5-Substituted-2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidines—Acyclic Nucleoside Phosphonate Analogues with Antiviral Activity

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2,4-Diamino-6-hydroxypyrimidines substituted in position 5 by an allyl, benzyl, cyanomethyl, ethoxycarbonylmethyl, phenyl, cyclopropyl, or methyl group were prepared either by C5-alkylation or by formation of the pyrimidine ring by cyclization. Their alkylation with 2-[(diisopropoxyphosphoryl)methoxy]ethyl tosylate afforded N1- and O6-regioisomers that were separated and converted to the free phosphonic acids by treatment with bromotrimethylsilane followed by hydrolysis. Reaction of 2,4-diamino-6-{[(diisopropoxyphosphoryl)methoxy]ethoxy}-pyrimidine with elemental bromine, N-chloro-, or N-iodosuccinimide gave the corresponding phosphorus-protected 5-bromo-, 5-chloro-, and 5-iodo derivatives, respectively. Their deprotection afforded 2,4-diamino-5-bromo- and -5-chloro-6-[2-(phosphonomethoxy)ethoxy]pyrimidines. 2,4-Diamino-5-methyl-6-[2-(phosphonomethoxy)ethoxy]pyrimidine was synthesized also by cross-coupling of the 5-bromo compound with $AlMe_3$, followed by deprotection. The compounds showed poor, if any, inhibitory activity against DNA viruses such as herpes simplex virus type 1 and type 2, cytomegalovirus, varicella-zoster virus, and vaccinia virus. In contrast, several 5-substituted 2,4-diaminopyrimidine derivatives markedly inhibited retrovirus replication in cell culture. The 5-methyl derivative was exquisitely inhibitory to human immunodeficiency virus and Moloney murine sarcoma virus-induced cytopathicity in cell culture ($EC_{50} \sim 0.00018$ μmol/mL) but also cytostatic to CEM cell cultures. In contrast, the 5-halogen-substituted derivatives showed pronounced antiretroviral activity ($EC_{50} = 0.0023-0.0110$ μmol/mL), comparable to that of the reference drugs adefovir and tenofovir, but were devoid of measurable toxicity at 0.3 μmol/mL.

Introduction

2-(Phosphonomethoxy)alkyl derivatives of purine and pyrimidine bases—acyclic nucleoside phosphonates (ANPs)—possess significant antiviral and cytostatic activity.[1] These nucleotide analogues contain an isopolar phosphonomethyl ether moiety instead of the nucleotide phosphate ester group that excludes their enzymatic degradation and/or eliminates problems with intracellular phosphorylation necessary for nucleoside activation. Among ANPs, particularly 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir, **1a**, Figure 1) is active against DNA viruses and retroviruses;[2] its prodrug, adefovir dipivoxil,[3] has been recently approved for hepatitis B therapy (Hepsera).[4] Structure−activity relationship studies in the series of PMEA congeners included modification of the parent molecule both in the side chain and in the heterocyclic moiety. Substitution of the 2-(phosphonomethoxy)ethyl chain in position 2 by a hydroxymethyl group leads to another structural type of active compound, (*S*)-*N*-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) derivatives, of which the adenine derivative HPMPA (**1e**) exhibits potent anti-DNA-viral activity.[5] Substitution of PMEA side chain in position 2 by the methyl group results in [(*R*)-2-phosphonomethoxypropyl]adenine (PMPA, tenofovir, **1c**) with very high potency and selectivity against HIV-1

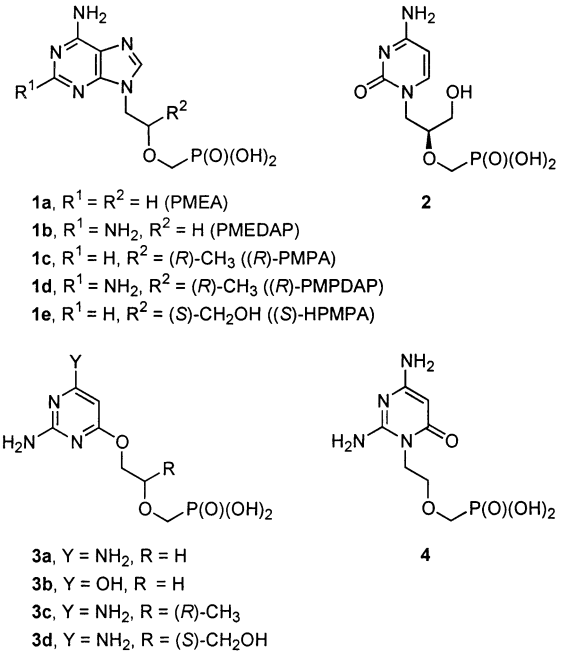

Figure 1.

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and HIV-2;[6] its oral prodrug, tenofovir disoproxil fumarate, has been approved for treatment of HIV infection (Viread).

The SAR studies demonstrated that the margins of structural alteration are very narrow.[7] The specifity of antiviral action is determined by the structure of the side chain. Except for the antiviral activity of the cytosine derivative (*S*)-HPMPC (cidofovir, Vistide, **2**, Figure 1),[8] the choice of the base is limited mostly to adenine, guanine, and 2,6-diaminopurine and to their 8-aza and 3-deaza congeners. The 2,6-diaminopurine and guanine ANP derivatives are often very potent antivirals[9] and/or exhibit antitumor properties.[10] The pharmacophore of purine acyclic nucleoside phosphonates is characterized by the presence of amino groups at the pyrimidine part of the purine system. While N6-substitution in adenine and 2,6-diaminopurine derivatives still preserves the (antiviral and/or cytostatic) activity in the PME and PMP series,[11] other alterations of amino groups generally result in complete loss of activity.[12]

Recently, we discovered a new type of antiviral acyclic nucleoside phosphonate[13] originating from 2-substituted 4-amino-6-hydroxypyrimidines. Alkylation of these bases by the appropriate phosphonate-protected synthon afforded a mixture of O6- and N1-regioisomers that were converted to the free phosphonates. While none of the isomeric 1-[2-(phosphonomethoxy)ethyl]pyrimidin-6-one derivatives **4** was antivirally active, among 6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives, compounds derived from 2,4-diaminopyrimidine (**3a**, **3c**, **3d**) and 2-amino-4-hydroxypyrimidine (**3b**) (Figure 1) significantly inhibited replication of retroviruses and herpesviruses in cell culture. Compounds **3** can be considered as analogues of 2,6-diaminopurine and guanine PME derivatives [PMEDAP (**1b**), PMEG] with an open imidazole ring in the purine moiety. This structural relation is strongly supported by the finding that the corresponding analogue of PMPDAP, i.e., 2,4-diamino-6-[(*R*)-2-(phosphonomethoxy)propyl]oxypyrimidine (**3c**), not only has the same selective antiretroviral activity as its 2,4-diaminopurine congener (*R*)-PMPDAP (**1d**) but this activity is also limited solely to the (*R*)-enantiomer; the (*S*)-enantiomer of **3c** is similarly devoid of antiviral activity as is (*S*)-PMPDAP.

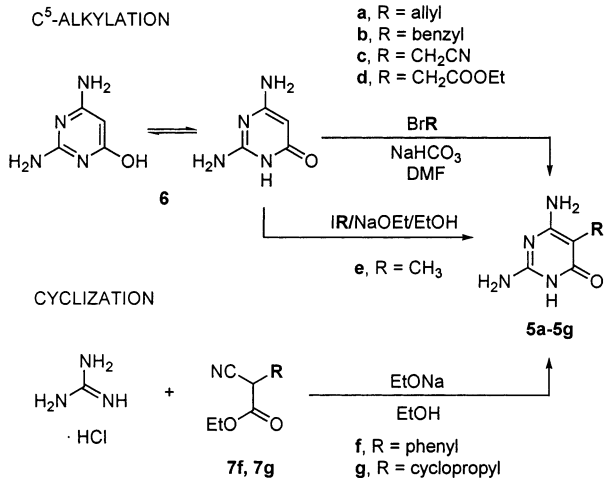
To further investigate the structure−activity relationship of this new group of antiviral compounds, in this paper we report on the synthesis and antiviral activity of 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives bearing various substituents at the C5-position of the parent compound **3a** and its N1-isomer **4**. The substituents were selected with regard to their spatial requirements and electronegativity: i.e., an allyl, benzyl, cyanomethyl, ethoxycarbonylmethyl, phenyl, chloro, bromo, cyclopropyl, and methyl group.

Chemistry

Two synthetic methods were used to prepare the starting 5-substituted-2,4-diaminopyrimidine bases **5a**−**g**: (a) direct C5-alkylation of pyrimidine base or (b) formation of pyrimidine ring by cyclization (Scheme 1).

Strong electrophiles, such as methyl bromides bearing an electron-withdrawing group, can regioselectively monoalkylate 2,4-diamino-6-hydroxypyrimidine (**6**) on

Scheme 1

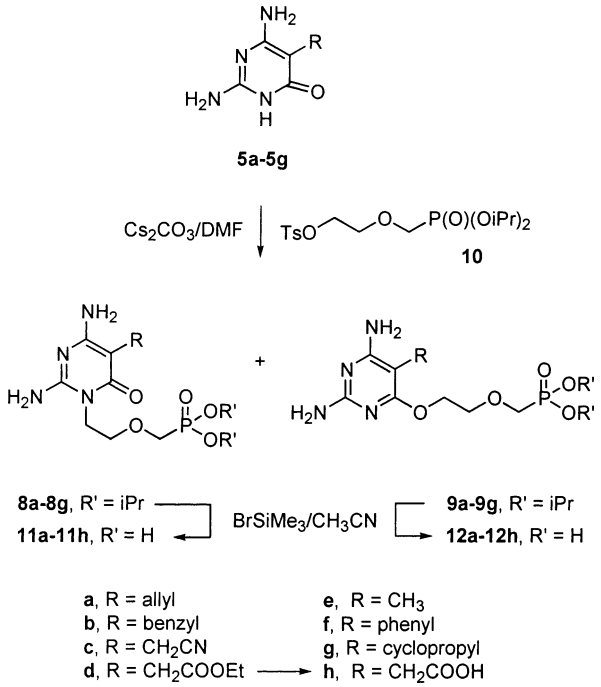


the C5-position.[14] This was the method of choice for synthesis of 5-allyl, benzyl, cyanomethyl, and ethoxycarbonylmethyl pyrimidines **5a**−**d**, using $NaHCO_3$ as a base and dimethylformamide as a solvent. The known procedure was applied for the C5-methylation of 2,4,-diamino-6-hydroxypyrimidine (**6**) (yield 22% of **5e**).[15] 5-Phenyl pyrimidine derivative **5f** was prepared by condensation[16] of commercially available ethyl phenylcyanoacetate (**7f**) with guanidine in the presence of sodium ethoxide. Analogously, 2,4-diamino-5-cyclopropyl-6-hydroxypyrimidine (**5g**) was obtained from ethyl cyclopropylcyanoacetate (**7g**); the latter compound was synthesized according to the literature[17] by condensation of diethyl carbonate with cyclopropaneacetonitrile using NaH as a base.

The ultimate aim of our study was the synthesis of 5-substituted 6-[2-(phosphonomethoxy)ethoxy]pyrimidines derived from the above bases. As expected, alkylation of **5a**−**g** with 2-(diisopropoxyphosphorylmethoxy)-ethyl tosylate **10** under standard conditions[18] (Cs_2CO_3, dimethylformamide, heating) afforded a mixture of N1-alkyl and O6-alkyl regioisomers in the ratio approximately from 1:1 to 1:2 (Scheme 2). Resulting 5-substituted 2,4-diamino-1-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}pyrimidin-6(1*H*)-ones **8a**−**g** and 5-substituted 2,4-diamino-6-{2-[(diisopropoxyphosphoryl)methoxy]-ethoxy}pyrimidines **9a**−**g** were separated by preparative HPLC. The isomeric diesters gave, on treatment with bromotrimethylsilane followed by hydrolysis, the free phosphonic acids **11a**−**g** and **12a**−**g**. 5-Ethoxycarbonylmethyl derivatives **8d** and **9d** afforded by the above procedure two products: in addition to the esters **11d** and **12d**, the free carboxylic acids **11h** and **12h** were isolated as a result of the ethyl ester hydrolysis. All compounds **11** and **12** were purified by ion-exchange column chromatography on Dowex 1 × 2 (OAc^-).

To extend this study, 5-halogeno derivatives (Br, Cl, I) were also prepared[13a] from the preformed diester-protected molecule of the O6-isomer **13**. Bromination of **13** with elemental bromine in DMF/CCl_4 gave smoothly the 5-bromo derivative **14a**; similarly, 5-chloro derivative **15a** and 5-iodo derivative **16a** were obtained from compound **13** on reaction with *N*-chlorosuccinimide or *N*-iodosuccinimide, respectively. Deprotection by bromotrimethylsilane in acetonitrile followed by hydrolysis gave the corresponding free phosphonates: 5-bromo

Scheme 2



derivative **14b** and its 5-chloro congener **15b**. However, the 5-iodo derivative **16a** gave under the conditions of this reaction and/or ion-exchange chromatography purification the 5-unsubstituted 2,4-pyrimidine derivative **3a** as the main product. Direct iodination of **3a** by elemental iodine in a dioxane−water mixture under the conditions described for pyrimidine nucleosides also failed.

As an alternative approach to the 5-C-substituted derivatives of compound **3a** we have also considered the cross-coupling of compound **14a** with organometallic reagents. This method has been widely applied to 5-halo pyrimidines[19] but not yet to 2,4-diamino-6-hydroxypyrimidine derivatives. In our study, trimethylaluminum was used for cross-coupling with bromo derivative **14a** as an alternative method for preparation of the 5-methyl derivative **9e** (Scheme 3). However, the main isolated product was monoester **17** (31%), diester **9e** was obtained in 8% yield only. Deprotection of the combined products **9e** and **17** gave phosphonic acid **12e** in 27% overall yield. Nevertheless, attempts to extend this method to the synthesis of the 5-ethyl derivative using triethylaluminum under the same conditions failed; only traces of the desired product were detected.

By analogy to the 5-unsubstituted compounds,[13a] the structure assignment of the N1- and O6-isomers of ANPs synthesized in this study is based on their ^{13}C and ^{1}H NMR spectra. The N1-isomers were characterized by the chemical shift of carbon atom C-1′ ($\delta \sim 40$ ppm) and C-6 ($\delta \sim 154$ ppm), while in the ^{13}C NMR spectra of O6-isomers there is a low-field shift of C-1′ carbon ($\delta \sim 64$ ppm), which is due to linkage to the oxygen atom, and a less significant low-field shift of the C-6 carbon ($\delta \sim 166$ ppm). We have also observed characteristic differences in ^{1}H NMR spectra in the chemical shifts of H-1′ (N1-isomers, $\delta \sim 4.0$ ppm; O6-isomers, $\delta \sim 4.3$ ppm). The presence of the phosphonate function at the side chain was manifested by splitting of the appropriate hydrogens and carbons due to spin−spin interactions J(H,P) and J(C,P), respectively.

Antiviral Activity. Contrary to the parent compound **3a**, none of the 5-substituted compounds **12**, **14b**, or **15b** were active against either HSV-1, HSV-2, a thymidine kinase (TK)-deficient HSV-1 strain, cytomegalovirus (CMV), or vaccinia virus (Table 1). 5-Methyl derivative **12e** was active against wild-type VZV and TK-deficient VZV strain. Among the newly synthesized congeners of **3a** and/or **4**, only three 5-substituted 6-[2-(phosphonomethoxy)ethoxy] (PMEO) pyrimidine derivatives (**12e**, **14b**, and **15b**) showed a pronounced antiviral activity in cell culture against retroviruses. Both the 5-methyl derivative **12e** and the 5-bromo and 5-chloro derivatives **14b** and **15b** were exquisitely inhibitory to Moloney murine sarcoma virus (MSV) in C3H/3T3 cell cultures [50% effective concentration (EC_{50}) ranging from 0.00016 to 0.007 μmol/mL, respectively]. Compound **12e** was also very effective against HIV-1 and HIV-2 in CEM cell cultures ($EC_{50} = 0.00023-0.00043$ μmol/mL). The 5-bromo and 5-chloro derivatives **14b** and **15b** were also active, though less efficient, against these viruses at EC_{50} values of 0.0031−0.0095 μmol/mL. These values were comparable with those recorded for adefovir and tenofovir.

To exclude the possibility that the antiviral effect of **12e** (Table 1, **12e** entry 1) could be due to contamination by traces of palladium originating from the catalyst, we have synthesized this compound also by alkylation of the 2,4-diamino-6-hydroxy-5-methylpyrimidine (**5e**); the thus obtained preparation of compound **12e** exhibited antiviral effects (Table 1, **12e** entry 2) comparable with the compound isolated from cross-coupling alkylation of the diester **13**.

A uniform interpretation of the above experimental data is difficult: the highly active methyl derivative **12e** contrasts with inactive analogue **12g**, bearing the slightly more sterically demanding cyclopropyl group. Nor is electronegativity the reason for the lack of antiviral activity of the cyanomethyl (**12b**), ethoxycarbonylmethyl (**12d**), or phenyl derivative (**12f**). Substitution at the position C-5 by the large electronegative bromine atom in **14b** and chloro atom in **15b** is consistent with preserving the antiviral activity. However, as evidenced by the limited stability of the analogous 5-iodo derivative, which is easily transformed to compound **3a**, it cannot be excluded that compounds **14b** and **15b** underwent a similar transformation inside the cell.[20]

All N1- (**11**) and O6-isomers (**12, 14b**) were inactive in vitro against all RNA viruses tested [i.e. vesicular stomatitis virus (VSV), parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus].

The majority of the compounds showed no appreciable cytotoxic activity against E_6SM, HEL, or CEM cell growth. The antivirally active compounds **12e** and **14b** did not affect microscopically visible cell morphology at 0.18 and 0.15 μmol/mL (HEL) or 1.4 and 1.2 μmol/mL (E_6SM), nor were they inhibitory to HEL cell proliferation at 0.07−0.18 μmol/mL. However, in sharp contrast with the 5-bromo- and 5-chloro-substituted compounds ($IC_{50} \geq 0.3$ μmol/mL), the 5-methyl derivative **12e** showed a pronounced cytostatic activity against proliferating CEM cells [50% inhibitory concentration (IC_{50})

Scheme 3

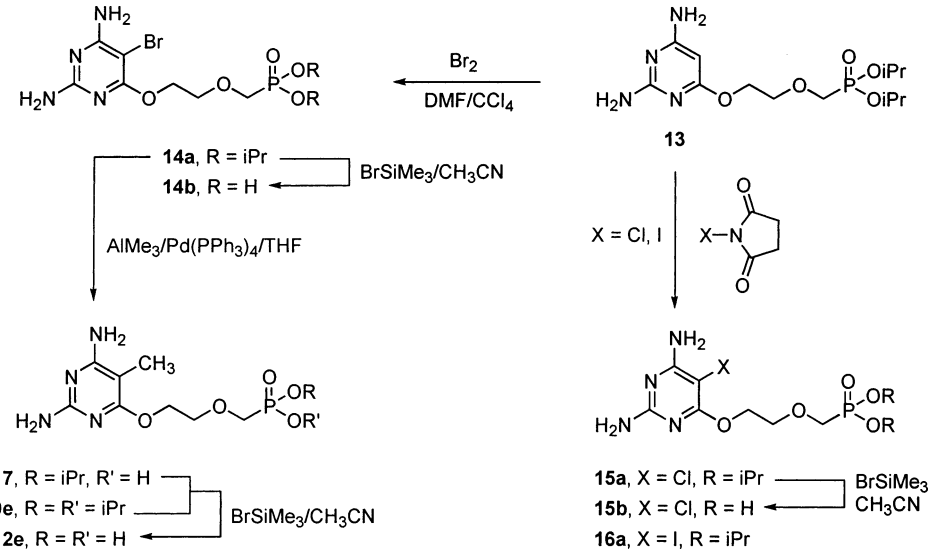


Table 1. Antiviral Activity of Test Compounds

	EC_{50}[a] (μmol/mL)										CC_{50}[b] (μmol/mL)
			CMV		VZV		HSV-1	HSV-2			
formula	HIV-1 (III_B)	HIV-2 (ROD)	AD169	Davis	OKA	07/1	(KOS)	(G)	VV	MSV	(CEM)
11a	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	0.77	>∼1.2	>∼0.12	>∼0.3
12a	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	0.79	>∼1.2	>∼0.12	>∼0.3
11b	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	>∼1.2	>∼1.2	>∼0.12	>∼0.3
12b	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	0.66	>∼1.2	>∼0.12	>∼0.3
11c	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	0.084	>∼1.2	>∼1.2	>∼1.2	0.3	>∼0.3
12c	>∼0.3	>∼0.3	>∼0.15	>∼0.15	0.14	0.11	>∼1.2	>∼1.2	>∼1.2	0.3	>∼0.3
11d	>∼0.3	0.11	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	>∼1.2	>∼1.2	0.011	>∼0.3
12d	>∼0.3	0.078	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	>∼1.2	>∼1.2	0.035	>∼0.3
11e	>∼0.3	>∼0.3	>∼0.12	>∼0.12			>∼1.2	>∼1.2	>∼1.2	>∼0.12	>∼0.3
12e	0.00043	0.00031	>∼0.15	>∼0.15	0.018	0.042	>∼1.2	>∼1.2	>∼1.2	0.00022	0.015
12e	0.00023	0.00023	>∼0.12	0.72			>∼1.2	>∼1.2	0.86	0.00016	0.0047
11f	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	>∼1.2	>∼1.2	>∼0.12	>∼0.3
12f	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	>∼1.2	>∼1.2	>∼0.12	>∼0.3
11g	>∼0.3	>∼0.3	>∼0.15	>∼0.15			>∼1.2	>∼1.2	>∼1.2	>∼0.12	>∼0.3
12g	>∼0.3	>∼0.3					>∼1.2	>∼1.2	>∼1.2	>∼0.12	>∼0.3
11h	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	>∼1.2	>∼1.2	0.082	>∼0.3
12h	>∼0.3	>∼0.3	>∼0.15	>∼0.15	>∼0.15	>∼0.15	>∼1.2	>∼1.2	>∼1.2	0.046	>∼0.3
14b	0.0073	0.0095	>∼0.15		0.15	> ∼0.15	0.7	0.70	0.7	0.007	>∼0.3
15b	0.0031	0.0044	>∼0.12	>0.6			>∼1.2	>∼1.2	>∼1.2	0.0028	0.3
					Reference Compounds						
3a	0.003	0.0016	>0.19	>0.19	0.0045	0.0095	0.025	0.033	0.18	0.00015	0.042
3d	>0.014	0.014	0.13	0.12	0.00017	0.00024	0.0048[c]	0.017[c]	0.0065	>0.002 7	0.023
PMEA	0.0033	0.0066	0.1	0.27	0.03	0.035	0.024	0.024	>0.17	0.0022	0.056
(R)PMPA	0.0012	0.0014	>0.17	>0.17	0.17	0.17	>0.17	>0.17	>0.17	0.004 6	0.41

[a] 50% effective concentration. [b] 50% cytostatic concentration. [c] EC_{50} values are the average for three HSV-1 strains (KOS, F, McIntyre) and three HSV-2 strains (G, 196, Lyons).

= 0.012−0.015 μmol/mL]. The molecular basis of this phenomenon is currently unclear.

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−methanol. 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 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).

Materials. Bromotrimethylsilane, *N*-chlorosuccinimide, *N*-iodosuccinimide, and cesium carbonate were purchased from Fluka (Switzerland); cyclopropylacetonitrile was from Lancaster (UK); and 2,4-diamino-6-hydroxypyrimidine, bromo derivatives used for its alkylations, trimethylaluminum, and ethyl phenylcyanoacetate were obtained from Sigma-Aldrich (Praha, Czech Republic). Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å).

2-[(Diisopropoxyphosphoryl)methoxy]ethyl Tosylate (10). Freshly prepared (2-acetoxyethoxy)methyl chloride[21] (153 g, 1 mol) was added dropwise under stirring at 140 °C to triisopropyl phosphite (212 g, 1.02 mol) at such a rate that the evolving isopropyl chloride still condensed. After the vigorous reaction subsided, the mixture was stirred at 140 °C for two more hours, and the volatiles were distilled off at 80 °C/15 Torr. Distillation of the residue in vacuo gave fraction 140−155 °C/1 Torr (242.8 g, 86%). This compound (211.7 g, 0.75 mol) was taken up in anhydrous methanol (2 L), and sodium methoxide (50 mL, 1 M solution in methanol) was added. After 2 h standing at the ambient temperature, the

reaction was complete. The solution was neutralized by adding Dowex 50 × 8 (H^+-form) prewashed with methanol. The suspension was filtered, made slightly alkaline by adding several drops of triethylamine, and the volatiles were taken down in vacuo. The oily residue was taken in ether (500 mL), washed with saturated NaCl solution, and dried, and the solvent was evaporated. The residue was dried at 50 °C/0.1 Torr to yield 130 g (0.54 mol, 72%) of diisopropyl (2-hydroxyethoxy)methanephosphonate.[22]

This product (60 g, 0.25 mol) in pyridine (500 mL) was treated portionwise with tosyl chloride (72 g, 0.3 mol) under cooling by ice. 4-(Dimethylamino)pyridine (1 g) was added, and the mixture was stirred for 3 h in an ice-bath and left to stand overnight at 5 °C. Methanol (20 mL) was added and the mixture was concentrated in vacuo at 30 °C (maximum bath temperature) to half of the original volume. The residue was diluted with ethyl acetate (700 mL) and the solution was washed successively with 100-mL portions of water (2×), 5% HCl (to acid reaction), water, saturated $KHCO_3$, and water. It was dried and taken down in vacuo. Chromatography of the residue in two portions on silica gel columns (1 L) in toluene gave the UV-absorbing product, which was collected and dried over P_2O_5 in vacuo to yield 69 g (70%) of compound **10** as a thick colorless oil that was used for further syntheses. ^{1}H NMR (DMSO-d_6): 7.78 d, 2H and 7.48 d, 2H, J = 8.3 (ar H); 4.56 m, 2H (P−OCH); 4.12 m, 2H and 3.70 m, 2H (O−CH_2); 3.70 d, 2H, J(P,CH) = 8.2 (P−CH_2); 2.42 s, 3H (CH_3); 1.23 d, 6H and 1.21 d, 6H, J(CH_3,CH) = 6.2 (CH_3). ^{13}C NMR (DMSO-d_6): 145.06, 132.51, 130.30 2C and 127.76, 2C (arom C); 70.36 d, 2C, J(P,C) = 6.4 (P−OC); 69.87, J(P,C) = 11.7 and 69.61 (O−CH_2); 64.87 d, J(P,C) = 164.1 (P−C); 23.94 d, 2C, J(P,C) = 3.9 and 23,81 d, 2C, J(P,C) = 4.9 (CH_3); 21.23 (CH_3).

C-5-Alkylation of 2,4-Diamino-6-hydroxypyrimidine (General Procedure). To a suspension of 2,4-diamino-6-hydroxypyrimidine (2.0 g, 16 mmol) and sodium hydrogen carbonate (1.24 g, 9.6 mmol, 1.1 equiv) in dimethylformamide (20 mL) was added bromo derivative (17.6 mmol, 1.1 equiv). The mixture was stirred at room temperature for 2 days. Solvent was evaporated in vacuo and the residue was codistilled with toluene and ethanol. Water (50 mL) was added and pH adjusted with acetic acid to 6−7. Crude product was filtered off and recrystallized from water−ethanol.

5-Allyl-2,4-diamino-6-hydroxypyrimidine (5a). Yield: 0.91 g, 31%. Mp: 255−256 °C. Anal. ($C_7H_{10}N_4O\cdot H_2O$) C, H, N. FABMS: 167 (MH^+) (30). ^{1}H NMR (DMSO-d_6): 9.88 brs, 1H (NH or OH); 6.00 brs, 2 H, and 5.63 brs, 2 H (NH_2); 5.68 ddt, 1 H, J(2′,1′) = 6.0, J(2′,3′) = 10.0 and 17.1, (H-2′); 4.99 brdt, 1 H, J(3_t′,2′) = 17.1, J(3_t′,1′) = J_{gem} = 1.5 (H-3′$_{trans}$); 4.84 brdt, 1 H, J(3_c′,2′) = 10.0, J(3_t′,1′) = J_{gem} = 1.5 (H-3′$_{cis}$); 2.91 d, 2 H, J(1′,2′) = 6.0 (H-1′). ^{13}C NMR: 162.45 (C-4), 161.90 (C-2), 53.44 (C-6); 137.07 (C-2′); 113.65 (C-3′); 85.09 (C-5); 26.82 (C-1′).

2,4-Diamino-5-benzyl-6-hydroxypyrimidine (5b). Yield: 1.6 g, 46%. Mp: 253−254 °C. Anal. ($C_{11}H_{12}N_4O$) C, H, N. FABMS: 217 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 9.96 brs, 1H (NH or OH); 7.24 d, 2 H, 7.19 t, 2H and 7.09 t, 1 H (arom H); 6.08 brs, 2 H, and 5.74 brs, 2 H (NH_2); 3.52 s, 2 H (CH_2Ph).

2,4-Diamino-5-cyanomethyl-6-hydroxypyrimidine (5c). Yield: 2.0 g, 72%. Mp: dec. Anal. ($C_6H_7N_5O\cdot{}^1/_2H_2O$) C, H, N. FABMS: 166 ($MH^+$) (55). ^{1}H NMR (DMSO-$d_6$): 10.06 brs, 1H (NH or OH); 6.24 brs, 2 H, and 6.19 brs, 2 H (NH_2); 3.36 s, 2 H (H-1′).

2,4-Diamino-5-ethoxycarbonylmethyl-6-hydroxypyrimidine (5d).[14] Yield: 1.97 g, 59%. Mp: dec. Anal. ($C_8H_{12}N_4O_3$) C, H, N. FABMS: 213 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 9.89 brs, 1H (NH or OH); 6.05 brs, 2 H, and 5.82 brs, 2 H (NH_2); 4.01 q, 2 H, J(CH_2,CH_3) = 7.1 (OCH_2); 3.17 s, 2 H (H-1′); 1.16 t, 3 H (CH_3).

2,4-Diamino-6-hydroxy-5-methylpyrimidine (5e) was prepared by monomethylation accoording to the literature and[15] crystallized from water−ethanol. Yield: 42%. Mp: 275−277 °C. Anal. ($C_5H_8N_4O\cdot{}^1/_2H_2O$) C, H, N. FABMS: 140 ($MH^+$) (60). ^{1}H NMR (DMSO-$d_6$): 10.00 brs, 1H (NH or OH); 5.98 brs, 2 H and 5.65 brs, 2 H (NH_2); 1.61 s, 3 H (CH_3).

Synthesis of 5-Substituted-2,4-diamino-6-hydroxypyrimidines by Cyclization (General Procedure). To a solution of EtONa (1.85 g, 27 mmol) in absolute ethanol (25 mL) were added guanidine hydrochloride (1.25 g, 12.5 mmol, 95%) and ethyl phenylcyanoacetate (2.3 mL, 12.5 mmol) or ethyl cyclopropylcyanoacetate (1.7 g, 12.5 mmol. The mixture was refluxed for 5 h under Ar atmosphere, stirred at room temperature overnight, and then evaporated in vacuo. Water was added and the mixture was neutralized by acetic acid. Crude product was filtered off and recrystalllized from water−ethanol.

2,4-Diamino-6-hydroxy-5-phenylpyrimidine (5f). Yield: 0.96 g, 38%), mp: 262−263 °C. Anal. ($C_{10}H_{10}N_4O$) C, H, N. FABMS: 203 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 10.20 brs, 1H (NH or OH); 7.30 m, 4 H and 7.15 t, 1 H (arom H); 6.22 brs, 2 H, and 5.55 brs, 2 H (NH_2).

2,4-Diamino-5-cyclopropyl-6-hydroxypyrimidine (5g). Yield: 1.62 g, 78%. Mp: dec. Anal. ($C_7H_{10}N_4O$) C, H, N. EIMS: 166 (M) (65). ^{1}H NMR (DMSO-d_6): 9.79 brs, 1H (NH or OH); 6.08 brs, 2 H and 5.79 brs, 2 H (NH_2); 1.01 m, 1 H, 0.66, 2, and 0.37 m, 2 H (cyclopropyl).

Alkylation of 5-ubstituted-2,4-diamino-6-hydroxypyrimidines (General Procedure). 5-Substituted-2,4-diamino-6-hydroxypyrimidine (4 mmol) was codistilled with toluene and the residue was sonicated with dimethylformamide (7 mL) and cesium carbonate (0.65 g, 2 mmol) and heated to 80 °C. Tosylate **10** (1.7 g, 4.4 mmol, 1.1 equiv) was added, and the reaction mixture was heated at 100 °C for 20−30 h, evaporated in vacuo, and codistilled with toluene and ethanol. The residue was treated with hot chloroform and filtered and the filtrate taken down in vacuo. Preparative HPLC afforded N- and O-isomers as colorless or yellow foams that were used in the next step without further purification.

5-Allyl-2,4-diamino-1-{2-[(diisopropoxyphosphoryl)-methoxy]ethyl}pyrimidin-6(1*H*)-one (8a). Yield: 0.25 g, 16%. FABMS: 389 (MH^+) (35). ^{1}H NMR (DMSO-d_6): 6.37 brs, 2 H, and 5.55 brs, 2 H (NH_2); 5.69 ddt, 1 H, J(2″,1″) = 6.1, J(2″,3″) = 10.0 and 17.1, (H-2″); 5.00 ddt, 1 H, J(3_t″,2″) = 17.1, J(3_t″,1″) = 1.5, J_{gem} = 2.3 (H-3″$_{trans}$); 4.83 ddt, 1 H, J(3_c″,2″) = 10.0, J(3_t″,1″) = 1.5, J_{gem} = 2.3 (H-3″$_{cis}$); 4.57 m, 2 H (P−OCH); 3.98 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.78 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.64 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.94 dt, 2 H, J(1″,3″) = 1.5, J(1″,2″) = 6.1 (H-1″); 1.23, 6, and 1.21 d, 6 H, J(CH_3,CH_2) = 6.1 (CH_3).^{13}C NMR: 161.62 (C-4); 159.93 (C-2); 153.83 (C-6); 136.99 (C-2″); 113.64 (C-3″); 85.06 (C-5); 70.39 d, 2 C, J(P,C) = 6.3 (P−OC); 70.26 d, J(P,C) = 11.2 (C-2′); 65.02 d, J(P,C) = 163.6 (P−C); 40.21 (C-1′); 27.81 (C-1″); 23.98 d, 2 C, J(P,C) = 3.9 and 23.86 d, 2 C, J(P,C) = 4.4 (CH_3).

5-Allyl-2,4-diamino-6-[2-(diisopropoxyphosphoryl-methoxy)ethoxy]pyrimidine (9a). Yield: 0.25 g, 16%. FABMS: 389 (MH^+) (20). ^{1}H NMR (DMSO-d_6): 5.79 brs, 2 H, and 5.70 brs, 2 H (NH_2); 5.72 ddt, 1 H, J(2″,1″) = 6.0, J(2″,3″) = 10.0 and 17.0, (H-2″); 5.00 ddt, 1 H, J(3_t″,2″) = 17.0, J(3_t″,1″) = 1.5, J_{gem} = 2.0 (H-3″$_{trans}$); 4.87 ddt, 1 H, J(3_c″,2″) = 10.0, J(3_t″,1″) = 1.5, J_{gem} = 2.0 (H-3″$_{cis}$); 4.59 m, 2 H (P−OCH); 4.26 m, 2 H (H-1′); 3.79 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.75 m, 2 H, (H-2′); 3.00 dt, 2 H, J(1″,3″) = 1.5, J(1″,2″) = 6.0 (H-1″); 1.24, 6, and 1.22 d, 6 H, J(CH_3,CH_2) = 6.2 (CH_3). ^{13}C NMR: 166.79 (C-6); 163.64 (C-4); 160.92 (C-2); 136.43 (C-2″); 114.16 (C-3″); 85.92 (C-5); 71.23 d, J(P,C) = 11.7 (C-2′); 70.28 d, 2 C, J(P,C) = 6.3 (P−OC); 65.23 d, J(P,C) = 164.1 (P−C); 64.23 (C-1′); 26.37 (C-1″); 23.98 d, 2 C, J(P,C) = 3.9 and 23.86 d, 2 C, J(P,C) = 4.4 (CH_3).

2,4-Diamino-5-benzyl-1-{2-[(diisopropoxyphosphoryl)-methoxy]ethyl}pyrimidin-6(1*H*)-one (8b). Yield: 0.25 g, 14%. FABMS: 439 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 7.23 d, 2 H, 7.18, 2, and 7.10 t, 1 H (arom H); 6.42 brs, 2 H, and 5.66 brs, 2 H (NH_2); 4.57 m, 2 H (P−OCH); 4.01 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.79 d, 2 H, J(P,CH) = 8.2 (P−CH_2); 3.66 t, 2 H, J(2′,1′) = 5.8 (H-2′); 3.54 s, 2 H (H-1″); 1.24, 6, and 1.21 d, 6 H, J(CH_3,CH_2) = 6.1 (CH_3).^{13}C NMR: 162.12 (C-4); 160.11 (C-2); 153.87 (C-6); 142.33, 128.27, 2 C, 127.90, 2 C, and 125.31 (arom C); 87.32 (C-5); 70.38 d, 2 C, J(P,C) = 6.3 (P−OC); 70.24 d, J(P,C) = 11.2 (C-2′); 65.03 d, J(P,C) = 164.1 (P−C); 40.30

(C-1′); 29.04 (C-1″); 23.98 d, 2 C, J(P,C) = 3.9 and 23.86 d, 2 C, J(P,C) = 4.4 (CH_3).

2,4-Diamino-5-benzyl-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (9b). Yield: 0.40 g, 23%. FABMS: 439 (MH^+) (35). ^{1}H NMR (DMSO-d_6): 7.30−7.10 m, 5 H (arom H); 5.88 brs, 2 H, and 5.74 brs, 2 H (NH_2); 4.58 m, 2 H (P−OCH); 4.28 m, 2 H (H-1′); 3.77 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.76 m, 2 H, (H-2′); 3.61 s, 2 H, (H-1″); 1.22, 6, and 1.20 d, 6 H, J(CH_3,CH_2) = 6.1 (CH_3).

2,4-Diamino-5-cyanomethyl-1-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}pyrimidin-6(1*H*)-one (8c). Yield: 0.25 g, 16%. FABMS: 388 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 6.66 brs, 2 H, and 6.17 brs, 2 H (NH_2); 4.56 m, 2 H (P−OCH); 4.00 t, 2 H, J(1′,2′) = 5.6 (H-1′); 3.78 d, 2 H, J(P,CH) = 8.1 (P−CH_2); 3.65 t, 2 H, J(2′,1′) = 5.6 (H-2′); 3.38 s, 2 H (H-1″); 1.22, 6, and 1.20 d, 6 H, J(CH_3,CH_2) = 6.2 (CH_3). ^{13}C NMR: 161.39 (C-4); 160.28 (C-2); 154.50 (C-6); 119.68 (CN); 77.53 (C-5); 70.40 d, 2 C, J(P,C) = 6.4 (P−OC); 70.00 d, J(P,C) = 11.2 (C-2′); 64.99 d, J(P,C) = 163.6 (P−C); 40.26 (C-1′); 23.98 d, 2 C, J(P,C) = 3.9 and 23.85 d, 2 C, J(P,C) = 4.9 (CH_3).

2,4-Diamino-5-cyanomethyl-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (9c). Yield: 0.26 g, 17%. FABMS: 388 (MH^+) (75). ^{1}H NMR (DMSO-d_6): 6.29 brs, 2 H, and 6.02 brs, 2 H (NH_2); 4.59 m, 2 H (P−OCH); 4.33 m, 2 H (H-1′); 3.81 d, 2 H, J(P,CH) = 8.5 (P−CH_2); 3.79 m, 2 H, (H-2′); 3.48 s, 2 H, (H-1″); 1.24, 6, and 1.23 d, 6 H, J(CH_3,-CH_2) = 6.1 (CH_3).^{13}C NMR: 166.93 (C-6); 163.38 (C-4); 161.84 (C-2); 119.00 (CN); 78.12 (C-5); 71.09 d, J(P,C) = 11.7 (C-2′); 70.32 d, 2 C, J(P,C) = 6.4 (P−OC); 65.17 d, J(P,C) = 163.6 (P−C); 64.52 (C-1′); 23.98 d, 2 C, J(P,C) = 3.9 and 23.85 d, 2 C, J(P,C) = 4.9 (CH_3).

2,4-Diamino-1-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-5-(ethoxycarbonylmethyl)-pyrimidin-6(1*H*)-one (8d). Yield: 0.25 g, 15%. FABMS: 435 (MH^+) (80), no further characterization, unstable.

2,4-Diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]-5-(ethoxycarbonylmethyl)-pyrimidine (9d). Yield: 0.22 g, 13%. FABMS: 435 (MH^+) (20), no further characterization, unstable.

2,4-Diamino-1-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-5-methylpyrimidin-6(1*H*)-one (8e). Yield: 0.39 g, 27%. FABMS: 363 (MH^+) (20). ^{1}H NMR (DMSO-d_6): 6.29 brs, 2 H, and 5.58 brs, 2 H (NH_2); 4.57 m, 2 H (P−OCH); 3.97 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.77 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.62 t, 2 H, J(2′,1′) = 5.8 (H-2′); 1.64 s, 3 H, (CH_3); 1.22, 6, and 1.20 d, 6 H, J(CH_3,CH_2) = 6.2 (CH_3).^{13}C NMR: 161.85 (C-4); 160.09 (C-2), 153.50 (C-6); 82.27 (C-5); 74.46 d, J(P,C) = 11.2 (C-2′); 70.37 d, 2 C, J(P,C) = 6.3 (P−OC); 64.98 d, J(P,C) = 164.1 (P−C); 40.17 (C-1′); 23.89 d, 2 C, J(P,C) = 4.9 and 23.85 d, 2 C, J(P,C) = 4.9 (CH_3); 9.39 (CH_3).

2,4-Diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]-5-methylpyrimidine (9e). Yield: 0.61 g, 42%. FABMS: 363 (MH^+) (35). ^{1}H NMR (DMSO-d_6): 6.41 brs, 2 H, and 6.33 brs, 2 H (NH_2); 4.59 m, 2 H (P−OCH); 4.31 m, 2 H (H-1′); 3.80 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.78 m, 2 H (H-2′); 1.74 s, 3 H (CH_3); 1.24, 6, and 1.22 d, 6 H, J(CH_3,CH_2) = 6.2 (CH_3).

2,4-Diamino-1-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-5-phenylpyrimidin-6(1*H*)-one (8f). Yield: 0.18 g, 10%. FABMS: 425 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 7.31 t, 2 H, 7.26 d, 2 H and 7.17 t, 1 H (arom H); 6.65 brs, 2 H, and 5.42 brs, 2 H (NH_2); 4.58 m, 2 H (P−OCH); 4.04 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.80 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.68 t, 2 H, J(2′,1′) = 4.6 (H-2′); 1.23, 6, and 1.22 d, 6 H, J(CH_3,CH_2) = 6.1 (CH_3).^{13}C NMR: 160.80 (C-4); 159.52 (C-2); 154.35 (C-6); 135.88, 131.03, 2 C, 128.12, 2 C, and 125.64 (arom C); 90.30 (C-5); 70.39 d, 2 C, J(P,C) = 6.4 (P−OC); 70.11 d, J(P,C) = 11.7 (C-2′); 65.02 d, J(P,C) = 164.1 (P−C); 40.15 (C-1′); 24.01 d, J(P,C) = 3.4, 23.98 d, J(P,C) = 3.4 and 23.86 d, 2 C, J(P,C) = 4.4 (CH_3).

2,4-Diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]-5-phenylpyrimidine (9f). Yield: 0.36 g, 21%. FABMS: 425 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 7.35 m, 2H and 7.25 m, 3 H (arom H); 6.00 brs, 2 H, and 5.55 brs, 2 H (NH_2); 4.52 m, 2 H (P−OCH); 4.26 m, 2 H (H-1′); 3.68 m, 2 H, (H-2′); 3.67 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 1.21, 6, and 1.18 d, 6 H, J(CH_3,CH_2) = 6.1 (CH_3). ^{13}C NMR: 166.09 (C-6); 163.11 (C-4); 161.51 (C-2); 134.07, 130.98, 2 C, 128.61, 2 C, and 126.52 (arom C); 91.31 (C-5); 71.18 d, J(P,C) = 12.2 (C-2′); 70.38 d, 2 C, J(P,C) = 6.4 (P−OC); 65.36 d, J(P,C) = 164.1 (P−C); 64.46 (C-1′); 24.11 d, J(P,C) = 3.9, 23.98 d, 2 C, J(P,C) = 4.9 (CH_3).

2,4-Diamino-5-cyclopropyl-1-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}pyrimidin-6(1*H*)-one (8g). Yield: 0.29 g, 19%. FABMS: 389 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 6.36 brs, 2 H, and 5.62 brs, 2 H (NH_2); 4.56 m, 2 H (P−OCH); 3.94 t, 2 H, J(1′,2′) = 4.8 (H-1′); 3.77 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.61 t, 2 H, J(2′,1′) = 4.8 (H-2′); 1.22, 6, and 1.21 d, 6 H, J(CH_3,CH_2) = 6.2 (CH_3); 1.07 m, 1 H, 0.67, 2, and 0.38 m 2 H (cyclopropyl).^{13}C NMR: 161.82 (C-4), 161.46 (C-2); 154.12 (C-6); 87.29 (C-5); 70.35 d, 2 C, J(P,C) = 6.4 (P−OC); 70.24 d, J(P,C) = 11.2 (C-2′); 64.97 d, J(P,C) = 163.6 (P−C); 39.85 (C-1′); 23.97 d, 2 C, J(P,C) = 3.9 and 23.85 d, 2 C, J(P,C) = 4.9 (CH_3); 6.79, 2 C and 5.91 (cyclopropyl).

2,4-Diamino-5-cyclopropyl-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (9g). Yield: 0.55 g, 35%. FABMS: 389 (MH^+) (95). ^{1}H NMR (DMSO-d_6): 5.84 brs, 2 H, and 5.70 brs, 2 H (NH_2); 4.59 m, 2 H (P−OCH); 4.26 m, 2 H (H-1′); 3.81 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.76 m, 2 H (H-2′); 1.24, 6, and 1.22 d, 6 H, J(CH_3,CH_2) = 6.2 (CH_3); 1.11 m, 1 H, 0.75, 2, and 0.38 m 2 H (cyclopropyl). ^{13}C NMR: 167.34 (C-6), 165.30 (C-4); 160.73 (C-2); 88.69 (C-5); 71.27 d, J(P,C) = 12.2 (C-2′); 70.27 d, 2 C, J(P,C) = 6.4 (P−OC); 65.24 d, J(P,C) = 164.5 (P−C); 63.95 (C-1′); 23.99 d, 2 C, J(P,C) = 3.9 and 23.865 d, 2 C, J(P,C) = 4.9 (CH_3); 7.02, 2 C and 4.38 (cyclopropyl).

5-Substituted-2,4-diamino-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-ones and 5-Substituted-2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidines (General Procedure). A mixture of diisopropyl ester (0.5 mmol), acetonitrile (10 mL), and $BrSiMe_3$ (1 mL) was stirred overnight at room temperature. After evaporation in vacuo and codistillation with acetonitrile, the residue was treated with water and concentrated aqueous ammonia was added to the alkaline reaction. The mixture was evaporated to dryness and the residue was applied onto a column of Dowex 50 × 8 (H^+-form, 20 mL) and washed with water. Elution with 2.5% aqueous ammonia and evaporation in vacuo afforded crude product as ammonium salt. This residue in a minimum volume of water was applied on a Dowex 1 × 2 (acetate) (25 mL) column, which was then washed with water followed by gradient of acetic acid (0−0.75 M for O-isomers, 0.5−2 M for N-isomers). The main UV-absorbing fraction was evaporated, the residue was codistilled three times with water and crystallized from water−ethanol to afford the product as a white solid.

5-Allyl-2,4-diamino-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (11a). Yield: 114 mg, 76%. Mp: 254−255 °C. Anal. ($C_{10}H_{17}N_4O_5P\cdot{}^1/_2H_2O$) C, H, N, P. FAB$^-$MS: 303 ($M-H^-$) (60). ^{1}H NMR (DMSO-$d_6$): 6.60 brs, 2 H, and 5.69 brs, 2 H (NH_2); 5.70 ddt, 1 H, J(2″,1″) = 6.2, J(2″,3″) = 10.0 and 17.1, (H-2″); 5.01 ddt, 1 H, J(3_t″,2″) = 17.1, J(3_t″,1″) = 1.5, J_{gem} = 2.3 (H-3″$_{trans}$); 4.84 ddt, 1 H, J(3_c″,2″) = 10.0, J(3_t″,1″) = 1.5, J_{gem} = 2.3 (H-3″$_{cis}$); 3.99 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.62 t, 2 H, J(2′,1′) = 6.0 (H-2′); 3.60 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 2.95 brd, 2 H, J(1″,2″) = 6.2 (H-1″).

5-Allyl-2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (12a). Yield: 113 mg, 75%. Mp: 240−241 °C. Anal. ($C_{10}H_{17}N_4O_5P$) C, H, N, P. FAB$^-$MS: 303 ($M-H^-$) (25). ^{1}H NMR (DMSO-d_6): 5.98 brs, 2 H, and 5.92 brs, 2 H (NH_2); 5.73 ddt, 1 H, J(2″,1″) = 6.1, J(2″,3″) = 10.0 and 17.1, (H-2″); 5.00 brd, 1 H, J(3_t″,2″) = 17.1 (H-3″$_{trans}$); 4.88 brd, 1 H, J(3_c″,2″) = 10.0 (H-3″$_{cis}$); 4.27 m, 2 H (H-1′); 3.73 m, 2 H, (H-2′); 3.57 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.00 brd, 2 H, J(1″,2″) = 6.1 (H-1″).

2,4-Diamino-5-benzyl-1-[2-(phosphonomethoxy)ethyl]-pyrimidin-6(1*H*)-one (11b). Yield: 125 mg, 70%. Mp: 252−253 °C. Anal. ($C_{14}H_{19}N_4O_5P\cdot 2H_2O$) C, H, N. FABMS: 355 ($MH^+$) (50).

^{1}H NMR (DMSO-d_6): 7.24 d, 2 H, 7.19, 2, and 7.09 t, 1 H (arom H); 6.57 brs, 2 H, and 5.75 brs, 2 H (NH_2); 4.00 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.63 t, 2 H, J(2′,1′) = 6.0 (H-2′); 3.60 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.54 s, 2 H (H-1″). ^{13}C NMR (D_2O): 164.10 (C-4); 161.18 (C-2); 155.56 (C-6); 140.10, 128.68, 2 C, 127.73, 2 C, and 126.19 (arom C); 89.33 (C-5); 70.95 d, J(P,C) = 10.7 (C-2′); 69.61 d, J(P,C) = 149.9 (P−C); 42.94 (C-1′); 28.66 (C-1″).

2,4-Diamino-5-benzyl-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (12b). Yield: 121 mg, 68%. Mp: 241−242 °C. Anal. ($C_{14}H_{19}N_4O_5P$·2/3H_2O) C, H, N. FABMS: 355 (MH^+) (50). ^{1}H NMR (DMSO-d_6): 7.23, 4, and 7.10 m, 1 H (arom H); 6.15 brs, 4 H (NH_2); 4.30 m, 2 H (H-1′); 3.74 m, 2 H (H-2′); 3.62 s, 2 H (H-1″); 3.58 d, 2 H, J(P,CH) = 8.6 (P−CH_2). ^{13}C NMR (D_2O): 168.05 (C-6); 163.70 (C-4); 160.70 (C-2); 139.95, 128.74, 2 C, 127.89, 2 C, and 126.32 (arom C); 90.38 (C-5); 70.66 d, J(P,C) = 8.8 (C-2′); 67.75 d, J(P,C) = 149.4 (P−C); 65.66 (C-1′); 27.50 (C-1″).

2,4-Diamino-5-cyanomethyl-1-[2-(phosphonomethoxy)-ethyl]pyrimidin-6(1*H*)-one (11c). Yield: 120 mg, 77%. Mp: 250−251 °C. Anal. ($C_9H_{14}N_5O_5P$·1/2H_2O) C, H, N. FABMS: 304 (MH^+) (50). ^{1}H NMR (DMSO-d_6): 6.71 brs, 2 H, and 6.20 brs, 2 H (NH_2); 3.99 t, 2 H, J(1′,2′) = 6.1 (H-1′); 3.62 t, 2 H, J(2′,1′) = 6.1 (H-2′); 3.60 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.39 s, 2 H (H-1″). ^{13}C NMR (D_2O + NaOD): 161.66 (C-2); 159.15 (C-4); 153.64 (C-6); 117.40 (CN); 81.09 (C-5); 68.10 d, J(P,C) = 11.3 (C-2′); 67.06 d, J(P,C) = 149.4 (P−C); 40.13 (C-1′).

2,4-Diamino-5-cyanomethyl-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine (12c). Yield: 123 mg, 81%. Mp: 258−259 °C, dec. Anal. ($C_9H_{14}N_5O_5P$) C, H, N. FABMS: 304 (MH^+) (60). ^{1}H NMR (DMSO-d_6): 6.28 brs, 2 H, and 6.02 brs, 2 H (NH_2); 4.31 m, 2 H (H-1′); 3.75 m, 2 H (H-2′); 3.53 d, 2 H, J(P,-CH) = 8.2 (P−CH_2); 3.49 s, 2 H (H-1″).

2,4-Diamino-5-(ethoxycarbonylmethyl)-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (11d) and 2,4-Diamino-5-(carboxymethyl)-1-[2-(phosphonomethoxy)ethyl]-pyrimidin-6(1*H*)-one (11h). Starting from a 2-fold amount (1 mmol) of diisopropyl ester **8d**, the Dowex 1 × 2 column chromatography gave two products:

11d: eluted by 1 M AcOH. Yield: 97 mg, 28%. Mp: dec. Anal. ($C_{11}H_{19}N_4O_7P$·H_2O) C, H, N, P. FABMS: 351 (MH^+) (20). ^{1}H NMR (DMSO-d_6): 6.63 brs, 2 H and 5.88 brs, 2 H (NH_2); 4.00 q, 2 H, J(CH_2,CH_3) = 7.1 (O−CH_2); 3.96 t, 2 H, J(1′,2′) = 6.1 (H-1′); 3.60 t, 2 H, J(2′,1′) = 6.1 (H-2′); 3.59 d, 2 H, J(P,-CH) = 8.2 (P−CH_2); 3.21 s, 2 H (H-1″); 1.17 t, 3 H, J(CH_3,-CH_2) = 7.1 (CH_3).

11h: eluted by 2 M AcOH. Yield: 90 mg, 27%. Mp: 235−236 °C. Anal. ($C_9H_{15}N_4O_7P$·H_2O) C, H, N, P. FABMS: 323 (MH^+) (10). ^{1}H NMR (DMSO-d_6): 6.73 brs, 2 H and 5.91 brs, 2 H (NH_2); 3.98 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.62 t, 2 H, J(2′,1′) = 6.0 (H-2′); 3.60 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.15 s, 2 H (H-1″).

2,4-Diamino-5-(ethoxycarbonylmethyl)-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (12d) and 2,4-Diamino-5-(carboxymethyl)-6-[2-(phosphonomethoxy)ethoxy]pyrimidin (12h). Starting from 2-fold amount (1 mmol) of diisopropyl ester **9d**, the Dowex 1 × 2 column chromatography gave two products:

12d: eluted by 0.25 M AcOH. Yield: 130 mg, 34%. Mp: 199−200 °C. Anal. ($C_{11}H_{19}N_4O_7P$·2H_2O) C, H, N, P. FABMS: 351 (MH^+) (20). ^{1}H NMR (DMSO-d_6): 6.21 brs, 2 H and 6.12 brs, 2 H (NH_2); 4.24 m, 2 H (H-1′); 4.04 q, 2 H, J(CH_2,CH_3) = 7.1 (O−CH_2); 3.70 m, 2 H (H-2′); 3.54 d, 2 H, J(P,CH) = 8.5 (P−CH_2); 3.30 s, 2 H (H-1″); 1.17 t, 3 H, J(CH_3,CH_2) = 7.1 (CH_3).

12h: eluted by 0.5−1.0 M AcOH. Yield: 72 mg, 22%. Mp: 228−229 °C. Anal. ($C_9H_{15}N_4O_7P$·H_2O) C, H, N, P. FABMS: 323 (MH^+) (20). ^{1}H NMR (DMSO-d_6): 6.21 brs, 2 H and 6.14 brs, 2 H (NH_2); 4.26 m, 2 H (H-1′); 3.70 m, 2 H (H-2′); 3.55 d, 2 H, J(P,CH) = 8.5 (P−CH_2); 3.21 s, 2 H (H-1″).

2,4-Diamino-5-methyl-1-[2-(phosphonomethoxy)ethyl]-pyrimidin-6(1*H*)-one (11e). Yield: 82 mg, 59%. Mp: 284−285 °C. Anal. ($C_8H_{15}N_4O_5P$) C, H, N, P. FABMS: 279 (MH^+) (20). ^{1}H NMR (D_2O + NaOD): 4.13 t, 2 H, J(1′,2′) = 5.2 (H-1′); 3.97 t, 2 H, J(2′,1′) = 5.2 (H-2′); 3.50 d, 2 H, J(P,CH) = 8.5 (P−CH_2); 1.79 s, 3 H (CH_3). ^{13}C NMR: 163.51 (C-4); 160.81 (C-2); 154.69 (C-6); 85.56 (C-5); 70.70 d, J(P,C) = 10.7 (C-2′); 69.33 d, J(P,C) = 150.4 (P−C); 42.27 (C-1′); 11.40 (CH_3).

2,4-Diamino-5-methyl-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (12e). Yield: 81 mg, 57%. Mp: 253−254 °C. Anal. ($C_8H_{15}N_4O_5P$) C, H, N, P. FABMS: 279 (MH^+) (70). ^{1}H NMR (D_2O + NaOD): 4.37 m, 2 H (H-1′); 3.91 m, 2 H (H-2′); 3.58 d, 2 H, J(P,CH) = 8.4 (P−CH_2); 1.84 s, 3 H (CH_3). ^{13}C NMR: 167.06 (C-6); 163.58 (C-4); 160.01 (C-2); 85.75 (C-5); 70.47 d, J(P,C) = 10.3 (C-2′); 68.96 d, J(P,C) = 148.9 (P−C); 65.42 (C-1′); 10.54 (CH_3).

2,4-Diamino-5-phenyl-1-[2-(phosphonomethoxy)ethyl]-pyrimidin-6(1*H*)-one (11f). Yield: 140 mg, 78%. Mp: 235−236 °C. Anal. ($C_{13}H_{17}N_4O_5P$·1/2H_2O) C, H, N, P. FABMS: 341 (MH^+) (30). ^{1}H NMR (DMSO-d_6): 7.32 t, 2 H, 7.26 d, 2 H and 7.18 t, 1 H (arom H); 6.81 brs, 2 H, and 5.49 brs, 2 H (NH_2); 4.03 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.66 t, 2 H, J(2′,1′) = 6.0 (H-2′); 3.62 d, 2 H, J(P,CH) = 8.3 (P−CH_2).

2,4-Diamino-5-phenyl-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (12f). Yield: 133 mg, 75%. Mp: 230−231 °C. Anal. ($C_{13}H_{17}N_4O_5P$·3/2H_2O) C, H, N, P. FABMS: 341 (MH^+) (40). ^{1}H NMR (DMSO-d_6): 7.36, 2, and 7.25 m, 3 H (arom H); 6.35 brs, 2 H and 5.79 brs, 2 H (NH_2); 4.27 m, 2 H (H-1′); 3.65 m, 2 H (H-2′); 3.48 d, 2 H, J(P,CH) = 8.4 (P−CH_2).

2,4-Diamino-5-cyclopropyl-1-[2-(phosphonomethoxy)-ethyl]pyrimidin-6(1*H*)-one (11g). Yield: 120 mg, 79%. Mp: 282−283 °C. Anal. ($C_{10}H_{17}N_4O_5P$) C, H, N, P. FABMS: 305 (MH^+) (95). ^{1}H NMR (D_2O + NaOD): 4.10 t, 2 H, J(1′,2′) = 5.3 (H-1′); 3.78 t, 2 H, J(2′,1′) = 5.3 (H-2′); 3.51 d, 2 H, J(P,-CH) = 8.6 (P−CH_2); 1.23 m, 1 H, 0.89, 2, and 0.40 m, 2 H (cyclopropyl).

2,4-Diamino-5-cyclopropyl-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine (12g). Yield: 125 mg, 82%. Mp: 255−256 °C. Anal. ($C_{10}H_{17}N_4O_5P$) C, H, N, P. FABMS: 305 (MH^+) (95). ^{1}H NMR (D_2O + NaOD): 4.38 m, 2 H (H-1′); 3.93 m, 2 H (H-2′); 3.62 d, 2 H, J(P,CH) = 8.4 (P−CH_2); 1.24 m, 1 H, 0.93, 2, and 0.45 m, 2 H (cyclopropyl).

Preparation of 2,4-Diamino-5-methyl-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (12e) by Cross-Coupling Reaction. To a solution of bromo derivative **14a** (0.85 g, 2 mmol) and Pd(PPh_3)$_4$ (0.23 g, 10 mol %) in dry tetrahydrofuran (35 mL) was added a 2 M solution of $AlMe_3$ in toluene (5 mL, 5 equiv) under Ar atmosphere. The resulting mixture was refluxed for 20 h and taken down in vacuo. The residue was treated with hot chloroform and filtered, and the filtrate was evaporated in vacuo. Chromatography on silica gel afforded unreacted starting material (**14a**, 0.12 g, 14%) and diisopropyl ester **9e** (0.06 g, 8%) FABMS: 363 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 6.41 brs, 2 H, and 6.33 brs, 2 H (NH_2); 4.59 m, 2 H (P−OCH); 4.31 m, 2 H (H-1′); 3.80 d, 2 H, J(P,CH) = 8.3 (P−CH_2); 3.78 m, 2 H (H-2′); 1.74 s, 3 H (CH_3); 1.24, 6, and 1.22 d, 6 H, J(CH_3,CH_2) = 6.2 (CH_3).

The solid that was filtered off from hot chloroform was triturated with a mixture of water, methanol, and aqueous NH_3 and filtered. The filtrate was taken down in vacuo and deionized on a column of Dowex 50 × 8. Further purification by Dowex 1 × 2 column chromatography (elution with gradient of acetic acid) afforded monoisopropyl ester **17**. Yield: 0.2 g, 31%. FABMS: 321 (MH^+) (100). ^{1}H NMR (DMSO-d_6): 6.98 brs, 4 H (NH_2); 4.41 m, 2 H (P−OCH); 4.26 m, 2 H (H-1′); 3.75 m, 2 H (H-2′); 3.55 d, 2 H, J(P,CH) = 8.4 (P−CH_2); 1.71 s, 3 H (CH_3); 1.15 d, 6 H, J(CH_3,CH_2) = 6.1 (CH_3).

Both intermediates **9e** and **17** were combined, dry acetonitrile (15 mL) and $BrSiMe_3$ (1.5 mL) were added, and the resulting mixture was stirred overnight at room temperature. Solvent was evaporated in vacuo and the residue was codistilled with acetonitrile. Water and aqueous NH_3 was added and the solution was evaporated to dryness. The residue was deionized on a Dowex 50 × 8 column and further purified by Dowex 1 × 2 column chromatography (elution with a gradient of acetic acid). Crystallization (water−ethanol) gave compound **12e**. Yield: 0.15 g, overall yield 27%. Mp: 253−255 °C. Anal. ($C_8H_{15}N_4O_5P$) C, H, N, P. FABMS: 279 (MH^+) (100). ^{1}H NMR

(D_2O + NaOD): 4.37 m, 2 H (H-1′); 3.91 m, 2 H (H-2′); 3.58 d, 2 H, J(P,CH) = 8.4 (P−CH_2); 1.84 s, 3 H (CH_3).

2,4-Diamino-5-bromo-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (14a). Compound **13** (4.3 g, 12.9 mmol) in DMF (40 mL) was treated with 0.7 M bromine solution in CCl_4 (20 mL). After 3 h of stirring at room temperature, the reaction mixture was made alkaline by addition of triethylamine and evaporated, and the residue **(14a)** was crystallized from ethanol. Yield: 3.9 g, 72%. Mp: 165 °C. Anal. ($C_{12}H_{22}BrN_4O_5P$): C, H, Br, N, P. FABMS: 427 (M^+, 59), 428 (57). ^{1}H NMR (DMSO-d_6): 6.33 br, 2H, and 6.13 s, 2H (NH_2); 4.59 m, 2H (P−OCH); 4.33 m, 2H (H-1′); 3.82 d, 2H, J(P,CH) = 8.4 (P−CH_2); 3.79 m, 2H (H-2′); 1.23 d, 6H, and 1.22 d, 6H J(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 164.65 (C-6); 162.04 (C-4); 161.10 (C-2)]; 71.64 (C-5); 70.84 d, J(P,C) = 12.2 (C-2′); 70.37 d, 2C, J(P,C) = 6.4 (P−O−C); 65.27 (C-1′); 65.23 d, J(P,C) = 164.9 (P−C); 24.03 d, 2C, J(P,C) = 3.9 and 23.91 d, 2C, J(P,C) = 4.4 (CH_3).

2,4-Diamino-5-chloro-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (15a). *N*-Chlorosuccinimide (1.18 g, 1.5 equiv) was added with stirring to the suspension of compound **13** (2.5 g, 7.5 mmol) in acetonitrile (70 mL). After 90 min at ambient temperature, the homogeneous reaction mixture was diluted with toluene (300 mL) and the solution was extracted successively (50 mL each) with 5% $NaHSO_3$, (3×), sat. $KHCO_3$, and water, dried, and evaporated. The crystalline residue was triturated with acetone (50 mL), filtered, and dried in vacuo. Yield: 2.60 g, 94.4%. Mp: 165 °C. Anal. ($C_{12}H_{22}ClN_4O_5P$): C, H, Cl, N, P. FABMS: 383 (M, 80). ^{1}H NMR (DMSO-d_6): 6.48 br, 2H, and 6.19 s, 2H (NH_2); 4.58 m, 2H (P−OCH); 4.34 m, 2H (H-1′); 3.81 d, 2H, J(P,CH) = 8.4 (P−CH_2); 3.79 m, 2H (H-2′); 1.23 d, 6H, and 1.21 d, 6H J(CH_3,CH) = 6.1 (CH_3). ^{13}C NMR (DMSO-d_6): 163.74 (C-6); 160.75 (C-4); 159.89 (C-2); 83.00 (C-5); 70.82 d, J(P,C) = 11.7 (C-2′); 70.39 d, 2C, J(P,C) = 6.4 (P−O−C); 65.17 (C-1′); 65.16 d, J(P,C) = 164.1 (P−C); 24.02 d, 2C, J(P,C) = 3.9 and 23.89 d, 2C, J(P,C) = 4.4 (CH_3).

2,4-Diamino-6-[2-(diisopropoxyphosphorylmethoxy)-ethoxy]-5-iodopyrimidine (16a). *N*-Iodosuccinimide (2.22 g, 1.5 equiv) was added under stirring to the solution of compound **13** (2.5 g, 7.5 mmol) in acetonitrile (70 mL). After 90 min at ambient temperature, the reaction mixture was diluted with toluene (300 mL) and the solution was extracted successively (50 mL each) with 5% $NaHSO_3$ (3×), sat. aqueous $KHCO_3$, and water, dried, and evaporated. The crystalline residue was triturated with ether (50 mL), filtered, and dried in vacuo. Yield: 3.0 g, 87.3%. Mp: 151 °C. Anal. ($C_{12}H_{22}IN_4O_5P$): C,H,I,N,P. FABMS: 475 (MH^+, 90), 349 (M−I, 55), 253 (base, 100), ^{1}H NMR (DMSO-d_6): 6.20 br, 2H, and 6.13 s, 2H (NH_2); 4.54 m, 2H (P−OCH); 4.31 m, 2H (H-1′); 3.84 d, 2H, J(P,CH) = 8.3 (P−CH_2); 3.78 m, 2H (H-2′); 1.23 d, 6H, and 1.33 d, 6H J(CH3,CH) = 6.3 (CH_3). ^{13}C NMR (DMSO-d_6): 167.31 (C-6); 164.56 (C-4); 162.45 (C-2)] 70.88 d, J(P,C) = 12.2 (C-2′); 70.35 d, 2C, J(P,C) = 6.3 (P−O−C); 65.53 (C-1′); 65.36 d, J(P,C) = 164.1 (P−C); 43.06 (C-5); 24.03 d, 2C, J(P,C) = 3.9 and 23.96 d, 2C, J(P,C) = 3.9 (CH_3).

2,4-Diamino-5-bromo-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (14b). The diester **14a** (3.5 g, 8.5 mmol) in acetonitrile (50 mL) was treated with bromotrimethylsilane (7 mL) overnight, the volatiles were evaporated in vacuo, and the residue codistilled with acetonitrile (2 × 25 mL). Water (50 mL) was added and the solution was set to pH 10 by addition of concentrated aqueous NH_3. After 10 min at this pH, the solution was taken down and the residue was applied on a Dowex 50 × 8 column (H^+-form) (100 mL). The column was then washed with water to the drop of acidity of the eluate. The resin was then suspended in water (150 mL) and concentrated aqueous NH_3 was added to pH 10.0 with stirring. After 10 min at this pH the suspension was filtered, the resin washed repeatedly with water (total, 400 mL), and the filtrate was evaporated in vacuo. The residue in water (20 mL) was alkalified by concentrated aqueous NH_3 to pH 10 and applied onto a column (100 mL) of Dowex 1 × 2. The column was washed with water, until the UV-absorption dropped, and then with a linear gradient of acetic acid (0−1 M, 1 L each); the UV-absorbing fraction was taken down in vacuo, the residue was codistilled with water, and the crystalline residue was filtered from the water supension. The crystalline product was washed with ethanol and ether and dried in vacuo. Yield: 1.7 g, 58%. Mp: 218 °C. Anal. ($C_7H_{12}BrN_4O_5P$): C, H, Br, N, P. FABMS: 343 (MH^+, 90), 349 (M − I, 55), 253 (base, 100), ^{1}H NMR (D_2O + NaOD): 4.14 m, 2H (H-1′); 3.93 m, 2H (H-2′); 3.61 d, 2H, J(P,CH) = 8.4 (P−CH_2); ^{13}C NMR (D_2O + NaOD): 165.32 (C-6); 161.84 (C-4); 160.79 (C-2); 73.64 (C-5); 70.47 d, J(P,C) = 10.3 (C-2′); 69.29 d, J(P,C) = 149.4 (P−C); 66.50 (C-1′).

2,4-Diamino-5-chloro-6-[2-(phosphonomethoxy)ethoxy]-pyrimidine (15b) was prepared from the diester **15a** (2.1 g, 5.7 mmol) analogously as described for compound **14b**. Yield: 1.6 g, 63%. Mp: 233 °C. Anal. ($C_7H_{12}ClN_4O_5P$): C, H, Cl, N, P. FABMS: 293 (MH^+, 90), ^{1}H NMR (D_2O + NaOD): 4.43 m, 2H (H-1′); 3.92 m, 2H (H-2′); 3.58 d, 2H, J(P,CH) = 8.5 (P−CH_2); ^{13}C NMR (D_2O + NaOD): 164.33 (C-6); 161.03 (C-4); 160.05 (C-2); 85.51 (C-5); 70.48 d, J(P,C) = 10.3 (C-2′); 69.29 d, J(P,C) = 149.4 (P−C); 66.40 (C-1′).

Deprotection of 2,4-Diamino-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]-5-iodopyrimidine (16a) was performed from 2.25 g (6.1 mmol) of compound **16a** in essentially the same manner as described for compound **14b**. The UV-absorbing fraction from Dowex 1 chromatography was codistilled twice with water and the residue boiled briefly with water (100 mL). The supension was left to stand at ambient temperature overnight, filtered, washed with acetone and ether, and dried. Yield: 1.57 g, 74%. Mp: 263 °C. Anal. ($C_7H_{13}N_4O_5P$): C, H, N, P. FABMS: 265 (MH^+), ^{1}H NMR (D_2O + NaOD): 5.39 s, 1H (H-5); 4.25 m, 2H (H-1′); 3.90 m, 2H (H-2′); 3.58 d, 2H, J(P,CH) = 8.4 (P−CH_2); ^{13}C NMR (D_2O + NaOD): 170.77 (C-6); 166.61 (C-4); 162.71 (C-2); 76.82 (C-5); 70.44 d, J(P,C) = 10.2 (C-2′); 69.17 d, J(P,C) = 150.4 (P−C); 66.18 (C-1′).

Antiviral Activity Assays. The antiviral, other than anti-HIV-1, assays were based on inhibition of virus-induced cytopathicity in either E_6SM (HSV-1, HSV-2, VV) or HEL (VZV, CMV) cell cultures, following previously established procedures.[21] 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- to 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.

Inhibition of HIV-1-Induced Cytopathicity in CEM Cells. The methodology of the anti-HIV assays has been described previously.[22] Briefly, human CEM (~3×10^5 cells.mL^{-1}) cells were infected with 100 $CCID_{50}$ HIV-1 ((III_B)) or HIV-2 (ROD) per mL and seeded in 200 μL-wells of 96-well microtiter plates, 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.[22] Murine C3H/3T3 embryo fibroblast cells were seeded at 5×10^5 cells mL^{-1} into 1-cm^2 wells of 48-well microplates. 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[23]) 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.

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