C,P) = 11.4 (C-2′); 75.02 (C-5); 161.92, 164.91 and 169.93 (C-2, C-4 and C-6).

Further elution of the silica gel column with chloroform−ethanol gradient afforded 2-amino-4-(dimethylamino)-1-[2-(diisopropylphosphorylmethoxy)ethyl]pyrimidin-6(1*H*)-one (**26a**) as a thick oil (2.0 g, 20.5%), which was treated with $BrSiMe_3$ (5 mL) and acetonitrile (50 mL) overnight and worked up similarly. After Dowex 1 chromatography, the product gave, on crystallization from water, 1.17 g (75.5%) of 2-amino-4-(dimethylamino)-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (**26b**), mp 235 °C. Anal. ($C_9H_{17}N_4O_5P$) C, H, N, P. ^{1}H NMR (DMSO): 2.89 s, 6H (N(CH_3)$_2$); 3.59 d, 2H, J(H,P) = 8.3 (P−CH_2); 3.61 t, 2H, J(2′,1′) = 6.0 (2 × H-2′); 3.98 t, J(1′,2′) = 6.0 (H-1′); 4.69 s, 1H (H-5); 6.69 bs, 2H (NH_2). ^{13}C NMR (DMSO): 36.93, 2C (N(CH_3)$_2$); 40.0 (C-1′); 66.76 d, J(C,P) = 158.7 (P−CH_2); 69.82 d, J(2′,P) = 10.3 (C-2′); 75.79 (C-5); 154.67, 161.94 and 162.37 (C-2, C-4 and C-6).

2-Amino-4-chloro-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (28) and 2-Amino-4-chloro-1-[2-(diisopropylphosphorylmethoxy)ethyl]pyrimidin-6(1*H*)-one (30). 2-Amino-4-chloro-6-hydroxypyrimidine monohydrate (**21**) (25 mmol) was codistilled with toluene (3 × 50 mL) in vacuo, and the residue was treated with DMF (50 mL), DBU (3.8 mL), and diisopropyl 2-(chloroethoxy)methylphosphonate (**10**) (7 mL). The mixture was stirred for 16 h at 100 °C, and the volatiles were evaporated at 50 °C/2 kPa. The residue was taken in chloroform (200 mL), filtered, and washed with saturated NaCl solution (100 mL). The aqueous wash was extracted with chloroform (5 × 50 mL), and the combined extracts were dried with magnesium sulfate and evaporated. Silica gel column (150 mL) chromatography by chloroform−ethanol gradient gave 2-amino-4-chloro-6-[2-(diisopropylphosphorylmethoxy)ethoxy]pyrimidine (**28**) (2.5 g, 27.2%), mp 89 °C. Anal. ($C_{13}H_{23}ClN_3O_5P$) C, H, Cl, N, P. MS: 368.3 (MH^+) (100). ^{1}H NMR (CD_3SOCD_3): 1.22 d, 6 H and 1.23 d, 6 H, J(CH_3,CH) = 6.1 (CH_3); 3.78 d, J(CH_2−P) = 8.3 (CH_2−P); 3.80 m, 2H (H-2′); 4.37 m, 2H (H-1′); 4.58 m, 2 H (P−OCH); 6.06 s, 1 H (H-5); 7.05 bs, 2 H (NH_2). ^{13}C NMR (CD_3SOCD_3): 23.83 d, 2C, J(CH_3,P) = 4.9 and 23.97 d, 2C, J(P,C) = 3.9 (CH_3); 65.00 d, J(P,C) = 164.1 (P−C); 65.15 (C-1′); 70.35 d, 2C, J(P,C) = 6.7 (P−OC); 70.52 d, 2C, J(P,C) = 11.7 (C-2′); 94.46 (C-5); 160.12 (C-2); 162.97 (C-4); 170.56 (C-6).

Further elution and crystallization from ethyl acetate−ether gave 2-amino-4-chloro-1-[2-(diisopropylphosphorylmethoxy)-ethyl]pyrimidin-6(1*H*)-one (**30**) (4.8 g, 52.4%), mp 95 °C. Anal. ($C_{13}H_{23}ClN_3O_5P$) C, H, Cl, N, P. MS: 368.4 (MH^+) (100). ^{1}H NMR (CD_3SOCD_3): 1.20 d, 6 H and 1.22 d, 6 H, J(CH_3,CH) = 6.1 (CH_3); 3.69 t, 2 H, J(2′,1′) = 5.5 (H-2′); 3.76 d, J(CH_2−P) = 8.1 (CH_2−P); 4.06 t, 2H, J(1′,2′) = 5.5 (H-1′); 4.55 m, 2 H (P−OCH); 5.67 s, 1 H (H-5); 7.60 bs, 2 H (NH_2). ^{13}C NMR (CD_3SOCD_3): 23.84 d, 2C, J(CH_3,P) = 3.9 and 23.97 d, 2C, J(P,C) = 3.9 (CH_3); 40.29 (C-1′); 65.03 d, J(P,C) = 164.1 (P−C); 69.01

d, J(P,C) = 11.7 (C-2′); 70.41 d, 2C, J(P,C) = 5.9 (P−OC); 98.28 (C-5); 155.73 (C-6); 157.87 (C-4); 161.48 (C-2).

2-Amino-4-hydroxy-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (29b). A mixture of compound **28** (5.7 g, 15.5 mmol), DABCO (3.6 g), and K_2CO_3 (9.0 g) in water (100 mL) was refluxed for 150 min under stirring, cooled, and acidified by addition of Dowex 50 × 8 (H^+-form). The suspension was alkalified with concentrated aqueous ammonia and, after 5 min stirring, filtered, and the resin was washed with 50% aqueous methanol (200 mL). The filtrate was evaporated to dryness, ethanol (50 mL) was added, and the mixture was evaporated to dryness. The residue gave by purification on silica gel column (150 mL) with chloroform−ethanol gradient crystalline 2-amino-6-[2-(diisopropylphosphorylmethoxy)ethoxy]-4-hydroxypyrimidine (**29a**) (4.2 g, 78%), mp 154 °C. Anal. ($C_{13}H_{24}N_3O_6P$) C, H, N, P. ^{1}H NMR (CD_3SOCD_3): 1.24 d, 6H and 1.23 d, 6 H, J(CH_3,CH) = 6.2 (4 × CH_3); 3.74 m, 2 H (H-2′); 3.76 d, J(CH_2−P) = 8.3 (CH_2−P); 4.19 m, 2H (H-1′); 4.59 m, 2 H (P−OCH); 4.75 s, 1 H (H-5); 6.65 bs, 2 H, 2H (NH_2); 10.45 s, 1H (OH). ^{13}C NMR (CD_3SOCD_3): 23.87 d, 2C, J(CH_3,P) = 4.9 and 24.01 d, 2C, J(P,C) = 3.9 (CH_3); 65.03 d, J(P,C) = 164.6 (P−C); 65.04 (C-1′); 70.37 d, 2C, J(P,C) = 6.3) (P−OC); 70.87 d, J(P,C) = 11.7 (C-2′); 79.95 (C-5); 155.68 (C-4); 164.25 (C-2); 171.01 (C-6).

This diester (4.0 g, 11.5 mmol) was treated with $BrSiMe_3$ (10 mL) in acetonitrile (80 mL) overnight and evaporated in vacuo, the residue was treated with water (50 mL) and alkalified with concentrated aqueous ammonia, and the mixture was evaporated in vacuo. The residue was deionized on a Dowex 50 × 8 column (100 mL), and the UV-absorbing ammonia eluate was evaporated to dryness. It was dissolved in minimum hot water by addition of concentrated aqueous ammonia and acidified with concentrated HCl to pH 3−3.5. The precipitate of compound **29b** was collected, washed with water and ethanol, and dried in vacuo. Yield, 0.7 g (23%), mp 227 °C. Anal. ($C_7H_{12}N_3O_6P$) C, H, N, P. ^{1}H NMR (CD_3SOCD_3): 3.58 d, J(CH_2−P) = 8.6 (CH_2−P); 3.76 m, 2 H (H-2′); 4.29 m, 2H (H-1′); 5.36 s, 1 H (H-5); 6.55 bs, 2 H, 2H (NH_2). ^{13}C NMR (CD_3SOCD_3): 64.81(C-1′); 66.86 d, J(CH_2,P) = 160.2 (CH_2−P); 70.70 d, J(2′,P) = 11.7 (C-2′); 78.60 (C-5); 162.76 (C-2); 171.30, 2C (C-4 and C-6).

Additional, somewhat impure compound **29b (**1.0 g**)** was obtained from the deionized filtrate by elution from Dowex 1 × 2 column with 1 M formic acid.

2-Amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (32b) and 2-Amino-4-[2-(phosphonomethoxy)ethoxy]-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (33b). A mixture of 2-amino-4,6-dihydroxypyrimidine (**31**) (12.7 g, 0.1 mol) and cesium carbonate (27.8 g (85 mmol) in DMF (200 mL) was stirred 1 h at 100 °C, and compound **10** (30 mL) was added. The mixture was stirred 16 h at 100 °C, filtered while hot, and evaporated in vacuo. Purification of the residue by silica gel column chromatography (200 mL) afforded 2.8 g of the oily residue of compound **32a** which was then treated with $BrSiMe_3$ (7 mL) in acetonitrile (50 mL) overnight. The residue was dissolved in water (100 mL), alkalified with concentrated aqueous ammonia and evaporated. The deionized residue was codistilled with ethanol and filtered from ethanol. Yield, 1.4 g (3.5%) 2-amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**32b**), mp 127 °C. Anal. ($C_{10}H_{19}N_3O_{10}P_2$) C, H, N, P. ^{1}H NMR (CD_3SOCD_3): 3.58 d, 2H, J(H,P) = 8.6 (P−CH_2); 3.76 m, 2H (2 × H-2′); 4.29 m, 2H (2 × H-1′); 5.36 s, 1H (H-5); 6.55 bs, 2H (NH_2). ^{13}C NMR (DMSO): 64.81, 2C (C-1′); 66.86 d, J(C,P) = 160.2 (P−CH_2); 70.70 d, 2C, J(C,P) = 11.7 (C-2′); 78.60 (C-5); 162.76 (C-2); 171.30 (C-4, C-6).

Further elution of the silica gel column gave a thick oil of diester **33a** (4.8 g), which was similarly treated with $BrSiMe_3$ (10 mL) in acetonitrile (70 mL) overnight and evaporated in vacuo. The residue was passed through Dowex 50 × 8 column (H^+-form) (150 mL), and the column was eluted with water. After washing out the inorganic acids, the UV-absorbing product fraction was eluted with water. It was evaporated in vacuo, and the residue was stirred with ethanol−acetone mixture (1:1, 100 mL) to give 1.8 g (4.5%) of 2-amino-4-[2-(phosphonomethoxy)ethoxy]-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (**33b**) as a yellowish amorphous product, mp 108 °C. Anal. ($C_{10}H_{19}N_3O_{10}P_2$) C, H, N, P. ^{1}H NMR (CD_3SOCD_3): 3.73 d, 2 H, J(CH_2,P) = 9.0 and 3.77 d, 2H, J(CH_2,P) = 9.0 (2 × CH_2−P); 3.85 bt, 2H, J(2′,1′) = 5.0 (H-2′); 3.95 m, 2H (H-2″); 4.17 bt, 2 H, J(1′,2′) = 5.0 (H-1′); 4.33 m, 2 H, 2H (H-1″); 5.90 s, 1H (H-5).

2,4-Diamino-6-{[2-(phosphonomethoxy)ethyl]sulfanyl}-pyrimidine (35b). Suspension of 2,4-diamino-6-sulfanylpyrimidine hemisulfate (**34**) (2.616 g, 13.7 mmol) in DMF (40 mL) was stirred with sodium hydride (1.0855 g, 27 mmol, 60% dispersion in paraffin oil) for 1 h, and compound **10** (9 mL, 17.2 mmol) was added. The mixture was stirred for 8 h at 80 °C, filtered through Celite pad, and evaporated in vacuo. The residue was purified on silica gel column (150 mL) in chloroform−ethanol (49:1) to afford 2,4-diamino-6-{[2-(diisopropylphosphorylmethoxy)ethyl]sulfanyl}pyrimidine (**35a**) (4.0 g, 80%), mp 109 °C. Anal. ($C_{13}H_{25}N_4O_4PS$) C, H, N, P, S. ^{1}H NMR (CD_3SOCD_3): 1.24 d, 6H and 1.25 d, 6 H, J(CH_3,CH) = 6.1 (CH_3); 3.17 t, 2H, J(1′,2′) = 6.6 (H-1′); 3.68 t, 2H, J(2′,1′) = 6.6 (H-2′); 3.77 d, 2H, J(CH_2−P) = 8.3 (CH_2−P); 4.60 m, 2 H (P−OCH); 5.60 s, 1 H (H-5); 5.95 brs, 2 H and 6.17 brs, 2H (NH_2).

This compound in acetonitrile (50 mL) was treated with $BrSiMe_3$ (5 mL) overnight, and the volatiles were evaporated in vacuo. The residue was treated with water (100 mL), alkalified with concentrated aqueous ammonia, and evaporated. The deionized residue was purified by Dowex 1 × 2 column (100 mL) chromatography [elution with water followed by linear gradient of acetic acid (0−0.5 M, 1.5 L each)]. The main UV-absorbing fraction was evaporated, and the residue was codistilled with water (3 × 50 mL) and crystallized from water. Yield, 2.8 g (91%) 2,4-diamino-6-{[2-(phosphonomethoxy)-ethyl]sulfanyl}pyrimidine (**35b**), mp 246 °C. Anal. ($C_7H_{13}N_4O_4PS$) C, H, N, P, S. ^{1}H NMR (D_2O + NaOD): 3.18t, 2H, J(1′,2′) = 6.8 (H-1′); 3.56 d, 2H, J(CH_2,P) = 8.7; (CH_2P); 3.68 t, 2H, J(2′,1′) = 6.8 (H-2′); 5.70 s, 1 H (H-5); 6.32 brs, 2H (2-NH_2); 6.48 brs, 2H (4-NH_2).

4-Amino-6-hydroxy-2-{[2-(phosphonomethoxy)ethyl]-sulfanyl}pyrimidine (36b). Compound **11** (monohydrate) (4.85 g, 30 mmol) in DMF (50 mL) was stirred with NaH (30 mmol) at room temperature till dissolution and synthon **10 (**8 mL**)** was added in one portion. The mixture was stirred at 80 °C for 6 h and evaporated at 50 °C in vacuo. The residue was extracted with hot chloroform (total 300 mL) and filtered through Celite pad, and the filtrate was purified on a silica gel column (300 mL) by chloroform−ethanol mixture. Yield, after crystallization from ethyl acetate−ether, 5.3 g (48.3%) of compound **36a**, mp 163−164 °C. Anal. ($C_{13}H_{24}N_3O_5PS$) C, H, N, P, S. ^{1}H NMR (CD_3SOCD_3): 1.23 d, 6 H, J(CH_3,CH) = 6.2 and 1.24 d, 6 H, J(CH_3,CH) = 6.2 (4 × CH_3); 3.25 t, 2H, J(1′,2′) = 6.3 (H-1′); 3.72 t, 2H, J(2′,1′) = 6.3 (H-2′); 3.77 d, 2H, J(CH_2−P) = 8.3 (CH_2−P); 4.59 m, 2 H (2 × OCH); 4.40 s, 1 H (H-5); 6.48 s, 2 H (NH_2); 11.55 bs, 1H (OH). ^{13}C NMR (CD_3SOCD_3): 23.94 d, 2C, J (P,C) = 4.4 and 24.04 d, 2C, J(P,C) = 3.9 (4 × CH_3); 28.85 (C-1′); 64.86 d, J(P,C) = 164.6 (P−C); 70.45 d, 2C, J(P,C) = 6.3 (2 × CHO); 71.20 d, J(2′,P) = 11.7 (C-2′); 81.54 (C-5); 162.48, 163.90 and 164.91 (C-2, C-4, and C-6).

This compound was treated with $BrSiMe_3$ (10 mL) in acetonitrile (70 mL) at ambient temperature overnight and worked up under standard conditions. The deionized crude reaction mixture was chromatographed on Dowex 1 × 2 column (150 mL) (elution with water followed by a linear gradient of formic acid (0−1 M, 1.5 L each). The product fraction was evaporated and codistilled with water, and the residue was crystallized from water. Yield, 3.1 g (76%) of compound **36b**, not melting <280 °C. Anal. ($C_7H_{12}N_3O_5PS$) C, H, N, P, S. ^{1}H NMR (D_2O + NaOD): 3.29 t, 2H, J(2′,1′) = 6.5 (H-2′); 3.54 d, 2H, J(CH_2−P) = 8.7 (CH_2−P); 3.80 t, 2H, J(1′,2′) = 6.5 (H-1′); 4.92 s, 1 H (H-5).

2,4-Diamino-6-(*S*)-[2-(phosphonomethoxy)propoxy]-pyrimidine [(*S*)-38b]. (*S*)-[2-(Diisopropylphosphorylmethoxy)propyl] tosylate [**(*S*)-37**] (25.7 g, 63 mmol) in DMF (40 mL) was added at 90 °C to a stirred mixture of 2,4-diamino-6-hydroxypyrimidine (**15**) (60 mmol), DMF (40 mL), and DBU

(10.6 mL, 60 mmol). The reaction mixture was stirred at 100 °C 24 h and evaporated in vacuo. The residue was taken in chloroform (200 mL), filtered, and washed with saturated NaCl (100 mL). The aqueous wash was extracted with chloroform (5 × 50 mL), and the combined chloroform solutions were dried with magnesium sulfate and evaporated. Chromatography on silica gel column (150 mL) by chloroform−ethanol gradient gave the O^6-isomer **38a** as an oily residue which was dried in vacuo over phosphorus pentoxide overnight. Acetonitrile (80 mL) and $BrSiMe_3$ (20 mL) were added to the residue, and the solution was left to stand overnight in a stoppered flask. The volatiles were evaporated in vacuo, the residue was treated with water (100 mL), alkalified with concentrated aqueous ammonia, and evaporated. The deionized residue was purified by Dowex 1 × 2 column (200 mL) chromatography [elution with water followed by linear gradient of acetic acid (0−0.5 M, 1.5 L each). The main UV-absorbing fraction was evaporated, and the residue was codistilled with water (3 × 50 mL) and crystallized from water. Yield, 3.5 g (19.7%) of compound **(*S*)-38b**, mp 281 °C. Anal. ($C_8H_{15}N_4O_5P\cdot H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): 1.26 d, 3H, J(3′,2′) = 6.4 (H-3′); 3.51 dd, 1H, $J(CH_b,P)$ = 9.4, J(gem) = 12.2 (CH_bP); 3.60 dd, 1H, $J(CH_a,P)$ = 9.4, J(gem) = 12.2 (CH_aP); 3.92 m, 2H (H-2′); 4.06 dd, 1H, J(1′b,2′) = 5.5, J(gem)= =10.5 (H-1′b); 4.14 dd, 1H, J(1′a,2′) = 3.7, J(gem) = 10.5 (H-1′a); 5.41 s, 1 H (H-5). ^{13}C NMR (D_2O): 15.84 (C-3′); 66.99 d, $J(CH_2-P)$ = 149.9 (P−C); 69.68 (C-1′); 75.14 d, J(2′,P) = 11.2 (C-2′); 76.84 (C-5); 166.74 (C-2); 162.83 (C-4); 171.05 (C-6).

2,4-Diamino-6-(*R*)-[2-(phosphonomethoxy)propoxy]-pyrimidine [(*R*)-38b]. This compound was prepared similarly from (*R*)-[2-(diisopropylphosphorylmethoxy)propyl] tosylate [**(*R*)-37**] (50 mmol), 2,4-diamino-6-hydroxypyrimidine (**15**) (60 mmol), and DBU (60 mmol) in DMF (70 mL). The reaction mixture was stirred at 100 °C for 24 h. Further workup followed the procedure described for the (*S*)-enantiomer. Yield, 3.55 g (24%) compound **(*R*)-38b**, not melting under 290 °C. Anal. ($C_8H_{15}N_4O_5P\cdot H_2O$) C, H, N, P. ^{1}H NMR and ^{13}C NMR spectra are identical with those of the (*S*)-enantiomer.

4-Amino-2-methylsulfanyl-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine (41b). Diethyl 2-hydroxyethylphosphonate (**40**) (5.3 g, 25 mmol) in DMF (40 mL) was treated at 0 °C with NaH (1.0 g, 60% dispersion in paraffin oil, 25 mmol) and, after 1 h stirring at 0 °C, 4-amino-6-chloro-2-methylsulfanylpyrimidine (**39**) (3.5 g, 20 mmol) was added in one portion. The mixture was stirred 16 h at 100 °C and evaporated to dryness in vacuo, and the residue was extracted with hot chloroform (total, 300 mL). The chloroform extract was evaporated in vacuo, and the residue containing compound **41a** was treated with $BrSiMe_3$ (10 mL) in acetonitrile (50 mL) at room temperature overnight. The mixture was evaporated to dryness in vacuo, and the residue was deionized on a column (100 mL) of Dowex 50 × 8. This product in water (20 mL) was dissolved by addition of concentrated aqueous ammonia and acidified by HCl to pH 3−3.5. The precipitate was collected, washed with water and ethanol, and dried in vacuo. Yield, 0.8 g (13.5%) of 4-amino-2-methylsulfanyl-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine (**41b**), mp 210−211 °C. Anal. ($C_8H_{14}N_3O_5PS$) C, H, N, P, S. ^{1}H NMR (D_2O): 2.48 s, 3H (SCH_3); 3.72 d, 2H, J(H,P) = 8.5 (P−CH_2); 3.94 m, 2H (2 × H-2′); 4.36 m, 2H (2 × H-1′); 5.68 s, 1H (H-5); ^{13}C NMR (D_2O): 16.02 (SCH_3); 69.20 (C-1′); 70.01 d, J(C,P) = 156.3 (P−CH_2); 73.53 d, J(C,P) = 10.7 (C-2′); 84.82 (C-5); 167.93, 172.13 and 173.70 (C-2, C-4 and C-6).

Acknowledgment. This study was performed as part of a research project of the Institute of Organic Chemistry and Biochemistry #4055905. It was supported by the State Grant Agency of the Czech Republic (#203/94/K001), by the program of targeted projects of the Academy of Sciences of the Czech Republic (#S4055109), by Gilead Sciences (Foster City, CA), by the Fifth Framework Programme of the European Commission, by ISEP/FORTIS, and by the Flemish "Geconcerteerde Onderzoeksacties Contract 00/12". Excellent technical assistance of Mrs. Běla Nováková, Mrs. Anita Van Lierde, Mrs. Frieda De Meyer, Miss L.Vandenheurck, Mrs. Ann Absillis, and Mrs. Lizette van Berckelaer is gratefully acknowledged. The authors' thanks are also due to the staff of the mass spectrometry and analytical departments of the Institute of Organic Chemistry and Biochemistry.

References

(1) Reviews: (a) Holý, A. Phosphonylmethyl analogues of nucleotides and their derivatives: chemistry and biology. *Nucleosides Nucleotides* **1987**, *6*, 147−155. (b) Holý A. Isopolar Phosphorus-Modified Nucleotide Analogues. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press: Greenwich, CT, 1994; pp 179−232. (c) Freeman, S.; Gardiner, J.M. Acyclic nucleosides as antiviral compounds. *Mol. Biotechnol.* **1996**, *5*, 125−137.

(2) (a) Naesens, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Neyts, J.; De Clercq, E. HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogues: A review of their pharmacology and clinical potential in the treatment of viral infections. *Antivir. Chem. Chemother.* **1997**, *8*, 1−23. (b) Ying, C.; De Clercq, E.; Neyts, J. Lamivudine, adefovir and tenofovir exhibit long-lasting anti-hepatitis B virus activity in cell culture. *J. Viral Hepat.* **2000**, *7*, 79−83.

(3) (a) Starrett, J. E., Jr.; Tortolani, D. R.; Hitchcock, M. J.; Martin, J. C.; Mansuri, M. M. Synthesis and in vitro evaluation of a phosphonate prodrug: bis(pivaloyloxymethyl) 9-(2-phosphonylmethoxyethyl)adenine. *Antiviral Res.* **1992**, *19*, 267−273. (b) Adefovir dipivoxil - Anti-HIV - Anti-HBV. *Drugs Future* **2000**, *25*, 844−847.

(4) Perrillo, R.; Schiff, E.; Yoshida, E.; Statler, A.; Hirsch, K.; Wright, T.; Gutfreund, K.; Lamy, P.; Murray, A. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *Hepatology* **2000**, *32*, 129−134. (b) Gilson, R. J.; Chopra, K. B.; Newell, A. M.; Murray-Lyon, I. M.; Nelson, M. R.; Rice, S. J.; Tedder, R. S.; Toole, J.; Jaffe, H. S.; Weller, I. V. A placebo-controlled phase I/II study of adefovir dipivoxil in patients with chronic hepatitis B virus infection. *J. Viral. Hepat.* **1999**, *6*, 387−395. (c) Torresi, J.; Locarnini, S. Antiviral chemotherapy for the treatment of hepatitis B virus infections. *Gastroenterology.* **2000**, *118*, S83−S103.

(5) (a) Otová, B.; Sladká, M.; Votruba, I.; Holý, A.; Křen, V. Cytostatic effect of 9-(2-phosphonomethoxyethyl)adenine (PMEA). I. Lymphatic leukemia KHP-Lw-I in Lewis rats. *Folia Biol. Prague* **1993**, *39*, 136−141. (b) Otová, B.; Sladká, M.; Blažek, K.; Schramlová, J.; Votruba, I.; Holý, A. Cytostatic effect of 9-(2-phosphonomethoxyethyl)adenine (PMEA). II. Lymphoblastic leukemia in Sprague−Dawley rats. *Folia Biol. Prague* **1993**, *39*, 142−149. (c) Otová, B.; Křenová, D.; Zidek, Z.; Holý, A.; Votruba, I.; Křen, V. Cytostatic effect of 9-(2-phosphonomethoxyethyl) adenine (PMEA). III. Rat and mouse carcinomas and sarcomas. *Folia Biol. Prague* **1993**, *39*, 311−314. (d) Hatse, S.; Balzarini, J.; De Clercq, E. Induction of erythroid differentiation of human leukemia K562 cells by the acyclic nucleoside phosphonate 9-(2-phosphonylmethoxyethyl)adenine (PMEA). *Nucleos. Nucleot.* **1995**, *14*, 649−652. (e) Hatse, S.; De Clercq, E.; Balzarini, J. Evidence for distinction of the differentiation-inducing activities and cytostatic properties of 9-(2-phosphonylmethoxyethyl)adenine and a variety of differentiation-inducing agents in human erythroleukemia K562 cells. *Mol. Pharmacol.* **1996**, *50*, 1231−1242. (f) Otová, B.; Zidek, Z.; Holý, A.; Votruba, I.; Sladká, M.; Marinov, I.; Lesková, V. Antitumor activity of novel purine acyclic nucleotide analogues PMEA and PMEDAP. *In Vivo.* **1997**, *11*, 163−167. (g) Hatse, S.; Naesens, L.; Degreve, B.; Segers, C.; Vandeputte, M.; Waer, M.; De Clercq, E.; Balzarini, J. Potent antitumor activity of the acyclic nucleoside phosphonate 9-(2-phosphonylmethoxyethyl)adenine in choriocarcinoma-bearing rats. *Int. J. Cancer* **1998**, *76*, 595−600. (h) De Clercq, E.; Andrei, G.; Balzarini, J.; Hatse, S.; Liekens, S.; Naesens, L.; Neyts, J.; Snoeck, R. Antitumor potential of acyclic nucleoside phosphonates. *Nucleosides Nucleotides* **1999**, *18*, 759−771.

(6) (a) Tsai, C. C.; Follis, K. E.; Sabo, A.; Beck, T. W.; Grant, R. F.; Bischofberger, N.; Benveniste, R. E.; Black, R. Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl)-adenine. *Science* **1995**, *270*, 1197−1199. (b) Van Rompay, K. K.; Cherrington, J. M.; Marthas, M. L.; Berardi, C. J.; Mulato, A. S.; Spinner, A.; Tarara, R. P.; Canfield, D. R.; Telm, S.; Bischofberger, N.; Pedersen, N. C. 9-[2-(Phosphonomethoxy)-propyl]adenine therapy of established simian immunodeficiency virus infection in infant rhesus macaques. *Antimicrob. Agents Chemother.* **1996**, *40*, 2586−2591. (c) Tsai, C. C.; Follis, K. E.; Beck, T. W.; Sabo, A.; Bischofberger, N.; Dailey, P. J. Effects of (R)-9-(2-phosphonylmethoxypropyl) adenine monotherapy on chronic SIV infection in macaques. *AIDS Res. Hum. Retroviruses.* **1997**, *13*, 707−712. (d) Srinivas, R. V.; Fridland, A. Antiviral

activities of 9-R-2-phosphonomethoxypropyl adenine (PMPA) and bis(isopropyloxymethylcarbonyl)PMPA against various drug-resistant human immunodeficiency virus strains. *Antimicrob. Agents Chemother.* **1998**, *42*, 1484−1487. (e) Suruga, Y.; Makino, M.; Okada, Y.; Tanaka, H.; De Clercq, E.; Baba, M. Prevention of murine AIDS development by (R)-9-(2-phosphonylmethoxypropyl)adenine. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **1998**, *18*, 316−322. (f) Van Rompay, K. K.; Cherrington, J. M.; Marthas, M. L.; Lamy, P. D.; Dailey, P. J.; Canfield, D. R.; Tarara, R. P.; Bischofberger, N.; Pedersen, N. C. 9-[2-(Phosphonomethoxy)propyl]adenine (PMPA) therapy prolongs survival of infant macaques inoculated with simian immunodeficiency virus with reduced susceptibility to PMPA. *Antimicrob. Agents Chemother.* **1999**, *43*, 802−812. (g) Van Rompay, K. K.; Miller, M. D.; Marthas, M. L.; Margot, N. A.; Dailey, P. J.; Canfield, D. R.; Tarara, R. P.; Cherrington, J. M.; Aguirre, N. L.; Bischofberger, N.; Pedersen, N. C. Prophylactic and therapeutic benefits of short-term 9-[2-(R)−(phosphonomethoxy)propyl]adenine (PMPA) administration to newborn macaques following oral inoculation with simian immunodeficiency virus with reduced susceptibility to PMPA. *J. Virol.* **2000**, *74*, 1767−1774.

(7) (a) Balzarini, J.; Holý, A.; Jindřich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. Differential anti-retrovirus effects of the (S)- and (R)-enantiomers of acyclic nucleoside phosphonates. Potent and selective in vitro and in vivo anti-retrovirus activity of the (R)-9-(2-phosphonomethoxypropyl) derivatives of heterocyclic bases. *Antimicrob. Agents Chemother.* **1993**, *37*, 332−338 (b) Gong, Y. F.; Marshall, D. R.; Srinivas, R. V.; Fridland, A. Susceptibilities of zidovudine-resistant variants of human immunodeficiency virus type 1 to inhibition by acyclic nucleoside phosphonates. *Antimicrob. Agents Chemother.* **1994**, *38*, 1683−1687. (c) Balzarini, J.; Aquaro, S.; Perno, C. F.; Witvrouw, M.; Holý, A.; De Clercq, E. Activity of the (R)-enantiomers of 9-(2-phosphonylmethoxypropyl)adenine and 9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine against human immunodeficiency virus in different human cell systems. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 337−341. (d) Balzarini, J.; Vahlenkamp, T.; Egberink, H. F.; Hartmann, K.; Witvrouw, M.; Pannecouque, C.; Casara, P.; Nave, J. F.; De Clercq, E. Antiretroviral activities of acyclic nucleoside phosphonates [9-(2-phosphonylmethoxyethyl)adenine, 9-(2-phosphonylmethoxyethyl)guanine, (R)-9-(2-phosphonylmethoxypropyl)adenine, and MDL 74,968] in cell cultures and murine sarcoma virus-infected newborn NMRI mice. *Antimicrob. Agents Chemother.* **1997**, *41*, 611−616.

(8) (a) Naesens, L.; Balzarini, J.; Rosenberg, I.; Holý, A.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP): a novel agent with anti-human immunodeficiency virus activity in vitro and potent anti-Moloney murine sarcoma virus activity in vivo. *Eur. J. Clin. Microbiol. Infect. Dis.* **1989**, *8*, 1043−1047. (b) Naesens, L.; Neyts, J.; Balzarini, J.; Holý, A.; Rosenberg, I.; De Clercq, E. Efficacy of oral 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) in the treatment of retrovirus and cytomegalovirus infections in mice. *J. Med. Virol.* **1993**, *39*, 167−172. (c) Neyts, J.; Stals, F.; Bruggeman, C.; De Clercq, E. Activity of the anti-HIV agent 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine against cytomegalovirus in vitro and in vivo. *Eur. J. Clin. Microbiol. Infect. Dis.* **1993**, *12*, 437−446. (d) Hartmann, K.; Balzarini, J.; Higgins, J.; De Clercq, E.; Pedersen, N. C. In vitro Activity of Acyclic Nucleoside Phosphonate Derivatives Against Feline Immunodeficiency Virus in Crandell Feline Kidney Cells and Feline Peripheral Blood Lymphocytes. *Antivir. Chem. Chemother.* **1994**, *5*, 13−19. (e) Andrei, G.; Snoeck, R.; De Clercq, E. Differential susceptibility of several drug-resistant strains of herpes simplex virus type 2 to various antiviral compounds. *Antivir. Chem. Chemother.* **1997**, *8*, 457−461.

(9) Otová, B.; Zidek, Z.; Holý, A.; Votruba, I.; Sladká, M.; Marinov, I.; Lesková, V. Antitumor activity of novel purine acyclic nucleotide analogs PMEA and PMEDAP. *In Vivo* **1997**, *11*, 163−167. (b) Otová, B.; Francová, K.; Franìk, F.; Koutník, P.; Votruba, I.; Holý, A.; Sladká, M.; Schramlová, J. 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine (PMEDAP) − a potential drug against hematological malignancies − induces apoptosis. *Anticancer Res.* **1999**, *19*, 3173−3182. (c) Bobková, K.; Otová, B.; Marinov, I.; Mandys, V.; Panczak, A.; Votruba, I.; Holý, A. Anticancer effect of PMEDAP − Monitoring of apoptosis. *Anticancer Res.* **2000**, *20*, 1041−1047.

(10) (a) Kreider, J. W.; Balogh, K.; Olson, R. O.; Martin, J. C. Treatment of latent rabbit and human papillomavirus infections with 9-(2-phosphonylmethoxy)ethylguanine (PMEG). *Antiviral Res.* **1990**, *14*, 51−58. (b) Ho, H. T.; Woods, K. L.; Konrad, S. A.; De Boeck, H.; Hitchcock, M. J. Cellular metabolism and enzymatic phosphorylation of 9-(2-phosphonylmethoxyethyl)guanine (PMEG), a potent antiviral agent. **1992**, *312*, 159−166. (c) Yu, K. L.; Bronson, J. J.; Yang, H.; Patick, A.; Alam, M.; Brankovan, V.; Datema, R.; Hitchcock, M. J.; Martin, J. C. Synthesis and antiviral activity of methyl derivatives of 9-[2-(phosphonomethoxy)ethyl]guanine. *J. Med. Chem.* **1992**, *35*, 2958−2969. (d) Balzarini, J.; Vahlenkamp, T.; Egberink, H. F.; Hartmann, K.; Witvrouw, M.; Pannecouque, C.; Casara, P.; Nave, J. F.; De Clercq, E. Antiretroviral activities of acyclic nucleoside phosphonates [9-(2-phosphonylmethoxyethyl)adenine, 9-(2-phosphonylmethoxyethyl)guanine, (R)-9-(2-phosphonylmethoxypropyl)adenine, and MDL 74,968] in cell cultures and murine sarcoma virus-infected newborn NMRI mice. *Antimicrob. Agents Chemother.* **1997**, *41*, 611−616.

(11) (a) Rose, W. C.; Crosswell, A. R.; Bronson, J. J.; Martin, J. C. In vivo antitumor activity of 9-[(2-phosphonylmethoxy)ethyl]guanine and related phosphonate nucleotide analogues. *J. Natl. Cancer Inst.* **1990**, *82*, 510−512. (b) Franìk, F.; Holý, A.; Votruba, I.; Eckschlager, T. Modulation of cell cycle progression and of antibody production in mouse hybridomas by a nucleotide analogue. *Cytotechnology* **1998**, *28*, 65−72. (c) Franìk, F.; Holý, A.; Votruba, I.; Eckschlager, T. Acyclic nucleotide analogues suppress growth and induce apoptosis in human leukemia cell lines. *Int. J. Oncol.* **1999**, *14*, 745−752.

(12) (a) Štěitina, R.; Votruba, I.; Holý, A.; Merta, A. The effect of purine phosphonomethoxyalkyl derivatives on DNA synthesis in CHO Chinese hamster cells. *Neoplasma.* **1993**, *40*, 373−378. (b) Otová, B.; Holý, A.; Votruba, I.; Sladká, M.; Bílá, V.; Mejsnarová, B.; Lesková, V. Genotoxicity of purine acyclic nucleotide analogues. *Folia Biol. Prague* **1997**, *43*, 225−229.

(13) (a) Zídek, Z.; Holý, A.; Franková, D. Immunomodulatory properties of antiviral acyclic nucleotide analogues: Cytokine stimulatory and nitric oxide costimulatory effects. *Int. J. Immunopharmacol.* **1997**, *19*, 587−597. (b) Zídek, Z.; Franková, D.; Holý, A.; Boubelík, M.; Dráber, P. Inhibition of murine macrophage nitric oxide synthase expression by a pivoxil prodrug of antiviral acyclic nucleotide analogue 9-(2-phosphonomethoxyethyl)adenine. *Biochem. Pharmacol.* **1997**, *54*, 855−861. (c) Zídek, Z.; Franková, D.; Holý, A. Macrophage activation by antiviral acyclic nucleoside phosphonates in dependence on priming immune stimuli. *Int. J. Immunopharmacol.* **2000**, *22*, 1121−1129. (d) Zídek, Z.; Franková, D.; Holý, A. Stimulation of cytokine and nitric oxide production by acyclic nucleoside phosphonates. *Nucleosides Nucleotides* **1999**, *18*, 959−961.

(14) (a) Zídek, Z.; Holý, A.; Franková, D.; Otová, B. Suppression of rat adjuvant arthritis by some acyclic nucleotide analogs. *Eur. J. Pharmacol.* **1995**, *286*, 307−310. (b) Zídek, Z.; Franková, D.; Holý, A. Chemokines, nitric oxide and antiarthritic effects of 9-(2-phosphonomethoxyethyl)adenine (Adefovir). *Eur. J. Pharmacol.* **1999**, *376*, 91−100.

(15) Holý, A.; Günter, J.; Dvořáková, H.; Masojídková, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. Structure-antiviral activity relationship in the series of pyrimidine and purine N-[2-(2-phosphonomethoxy)ethyl] nucleotide analogues. 1. Derivatives substituted at the carbon atoms of the base. *J. Med. Chem.* **1999**, *42*, 2064−2086.

(16) (a) Dvořáková, H.; Holý, A. Synthesis and Biological Effects of N-(2-Phosphonomethoxyethyl) Derivatives of Deazapurine Bases. *Collect. Czech. Chem. Commun.* **1993**, *58*, 1419−1429. (b) Franchetti, P.; Sheikha, G. A.; Cappellacci, L.; Messina, L.; Grifantini, M.; Loi, A. G.; Demontis, A.; Spiga, M. G.; La Colla, P. 8-Aza-analogues of PMEA and PMEG: Synthesis and in vitro anti-HIV activity. *Nucleosides Nucleotides* **1994**, *13*, 1707−1719. (c) Franchetti, P.; Abusheikha, G.; Cappellacci, L.; Grifantini, M.; Demontis, A.; Piras, G.; Loi, A. G.; La Colla, P. Synthesis and antiviral activity of 8-aza analogs of chiral [2-(phosphonomethoxy)propyl]guanines. *J. Med. Chem.* **1995**, *38*, 4007−4013. (d) Holý, A.; Dvořáková, H.; Jindřich, J.; Masojídková, M.; Buděšínský, M.; Balzarini, J.; Andrei, G.; De Clercq, E. Acyclic nucleotide analogues derived from 8-azapurines: synthesis and antiviral activity. *J. Med. Chem.* **1996,** *39*, 4073−4088.

(17) (a) Holý, A.; Snoeck, R.; Balzarini, J.; Andrei, G.; De Clercq, E. Antiviral activity of 2-phosphonomethoxyalkyl derivatives of N6-substituted 6-aminopurines. *Antiviral Res.* **1995**, *26*, A231−A231. (b) Meerbach, A.; Holý, A.; Wutzler, P.; De Clercq, E.; Neyts, J. Inhibitory effects of novel nucleoside and nucleotide analogues on Epstein−Barr virus replication. *Antivir. Chem. Chemother.* **1998**, *9*, 275−282.

(18) (a) Holý, A.; Zídek, Z.; Votruba, I. Inhibition of murine lymphocyte proliferation by N-6-substituted acyclic purine nucleoside phosphonates. *Collect. Czech. Chem. Commun.* **1996**, *61*, S182−S187. (b) Hatse, S.; Naesens, L.; De Clercq, E.; Balzarini, J. N-6-cyclopropyl-PMEDAP: A novel derivative of 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) with distinct metabolic, antiproliferative, and differentiation-inducing properties. *Biochem. Pharmacol.* **1999**, *58*, 311−323.

(19) (a) Compton, M. L.; Toole, J. J.; Paborsky, L. R. 9-(2-Phosphonylmethoxyethyl)-N6-cyclopropyl-2,6-diaminopurine (cpr-PMEDAP) as a prodrug of 9-(2-phosphonylmethoxyethyl)guanine (PMEG). *Biochem. Pharmacol.* **1999**, *58*, 709−714.

(20) Hocek, M.; Masojídková, M.; Holý, A. Synthesis of acyclic nucleotide analogues derived from 2-(aminomethyl)adenine and 2-(aminomethyl)hypoxanthine. *Collect. Czech. Chem. Commun.*

1995, *60*, 875−882. (b) Hocek, M.; Masojídková, M.; Holý, A.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. Synthesis and antiviral activity of acyclic nucleotide analogues derived from 6-(aminomethyl)purines and purine-6-carboxamidines. *Collect. Czech. Chem. Commun.* **1996**, *61*, 1525−1537. (c) Hocek, M.; Masojídková, M.; Holý, A. Synthesis of acyclic nucleotide analogues derived from 6-hetarylpurines via cross-coupling reactions of 9-[2-(diethoxyphosphonylmethoxy)ethyl]-6-iodopurine with hetaryl organometallic reagents. *Collect. Czech. Chem. Commun.* **1997**, *62*, 136−146.

(21) For review, see: (a) De Clercq, E. Towards an effective chemotherapy of virus infections: Therapeutic potential of cidofovir [(S)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine, HPMPC] for the treatment of DNA virus infections. *Collect. Czech. Chem. Commun.* **1998**, *63*, 480−506. (b) Safrin, S.; Cherrington, J.; Jaffe, H. S. Cidofovir. Review of current and potential clinical uses. *Adv. Exp. Med. Biol.* **1999**, *458*, 111−120.

(22) (a) Andrei, G.; Snoeck, R.; Piette, J.; Delvenne, P.; De Clercq, E. Inhibiting effects of cidofovir (HPMPC) on the growth of the human cervical carcinoma (SiHa) xenografts in athymic nude mice. *Oncol. Res.* **1998**, *10*, 533−539. (b) De Clercq, E.; Andrei, G.; Balzarini, J.; Hatse, S.; Liekens, S.; Naesens, L.; Neyts, J.; Snoeck, R. Antitumor potential of acyclic nucleoside phosphonates. *Nucleosides Nucleotides* **1999**, *18*, 759−771. (c) Snoeck, R.; Noel, J. C.; Muller, C.; De Clercq, E.; Bossens, M. Cidofovir, a new approach for the treatment of cervix intraepithelial neoplasia grade III (CIN III). *J. Med. Virol.* **2000**, *60*, 205−209.

(23) Holý, A.; Dvořáková, H.; Masojídková, M. Synthesis of enantiomeric N-(2-phosphonomethoxypropyl) derivatives of heterocyclic bases. 2. Synthon approach. *Collect. Czech. Chem. Commun.* **1995**, *60*, 1390−1409.

(24) Holý, A.; Buděšínský, M.; Podlaha, J.; Císařová, I. Synthesis of N1-[2-(phosphonomethoxy)ethyl] derivatives of 2,4-diaminopyrimidine and related acyclic nucleotide analogs. *Collect. Czech. Chem. Commun.* **1999**, *64*, 242−256.

(25) Linn, J. A.; McLean, E.; Kelley, J. L. 1,4-Diazabicyclo[2,2,2]-octane (DABCO)-catalyzed hydrolysis and alcoholysis reactions of 2-amino-9-benzyl-6-chloro-9H-purine. *J. Chem. Soc., Perkin Trans. 1.* **1994**, *1994*, 913−914.

(26) (a) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. *J. Infect. Dis.* **1980**, *141*, 563−574. (b) De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holý, A. Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. *Antiviral Res.* **1987**, *8*, 261−272.

(27) Baba, M.; Mori, S.; Shigeta, S.; De Clercq, E. Selective inhibitory effect of (S)-9-(3-hydroxy-2- phosphonylmethoxypropyl)adenine and 2′-nor-cyclic GMP on adenovirus replication in vitro. *Antimicrob. Agents Chemother.* **1987**, *31*, 337−339.

(28) Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holý, A.; Schellekens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) effectively inhibits simian immunodeficiency virus (SIV) infection in Rhesus monkeys. *AIDS* **1991**, *5*, 21−28.

(29) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holý, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. Marked in vivo antiretrovirus activity of 9-(2-phosphonylmethoxyethyl)-adenine, a selective anti-human immunodeficiency virus agent. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332−336.

(30) Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. Synthesis and cytostatic activity of substituted 6-phenylpurine bases and nucleosides: Application of the Suzuki-Miyaura cross-coupling reactions of 6-chloropurine derivatives with phenylboronic acids. *J. Med. Chem.* **2000**, *43*, 1817−1825.

(31) Roth, B.; Strelitz, J. Z. The protonation of 2,4-diaminopyrimidines I. Dissociation constants and substituents effects. *J. Org. Chem.* **1969**, *34*, 824−836.

(32) Brown, D. J.; Jacobsen, N. W. Pyrimidine reactions. Part IV. The methylation of 2,4- and 4,5-diaminopyrimidine and related compounds. *J. Chem. Soc.* **1962**, 3172−3179.

(33) De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A novel selective broad-spectrum anti-DNA virus agent. *Nature* **1986**, *323*, 464−467.

JM011095Y