Articles

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

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A series of dialkyl esters of purine and pyrimidine *N*-[2-(phosphonomethoxy)ethyl] derivatives substituted at position 2, 6, or 8 of the purine base or position 2, 4, or 5 of the pyrimidine base were prepared by alkylation of the appropriate heterocyclic base with 2-chloroethoxymethylphosphonate diester in the presence of sodium hydride, cesium carbonate, or 1,8-diazabicyclo-[5,4,0]undec-7-ene (DBU) in dimethylformamide. Additional derivatives were obtained by the transformations of the bases in the suitably modified intermediates bearing reactive functions at the base moiety. The diesters were converted to the corresponding monoesters by sodium azide treatment, while the free acids were obtained from the diester by successive treatment with bromotrimethylsilane and hydrolysis. None of the PME derivatives in the pyrimidine series, their 6-aza or 3-deaza analogues, exhibited any activity against DNA viruses or retroviruses tested, except for the 5-bromocytosine derivative. Substitution of the adenine ring in PMEA at position 2 by Cl, F, or OH group decreased the activity against all DNA viruses tested. PMEDAP was highly active against HSV-1, HSV-2, and VZV in the concentration range (EC_{50}) of 0.07−2 μg/mL. Also the 2-amino-6-chloropurine derivative was strongly active (EC_{50} = 0.1−0.4 μg/mL) against herpes simplex viruses and (EC_{50} = 0.006−0.3 μg/mL) against CMV and VZV. PMEG was the most active compound of the whole series against DNA viruses (EC_{50} ∼ 0.01−0.02 μg/mL), though it exhibited significant toxicity against the host cells. The base-modified compounds did not show any appreciable activity against DNA viruses except for 7-deazaPMEA (IC_{50} ∼ 7.5 μg/mL) against HIV-1 and MSV. The neutral (diisopropyl, diisooctyl) diesters of PMEA were active against CMV and VZV, while the corresponding monoesters were inactive. The diisopropyl ester of the 2-chloroadenine analogue of PMEA showed substantially (10−100×) higher activity against CMV and VZV than the parent phosphonate. Also, the diisopropyl and diisooctyl ester of PMEDAP inhibited CMV and VZV, but esterification of the phosphonate residue did not improve the activity against either MSV or HIV.

Introduction

9-[2-(Phosphonomethoxy)ethyl]adenine (**1**) (PMEA, CAS 106941-25-7 (registry numbers provided by author)) exhibits antiviral activity in vitro directed against some DNA viruses[1] as well as against retroviruses.[2] Its activity in the Moloney murine sarcoma virus model in vivo is comparable to—or in some aspects prevails over—that of AZT.[3] Also, diseases caused by other related mammalian retroviruses—murine acquired immunodeficiency disease,[4] FIV and FeLV,[5] Visna virus,[6] and simian immunodeficiency virus[3b,7]—were successfully treated with this drug. The in vitro activity against HIV-1 and HIV-2 as well as the expected therapeutic effect in AIDS-related viral diseases[8] predetermined this compound [Adefovir][9] and its bis(pivaloyloxymethyl) ester [Bis-(POM)-PMEA, Adefovir dipivoxil, Preveon] with an increased oral absorption and increased transport into the cell[10] for clinical studies against AIDS[11] and hepatitis B.[12] Molecular biological investigation[13] demonstrated phosphorylation of PMEA to its mono- and diphosphate (analogues of nucleoside di- and triphosphates) catalyzed by nucleotide kinases[14] or 5-phosphoribosyl 1-pyrophosphate synthetase;[15] the diphosphate (PMEApp) is inhibitory to cellular DNA polymerases.[16] In addition, it acts as a substrate/inhibitor for reverse transcriptases[17] and its incorporation into the growing DNA chain results in chain termination. Adefovir also exhibits cytostatic activity in rat and mouse carcinomas and sarcomas.[18] Moreover, it has significant immunomodulatory and tumor cell differentiating properties.[19]

Structure−biological activity 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 at the position 2 by a hydroxymethyl group led to three novel structural types of potent antivirals: (*S*)-

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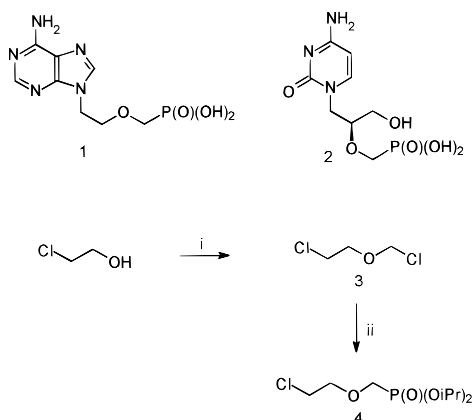
Scheme 1[a]



[a] Reagents and conditions: (i) $(CH_2O)_3$/HCl; (ii) $P(OiPr)_3$, 100 °C.

3-hydroxy-2-phosphonomethoxypropyl (HPMP) compounds with anti-DNA-viral activity, comprising among others the adenine derivative HPMPA (CAS 92999-29-6),[1,2,20] its guanine counterpart HPMPG,[21] and the cytosine derivative HPMPC (**2**) (CAS 113852-37-2, Cidofovir)[22]—an active constituent of Vistide which has been approved for treatment of CMV retinitis in AIDS patients.[23] Cidofovir is also efficacious in the therapy of acyclovir- and foscarnet-resistant HSV-1 lesions and in other viral diseases, e.g., papillomavirus-induced warts, nasopharyngeal carcinoma, etc.[24] Introduction of a fluoromethyl group at the side chain results in (*S*)-(3-fluoro-2-phosphonomethoxypropyl) (FPMP) derivatives with prominent antiretroviral activity.[5d,25] Finally, substitution at the position 2 of the side chain by a methyl group affords PMP [(*R*)-2-phosphonomethoxypropyl] derivatives with very high potency against HIV-1 and HIV-2 in vitro[26] and with therapeutic and prophylactic activity in the simian model of immunodeficiency disease.[27] Interestingly, any other substitution at this position of the side chain results in total loss of antiviral activity.[28]

We have systematically investigated the relation between the structure of the heterocyclic base and the antiviral activity of PME derivatives: our early papers define the pharmacophore as 9-substituted purine derivatives bearing an amino group at the position 2 or 6 of the base. All other purine as well as all pyrimidine (thymine, uracil, cytosine) derivatives in the PME series are inactive.[1b,2a,29] Thus, while the 2-aminopurine derivative manifests a modest in vitro antiviral activity, the antiviral potency of the 2,6-diaminopurine (PMEDAP) and guanine (PMEG) derivatives is similar or higher than that of PMEA. PMEDAP is highly active in vivo against mammalian retroviruses,[30] whereas the interest in PMEG concentrates chiefly on its effect upon papillomaviruses[31] and/or its anticancer activity.[32] These phosphonates are also phosphorylated by cellular nucleotide kinases.[29b,33] In addition to the action of their diphosphates upon DNA polymerases,[16] PMEG and its anabolites efficiently inhibit purine nucleoside phosphorylases.[34] Disturbances in the cellular purine nucleoside pool due to this inhibition may explain the high cytotoxicity of the guanine derivative.

Studies of aza- and deazapurine analogues derived from PMEA and its base-modified congeners showed that the 8-aza derivatives preserve their antiviral effect,[35] while the activity in the deazapurine series is limited to the 3-deazaadenine derivative only.[36] The present paper describes synthesis and antiviral activity of additional *N*-[2-(phosphonomethoxy)ethyl] derivatives substituted and/or modified at the pyrimidine or purine base.

Chemistry

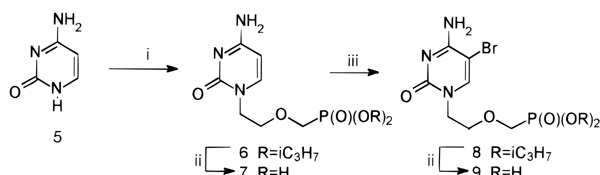
There are two approaches for the preparation of the *N*-[2-(phosphonomethoxy)ethyl] derivatives of heterocyclic bases: condensation of the appropriate *N*-(2-hydroxyethyl) derivatives with dialkyl *p*-toluenesulfonyloxymethanephosphonates in the presence of sodium or lithium hydride or alkylation of the heterocyclic base with an appropriate organophosphorus synthon bearing the features of the side chain, in the presence of sodium hydride, potassium carbonate, or preferentially, cesium carbonate. In the present study, we are using mainly the latter approach following the conditions described in our earlier papers.[37] The synthon **4** used for the purpose is dialkyl 2-chloromethoxyethanephosphonate. However, we are using the isopropyl ester group for protection of the phosphonate residue instead of the ethyl group used in our earlier studies. This alteration minimizes the formation of *N*-ethyl derivatives of the bases which often takes place at higher reaction temperatures or prolonged reaction times.[37]

The required synthon **4** is easily available from 2-chloroethanol: its reaction with 1,3,5-trioxane/HCl gives 2-chloroethyl chloromethyl ether (**3**) which is transformed to the compound **4** by the reaction with triisopropyl phosphite (Scheme 1). This procedure can be easily performed on a large scale, and the resulting synthon **4** is very stable under ordinary conditions. The regioisomeric diester intermediates which are formed during the alkylations with this synthon can be easily purified by silica gel chromatography or crystallization. They were converted to the free phosphonic acids by transsilylation with bromotrimethylsilane followed by alkaline hydrolysis of the intermediary bis(trimethylsilyl) ester.[37] Additional acyclic nucleotide analogues of the PME-type which are described in this paper were prepared by transformations (halogenation, transformation of halogeno derivatives, and/or deamination reactions of the heterocyclic bases) of the appropriate *N*-[2-(diisopropylphosphonylmethoxy)ethyl] intermediates or the free 2-(phosphonomethoxy)ethyl derivatives.

Pyrimidine Derivatives. Our first synthesis of the cytosine derivative **7** was achieved by the alkylation of N^4-benzoylcytosine, followed by methanolysis and transsilylation.[37] However, direct alkylation of cytosine with synthon **4** in the presence of cesium carbonate affords the required diester **6** directly in a fair yield. The treatment of this compound with bromotrimethylsilane followed by hydrolysis yielded the free phosphonate **7** (PMEC), which was isolated by ion exchange chromatography. The reaction of the intermediate **6** with bromine in dimethylformamide (DMF)/carbon tetrachloride gave 5-bromocytosine derivative **8** which was also subsequently converted to the free phosphonate **9** (Scheme 2).

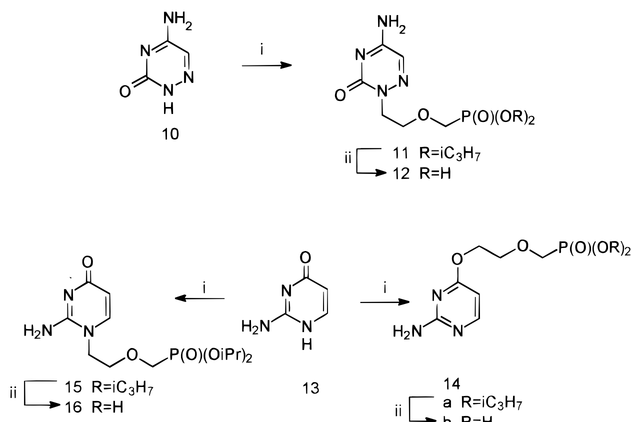
The related 6-azacytosine (**10**) gave, in the alkylation reaction with synthon **4** in the presence of Cs_2CO_3, a single regioisomer **11** which, in turn, gave the free phosphonate **12** (Scheme 3). In contrast to the alkylation

Scheme 2[a]



[a] Reagents and conditions: (i) **4**/Cs_2CO_3; (ii) TMSBr/CH_3CN, H_2O; (iii) Br_2/DMF/CCl_4.

Scheme 3[a]

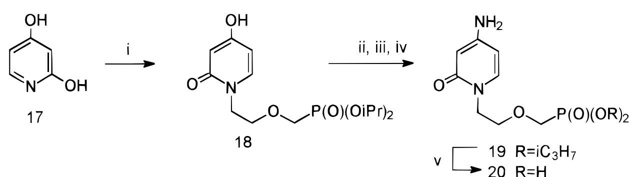


[a] Reagents and conditions: (i) **4**/Cs_2CO_3/DMF; (ii) TMSBr/acetonitrile.

of cytosine and its 6-aza analogue, isocytosine (2-amino-4-hydroxypyrimidine, **13**) afforded, on treatment with synthon **4** and Cs_2CO_3, followed by the standard bromotrimethylsilane procedure, a mixture of the desired N^1-isomer **16** and the O^4-substituted side product **14b** with characteristic UV and ^{13}C NMR spectra: while the chemical shift of C-1′ in compound **16** (d 44.34) clearly corresponds to N^1-substitution, the significant low-field shift of C-1′ in compound **14b** (d 66.10) and the observed alkylation shifts of the carbon atoms of the base indicate the linkage of the side chain to oxygen atom O^2 (Scheme 3).

We have also prepared the deaza analogue of PMEC (**8**), 4-amino-1-[2-(phosphonomethoxy)ethyl]pyridin-2-one (**20**), by the sequence depicted in Scheme 4: 2,4-dihydroxypyridine (**17**) was first transformed to the 1-substituted diester **18** by treatment with synthon **4** in the presence of Cs_2CO_3. The intermediate **18** gave, on successive treatment with $POCl_3$/triazole and ammonolysis, the compound **19,** which ultimately afforded the free phosphonate **20** (PME-3-deazaC) by the trans-silylation reaction.

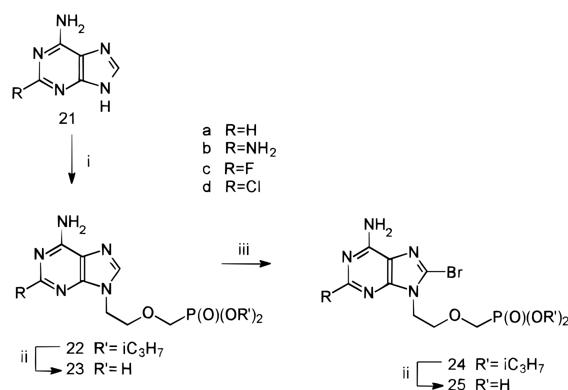
Scheme 4[a]



[a] Reagents and conditions: (i) **4**/Cs_2CO_3/DMF; (ii) $POCl_3$/pyridine; (iii) triazole/pyridine; (iv) aq NH_3; (v) TMSBr/acetonitrile; H_2O.

Purine Derivatives. Several variants of adenine alkylation with the synthon **4** or its analogues were already described in our earlier papers.[37,38] While the use of adenine sodium salt is restricted by its limited solubility in dimethylformamide, the application of potassium carbonate as a base requires a higher reaction temperature (boiling dimethylformamide); the most convenient procedure is its replacement by cesium carbonate. However, this rather expensive reagent must be used in equimolar quantity. Furthermore, a possible contamination of the products with cesium ions might be undesirable for compounds targeted at biological systems. Therefore, we have examined the possibility of using hindered tertiary organic amines as the bases in the alkylation of adenine by synthons of the type **4** performed in dimethylformamide at temperatures around 100 °C. While no reaction took place with equimolar amounts of diisopropylethylamine (Hünig base), 1,8-bis-(dimethylamino)naphthalene (proton sponge), and/or DABCO (1,4-diazabicyclo[2.2.2]octane) under these conditions, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) proved to be the reagent of choice. The preheated mixture of equimolar amounts of adenine and DBU in dimethylformamide reacted smoothly with synthon **4** and afforded the expected diester **22a** with only a minor impurity of its (tentatively) N^3-isomer. This reaction proceeds faster than the same process mediated by Cs_2CO_3 and reaches an equilibrium after 4−5 h at 100 °C. After the evaporation of the solvent, the salts can be extracted from organic solutions by water and the diisopropyl ester of PMEA (**22a**) isolated by silica gel chromatography. As the overall yield of this product is comparable with those obtained by the alternate procedures mentioned, this process variant offers unequivocal advantages.[39] The subsequent cleavage of diester **22a** with bromotrimethylsilane, hydrolysis, and deionization afforded PMEA (**1, 23a**) identical with an authentic preparation (Scheme 5).

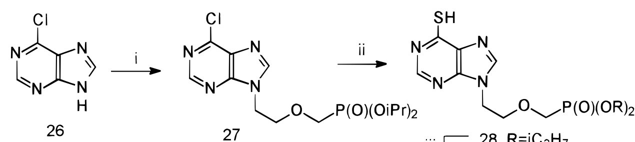
Scheme 5[a]



[a] Reagents and conditions: (i) **4**/Cs_2CO_3, NaH or DBU in DMF; (ii) TMSBr/CH_3CN, H_2O; (iii) Br_2/DMF/CCl_4.

The 2,6-diaminopurine analogue (PMEDAP, **23b**) was also synthesized by the same procedure (Scheme 5), using both Cs_2CO_3 and DBU as a base. Though the yield of the diester intermediate **22b** gained by the latter variant was slightly inferior to the former one [probably due to the extremely low solubility of 2,6-diaminopurine (**21b**) in DMF], the short duration and easy workup also makes, in this case, the DBU variant competitive with the others. The diester **22b** was converted by the standard procedure to PMEDAP (**23b**) which was

Scheme 6[a]



[a] Reagents and conditions: (i) **4**/NaH/DMF; (ii) $H_2NC(S)NH_2$; (iii) TMSBr/CH_3CN, H_2O.

identified by the usual parameters and by comparison with the authentic sample.

Several bioactive nucleosides originate from 2-chloroadenine (**21d**): the 2-deoxy-β-D-riboside (Cladribine) is widely used in cancer treatment; the 2-fluoroadenine (**21c**) pharmacophore is present in the anticancer β-D-arabinoside (Fludarabine) and its 5′-phosphate. To investigate the influence of the substitution at the position 2 of the purine ring on the biological activity, we have also synthesized the appropriate 2-halogeno derivatives of PMEA—**23c** and **23d**. In these cases, the Cs_2CO_3 variant afforded satisfactory yields of the 2-fluoro- (**22c**) and 2-chloroadenine intermediates (**22d**) which were isolated, fully characterized by NMR, and finally converted by the bromotrimethylsilane (TMSBr) procedure to the free phosphonates **23c** and **23d** (Scheme 5). The bromination of the phosphonate diesters derived from adenine and 2,6-diaminopurine (**22a,b**) followed by diester cleavage gave the PME derivatives of 8-bromoadenine (**24a**) and 8-bromo-2,6-diaminopurine (**24b**). These compounds were (similar to the 5-bromocytosine derivative **9)** used also for the preparation of tritium (8-[3H])-labeled PME derivatives.

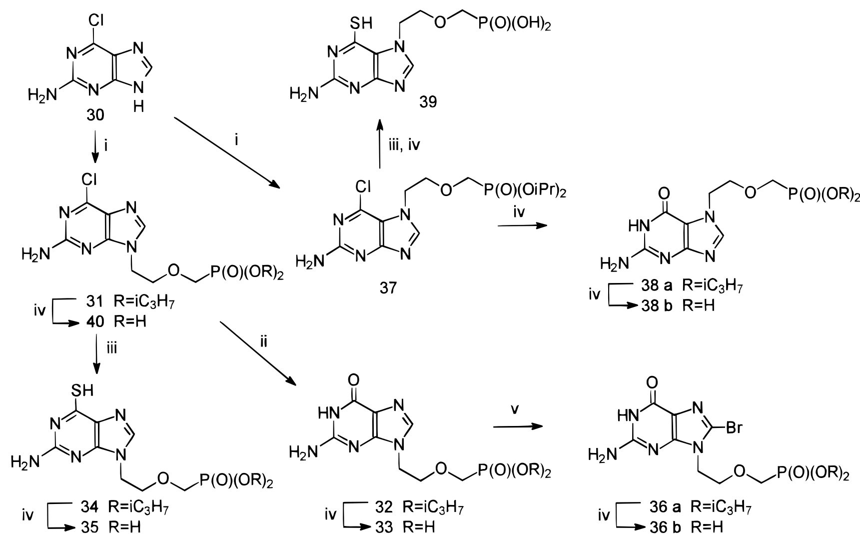
6-Chloropurine (**26**) gave, on treatment with NaH in dimethylformamide at 0 °C, the solution of its sodium salt. On treatment with synthon **4** at elevated temperature it is smoothly converted to the N^9-isomer **27** described earlier[37,40] (the same compound was obtained as the only product in the Cs_2CO_3 variant of the reaction). Reflux with thiourea in ethanol solution gave the 6-mercaptopurine diester **28** which was ultimately converted to the free phosphonate **29** (Scheme 6).

Owing to the extreme insolubility of guanine, direct alkylations of this base are difficult. This obstacle is usually circumvented by the use of well soluble 2-amino-6-chloropurine or 6-alkoxy-2-aminopurines and subsequent transformations of the 6-substituents. In contrast to the regioselective reaction of 6-chloropurine with synthon **4** which proceeds nearly exclusively at the position N^9 both with NaH and with Cs_2CO_3, 2-amino-6-chloropurine (**30**) affords, on treatment by this reagent either with the preformed sodium salt or in the presence of Cs_2CO_3, the mixture of 9-isomer **31** and 7-isomer **37** in which the 9-isomer strongly predominates (Scheme 7).[40] Both compounds were easily separated by silica gel chromatography and characterized by analysis and NMR spectra. In this case, the use of DBU in the alkylation reaction offers many advantages compared to the standard procedures: it proceeds much faster and significantly favors the formation of the 9-isomer **31.** The overall yield is slightly better or comparable with the former procedures, the workup is simpler and, eventually, pure **31** can be obtained from the crude reaction mixture by crystallization.

The hydrolysis of the diester **31** and **37**, either by K_2CO_3 in the presence of DABCO[41] or, preferentially, by brief reflux in 1 M HCl, gave smoothly the diisopropyl ester of PMEG (**32**) which was then easily converted to PMEG (**33**). Similarly, the 7-isomer **37** afforded the 7-isomer of PMEG, compound **38.** Both compounds **33** and **38** were identical with the authentic samples by HPLC, and also their ^1H NMR spectra corresponded to those of the reference materials.[37]

Treatment of the diester **31** with thiourea afforded 2-amino-6-mercaptopurine intermediate **34** which was deprotected to the free phosphonate **35** (6-thio-PMEG). Analogously, treatment of the 7-isomer **37** with thiourea and subsequent deprotection led to the 7-isomer **39**. The structure of all these compounds was verified by the standard methods (NMR, MS, analysis). The 8-bromo derivative of PMEG, compound **36b**, was synthesized from the diester (**32**) by bromination in DMF/CCl_4 followed by transsilylation of the 8-bromo intermediate **36a**. We have also attempted the preparation of 2-amino-

Scheme 7[a]

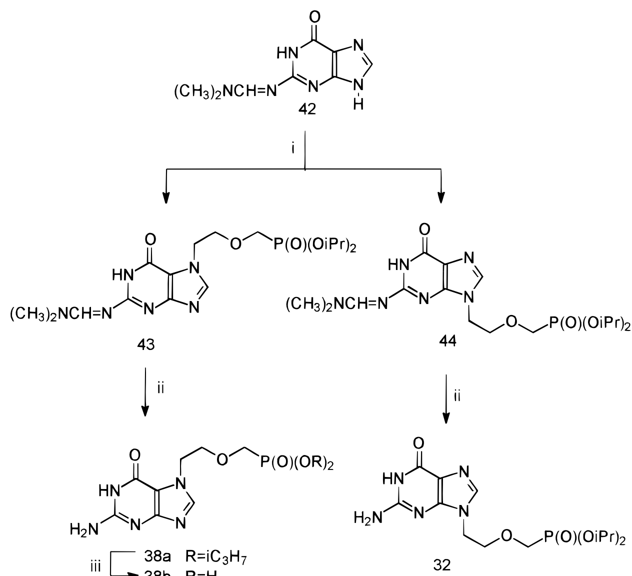


[a] Reagents and conditions: (i) **4**/Cs_2CO_3 or NaH or DBU in DMF; (ii) HCl/H_2O; (iii) H_2NCSNH_2/C_2H_5OH; (iv) TMSBr in CH_3CN; (v) Br_2 in DMF/CCl_4.

6-chloro-9-[2-(phosphonomethoxy)ethyl]purine **40** by the cleavage of ester linkages in the diester **31**. Despite the comparatively high reactivity of the C−Cl linkage in 6-chloropurine derivatives, careful workup of the reaction mixture after transsilylation afforded the free acid **40** which was, according to HPLC, only slightly contaminated with PMEG and gave expected MS and NMR characteristics (Scheme 7). Finally, the diaminopurine derivative **23b** was converted by the action of isoamyl nitrite in 80% acetic acid to 2-hydroxy-9-[2-(phosphonomethoxy)ethyl]adenine diester (**41a**) which gave by deprotection the phosphonate **41b**.

We have also examined the possibility of replacing guanine in the alkylation by synthon **4** in the presence of DBU by well soluble and easily available *N*²-dimethylaminomethyleneguanine (**42**). However, this reaction affords the 7-isomer **43** as the main product. The guanine derivative **38a** which arose by alkaline hydrolysis was finally transformed to 7-PMEG (**38b**) (Scheme 8).

Scheme 8[a]



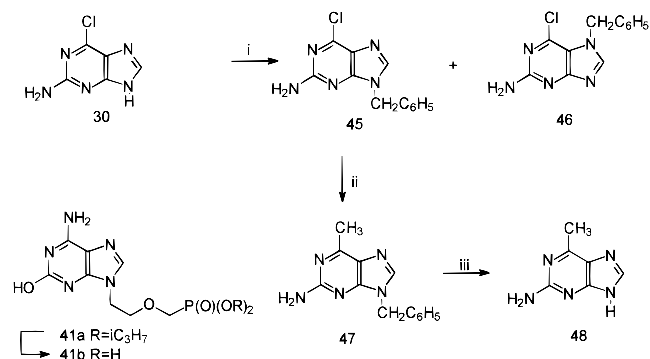
[a] Reagents and conditions: (i) **4**/DBU in DMF; (ii) NH_3/H_2O; (iii) $TMSBr/CH_3CN$.

The desired 9-isomer **44** which was present in the alkylation mixture as a minor component (ratio **43:44** ~ 6:1) was converted to the compound **32** described above by treatment with aqueous ammonia. Thus, compound **42** can be recommended for N⁷-alkylations of guanine only. (A similar change of regiospecificity was observed in *N*⁶-(dimethylaminomethylene)adenine which gives exclusively the *N*⁷-alkyl derivatives in contrast to the predominant N⁹-alkylations of adenine.[42])

In our earlier papers we have demonstrated the antiviral activity of 2-amino-9-[2-(phosphonomethoxy)ethyl)-purine (**51**). This compound can be prepared from the easily available 2-amino-6-chloropurine intermediate **31** by catalytic hydrogenation followed by transsilylation.

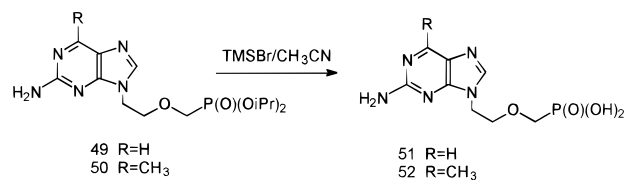
As a part of the structure−activity relationship investigation we also investigated the influence of the 6-alkyl substitution at the purine ring in the 2-amino-6-methylpurine derivative **50b.** The starting 9-benzyl derivative **45** was obtained by benzylation of 2-amino-6-chloropurine (**30**) together with its 7-isomer **46.** Palladium-catalyzed methylation of compound **45** with trimethylaluminum[43] gave compound **47** which was transformed to 2-amino-6-methylpurine (**48**) by palladium-catalyzed hydrogenolysis (Scheme 9). Treatment of this base with synthon **4** in the presence of Cs_2CO_3 afforded the diester intermediate **50** and the free phosphonate **52** as the final product (Scheme 10).

Scheme 9[a]



[a] Reagents and conditions: (i) $NaH/C_6H_5CH_2Br$; (ii) $Me_3Al/THF/Pd(0)$; (iii) $H_2/Pd/HCl$.

Scheme 10

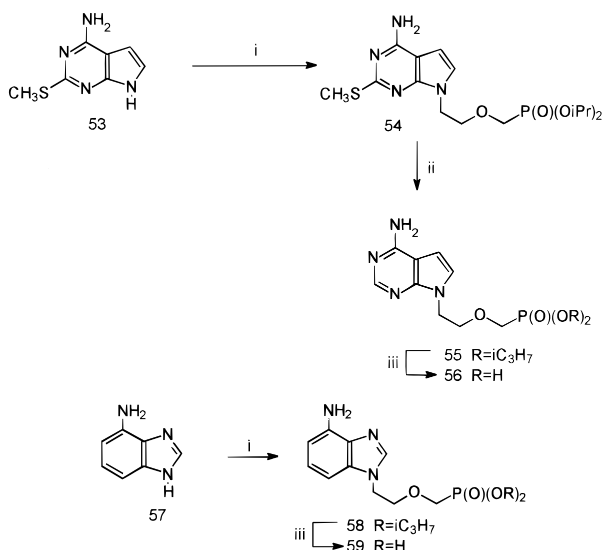


Deazapurine Derivatives and Related Compounds. The presence of nitrogen atoms at different positions of the purine system plays an important role in the interaction with numerous enzymes. Thus, the nitrogen atom N⁷ in the imidazole part of the purine ring frequently plays a key role in the biological activity of purine nucleoside analogues: Derivatives of tubercidine and related pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics display a broad spectrum of biological effects.[44] We have synthesized the 7-deazaadenine derivative **56** by the reaction sequence depicted in Scheme 11: 4-Amino-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**53**) gave, by alkylation with synthon **4** in the presence of Cs_2CO_3, compound **54** which was subsequently desulfurized with Raney-Ni to the diester **55**. Treatment of this compound with bromotrimethylsilane followed by hydrolysis led to 7-deaza-PMEA (**56**).

4-Aminobenzimidazole [1-(3)*H*-benzoimidazol-4-ylamine] (**57**) can be formally regarded as 6-amino-1,3-dideazapurine. It was prepared by modification of described procedures and converted to the phosphonate **59** by the standard alkylation procedure via diester **58** (Scheme 11).

In our earlier papers we have already described the synthesis and antiviral properties of 9-[2-(phosphonomethoxy)ethyl] derivatives of both 1-deaza- and 3-deazaadenine.[36] This series is now completed by PME-compounds derived from 1-deaza and 3-deaza-2,3-diaminopurines. 1-Deaza-2,6-diaminopurine (**60a**) and 3-deaza-2,6-diaminopurine (**60b**) were prepared by modification of procedures described in the literature (vide

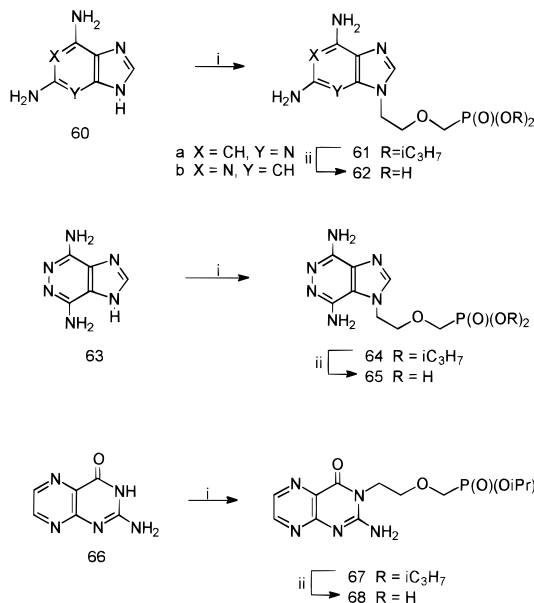
Scheme 11[a]



[a] Reagents and conditions: (i) **4**/Cs_2CO_3 in DMF; (ii) Raney-Ni; (iii) TMSBr in CH_3CN.

infra) and alkylated by synthon **4** in the presence of Cs_2CO_3. In both cases, the 9-isomers (**61**) which were isolated as the main products were converted to the free phosphonates **62** by transsilylation (Scheme 12).

Scheme 12[a]



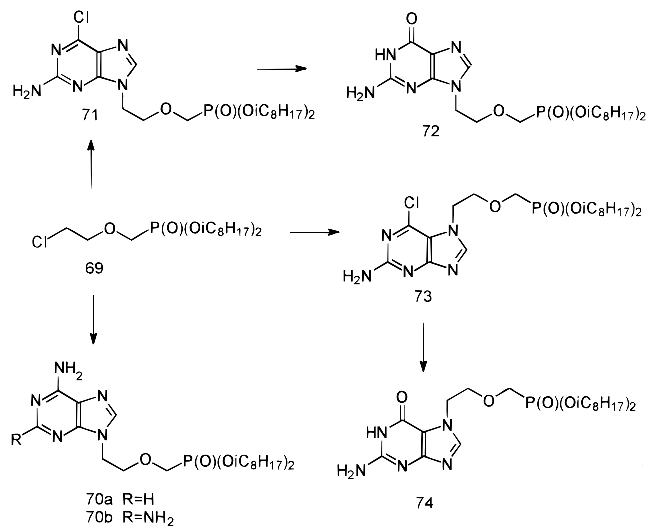
[a] Reagents and conditions: (i) **4**/Cs_2CO_3 in DMF; (ii) $BrSiMe_3$ in CH_3CN.

The obvious similarity of the imidazo[4,5-*d*]pyrazine ring to the purine system led us to synthesize also the diaminopurine analogue **65**. The procedure started from 4,7-diaminoimidazo-[4,5-*d*]pyrazine (**63**) and proceeded by alkylation with synthon **4** in the presence of Cs_2CO_3 analogously as in purine alkylations. The diester **64** which was obtained as the only reaction product was transformed to the free phosphonate **65,** an isomer of PMEDAP, by transsilylation (Scheme 12).

2-Amino-4-hydroxypteridine (**66**) could be considered an analogue of guanine. The alkylation of this base was directed to the position N^3 and gave compound **67** which was then converted to the free phosphonate **68**. Contrary to the glycosylation reactions,[45] no other regioisomers were identified among the alkylation products (Scheme 12).

Synthesis of Hydrophobic Ester Derivatives of Selected Acyclic Nucleotide Analogues. Our preliminary data demonstrated the occurrence of antiviral activity not only in free phosphonates but in some cases in their monoester and to a certain extent also in their diester. This fact witnesses that such compounds (a) adhere and/or penetrate through cellular membrane and (b) are evidently substrates for nucleolytic enzymes which are present there or in the cytoplasm. The interaction with the membrane could be enhanced by hydrophobicity of the compound. Therefore, we have also prepared and involved in this study the di(isooctyl)-ester of selected representatives of biologically active acyclic nucleoside phosphonates: PMEA (**23a**), PMEDAP (**23b**), PMEG (**33**), and its 7-isomer **38**. The synthetic routes were similar to those described above: the required synthon **69** was obtained from tri(isooctyl)-phosphite and 2-(chloroethyl) chloromethyl ether. Its reaction with adenine, 2,6-diaminopurine, or 2-amino-6-chloropurine in the presence of cesium carbonate gave the required diisooctyl esters **70, 71**, and **73**. The isomeric 2-amino-6-chloropurine derivatives **71** and **73** were further transformed to isomeric 9- and 7-PMEG diisooctyl ester (**72** and **74**, respectively) (Scheme 13).

Scheme 13



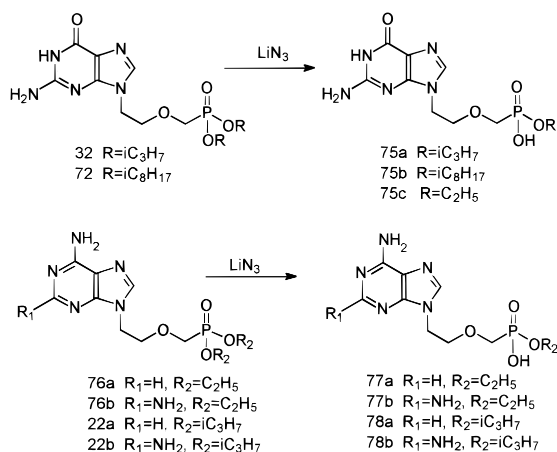
All compounds were easily purified by silica gel chromatography; their extremely hydrophobic properties are reflected by their waxy character.

We have also synthesized the isopropyl **75a** and isooctyl ester of PMEG **75b** by the specific cleavage of their diesters **32** and **72** with lithium azide in DMF. The products were isolated by ion exchange chromatography. This method was recently described[46] also for the preparation of the ethyl (**77**) and isopropyl (**78**) esters of PMEA and PMEDAP from their diethyl (**76**) and diisopropyl esters (**22**) (Scheme 14).

Biological Activity

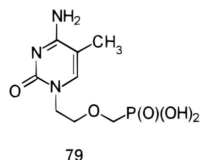
The free nucleotide analogues as well as their mono- and diesters were evaluated for their inhibitory effect on the DNA viruses herpes simplex virus type 1 (HSV-

Scheme 14



1), HSV-2, cytomegalovirus (CMV), varicella-zoster virus (VZV), and vaccinia virus (Table 1) and against the retroviruses Moloney murine sarcoma virus (MSV) and human immunodeficiency virus type 1 (HIV-1) and HIV-2 (Table 2).

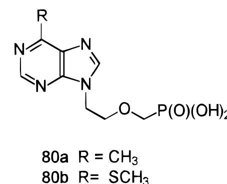
In the pyrimidine series, none of the earlier reported PME derivatives (cytosine, uracil, thymine, or 5-methylcytosine derivative **79**) exhibited any activity against DNA viruses.[1b] This fact was repeatedly confirmed for the cytosine derivative **7**. However, the 5-bromocytosine derivative **9** showed a moderate activity against both



TK^+ and TK^- VZV strains (3−8 μg/mL), while its effect on HSV-1, the thymidine kinase (TK^-) deficient HSV-1 strain VMW1837, HSV-2, and CMV was only marginal (20−40 μg/mL). Other structural alterations including −CH= → −N= replacement in 6-azacytosine derivative **12**, or *vice versa* −N= → −CH= replacement in the 3-deazacytosine derivative **20**, resulted in inactive compounds. Interestingly, the isocytosine derivative **16** which is structurally related to the pyrimidine part of the guanine system in PMEG (**33**) also lacks any antiviral effect.

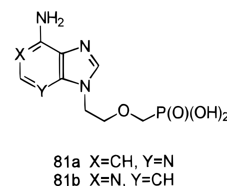
The structure−activity studies in the purine acyclic nucleotide series comprise alterations of the heterocyclic base in three major lead structures PMEA (**23a**), PMEDAP (**23b**), and PMEG (**33**). Substitution of the adenine ring by chlorine or fluorine atoms at the position 2 in compounds **23c,d** as well as the introduction of a hydroxyl group at this position (**41b**) decreases the antiviral activity against all DNA viruses tested. In our earlier papers on PME derivatives we have reported that the hypoxanthine derivative is devoid of activity against DNA viruses.[1b] Replacement of the oxygen atom at position 6 by sulfur in compound **29** does not result in an antiviral effect. Neither the introduction of methyl (**80a**) nor methylthio groups (**80b**) at position 6 of the purine moiety in the compounds prepared earlier[2a,13a,22] affords such an antiviral activity.

2-Aminopurine derivative **51** (PMEMAP) is a parent structure of another series: it has marginal activity on



its own (EC_{50} between 20 and 150 μg/mL) against HSV-1 and VZV. Its 6-methyl congener **52** is devoid of any activity against DNA viruses. However, the introduction of a 6-amino function affords the antiviral molecule PMEDAP (**23b**) which is highly active against herpes viruses within the concentration (EC_{50}) ranges of 0.07−2 μg/mL for HSV-1 and HSV-2 and 1−3 μg/mL for VZV and which is less active (EC_{50} = 10 μg/mL) against CMV. Much more pronounced activity (EC_{50} of 0.1−0.4 μg/mL against herpes simplex viruses and between 0.006 and 0.3 μg/mL against CMV and VZV) was encountered with 2-amino-6-chloropurine derivative **40.** However, it cannot be excluded that this effect could be interpreted by its partial conversion to the guanine derivative PMEG (**33**) in the host cell. Such conversions have been observed in other types of 2-amino-6-chloropurine derivatives,[47] and PMEG is the most active compound of the whole series against DNA viruses (EC_{50} ∼ 0.01−0.02 μg/mL), though it exhibits significant toxicity against the host cells. Replacement of the 6-oxo by a 6-thioxo group affords the 6-thioguanine derivative **35** with lower but marked activity against herpes viruses, particularly evident for VZV (EC_{50} ∼ 0.01−0.02 μg/mL). The introduction of a bromine atom at the position 8 diminishes the antiviral effect of PMEA, PMEDAP, and PMEG: whereas a moderate activity was still encountered with the 8-bromo-2,6-diaminopurine derivative **25b** (5−12 μg/mL against VZV and CMV, ∼20 μg/mL against herpes simplex viruses) and with the 8-bromoguanine derivative (**36b**), the 8-bromoadenine derivative **25a** was virtually inactive (>50 μg/mL for VZV).

The purine base in the PME derivatives can be modified by replacements of −CH= vs −N= groups. During our studies we have thoroughly investigated these structural alterations in three principal structural types derived from adenine, 2,6-diaminopurine, and guanine. The replacement of the nitrogen atom at the position 1 of the six-membered ring of adenine by a methine group resulted in the inactive 1-deazaadenine derivative **81a**.[36] Also the 2,6-diaminopurine analogue **62a** described in this paper had no appreciable antiviral activity. The situation is different for the 3-deaza analogues: while the 3-deaza counterpart of PMEA (**81b**)[36] still has no marked antiviral activity in the

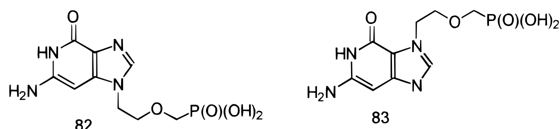


systems studied and the activity of its PMEDAP congener **62b** against the herpes simplex viruses can hardly be recognized, the 3-deazaguanine analogue of PMEG (**82**)[36] is endowed with substantial antiviral potency; as

in PMEG (**33**) it is directed against HSV-1, HSV-2, VZV, and CMV. Obviously, the order of activity PMEG > PMEDAP > PMEA also applies for the 3-deazapurine series. It should be noted that the 3-deaza analogue of the structurally closely related antiviral compound 9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (HPMPA) is comparable with its parent compound.[36a] As expected, the exchange of both nitrogen atoms in the pyrimidine part of adenine in PMEA (compound **59**) resulted in a complete loss of activity against herpes viruses. From the 7-deazapurine series we have only studied compound **56;** its activity was only marginal, unlike its adenine counterpart PMEA (**23a**).

In the course of the base modification, we have also prepared PME derivatives containing nonpurine systems structurally closely related to 2,6-diaminopurine and guanine: while the isomer of PMEDAP, compound **65**, did not show any appreciable activity against the DNA viruses tested, the guanine-related pterine derivative **68** was endowed with marginal activity against herpes simplex viruses ($EC_{50} \sim 70-150$ μg/mL) and a moderate effect ($EC_{50} \sim 8-30$ μg/mL) against VZV.

The antiviral activity in the PME series is strictly regiospecific: all antivirally active purine derivatives bear the acyclic chain at the N^9-position. In contrast to PMEG (**33**) and its 6-thio analogue **35**, the 7-isomer of PMEG (**38b**) and its 6-thio derivative (**39**) are inactive against all DNA viruses tested. Also, the above-mentioned activity of the 3-deaza analogue **82** is lost in its 7-regioisomer **83**.



It ought to be mentioned that, in contrast to herpes viruses, vaccinia virus was not sensitive to the PME derivatives that were examined in this study. A notable effect was recorded only for PMEDAP (**23b**) and PMEG (**33**); even in those cases, the efficacy was 1−2 orders of magnitude lower (20 μg/mL or 2 μg/mL, respectively) compared to the activity of these compounds against herpes viruses.

Our present study of the mono- and diesters of the acyclic nucleoside phosphonates is a continuation of our earlier work.[48] It focuses again on selected prototypes of the antivirally active compounds PMEA, PMEDAP, and PMEG and the effect of esterification with an ethyl, isopropyl, and isobutyl group on their antiviral activity. In the series of PMEA derivatives, the monoesterification of the parent compound (**23a**) by either the ethyl (**77a**) or the isopropyl group (**78a**) abolished the activity. On the other hand, neutral diesters are active against CMV and VZV: diisopropyl ester **22a** in the range of 8−40 μg/mL and the lipophilic diisooctyl ester **70a** at $EC_{50} \sim 1-3$ μg/mL. Unlike the parent PMEA (**23a**) they were inactive against herpes simplex viruses. In the group of PMEDAP ester derivatives, the ethyl ester **77b** shows activity against VZV ($EC_{50} \sim 2-6$ μg/mL) comparable to that of the parent compound **23b**. The ester **77b** does not affect either herpes simplex viruses or CMV. However, the isopropyl ester **78b** is devoid of activity against DNA viruses. PMEDAP diethyl ester (**76b**) is inactive, while the diisopropyl (**22b**) and diisooctyl ester (**70b)** inhibit CMV ($EC_{50} \sim 0.8-2$ μg/mL) and VZV (strain YS) ($EC_{50} \sim 36$ or 3.2 μg/mL, respectively). Owing to the extremely potent antiviral activity of the parent compound, both mono- and diesters of PMEG are endowed with a strong antiviral activity: in the monoester series it declines in the order of ethyl (**75c**) > isopropyl (**75a**) ≫ isooctyl (**75b**). Compared to the activity of the first two monoesters, which is comparable to or at the worst 1 order of magnitude lower than that of the parent compound, the effect of the lipophilic monoester **75b** is marginal and only targeted to VZV. In the diester group we have compared the diisopropyl ester **32** with the diisooctyl ester **72:** the antiviral effect is several orders of magnitude lower compared to the parent nucleotide **33** and expressed predominantly against CMV and, to a minor extent, VZV. Generally, the esterification deteriorates the antiviral activity in the PMEG series. Evidently, the antiviral effect of the esters derived from acyclic nucleoside phosphonate analogues is a combination of structural parameters governing their penetration through the cell membrane, their intracellular cleavage to the free phosphonate molecule, and the activation of the latter to the diphosphate metabolites. In the monoester series, the permeation will be affected simultaneously by the polar character of the molecule and the hydrophobicity of the ester group. It is not clear which enzymes are responsible for the hydrolysis of the ester functions nor what is their base specificity. However, we know that the parent compounds can be activated by different nucleotide kinases.[14,33] Therefore, we compared the activity of other pairs of base-substituted phosphonates with their diesters: The most impressive improvement due to the esterification was encountered in 2-chloroadenine derivatives, where the diisopropyl ester **22d** showed substantially (10−100×) higher activity compared to the parent phosphonate **23d** against CMV ($EC_{50} = 0.7$ vs 88 μg/mL) and VZV ($EC_{50} = 0.5-3.7$ vs 19−40 μg/mL). However, no additional improvements of this sort were observed in those cases where the parent phosphonate itself was devoid of any antiviral activity (derivatives of 6-azacytosine **11** and **12** or 6-thiopurine **28** and **29**) or even the opposite (i.e., 8-bromoguanine **36a** and **36b**, 8-bromo-2,6-diaminopurine **24b** and **25b**). Most strikingly in the 2-amino-6-chloropurine derivatives, the diisopropyl ester **31** proved considerably less active compared to the free phosphonate **40.** It is plausible that in those cases where the free acids are comparatively easily transported through the membrane, the esterification can hardly improve the activity or, alternatively, the enzymes that are responsible for the release of the free phosphonates are rather specific in their action.

With respect to the antiretroviral activity of base-modified PME derivatives against MSV-induced transformation, marked activity of PMEA (**23a**), PMEDAP (**23b**), PMEMAP (**51**), and PMEG (**33**) was already reported earlier.[3a,30] From the data in Table 2 it is evident that the substitution of the adenine base in PMEA by a fluorine (**23c**) or a chlorine atom (**23d**) or by a hydroxyl group (**41b**) annihilates the antiviral activity. Our present studies revealed that replacement

Table 1. Activity (EC_{50}, μg/mL) of *N*-[2-(Phosphonomethoxy)ethyl] Derivatives against Herpes Viruses and Vaccinia Virus[a,b]

	HSV-1			HSV-2			HSV-1 TK⁻		CMV		VZV TK⁺		VZV TK⁻		
no.	KOS	F	McIntyre	G	196	Lyons	B2006	VMW 1837	AD 169	Davis	OKA	YS	07/1	YS/R	VV
							1. Free Acids								
7	300	300	NA	300	300	NA	NA	100	75	70	150	400	150	150	NA
9	20	40	70	150	100	70	300	20	30	33	6	5	3	8	>400
12	NA	NA	NA	NA	NA	NA	–	NA	>100	>100	>100	>100	>100	>100	NA
14b	>200	>200	300	>200	>200	300	–	>200	>100	>100	>100	>100	>100	NA	NA
16	>400	NA	NA	NA	NA	NA	–	NA	>100	>100	>100	40	>100	NA	NA
20	NA	NA	NA	NA	NA	NA	–	NA	100	>100	>100	65	75	86	>40
23a	3.6	–	–	1.1	–	–	13	5	30	77	8.5	5.4	NA	18	–
23b	2	2	2	0.2	0.07	0.07	–	2	10	10	1	2	2.5	3	20
23c	70	–	–	70	–	–	20	70	>50	>50	25	29	33	50	150
23d	NA	NA	NA	NA	NA	NA	–	NA	88	85	40	32	19	33	>40
24b	>400	>400	>400	>400	>400	300	>400	>400	25	20	26	>50	>50	>50	>400
25a	NA	NA	NA	NA	NA	NA	NA	NA	>50	>50	>50	>50	>50	>50	NA
25b	20	7	20	20	70	20	20	20	8	12	3.2	8.4	6	5	300
29	>400	>400	>400	>400	>400	>400	>400	>400	>50	>50	>50	>50	NA	>50	>400
36b	240	240	240	240	48	>400	>400	48	43	>50	4	18	28	8	–
38a	>200	>200	>200	>200	>200	>200	>200	>200	>50	>50	>50	>50	NA	>50	>200
38b	300	200	150	300	200	>400	>200	>200	>50	>50	44	>50	NA	25	>400
39	>100	–	–	>100	–	–	>100	20	>50	>50	37	30	20	25	>100
40	0.23	0.13	0.22	0.38	0.38	0.38	0.15	0.23	0.05	0.30	0.006	0.07	0.02	0.02	–
41b	>400	–	–	>400	–	–	>400	>400	>50	>50	>50	>50	>50	>50	>400
51	70	20	20	>400	150	70	–	70	>100	>100	60	28	27	NA	NA
52	>200	–	–	400	–	–	100	200	>50	>50	>50	>50	>50	>50	>400
56	200	150	100	70	200	100	300	150	>50	>50	50	20	NA	40	150
59	>400	–	–	>400	–	–	>400	>400	>50	>50	>50	>50	>50	>50	>400
62a	300	>400	>400	300	>400	300	150	70	>50	>50	>50	>50	>50	>50	>400
62b	150	70	100	70	100	70	400	70	>50	>50	>5	>5	>5	>5	150
65	>200	>200	>200	>200	>200	>200	>200	300	>50	>50	>50	>50	>50	>50	>200
68	70	150	150	70	150	70	70	70	>50	>50	8	35	30	>50	200
79	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
80a	NA	NA	NA	NA	NA	NA	NA	NA	100	200	NA	NA	NA	NA	NA
80b	NA	NA	NA	NA	NA	NA	NA	NA	>400	>400	50	>400	>400	>400	NA
81a	>400	–	–	>400	–	–	200	150	>400	>400	>400	200	300	200	>400
81b	NA	NA	NA	NA	NA	NA	NA	NA	70	400	50	100	70	40	NA
82	4.5	7	4	12	7	7	7	2	2	1.5	0.74	1.55	0.93	0.51	150
83	>200	>200	>200	>200	>200	>200	>200	>200	>100	>100	>40	>40	>40	>40	NA
							2. Mono- and Diesters								
11	NA	NA	NA	NA	NA	NA	–	NA	>100	>100	>100	97	80	80	NA
18	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA	NA	NA
22a	>400	–	–	>400	–	–	>400	>400	8	30	7	40	20	10	>400
22b	240	240	89	>400	240	240	240	80	2	2	>50	36	>50	32	–
22d	NA	NA	NA	NA	NA	NA	–	NA	0.65	0.70	0.50	3.70	NA	2.1	NA
28	>400	–	–	>400	–	–	>400	>400	>50	>50	>50	>50	>50	>50	>400
31	NA	NA	NA	NA	NA	NA	–	NA	40	10	13	10	9	6	NA
32	240	240	80	400	240	240	240	240	>50	>50	20	14	31	17	
36a	>400	240	240	>400	240	240	>400	240	>50	>50	>50	>50	>50	>50	
70a	>3.2	–	–	3.2	–	–	>3.2	>3.2	1	3	2.4	2	3.3	34	>3.2
70b	>16	–	–	>16	–	–	>16	>16	0.86	0.80	>5	3.20	>20	>2	>16
71	>16	–	–	>16	–	–	>16	>16	0.86	1	>2	>2	>2	>2	>16
72	>80	–	–	>80	–	–	>80	>80	1.2	1.1	10.5	4.2	18	4.3	>80
73	>16	–	–	>16	–	–	>16	>16	5	>5	>5	>5	>5	>5	>16
74	>16	–	–	>16	–	–	>16	>16	11	11	31.8	30.2	26.2	20	>16
75a	5.7	0.64	0.64	5.3	1.9	1.9	5.7	5.7	0.35	0.3	0.004	0.01	0.005	0.009	–
75b	240	240	240	240	240	240	240	240	>50	>50	1.6	13	35	2.4	–
75c	2	2	2	0.7	2	2	4	2	0.15	0.2	0.008	0.01	0.004	0.007	20
76b	NA	NA	NA	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA
77a	NA	150	300	70	150	150	150	150	NA	NA	40	60	45	80	NA
77b	300	200	300	70	300	150	200	150	NA	NA	4	5	2	6	>400
78a	200	–	–	300	–	–	>400	300	>50	>50	>50	>50	>50	>50	>400
78b	150	–	–	>400	–	–	>400	>400	>50	>50	32.	>50	>50	>50	>400

[a] Abbreviations: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; CMV, cytomegalovirus; VZV, varicella zoster virus; VV, vaccinia virus; TK, thymidine kinase; NA, not active; -, not determined. [b] For host cell type, see Experimental Section.

of the 6-oxo function in the guanine residue of compound **35** by sulfur preserves the activity: the 2-amino-6-chloro derivative of PMEG **40** belongs to the most active compounds of this series, although its toxicity is considerable. As mentioned above, this activity could be related to its transformation to PMEG. On the other hand, the 6-methyl-2-aminopurine derivative **52** has a very poor activity. The substitution of the purine base by a bromine atom at the position 8 diminishes but does not abolish the anti-MSV activity in PMEDAP (**25b**) or PMEG (**36b**) congeners, whereas the 8-bromo derivative of PMEA (**25a**) is inactive. These data suggest for anti-MSV activity a common pharmacophore of the type **86**. This hypothesis is corroborated by the earlier reported anti-MSV activity of the 3-deaza analogue of PMEG (**82**)[36] as well as by the anti-MSV activities of the 8-aza analogues of PMEDAP (**84**) and PMEG (**85**) which bear similar structural features.[35] It is interesting to note that none of the pyrimidine derivatives exhibit any activity against MSV except for the compound **14b**

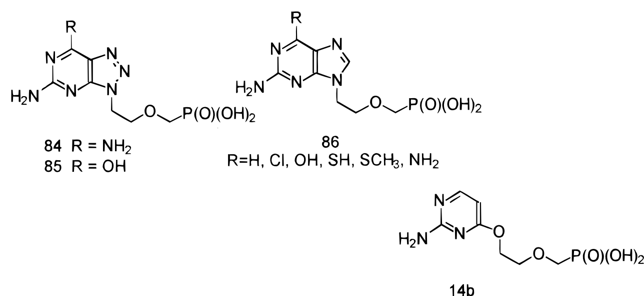
Table 2. Antiviral Activity (EC_{50}, μg/mL) of *N*-[2-(Phosphonomethoxy)ethyl] Derivatives against Retroviruses[a,b]

no	MSV	HIV-1		HIV-2	
		MT-4	CEM	MT-4	CEM
1. Free Acids					
7	>200	NA			
9	19.2 ± 12.9		>100		>100
12	>100	>100		>100	
14b	2.33 ± 0.31	>100		>100	
16	>100	>100		>100	
20	>100	>100		>100	
23a	1.14 ± 0.04				
23b	0.60 ± 0.33	2.67 ± 1.53			
23c	22.3 ± 6.1		>100		40.0 ± 0.0
23d	18.2 ± 6.5	>100		>100	
25a	>200	>100	>100	>100	>100
25b	2.43 ± 1.86		>100		>100
29	>200		>100		>100
33	0.0006 ± 0.0003	>0.2		>0.2	0.03
35	1 ± 0.5	9.50		8.90	
36a	>40		>100		>100
36b	>0.16		>0.16		>0.16
38a	>200		>100		>100
38b	96 ± 14.8		>100		>100
39	31 ± 10	>100	>100	>100	>100
40	>0.16		>0.16		>0.16
41b	123 ± 23		>100		>100
51	0.10 ± 0.05	40.9 ± 4.4		50.0 ± 0.1	
52	30 ± 2.5		>100		>100
56	4.10	7.56 + 2.04	4	8.27 ± 0.26	2.5 ± 2.1
59	>200		>100		>100
62a	142 ± 16		>100		>100
62b	144 ± 27	51.0 ± 1.7	30.0 ± 14.1	78.5 ± 18	16 ± 5.7
65	124 ± 32		>100		>100
68	26.5 ± 1.6		>100		>100
79	>200	>100			
80a	>200	>400			
80b	>200	>200		>400	
81a	13.8 ± 1.55	NA			
81b	>100	>200			
82	2.9 ± 0.3	>20		>20	
83	>100	>100		>100	
2. Mono- and Diesters					
11	>100	NA			
18	ND	>200		>200	
22a	>40	171 ± 43			
22b	>40		>100		>100
22d	>100	>20			
24b	>40		>100		>100
28	>40		>100		>100
31	>100	>100		>100	
32	13.5 ± 8.2		>100		>100
70a			>4		>4
70b	>8		>4		>4
71	>8		>4		>4
72	>40		>4		>4
73	>8		>4		>4
74	>8		>20		>20
75a	>0.064		>20		11.0 ± 1.4
75b			>100		>100
75c	0.24 ± 0.06	2.6 ± 0.01			
76a	>40		>100		>100
76b	>200	>400		>400	
77a	120 ± 7		>100		>100
77b	>200	>100		>400	
78a	>40		>100		>100
78b	>200		>100		>100

[a] Abbreviations: HIV, human immunodeficiency virus; MSV, Moloney sarcoma virus; NA, not active. [b] For details, see Experimental Section.

whose structure bears a strong resemblance to the mentioned pharmacophore.

Marked activity against HIV-1 was observed for the parent members of the series, PMEA (**23a**), PMEDAP (**23b**), and PMEG (**33**)[2]. None of the substituted congeners of these compounds nor any of the pyrimidine derivatives revealed activity against HIV. Only the O~S exchange at the position 6 of guanine retained moderate activity (**35,** $IC_{50} \sim 9$ μg/mL); this activity, however, is by several orders of magnitude lower than that of the parent PMEG (**33**). The 7-deaza analogue of PMEA (**56**) exhibits pronounced activity ($IC_{50} \sim 7.5$ μg/mL) which might indicate lesser importance of the N7-nitrogen atom for the activity compared to the role of the nitrogen atoms in positions 1 and 7 whose absence totally abolishes the antiretroviral activity in the 1-deaza-2,6-



diaminopurine (**62a**), 3-deaza-2,6-diaminopurine (**62b**), and 6-amino-1,3-dideazapurine (**59**) derivatives. We have observed such a consequence of the structural alteration of the base already for 1-deaza (**81a**) and 3-deaza analogues of PMEA (**81b**),[36]while the N∼C exchange in the 8-azapurine derivatives generally does not extinguish the antiviral activity.[35]

The esterification of the phosphonate residue by one or two ester groups does not improve the antiretroviral activity. As demonstrated in the PMEA series, the ethyl (**77a**) or isopropyl ester (**78a**) does not inhibit either MSV or HIV; the same was observed in the group of PMEDAP monoesters where the ethyl (**77b**) and isopropyl ester (**78b**) are inactive. In contrast to the isopropyl (**75a**) and isooctyl ester of PMEG (**75b**) which are totally inactive against HIV and MSV, some activity (EC_{50} ∼ 2.6 or 0.24 μg/mL, respectively) was observed for the PMEG ethyl ester (**75c**). However, taking in account the extreme sensitivity of MSV against PMEG (EC_{50} ∼ 0.6 ng/mL), the above value could be explained by a contamination with <0.04% PMEG in the test compound. The dialkyl esters of PMEA (**76a**, **22a**, **70a**), PMEDAP (**76b**, **22b**, **70b**), or PMEG (**32**, **72**) were devoid of any appreciable antiretroviral activity, as were the diesters derived from other acyclic phosphonates with anti-HIV activity, i.e., the 2-amino-6-chloropurine (**31, 71**), 8-bromoguanine (**36a**), and 8-bromo-2,6-diaminopurine (**24b**) derivatives.

The above data also show that the activity against the Moloney murine sarcoma virus in the series of PME derivatives is markedly higher compared to the effect of the same compounds against both HIV-1 and HIV-2 in CEM or MT-4 cell cultures. The reason for this phenomenon is unclear so far, but it has been observed already within the series of acyclic 8-azapurine nucleotide analogues.[35] It could be related to a differential uptake and/or metabolism in the murine versus human cells. Alternatively, MSV reverse transcriptase (RT) might be more susceptible to the inhibitory effects of these test compounds (in their diphosphate form) than HIV RT.

Experimental Section

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried overnight at 2 kPa over P_2O_5. Melting points were determined on a Kofler block and are uncorrected. TLC was performed on Silufol UV254 plates (Kavalier Votice, Czech Republic) in systems S1, chloroform−ethanol (95:5); S2, chloroform−ethanol (9:1); S3 chloroform−ethanol (4:1); S4, ethyl acetate−acetone−ethanol−water (4:1:1:1). Preparative TLC was carried out on 40 × 17 × 0.4 cm loose layer plates of silica gel containing UV indicator (made in the Service Laboratory of the Institute). HPLC was performed on Separon SGX RPS column (200 × 4 mm) in 0.05 M triethylammonium hydrogen carbonate, pH 7.5 (S5), containing varying concentrations of acetonitrile (0−20%). Paper electrophoresis was performed on a Whatman No. 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogen carbonate (TEAB) at pH 7.5; the electrophoretical mobilities were referenced to uridine 3′-phosphate.

Proton NMR spectra were taken on Varian UNITY-200 (at 200 MHz) and/or Varian UNITY-500 (at 500 MHz) instruments in CD_3SOCD_3, D_2O, or D_2O + NaOD solutions with tetramethylsilane (TMS) or sodium disilapentanesulfonate (DSS) as the respective internal standards. Proton chemical shifts and coupling constants were obtained by the first-order analysis of the spectra. Coupling constants (*J*) are recorded in hertz. ^{13}C NMR spectra were measured on a Varian UNITY-500 (at 125.7 MHz) instrument in D_2O solutions and referenced externally by secondary standardization to the determined position of the dioxane signal in D_2O [*d*(dioxane) = 66.86 ppm].

Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). UV absorption spectra were measured on a Beckman DU-65 spectrometer in aqueous solutions.

Materials. Bromotrimethylsilane, 6-chloropurine, 2,4-dihydroxypyridine, benzylamine, 2-chloroethanol, and cesium carbonate were purchased from Fluka (Switzerland); 2-fluoroadenine and 2,6-dichloropurine were obtained from Sigma (Germany); 2-amino-6-chloropurine was purchased from Mack (Germany); 2,6-diaminopurine was obtained from Tokyo Kasei Co (Japan). DBU, trimethylaluminum, and triisopropyl phosphite were purchased from Aldrich (Germany). Dimethylformamide dimethylacetal was purchased from BASF (Germany), dimethylformamide dineopentylacetal was prepared according to ref 49. 6-Azacytosine was kindly donated by Dr. A. Pískala from this Institute, tri(isooctyl) phosphite by Albright & Wilson UK Inc. Dimethylformamide was distilled from P_2O_5 and stored over molecular sieves (4 Å). Acetonitrile was refluxed with CaH_2 and distilled over molecular sieves (4 Å). Tetrahydrofuran was distilled before use from sodium hydride.

General Methods. Deionization of the Reaction Mixture. The solution of reaction products in water (20−25 mL) was applied on 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) until the drop of the UV absorption (254 nm) and acid reaction of the eluate. The standard elution rate was 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) were used. The sample was dissolved in water (20−25 mL), alkalified with concentrated aqueous ammonia to pH 9−9.5, and applied on the column. Elution with water (3 mL/min) was continued until the drop of the initial UV absorption (254 nm) of the eluate. The column was then eluted either with the linear gradient of acetic acid or with 1 M acetic acid (3 mL/min, fractions 30 mL) as indicated.

Di(2-propyl) 2-Chloroethoxymethylphosphonate (4). Tri(2-propyl) phosphite (246 mL, 1 mol) in a 500 mL flask equipped with Vigreux column and dropping funnel was heated at 100 °C, and 2-chloroethyl chloromethyl ether (128 g, 1.05 mol) was added dropwise under stirring at such a rate that the formed 2-iodopropane continually distilled off. After the addition ended, the mixture was heated for an additional 3 h and distilled in vacuo: bp 120 °C, yield 241 g (93%). Anal. ($C_9H_{20}ClO_4P$) C, H, Cl, P.

Bis(2-propyl) 1-[2-(Phosphonomethoxy)ethyl]cytosine (6). The mixture of cytosine (4.4 g, 40 mmol) and cesium carbonate (6.6 g, 20 mmol) in DMF (100 mL) was stirred at 100 °C for 1 h with exclusion of moisture, and after the addition of bis(2-propyl) 2-chloroethoxymethylphosphonate (**4**) (16 g, 50 mmol) the mixture was heated under stirring at 100 °C for an additional 12 h. The mixture was filtered while hot and taken down to dryness at 50 °C/13 Pa. The residue in

methanol (200 mL) was treated with silica gel (100 g), and the slurry was evaporated in vacuo. This material was applied on a column of silica gel (500 mL) in chloroform, and the column was eluted with chloroform and then with chloroform–methanol mixture (4:1). The product was collected by filtration from an ether suspension and dried: yield 6.25 g (47.3%) of compound **6**, mp 151 °C. Anal. ($C_7H_{11}N_6O_4P$) C, H, N, P. ^1H NMR ($(CD_3)_2SO$): δ 7.47 d, 1H (H-6), J(6,5) = 7.1; 5.61 d, 1H (H-5), J(5,6) = 7.1; 4.55 dsept, 2H (P–OCH), J(CH,CH_3) = 6.1, J(P,CH) = 7.1; 3.80 t, 2H (H-1′), J(1′,2′) = 5.0; 3.74 d, 2H (P–CH_2), J(P–CH) = 8.6; 3.67 t, 2H (H-2′), J(2′,1′) = 5.0; 1.22 and 1.20 2 × d, 6 H (4 × CH_3), J(CH_3,CH) = 6.1.

1-[2-(Phosphonomethoxy)ethyl]cytosine (7). The mixture of compound **6** (4.0 g, 12 mmol), acetonitrile (40 mL), and TMSBr (6 mL) was left to stand overnight at room temperature and taken down to dryness in vacuo. The residue in water (50 mL) was alkalified with concentrated aqueous ammonia and again evaporated. The product in water (50 mL) was deionized on the column (200 mL) Dowex 50 × 8 (H^+-form). The residue was purified on a Dowex 1 × 2 column (150 mL). The column was washed with water until the drop of UV absorption of the eluate; the resin was stirred in 1 M acetic acid (200 mL), filtered, and washed with boiling water (total, 1 L). The filtrate was evaporated in vacuo and the residue crystallized from water: yield 2.4 g (80%) of compound **7**, mp 167–168 °C. Anal. ($C_7H_{12}N_3O_5P$) C, H, N, P. ^1H NMR spectra and other physicochemical parameters were identical with the sample prepared by the procedure described earlier. UV spectrum: (pH 2) λ_{max} = 283 nm (ϵ_{max} = 8300).

5-Bromo-1-[2-(phosphonomethoxy)ethyl]cytosine (9). Bromine solution in carbon tetrachloride (0.3 M, 25 mL) was added to the solution of compound **6** (1.5 g, 4.54 mmol) in DMF (25 mL), and the mixture was stirred overnight in a closed flask. The solvents were evaporated in vacuo, and the residue was deionized on Dowex 50 × 8 (H^+-form) under the standard conditions with 20% methanol. The resin was suspended in 20% methanol (200 mL), alkalized with ammonia (pH 9–10), filtered, and washed with methanol (100 mL), and the filtrate was evaporated in vacuo. Compound **8** was obtained by chromatography on a silica gel plate (see above) in chloroform–ethanol (4:1). The product was eluted with methanol (200 mL) and dried in vacuo. Acetonitrile (20 mL) and TMSBr (2 mL) were added, and the mixture was left to stand overnight at room temperature. The volatiles were evaporated in vacuo, and the residue was treated with water (50 mL), alkalized with aqueous ammonia, and evaporated. The residue was deionized on a Dowex 50 × 8 column (50 mL) and purified on a Dowex 1 × 2 column (50 mL), and the product was eluted with 1 M acetic acid. After evaporation in vacuo and codistillation with water to remove residual acetic acid, compound **9** was obtained by crystallization from water: yield 0.70 g (47%), mp 142–146 °C. Anal. ($C_7H_{11}BrN_3O_5P$) C, H, N, P. ^1H NMR (D_2O + NaOD): δ 8.07 s, 1H (H-6); 4.01 t, 2H, (H-1′) J(1′,2′) = 5.1; 3.82 t, 2H, (H-2′), J(1′,2′) = 5.1; 3.50 d, 2H (P–CH_2), J(P–CH) = 8.3. UV spectrum: (pH 1) λ_{max} = 302 nm (ϵ_{max} = 8700); (pH 7) λ_{max} = 290 nm (ϵ_{max} = 6100); (pH 12): λ_{max} = 291 nm (ϵ_{max} = 6500).

Bis(2-propyl) 1-[2-(Phosphonomethoxy)ethyl]-6-azacytosine (11). A mixture of 6-azacytosine (**10**) (1.12 g, 10.0 mmol), DMF (20 mL), cesium carbonate (1.65 g, 5.0 mmol), and bis(2-propyl) 2-chloroethoxymethylphosphonate (**4**) (2.7 g, 10.5 mmol) was heated at 120 °C under stirring and exclusion of moisture for 4 h until the starting compound disappeared (TLC in S3). After evaporation of the solvent in vacuo and codistillation with toluene (3 × 50 mL), the residue was extracted with boiling chloroform, evaporated and the residue chromatographed on the column (50 g) of silica gel in chloroform. The product was eluted by a chloroform–methanol mixture, 95:5, the relevant fraction evaporated, and the residue crystallized from ethyl acetate (ether added to turbidity) to afford 1.60 g (47.8%) of compound **11**, mp 114 °C. R_f = 0.41 (S3) (starting **10**, 0.15). Anal. ($C_{13}H_{24}N_7O_4P$) C, H, N, P. ^1H NMR ($(CD_3)_2SO$): δ 7.80 brs 1H and 7.70 brs, 1H (NH_2); 7.41 s 1H (H-5); 4.54 dsept, 2H (P–OCH), J(CH–CH_3) = 6.1; 4.02 t (H-1′), J(CH_2–CH_2) = 5.4; 3.78 t (H-2′), J(1′,2′) = 5.4; 3.73 d, 2H (P–CH_2), J(P–CH) = 8.3; 1.21 and 1.19 2 × d, 2 × 6H (4 × CH_3), J(CH_3,CH) = 6.1.

1-[2-(Phosphonomethoxy)ethyl]-6-azacytosine (12). TMSBr (4 mL) was added to the bis(2-propyl)ester **11** (1.35 g, 4 mmol) in acetonitrile (40 mL), and the mixture was left to stand overnight in a stoppered flask at room temperature. The volatiles were evaporated in vacuo, water (20 mL) was added, and after standing for 30 min at room temperature, the mixture was alkalified by aqueous ammonia and evaporated. The residue in water (20 mL) was deionized on Dowex 50 × 8 under the standard conditions. The UV-absorbing fraction of the product was evaporated in vacuo and crystallized from 70% aqueous ethanol (ether added to turbidity): yield 0.70 g (70%) of compound **12**, mp 228 °C. Anal. ($C_6H_{11}N_4O_5P$) C, H, N, P. ^1H NMR (D_2O + NaOD): δ 7.63 s, 1H (H-5); 4.19 t, 2H (H-1′), J(1′,2′) = 5.4; 3.89 t, 2H (H-2′), J(1′,2′) = 5.4; 3.49 d, 2H (P–CH_2), J(P–CH) = 8.55. UV spectrum (pH 2) λ_{max} = 284 nm (ϵ_{max} = 7200).

1-[2-(Phosphonomethoxy)ethyl]isocytosine (16) and 2-Amino-4-[2-(phosphonomethoxy)ethoxy]pyrimidine (14b). A mixture of isocytosine (**13**) (2.2 g, 20.0 mmol), DMF (40 mL), cesium carbonate (3.30 g, 10.0 mmol), and compound **4** (5.4 g, 21 mmol) was heated at 130 °C under stirring and exclusion of moisture for 12 h and evaporated in vacuo. The residue was codistilled with toluene (2 × 50 mL) and extracted with boiling chloroform (2 × 50 mL). The residue of the extract was chromatographed on the column (50 g) of silica gel in chloroform to afford crude diester fraction (**14a, 15**) (3.3 g, 49.5%): R_f = 0.58 (S3) (starting **13**, 0.20).

TMSBr (3 mL) was added to the solution of this product (3.3 g, 10 mmol) in acetonitrile (30 mL), and the mixture was left to stand overnight in a stoppered flask at room temperature. The mixture was evaporated in vacuo, water (20 mL) was added, and after standing for 30 min at room temperature, the mixture was alkalified by aqueous ammonia and evaporated. The residue was desalted on a Dowex 50 × 8 column under the standard conditions. The product was purified on Dowex 1 × 2 (acetate) by elution with linear gradient of acetic acid (0–0.3 M, 1 L each). Two UV– absorbing product fractions were evaporated in vacuo and the residues crystallized from water. The former fraction afforded compound **14b** (0.50 g, 20%): mp 253 °C. Anal. ($C_7H_{11}N_6O_4P$) C, H, N, P. k (S4) = 6.73. ^1H NMR (D_2O + NaOD): δ 8.04 s, 1H (H-6), J(6,5) = 6.0; 6.24 s, 1H (H-5), J(5,6) = 6.0; 4.42 t, 2H (H-1′), J(1′,2′) = 4.9; 3.93 t, 2H (H-2′) J(1′,2′) = 4.9; 3.58 d, 2H (P–CH2), J(P–CH) = 8.5. ^{13}C NMR (D_2O + NaOD): δ 170.51 (C-4), 162.99 (C-2); 158.88 (C-6); 97.59 (C-5); 70.37 d (C-2′), J(P,C) = 10.1; 69.31 d (P–C), J(P,C) = 150.2; 66.10 (C-1′). (UV spectrum: (pH 2) λ_{max} = 269 nm.

The latter fraction gave compound **16** (0.75 g, 30%): mp 234–236 °C. Anal. ($C_7H_{11}N_6O_4P$) C, H, N, P. k (S5) = 3.53. ^1H NMR (D_2O + NaOD): δ 7.71 s, 1H (H-6), J(6,5) = 6.4; 5.95 d, 1H (H-5), J(5,6) = 6.4; 4.24 t, 2H (H-1′), J(1′,2′) = 5.2; 3.89 t, 2H (H-2′), J(2′,1′) = 5.2; 3.55 d, 2H (P–CH_2), J(P–CH) = 8.5. ^{13}C NMR (D_2O + NaOD): δ 165.44 (C-4); 157.81 (C-2); 155.53 (C-6); 102.33 (C-5); 70.50 d (C-2′), J(P,C) = 11.0; 69.72 d (P–C), J(P,C) = 149.3; 44.34 (C-1′). UV spectrum: (pH 2) λ_{max} = 260 nm.

1-[2-(Phosphonomethoxy)ethyl]-3-deazacytosine (20). A mixture of 2,4-dihydroxypyridine (0.55 g, 5 mmol), compound **4** (2 g, 7.7 mmol), cesium carbonate (1.0 g, 3 mmol), and DMF (15 mL) was heated at 100 °C under stirring and exclusion of moisture for 16 h and evaporated in vacuo. The residue gave, on silica column (100 mL) chromatography and crystallization from ether, diester **18** (0.70 g, 42%): mp 83 °C, R_f = 0.46 (S3). Anal. ($C_7H_{11}N_6O_4P$) C, H, N, P. ^1H NMR ($(CD_3)_2SO$): δ 11.10 br s, 1H (OH); 7.25 d, 1H (H-6), J(5,6) = 7.30; 5.84 dd, 1H (H-5), J(5,6) = 7.30; 5.71 d, 1H (H-3), J(3,5) = 2.40; 4.63 and 4.58 dsept, 2H (P–OCH), J(CH,CH_3) = 6.1; 4.08 m, 2H (H-1′); 3.83 m, 2H (H-2′); 3.82 d, 2H (P–CH_2), J(P–CH) = 8.01; 1.25 and 1.245 2 × d, 2 × 6 H (4 × CH_3), J(CH_3,CH) = 6.1. UV spectrum (CH_3OH): λ_{max} = 280 nm; (pH 2) λ_{max} = 274 nm (ϵ_{max} = 4000), 319.5 (ϵ_{max} = 4000).

This material (0.70 g, 2.1 mmol) was dissolved in pyridine (10 mL) and treated with phosphoryl chloride (0.343 g, 2.24 mmol) and 1,2,4-triazole (0.30 g, 4.35 mmol); the mixture was stirred overnight in a closed flask, and methanolic ammonia (30%, 20 mL) was added. After standing for another 24 h at room temperature the mixture was evaporated in vacuo and deionized on a column of Dowex 50 × 8 under the standard conditions. Compound **19** (R_f = 0.20, S3) was codistilled with ethanol (2 × 25 mL) and dried overnight in vacuo over phosphorus pentoxide. Acetonitrile (30 mL) and TMSBr (3 mL) were added to the residue, and the solution was left to stand overnight at room temperature. The mixture was evaporated and further processed as described for compound **16**. The deionized product was eluted from a column (50 mL) of Dowex 1 × 2 (acetate) by 1 M formic acid. The UV-absorbing eluate was evaporated with water (5 × 25 mL) and crystallized from 80% aqueous ethanol (ether added to turbidity): yield 300 mg (57.6%) of compound **20**, mp 262 °C. Anal. ($C_7H_{11}N_6O_4P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 7.47 d, 1H, (H-6), *J*(6,5) = 7.3; 6.31 dd, 1H (H-5), *J*(3,5) = 2.4, *J*(5,6) = 7.3; 6.04 d, 1H (H-3), *J*(3,5) = 2.4; 4.26 m, 2H (H-1′); 3.97 m, 2H (H-2′); 3.63 d, 2H (P−CH_2), *J*(P−CH) = 8.5. UV spectrum: (pH 2) λ_{max} = 271 nm (ϵ_{max} = 4300).

Bis(2-propyl) 9-[2-(Phosphonomethoxy)ethyl]adenine (22a). Method A. Adenine (13.5 g, 0.1 mol) and sodium hydride (2.4 g, 0.1 mol) in DMF (350 mL) were stirred for 1 h at 80 °C with exclusion of moisture, and compound **4** (28 g, 0.108 mol) in DMF (100 mL) was added dropwise over 15 min. The mixture was stirred for 16 h at 100 °C and evaporated in vacuo. The residue was codistilled with toluene (3 × 100 mL), and extracted with boiling chloroform (total, 500 mL). The extract was concentrated in vacuo and applied on a column of silica gel (300 mL) in chloroform. The column was eluted first with chloroform and then with chloroform−methanol (95:5) to give compound **22a** which was crystallized from ethyl acetate−petroleum ether: yield 21.5 (59.5%), mp 138 °C, R_f = 0.47 (S3). Anal. ($C_{14}H_{24}N_5O_4P$) C, H, N, P. ^{1}H NMR (($CD_3)_2$SO): δ 8.15 s, 1H (H-2); 8.09 s, 1H (H-8); 7.19 br s, 2H (NH_2); 4.46 dsept, 2H (P−OCH), *J*(CH,CH_3) = 6.1, *J*(P−OCH) = 7.7; 4.34 t, 2H (H-1′), *J*(1′,2′) = 5.0; 3.91 t, 2H (H-2′), *J*(2′,1′) = 5.0; 3.79 d, 2H (P−CH_2), *J*(P−CH) = 8.4; 1.18 and 1.13 2 × d, 2 × 6 H (4 × CH_3), *J*(CH_3,CH) = 6.1.

Method B. Adenine (2.7 g, 20 mmol) and DBU (3.2 mL, 20.6 mmol) in DMF (40 mL) were heated under stirring at 80 °C for 30 min, and synthon **4** (7.5 mL) was added. The mixture was stirred for 5 h at 100 °C and taken down in vacuo. The residue in ethyl acetate (200 mL) was washed with water (2 × 25 mL), dried with $MgSO_4$, filtered, and evaporated. The residue gave, on silica gel column (200 g) chromatography, product **22a** which was crystallized from ethyl acetate−petroleum ether: yield 3.8 g (53%); mp 138 °C.

Bis(2-propyl) 2,6-Diamino-9-[2-(phosphonomethoxy)-ethyl]purine (22b). Method A. A mixture of 2,6-diaminopurine (6 g, 40 mmol), cesium carbonate (6.6 g, 20 mmol), and DMF (100 mL) was stirred for 30 min at 100 °C, and synthon **4** (13 g, 50 mmol) was added. The mixture was stirred for 8 h at 100 °C and evaporated in vacuo. The residue was worked up as described for compound **22a** and the product crystallized from ethanol−petroleum ether: yield 8.9 g (59.7%) of compound **22b**, mp 193−194 °C. Anal. ($C_{14}H_{25}N_6O_4P$) C, H, N, P. ^{1}H NMR (($CD_3)_2$SO): δ 7.65 s, 1H (H-8); 6.67 s, 2H and 5.78 s, 2H (NH_2); 4.52 dsept, 2H (P−OCH), *J*(CH,CH_3) = 6.2, *J*(P−OCH) = 7.8; 4.12 t, 2H (H-1′), *J*(1′,2′) = 5.2; 3.82 t, 2H (H-2′), *J*(2′,1′) = 5.2; 3.77 d, 2H (P−CH_2), *J*(P−CH) = 8.3; 1.19 d, 6 H (CH_3), *J*(CH_3,CH) = 6.1; 1.16 d, 6 H (CH_3), *J*(CH_3,CH) = 6.3.

Method B. The reaction was performed essentially as described for compound **22a** (method B) with 20 mmol of 2,6-diaminopurine and 20.6 mmol of DBU: Yield 3.20 g (54%) of compound **22b**, mp 194 °C.

Bis(2-propyl) 9-[2-(Phosphonomethoxy)ethyl]-2-chloroadenine (22d). The mixture of 2-chloroadenine (**21d**)(1.70 g, 10 mmol), cesium carbonate (1.65 g, 5 mmol), bis(2-propyl) 2-chloroethoxymethylphosphonate (**4**) (3.05 g, 11.8 mmol), and DMF (20 mL) was stirred at 120 °C for 16 h with exclusion of moisture. After evaporation of the solvent in vacuo, the residue was extracted with boiling chloroform (total, 300 mL), and the filtrate was concentrated and applied on a column (300 mL) of silica gel in chloroform. The column was first eluted with the same solvent followed by chloroform−ethanol mixture, 97.5:2.5. The product was obtained by crystallization from ethyl acetate−petroleum ether: yield 3.0 g (76.5%) of compound **22d**, mp 103 °C, R_f = 0.63 (S3). Anal. ($C_7H_{11}ClN_6O_4P$) C, H,Cl, N, P. ^{1}H NMR (($CD_3)_2$SO): δ 8.09 s, 1H (H-8); 7.74 br s, 2H (NH_2); 4.48 dsept, 2H (P−OCH), *J*(CH,CH_3) = 6.1; 4.29 t, 2H (H-1′), *J*(1′,2′) = 5.0; 3.87 t, 2H (H-2′), *J*(2′,1′) = 5.0; 3.78 d, 2H (P−CH_2), *J*(P−CH) = 8.6; 1.16 and 1.11 2 × d, 2 × 6 H (4 × CH_3), *J*(CH_3,CH) = 6.1.

9-[2-(Phosphonomethoxy)ethyl]adenine (23a). Compound **22a** (25 g, 70 mmol) in acetonitrile (200 mL) was treated with bromotrimethylsilane (20 mL) overnight in a closed flask. The solution was evaporated and the residue codistilled with acetonitrile (2 × 50 mL). Water (200 mL) was added, and the emulsion was alkalified with ammonia and evaporated. The residue in water (300 mL) was acidified by addition of Dowex 50 × 8 (H^+-form) and the suspension applied onto a column (500 mL) of the same ionex. The column was washed with water until neutral, and the resin suspended in water (1 L) was treated batchwise with ammonia to pH 9−10. The suspension was filtered and washed with water (2 L), and the combined filtrates were evaporated in vacuo. The residue in boiling water (200 mL) was acidified with concentrated hydrochloric acid to pH 3−3.5, ethanol (200 mL) was added, and the solution was set aside in the refrigerator to crystallize. The product was collected and washed with 50% aqueous ethanol, ethanol, and ether: yield 17.0 g (89%) of compound **23a**, identical with the authentic material, mp 301 °C. Anal. ($C_8H_{12}N_5O_4P$) C, H, N, P.

2,6-Diamino-9-[2-(phosphonomethoxy)ethyl]purine (23b). Diester **22b** (6 g, 16.1 mmol) in acetonitrile (150 mL) was treated with TMSBr (15 mL) in a closed flask overnight at room temperature and evaporated in vacuo. Water (100 mL) was added and the solution alkalized with ammonia. The emulsion was evaporated in vacuo, and the residue was dissolved in boiling water (200 mL). The hot solution was acidified under stirring with concentrated HCl to pH 3.5 and left to crystallize at room temperature overnight. The crystalline product was filtered, washed with water, ethanol, and ether, and dried: yield 4.5 g (97%), not melting below 280 °C, of compound **23b** identical with the authentic material. Anal. ($C_8H_{13}N_6O_4P$) C, H, N, P.

2-Fluoro-9-[2-(phosphonomethoxy)ethyl]adenine (23c). A mixture of 2-fluoroadenine (0.92 g, 6 mmol) and sodium hydride (144 mg, 6 mmol) in DMF (20 mL) was stirred for 1 h at 80 °C, and synthon **4** (7 mmol) was added. After being stirred for 16 h at 80 °C, the mixture was evaporated and the residue in methanol applied on a preparative plate of silica gel. The plate was developed by chloroform−methanol (9:1), and the band of the product (R_f = 0.48, S2; starting compound 0.20) was eluted with methanol (total, 300 mL). The eluate was evaporated to dryness and dried in vacuo. The residue of compound **22c** in acetonitrile was treated with TMSBr overnight at room temperature and evaporated. The residue in water (25 mL) was alkalized with ammonia, evaporated, and deionized on a column (50 mL) of Dowex 50 × 8 (H^+-form) under the standard conditions, and the product was purified on a Dowex 1 × 2 column; the column was first washed with 1 M acetic acid, and the ionex on the filter was washed with boiling water (500 mL). The filtrate was evaporated in vacuo, and the residue was recrystallized from water yielding pure compound **23c** (0.66 g, 38%), not melting below 300 °C. Anal. ($C_8H_{11}FN_5O_4P$) C, H, F, N, P. ^{1}H NMR (D_2O + NaOD): δ 7.87 s, 1H (H-8); 4.33 t, 2H (H-1′) *J*(1′,2′) = 5.1; 3.90 t, 2H (H-2′), *J*(1′,2′) = 5.1; 3.50 d, 2H (P−CH_2), *J*(P−CH) = 8.3. UV spectrum: (pH 2) λ_{max} = 262 nm (ϵ_{max} = 4300), 319.5 (ϵ_{max} = 4000).

2-Chloro-9-[2-(phosphonomethoxy)ethyl]adenine (23d). Compound **22d** (1.0 g, 2.55 mmol) and TMSBr (4 mL) in

acetonitrile (40 mL) were left to stand overnight at ambient temperature. The mixture was evaporated, taken up in water (50 mL), alkalified by concentrated aqueous ammonia, and evaporated. The crude product was deionized on a column of Dowex 50 × 8 (H^+-form) under the standard conditions and finally purified on a column (50 mL) of Dowex 1 × 2 (acetate). The column was washed first with water until the drop of the UV absorption of the eluate, and the resin was stirred with 2 M formic acid (100 mL). The slurry was filtered and the resin washed with several portions of boiling water (total, 1 L). After evaporation, the residue was codistilled with water (3 × 25 mL) to remove formic acid and filtered with ethanol. The product was washed with ether and dried: yield 0.4 g (51.0%) of compound **23d**, not melting below 280 °C. R_f = 0.63 (S3). Anal. ($C_7H_{11}ClN_6O_4P$) C, H,Cl, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.16 s, 1H (H-8); 4.32 t, 2H (H-1′) J(1′,2′) = 5.0; 3.93 t, 2H (H-2′), J(1′,2′) = 5.0; 3.58 d, 2H (P−CH_2), J(P−CH) = 8.55. UV spectrum: (pH 2) λ_{max} = 265 nm (ϵ_{max} = 10 600).

Bis(2-propyl) 8-Bromo-2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purine (24b). Bromine solution in carbon tetrachloride (0.3 M, 50 mL) was added to the solution of compound **22b** (3.1 g, 8.3 mmol) in DMF (25 mL), and the mixture was stirred in a closed flask overnight at room temperature. The solvents were evaporated in vacuo, and the residue was codistilled with toluene and deionized in 20% aqueous methanol on a Dowex 50 × 8 (H^+-form) column (100 mL) under the standard conditions. The UV-absorbing ammonia eluate was evaporated in vacuo, codistilled with ethanol, and crystallized from the same solvent: yield 2.55 g (68%) of compound **24b**, mp 207 °C, R_f = 0.25 (S2) (starting compound **22b**, 0.17). Anal. ($C_{14}H_{24}BrN_6O_4P$) C, H, Br, N, P. ^{1}H NMR (($CD_3)_2SO$): δ 6.84 s, 2H and 5.94 s, 2H (NH_2); 4.49 dsept, 2H (P−OCH), J(CH,CH_3) = 6.1, J(P−OCH) = 7.6; 4.12 t, 2H (H-1′), J(1′,2′) = 5.5; 3.84 t, 2H (H-2′), J(2′,1′) = 5.5; 3.75 d, 2H (P−CH_2), J(P−CH) = 8.3; 1.16, 6H, and 1.13 d, 6 H (4 × CH_3), J(CH_3,CH) = 6.3.

8-Bromo-9-[2-(phosphonomethoxy)ethyl]adenine (25a). A solution of $Na_2HPO_4 \cdot 12H_2O$ (8 g) in water (80 mL) was added to the solution of compound **22a** (2.15 g, 6 mmol) in dioxane (130 mL), and the mixture was treated with bromine (0.4 mL, 7.85 mmol). The mixture was stirred for 3 h (the conversion was complete: TLC in S2, R_f = 0.65, **22a** 0.48). The reaction mixture was taken down to dryness in vacuo, the residue was extracted with chloroform (total, 300 mL), the extract was evaporated, and the residue of compound **24a** was dried in vacuo. Acetonitrile (30 mL) and TMSBr (3 mL) were added, and the mixture was stirred in a closed flask overnight. The mixture was evaporated in vacuo, water (100 mL) was added, and the solution was alkalified with ammonia. After evaporation in vacuo, the residue was deionized on a column of Dowex 50 × 8 under the standard conditions. The crude product was purified by chromatography on a Dowex 1 × 2 column (100 mL), and the product was eluted with 1 M acetic acid. The UV-absorbing fraction gave, on evaporation and codistillation with water (3 × 25 mL), pure compound **25a** which was recrystallized from water−ethanol−ether: yield 1.45 g (68.3%), mp 235 °C. Anal. ($C_8H_{11}BrN_5O_4P$) C, H, Br, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.05 s, 1H (H-2); 4.35 t, 2H (H-1′), J(1′,2′) = 5.5; 3.96 t, 2H (H-2′), J(1′,2′) = 5.5; 3.65 d, 2H (P−CH_2), J(P−CH) = 8.5. UV spectrum: (pH 2) λ_{max} = 264 nm (ϵ_{max} = 16 600).

8-Bromo-2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purine (25b). Compound **24b** (2.27 g, 5 mmol) in acetonitrile (25 mL) was treated with TMSBr (2.5 mL) overnight, and the mixture was evaporated in vacuo. Water (50 mL) was added, and the solution was alkalized with ammonia and evaporated. The residue was deionized on a column (50 mL) of Dowex 50 × 8 under the standard conditions, and the product was purified on a column (50 mL) of Dowex 1 × 2 (acetate) and eluted with 1 M acetic acid. The UV-absorbing eluate was evaporated, and the residue was codistilled with water (3 × 25 mL) and crystallized from 80% ethanol (ether added to turbidity): yield 1.05 g (57.2%) of compound **25b**, not melting below 280 °C. Anal. ($C_8H_{12}BrN_6O_4P$) C, H, Br, N, P. ^{1}H NMR (D_2O + NaOD): δ 4.21 t, 2H (H-1′), J(1′,2′) = 5.1; 3.90 t, 2H (H-2′), J(1′,2′) = 5.1; 3.59 d, 2H (P−CH_2), J(P−CH) = 8.3. UV spectrum: (pH 2) λ_{max} = 265 nm (ϵ_{max} = 6600), λ_{infl} = 282 nm.

Bis(2-propyl) 6-Chloro-9-[2-(phosphonomethoxy)ethyl]purine (27). Method A. 6-Chloropurine (7.6 g, 50 mmol) was added to a suspension of sodium hydride (1.2 g, 50 mmol) in DMF (180 mL) precooled to 0 °C. The reaction mixture was stirred at 0 °C for 30 min and compound **4** (15.6 g, 60 mmol) was added in one portion. The mixture was stirred for 24 h at 80 °C and evaporated in vacuo. The residue was codistilled with toluene (2 × 25 mL portion) and chromatographed on a column of silica gel (500 mL) in chloroform. Elution with chloroform followed by chloroform−methanol mixture (95:5) gave the oily product **27** (8.8 g, 46.7%). Anal. ($C_{14}H_{22}ClN_4O_4P$) C, H, Cl, N, P. ^{1}H NMR (($CD_3)_2SO$): δ 8.78 s, 1H and 8.65 s, 1H (H-2 and H-8); 4.50 t, 2H (H-1′), J(1′,2′) = 5.0; 4.43 m, 2H (P−OCH), 3.95 t, 2H (H-2′), J(2′,1′) = 5.0; 3.77 d, 2H (P−CH_2), J(P−CH) = 8.3; 1.13 d, 6H and 1.06 d, 6 H (4 × CH_3), J(CH_3,CH) = 6.1.

Method B. DBU (6.4 mL, 41.2 mmol) was added to a suspension of 6-chloropurine (6.18 g, 40 mmol) in DMF (80 mL) under stirring, and the solution was treated with the synthon **4** (15 mL, 53 mmol). The mixture was stirred at 80 °C for 6 h with exclusion of moisture, and the solvent was stripped off in a vacuum. The residue was taken up in ethyl acetate (500 mL) and extracted with water (three 100 mL portions). The organic layer was dried with magnesium sulfate, filtered, and taken down in vacuo. The residue afforded, by chromatography (silica gel column, 300 mL) under the conditions described in method A, compound **27** (8.0 g, 49%).

Bis(2-propyl) 9-[2-(Phosphonomethoxy)ethyl]-6-mercaptopurine (28). A solution of compound **27** (5.0 g, 13.3 mmol) and thiourea (4.0 g) in ethanol (100 mL) was refluxed with exclusion of moisture for 1 h, cooled, and made alkaline with triethylamine. The mixture was evaporated in vacuo, refluxed in chloroform (300 mL), and filtered while hot. The filtrate was evaporated and purified on a column (300 mL) of silica gel in chloroform. The product-containing fractions were evaporated, and the product was crystallized from ethyl acetate−ether: yield 3.4 g (68.5%) of compound **28**, mp 192−193 °C. Anal. ($C_{14}H_{23}N_4O_4PS$) C, H, N, P, S. ^{1}H NMR (($CD_3)_2SO$): δ 13.70 br s, 1H (NH); 8.24 s, 1H and 8.20 s, 1H (H-2 and H-8); 4.46 dsept, 2H (P−OCH), J(CH,CH_3) = 6.3, J(P,CH) = 7.6; 4.36 t, 2H (H-1′), J(1′,2′) = 5.0; 3.89 t, 2H (H-2′), J(2′,1′) = 5.0; 3.77 d, 2H (P−CH_2), J(P−CH) = 8.3; 1.16 and 1.12 2 × d, 2 × 6H (4 × CH_3), J(CH_3,CH) = 6.3.

9-[2-(Phosphonomethoxy)ethyl]-6-mercaptopurine (29). Compound **28** (1.2 g, 3.2 mmol) in acetonitrile (40 mL) was treated with bromotrimethylsilane (4 mL) overnight and evaporated. The residue was codistilled with acetonitrile (3 × 20 mL), and water (25 mL) was added. After standing for 30 min at room temperature, the solution was slightly alkalized with triethylamine and evaporated. The residue in water (20 mL) was applied on a column of Dowex 50 × 8 (H^+-form), and the column was washed with water. The product was eluted with considerable retention. Evaporation in vacuo and crystallization from aqueous ethanol (ether added to turbidity) gave white crystalline compound **29** (0.50 g, 54%): mp 206 °C. Anal. ($C_8H_{11}N_4O_4PS$) C, H, N, P, S. ^{1}H NMR (D_2O + NaOD): δ 8.31 s, 1H and 8.29 s, 1H (H-2 and H-8); 4.64 t, 2H (H-1′), J(1′,2′) = 4.9; 3.96 t, 2H (H-2′), J(1′,2′) = 4.9; 3.62 d, 2H (P−CH_2), J(P−CH) = 8.8. UV spectrum: (pH 2) λ_{max} = 320 nm (ϵ_{max} = 18 800).

Bis(2-propyl) 9-[2-(Phosphonomethoxy)ethyl]-2-amino-6-chloropurine (31) and Bis(2-propyl) 7-[2-(Phosphonomethoxy)ethyl]-2-amino-6-chloropurine (37). Method A. 2-Amino-6-chloropurine (5.1 g, 30 mmol), cesium carbonate (4.9 g, 15 mmol), and synthon **4** (9.0 g, 35 mmol) in DMF (120 mL) were stirred for 3 h at 120 °C with exclusion of moisture (the reaction was complete according to TLC in S2). The solvent was stripped down in vacuo, the residue codistilled with toluene (3 × 50 mL) and extracted with boiling chloroform (total, 500 mL), and the extract chromatographed on a column (300 mL) of silica gel in chloroform. Elution with the same

solvent afforded 9-isomer **31** which was crystallized from ethyl acetate (an equal volume of ether was added followed by petroleum ether until turbidity) to afford pure compound **31** (6.3 g, 53%): mp 93 °C. Anal. ($C_{14}H_{23}ClN_5O_4P$) C, H, Cl, N, P. ^1H NMR ($(CD_3)_2SO$): δ 8.06 s, 1H (H-8); 6.89 br s, 2H (NH_2); 4.48 dsept, 2H (P−OCH), $J(CH,CH_3)$ = 6.1; 4.24 t, 2H (H-1′), $J(1',2')$ = 5.0; 3.87 t, 2H (H-2′), $J(2',1')$ = 5.0; 3.77 d, 2H (P−CH_2), J(P−CH) = 8.3; 1.16d, 6H and 1.11 d, 6 H (4 × CH_3), $J(CH_3,CH)$ = 6.1.

Further elution of the column with chloroform−ethanol (95:5) gave, after crystallization from ethyl acetate−petroleum ether, compound **37** (1.8 g, 15%): mp 152 °C. Anal. ($C_{14}H_{23}ClN_5O_4P$) C, H, Cl, N, P. ^1H NMR ($(CD_3)_2SO$): δ 8.27 s, 1H (H-8); 6.60 br s, 2H (NH_2); 4.47 m, 4H (P−OCH and 1′-CH_2); 3.86 t, 2H (H-2′), $J(2',1')$ = 4.6; 3.75 d, 2H (P−CH_2), J(P−CH) = 8.1; 1.13 and 1.10 2 × d, 2 × 6H (4 × CH_3), $J(CH_3,CH)$ = 6.6.

Method B. DBU (16 mL, 103 mmol) was added to a solution of 2-amino-6-chloropurine (**30**) (17.05 g, 100 mmol) in DMF (200 mL) under stirring, and the solution was treated with synthon **4** (22.5 mL, 100 mmol). The mixture was stirred at 80 °C for 6 h with exclusion of moisture, and the solvent was stripped off in a vacuum. The residue was taken up in ethyl acetate (500 mL) and extracted with water (three 100 mL portions). The organic layer was dried with $MgSO_4$, filtered, and taken down in vacuo. The residue afforded on chromatography (silica gel column, 300 mL) under the conditions described in method A 18.8 g (47.5%) of compound **31** and 2.50 g (6.4%) of the 7-isomer **37**, both identical with the above preparations.

Bis(2-propyl) 9-[2-(Phosphonomethoxy)ethyl]guanine (32). Method A. A mixture of compound **31** (5.0 g, 12.8 mmol), potassium carbonate (7.06 g, 51.1 mmol), and DABCO (2.8 g, 25 mmol) in water (40 mL) was stirred at reflux for 3 h and neutralized by addition of Dowex 50 × 8 (H^+-form). The mixture was filtered, the resin was washed with water (100 mL) and methanol (200 mL), and the filtrate was evaporated in vacuo. The residue afforded on crystallization from water compound **32** (3.7 g, 77.4%): mp 228 °C. Anal. ($C_{14}H_{24}N_5O_5P$) C, H, N, P. ^1H NMR ($(CD_3)_2SO$): δ 10.70 s, 1H (NH); 7.64 s, 1H (H-8); 6.49 br s, 2H (NH_2); 4.51 m, 2H (P−OCH); 4.11 t, 2H (H-1′), $J(1',2')$ = 5.1; 3.81 t, 2H (H-2′), $J(2',1')$ = 5.1; 3.77 d, 2H (P−CH_2), J(P−CH) = 8.3; 1.19 and 1.16 2 × d, 2 × 6H (4 × CH_3), $J(CH_3,CH)$ = 6.1.

Method B. A solution of compound **31** (5.0 g, 12.8 mmol) in 1 M HCl (120 mL) was refluxed for 1 h, cooled, and neutralized with concentrated aqueous ammonia. The mixture was concentrated to a thick paste and recrystallized from water at room temperature. The crystalline compound **32** was collected by filtration, washed with water, acetone, and ether, and dried in vacuo: yield 3.9 g (81.7%), mp 228 °C, identical according to TLC and ^1H NMR spectrum with material prepared by method A.

Method C. Compound **44** (300 mg, 0.7 mmol) in aqueous ammonia (1:4, 5 mL) was left to stand overnight at ambient temperature and evaporated. The product was codistilled with ethanol and precipitated from methanol (2 mL) with ether: yield 200 mg (66%) of compound **32**, the ^1H NMR spectrum was identical with the specimen prepared by method A.

9-[2-(Phosphonomethoxy)ethyl]guanine (33). Method A. Compound **31** (3.5 g, 9 mmol) in 1 M HCl (100 mL) was refluxed for 1 h, cooled, neutralized with concentrated aqueous ammonia, and evaporated. The residue was deionized on a Dowex 50 × 8 (H^+-form) column (50 mL), and the UV-absorbing ammonia eluate was evaporated and dried in vacuo. The residue in acetonitrile (50 mL) was treated with TMSBr (4 mL), and the mixture was stirred overnight at room temperature in a closed flask. The mixture was taken down to dryness, water (50 mL) was added, and the solution was alkalized with ammonia and evaporated in vacuo. The residue in water (100 mL) was acidified with 0.25 M sulfuric acid to pH 3.5 and left to crystallize at room temperature. The product was collected, washed with water, ethanol, and ether, and dried in vacuo: yield 1.70 g (61.5%) of compound **33** (monohydrate), not melting below 280 °C. Anal. ($C_8H_{12}N_5O_5P$) C, H, N, P. ^1H NMR (D_2O + NaOD): δ 7.87 s, 1H (H-8); 4.21 t, 2H (H-1′), $J(1',2')$ = 5.1; 3.93 t, 2H (H-2′), $J(1',2')$ = 5.1; 3.62 d, 2H (P−CH_2), J(P−CH) = 8.5. UV spectrum: (pH 1) λ_{max} = 254 nm (ϵ_{max} = 10 100), sh 277 (ϵ_{max} = 6500) (pH 7) λ_{max} = 252 nm (ϵ_{max} = 10 700), sh 270 nm (ϵ_{max} = 7200); (pH 12) λ_{max} = 257 nm (ϵ_{max} = 9300); sh 268 nm (ϵ_{max} = 9000).

Method B. Compound **32** (2.0 g, 5.4 mmol) in acetonitrile (40 mL) was treated with bromotrimethylsilane (4 mL) at room temperature overnight and evaporated. Water (50 mL) was added, and the solution was alkalized with ammonia and evaporated. The residue in water (100 mL) was acidified with 0.25 M sulfuric acid to pH 3.5 and left to crystallize at room temperature. The product was collected, washed with water, ethanol, and ether, and dried in vacuo: yield 1.1 g (66.3%) of compound **33** (monohydrate), identical with the product obtained by method A.

2-Amino-6-mercapto-9-[2-(phosphonomethoxy)ethyl]-purine (35). A mixture of 6-chloro derivative **31** (2.2 g, 5.6 mmol) and thiourea (1.5 g, 18.8 mmol) in ethanol (100 mL) was heated for 1 h in reflux, alkalized with triethylamine, and evaporated. The residue was extracted with boiling chloroform (total, 300 mL), the filtrate was evaporated, and the crude diester **34** was dried in vacuo. Acetonitrile (40 mL) and TMSBr (4 mL) were added, and the mixture was stirred in a closed flask overnight. The suspension was evaporated to dryness, water (100 mL) was added, and the solution was alkalized with ammonia. The solution was evaporated to dryness, the residue was redissolved in water (80 mL), and the solution was acidified with 0.5 M sulfuric acid to pH 3.5−4.0. The product which crystallized overnight in the refrigerator was collected, washed with water, and recrystallized from water: yield 1.4 g (82%) of yellow crystalline compound **35**, not melting below 280 °C. Anal. ($C_8H_{12}N_5O_4PS$) C, H, N, P. ^1H NMR (D_2O + NaOD): δ 8.03 s, 1H (H-8); 4.26 t, 2H (H-1′), $J(1',2')$ = 5.0; 3.94 t, 2H (H-2′), $J(1',2')$ = 5.0; 3.64 d, 2H (P−CH_2), J(P−CH) = 8.6.

Bis(2-propyl) 8-Bromo-9-[2-(phosphonomethoxy)ethyl]guanine (36a). Compound **32** (3.0 g, 8 mmol) in DMF (25 mL) was treated with bromine solution in CCl_4 (0.3 M, 30 mL). The resulting solution was left to stand for 4 h at room temperature, CCl_4 was evaporated in vacuo at 30 °C, and another portion of bromine solution in CCl_4 (0.3 M, 20 mL) was added. After 1 h at room temperature, the conversion was complete (S3, R_f = 0.28 (**32**), 0.38 (**36a**)). The mixture was concentrated in vacuo at 45 °C and applied in water (50 mL) on a column (150 mL) of Dowex 50 × 8 in H^+-form. The column was washed with water until the drop of acidity and UV absorption of the eluate and then with diluted (1:10) ammonia in 20% aqueous methanol. The UV-absorbing fraction was evaporated in vacuo and recrystallized from ethyl acetate/petroleum ether: yield 3.4 g (94%) of compound **36a**, mp 225 °C. Anal. ($C_{14}H_{23}BrN_5O_5P$) C, H, Br, N, P. ^1H NMR ($(CD_3)_2SO$): δ 10.69 s, 1H (NH); 6.57 br s, 2H (NH_2); 4.50 m, 2H (P−OCH); 4.10 t, 2H (H-1′), $J(1',2')$ = 5.2; 3.83 t, 2H (H-2′), $J(2',1')$ = 5.2; 3.75 d, 2H (P−CH_2), J(P−CH) = 8.3; 1.17 and 1.14 2 × d, 6 H (2 × CH_3), $J(CH_3,CH)$ = 6.1. UV spectrum: (pH 1) λ_{max} = 257 nm (ϵ_{max} = 14 600); sh 275 (ϵ_{max} = 11 000).

8-Bromo-9-[2-(Phosphonomethoxy)ethyl]guanine (36b). Compound **36a** (1.6 g, 3.5 mmol) in acetonitrile (40 mL) was treated with TMSBr (4 mL) overnight and taken down to dryness. The residue in water (50 mL) was alkalized with ammonia and evaporated. The residue in water (25 mL) was acidified by addition of Dowex 50 × 8 (H^+-form), and the suspension was applied on a column (100 mL) of the same resin. The column was washed with water. The UV-absorbing fraction which eluted with retention was evaporated and crystallized from water: yield 1.0 g (77%) of compound **36b**, not melting at <260 °C. Anal. ($C_{14}H_{23}BrN_5O_5P$) C, H, Br, N, P. ^1H NMR (D_2O + NaOD): δ 4.25 t, 2H (H-1′), $J(1',2')$ = 5.5; 3.92 t, 2H (H-2′), $J(1',2')$ = 5.5; 3.51 d, 2H (P−CH_2), J(P−CH) = 8.3.

Bis(2-propyl) 7-[2-(Phosphonomethoxy)ethyl]guanine (38a). Method A. A mixture of compound **37** (1.2 g, 3.1 mmol),

potassium carbonate (0.42 g, 3.1 mmol), and DABCO (0.42 g, 3.8 mmol) in water (35 mL) was stirred at reflux for 3 h, and the crystalline product **38a** was filtered, washed with water, and recrystallized from water: yield 0.90 g (77.5%), mp 240 °C. Anal. ($C_{14}H_{24}N_5O_5P$) C, H, N, P. ^{1}H NMR ($(CD_3)_2SO$): δ 10.88 brs, 1H (NH); 7.86 s, 1H (H-8); 6.17 br s, 2H (NH_2); 4.49 m, 2H (P−OCH); 4.35 t, 2H (H-1′), J(1′,2′) = 4.9; 3.85 t, 2H (H-2′), J(2′,1′) = 4.9; 3.75 d, 2H (P−CH_2), J(P−CH) = 8.6; 1.18 d, 6H and 1.11d, 6 H (4 × CH_3), J(CH_3,CH) = 6.1.

Method B. A solution of compound **43** (2.4 g, 5.6 mmol) in methanol (50 mL) and concentrated aqueous ammonia (50 mL) was left to stand overnight at room temperature and evaporated in vacuo. The residue gave, on crystallization from ethanol−ether, chromatographically pure compound **38a** (1.8 g, 86%), with a ^{1}H NMR spectrum identical with the sample prepared by method A.

7-[2-(Phosphonomethoxy)ethyl]guanine (38b). Compound **38a** (0.7 g, 1.9 mmol) in acetonitrile (30 mL) was treated with TMSBr (3 mL) at room temperature overnight and evaporated. Water (50 mL) was added, the mixture deionized on a Dowex 50 column, and the residue of the ammonia eluate was chromatographed on a Dowex 1 × 2 (acetate) column (100 mL) with acetic acid (0−1 M, 1 L each) gradient. The product-containing fraction (0.5−0.6 M) was evaporated, and the residue was codistilled with water (3 × 25 mL) and crystallized from water/ethanol: yield 0.35 g (61%) of the monohydrate of compound **38b**, not melting below 270 °C. Anal. ($C_8H_{12}N_5O_5P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.03 s, 1H (H-8); 4.44 t, 2H (H-1′), J(1′,2′) = 4.9; 3.97 t, 2H (H-2′), J(1′,2′) = 4.9; 3.57 d, 2H (P−CH_2), J(P−CH) = 8.8. UV spectrum: (pH 1) λ_{max} = 250 nm (ϵ_{max} = 10 100), sh 274 (ϵ_{max} = 6500); (pH 7) λ_{max} = 284 nm (ϵ_{max} = 7400); (pH 12) λ_{max} = 281 nm (ϵ_{max} = 7400).

2-Amino-6-mercapto-7-[2-(phosphonomethoxy)ethyl]purine (39). A solution of compound **37** (2.1 g, 5.4 mmol) and thiourea (2.5 g) in ethanol (100 mL) was refluxed for 2 h, alkalized with triethylamine, and evaporated. The mixture was extracted with chloroform (2 × 100 mL) and the filtrate evaporated. Acetonitrile (40 mL) and TMSBr (4 mL) were added, and the mixture was stirred overnight in a closed flask. The mixture was evaporated in vacuo and the residue deionized under the standard conditions. The product was purified on a Dowex 1 × 2 (acetate) column (50 mL), the column was washed with water and 1 M acetic acid (200 mL each). The resin was stirred in 10% aqueous formic acid (150 mL), filtered, and washed with boiling water (total, 1 L). The combined eluates gave, on evaporation and filtration from water, 0.50 g (30.4%) compound **39**, not melting below 280 °C. Anal. ($C_8H_{12}N_5O_4PS$) C, H, N, P, S. ^{1}H NMR (D_2O + NaOD): δ 8.18 s (H-8); 4.77 t, 2H, (H-1′) J(1′,2′) = 5.0; 4.01 t, 2H (H-2′), J(1′,2′) = 5.0; 3.61 d, 2H (P−CH_2), J(P−CH) = 8.8. UV spectrum: (pH 2) λ_{max} = 259 nm (ϵ_{max} = 14 700).

2-Amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (40). Compound **31** (1.96 g, 5 mmol) in acetonitrile (30 mL) was treated with bromotrimethylsilane (5 mL) overnight and evaporated in vacuo, and the residue was codistilled with acetonitrile (2 × 25 mL) and toluene (2 × 25 mL). Water (50 mL) was added to the residue, and the solution was left to stand 30 min at ambient temperature and neutralized with lithium hydroxide. The solution was evaporated in vacuo to dryness and codistilled with ethanol (2 × 25 mL). The residue was stirred with an ethanol−ether mixture (1:2) (50 mL) and then decanted, and the washing continued under the same conditions. The residue was filtered, washed with the same mixture (20 mL), and dried in vacuo: yield 1.12 g (56%) of compound **40**. Anal. ($C_8H_9ClLi_2N_5O_4P{\cdot}HBr$) C, H, N, P, Cl, Br. ^{1}H NMR (D_2O): δ 8.19 s, 1H (H-8); 4.31 t, 2H (H-1′), J(1′,2′) = 5.1; 3.94 t, 2H (H-2′), J(1′,2′) = 5.1; 3.61 d, 2H (P−CH_2), J(P−CH) = 8.6.

2-Hydroxy-9-[2-(phosphonomethoxy)ethyl]adenine (41b). 2-Methylpropyl nitrite (15 mL) was added to a solution of compound **22b** (5.0 g, 13.4 mmol) in 80% acetic acid (75 mL) under Ar, and the mixture was left to stand at 0 °C overnight. The mixture was evaporated, the residue codistilled with toluene (three 20 mL portions), and the residue chromatographed on a silica gel column (300 mL) by chloroform/methanol (95:5) gradient. The product was evaporated and dried in vacuo. Crystallization from ethyl acetate (ether added until turbidity) afforded crude crystalline compound **41a** (3.7 g) containing, according to ^{1}H NMR, approximately 15% of a contaminant. ^{1}H NMR ($(CD_3)_2SO$): δ 10.30 br, 1H (OH); 8.20 br, 2H (NH_2); 7.72 s, 1H (H-8); 4.52 m, 2H (P−OCH); 4.11 t, 2H (H-1′), J(1′,2′) = 5.0; 3.81 t, 2H (H-2′), J(2′,1′) = 5.0; 3,78 d, 2H (P−CH_2), J(P−CH) = 8.3; 1.19, 6 H and 1.15 d, 6H (4 × CH_3), J(CH_3,CH) = 6.1.

This compound (2.5 g) in acetonitrile (40 mL) was treated with TMSBr (4 mL) overnight and evaporated in vacuo. The residue was codistilled with toluene (25 mL) and dissolved with water (50 mL) by the addition of concentrated aqueous ammonia (10 mL). The solution was evaporated, and the residue applied on a Dowex 50 × 8 (H^+) column (100 mL). The column was extensively eluted with water until the drop of UV absorption. Further elution of the column with 2.5% ammonia afforded a crude material which was purified by chromatography on a Dowex 1 × 2 (acetate) column with acetic acid (0−0.4 M, 1 L each) gradient. The main fraction was evaporated, and the residue was crystallized from water: yield 0.90 g (46%) of compound **41b**, not melting below 260 °C. Anal. ($C_8H_{12}N_5O_6P{\cdot}H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 7.89 s, 1H (H-8); 4.19 t, 2H (H-1′), J(1′,2′) = 4.9; 3.95 t, 2H (H-2′), J(1′,2′) = 4.9; 3.67 d, 2H (P−CH_2), J(P−CH) = 8.8. UV spectrum: (pH 1) λ_{max} = 283 nm (ϵ_{max} = 12 400); (pH 7) λ_{max} = 292 nm (ϵ_{max} = 10 200); (pH 12) λ_{max} = 278 nm (ϵ_{max} = 7800), sh 246 nm (ϵ_{max} = 6500).

Bis(isopropyl) N_2-Dimethylaminomethylene-7-[2-(phosphonomethoxy)ethyl]guanine (43) and Bis(isopropyl) N_2-Dimethylaminomethylene-9-[2-(phosphonomethoxy)ethyl]guanine (44). N_2-Dimethylaminomethyleneguanine[50] (**42**) (4.1 g, 20 mmol) in DMF (40 mL) was treated under stirring at room temperature with DBU (3.2 mL, 20.6 mmol), and after 20 min of stirring, synthon **4** (7.5 mL, 33 mmol) was added. The mixture was heated for 5 h at 100 °C and taken down in vacuo. The residue was codistilled with toluene (3 × 50 mL) and chromatographed on a column (250 mL) of silica gel in chloroform (stepwise methanol gradient of 2.5%). Elution afforded compound **43** (4.3 g, 57.5%) as an amorphous solid. Anal. ($C_{17}H_{29}N_6O_5P$) C, H, N, P. ^{1}H NMR spectrum: ($(CD_3)_2SO$): δ 11.37 s, 1H (NH); 8.61 s, 1H (N=CH−N); 7.94 s, 1H (H-8); 4.50 dsept, 2H (P−OCH), J(P,OCH) = 7.6, J(CH,CH_3) = 6.1; 4.41 t, 2H (H-1′), J(1′,2′) = 5.1; 3.87 t, 2H (H-2′), J(2′,1′) = 5.1; 3.76 d, 2H (P−CH_2), J(P−CH) = 8.3; 3.14 s, 3H and 3.01 s, 3H (N−CH_3); 1.18 d, 6H and 1.14d, 6H (4 × CH_3), J(CH_3,CH) = 6.1.

Further elution of the column gave compound **44** (0.45 g, 5.3%) (crystallized from ethyl acetate−petroleum ether), not melting under 250 °C. Anal. ($C_{17}H_{29}N_6O_5P$) C, H, N, P. ^{1}H NMR spectrum: ($(CD_3)_2SO$): δ 11.23 s, 1H (NH); 8.57 s, 1H (N=CH−N); 7.77 s, 1H (H-8); 4.50 dsept, 2H (P−OCH), J(P,OCH) = 7.6, J(CH,CH_3) = 6.1; 4.21 t, 2H (H-1′), J(1′,2′) = 5.1; 3.87 t, 2H (H-2′), J(2′,1′) = 5.1; 3.79 d, 2H (P−CH_2), J(P−CH) = 8.3; 3.15 s, 3H and 3.02 s, 3H (N−CH_3); 1.18 d, 6H and 1.14 d, 6 H (4 × CH_3), J(CH_3,CH) = 6.1.

2-Amino-9-benzyl-6-chloropurine (45) and 2-Amino-7-benzyl-6-chloropurine (46). Benzyl bromide (9 mL, 75 mmol) was added to a mixture of 2-amino-6-chloropurine (8.5 g, 50 mmol), potassium carbonate (20.8 g, 150 mmol), and DMF (300 mL), and the mixture was stirred overnight with exclusion of moisture. The suspension was filtered and the filtrate taken down to dryness in vacuo. The residue was extracted with boiling ethyl acetate (400 mL) and filtered, the extract was evaporated in vacuo, and the product was filtered with ether: yield 8.4 g (64.7%) of compound **45**, mp 204 °C. Anal. ($C_{12}H_{10}ClN_5$) C, H, Cl, N. ^{1}H NMR ($(CD_3)_2SO$): δ 8.24 s, 1H (H-8); 7.20−7.40 m, 5H (arom H); 6.97 s, 2H (NH_2); 5.30 s, 2H (N−CH_2).

The residue after ethyl acetate extraction was crystallized from ethanol to afford the N7-isomer **46** (3.7 g, 28.5%), not melting below 260 °C. Anal. ($C_{12}H_{10}ClN_5$) C, H, Cl, N. ^{1}H NMR

($(CD_3)_2SO$): δ 8.61 s, 1H (H-8); 7.25–7.40 m, 5H (arom H); 6.65 s, 2H (NH_2); 5.57 s, 2H ($N-CH_2$).

2-Amino-6-methylpurine (48). A suspension of 6.4 g (25 mmol) of compound **45** and tetrakis(triphenylphosphino)-palladium (0.7 g) in tetrahydrofuran (THF) (200 mL) in a 1 L flask equipped with reflux condenser and septum was treated in an Ar atmosphere under stirring dropwise via a syringe with trimethylaluminum (2 M solution in toluene, 30 mL), and the syringe was subsequently washed with THF (20 mL). The thus-obtained clear solution was boiled for 3 h in reflux in an Ar atmosphere, cooled, and diluted with toluene (300 mL), and methanol (20 mL) was added dropwise. Solid ammonium chloride (2 g) was added, and the mixture was boiled for 2 h in reflux and filtered while hot over Celite. The filtrate was evaporated in vacuo, the residue dissolved in boiling ethyl acetate, and the crystalline material which promptly formed was filtered: yield 3.1 g (52%) of chromatographically pure (S3) compound **47**, mp 251 °C.

Compound **47** (3.1 g, 13 mmol) was hydrogenated in methanol (400 mL) containing concentrated HCl (20 mL) over 10% Pd/C (1.2 g) and palladium chloride (1.0 g) at room temperature and slight overpressure. The hydrogenation proceeded sluggishly over 2 days. The mixture was filtered over Celite, and the filtrate was made alkaline with ammonia and taken down to dryness. The residue was desalted on a Dowex 50 × 8 column (150 mL) (see above). Aqueous ammonia (2.5%) eluted crude product which was, after evaporation, triturated with ether and filtered. This material afforded on continuous extraction (2 days) with chloroform pure compound **48** (1.55 g, 80%): mp 251 °C. Anal. ($C_6H_7N_5$) C, H, N.

Bis(2-propyl) 2-Amino-6-methyl-9-[2-(phosphonomethoxy)ethyl]purine (50). A mixture of 2-amino-6-methylpurine (**48**) (1.2 g, 8 mmol) and cesium carbonate (1.5 g, 4.6 mmol) in DMF (20 mL) was heated under stirring for 30 min at 80 °C, and compound **4** (2.8 g, 10.8 mmol) was added. The reaction mixture was stirred for 8 h at 100 °C and evaporated in vacuo. The residue was extracted with boiling chloroform (total, 150 mL), and the extract was evaporated. The residue was separated on two preparative plates of silica gel in the system chloroform–ethanol (93:7), and the bands of products were eluted with methanol. The product **50** was obtained as a thick oil (0.65 g, 22%): R_f = 0.52 (S3). ^{1}H NMR ($(CD_3)_2SO$): δ 7.93 s, 1H (H-8); 6.41 br s, 2H (NH_2); 4.49 dsept, 2H (P–OCH), $J(CH,CH_3)$ = 6.1, J(P,CH) = 7.6; 4.20 t, 2H (H-1′), J(1′,2′) = 5.1; 3.85 t, 2H (H-2′), J(2′,1′) = 5.1; 3,76 d, 2H ($P-CH_2$), J(P–CH) = 8.3; 1.17 and 1.12 2 × d, 2 × 6 H (4 × CH_3), $J(CH_3,CH)$ = 6.1.

2-Amino-9-[2-(phosphonomethoxy)ethyl]purine (51). A solution of compound **31** (3.9 g, 10 mmol) in methanol (250 mL) containing concentrated HCl (1 mL) was hydrogenated over 10% Pd/C (1.0 g) at room temperature overnight. The suspension was filtered over Celite and washed with methanol (100 mL), and the filtrate was alkalized with triethylamine. The solvent was evaporated and the residue deionized on a Dowex 50 × 8 (H^+-form) column (100 mL) (elution with 20% aqueous methanol until the neutral reaction of the eluate and then with 2.5% ammonia in 20% aqueous methanol). The UV-absorbing ammonia eluate was evaporated and the residue of compound **49** (R_f = 0.10 in S2; starting **31**, 0.42) dried in vacuo. Acetonitrile (40 mL) and TMSBr (4 mL) were added and the mixture was left to stand overnight at room temperature. The mixture was evaporated in vacuo, water (50 mL) was added, and the solution was alkalized with ammonia. After evaporation, the residue was deionized on Dowex 50 under the standard conditions, and the crude product was purified on Dowex 1 × 2 (100 mL, acetate) by elution with a linear gradient of acetic acid (0–0.3 M, 1 L each). The product-containing fractions were evaporated, the residue was codistilled with water (3 × 25 mL) and dissolved in boiling water, and ethanol (3 vol.) was added. The crystalline compound **51** which separated in the refrigerator was collected and dried in vacuo: yield 1.25 g (45.7%), mp 266 °C. Anal. ($C_8H_{12}N_6O_4P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.57 s, 1H (H-8); 8.23 s, 1H (H-6); 4.33 t, 2H (H-1′), J(1′,2′) = 5.2; 3.92 t, 2H (H-2′), J(1′,2′) = 5.2; 3.49 d, 2H ($P-CH_2$), J(P–CH) = 8.4. UV spectrum: (pH 2) λ_{max} = 246 nm (ϵ_{max} = 3100), 314 (ϵ_{max} = 3500).

2-Amino-6-methyl-9-[2-(phosphonomethoxy)ethyl]purine (52). Compound **50** (0.60 g, 1.6 mmol) in acetonitrile (30 mL) was treated with TMSBr (3 mL), and the solution was left overnight in a closed flask at room temperature. The mixture was evaporated and further worked up as described for compound **51**. The deionized product was applied in slightly alkaline solution (pH 8–9) on a Dowex 1 × 2 column (50 mL), and the ionex was washed with water. The resin was treated batchwise with 1 M acetic acid (100 mL), filtered, and washed with boiling 1 M acetic acid (500 mL). The combined filtrates were evaporated and the residue was codistilled with water (5 × 20 mL) to afford product which was collected from ethanol and dried in vacuo: yield 0.25 g (54.5%) of compound **52**, not melting below 270 °C. Anal. ($C_9H_{14}N_5O_4P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.18 s, 1H (H-8); 4.29 t, 2H (H-1′), J(1′,2′) = 4.9; 3.94 t, 2H (H-2′), J(1′,2′) = 4.9; 3.66 d, 2H ($P-CH_2$), J(P–CH) = 8.8; 2.53 s, 3H (CH_3). UV spectrum: (pH 2) λ_{max} = 248 nm (ϵ_{max} = 4400), 310 (ϵ_{max} = 5800).

Bis(2-propyl) 4-Amino-2-methylthio-7-[2-(phosphonomethoxy)ethyl]pyrrolo[2,3-*d*]pyrimidine (54). A mixture of 4-amino-2-methylthio-7H-pyrrolo[2,3-*d*]pyrimidine (**53**) (1.8 g, 10 mmol), DMF (50 mL), cesium carbonate (1.9 g, 6 mmol), and compound **4** (4.6 g, 14 mmol) was heated at 120 °C under stirring and exclusion of moisture until the base disappeared (12 h, TLC chloroform–methanol, 85:15). The solvent was evaporated in vacuo and the residue was codistilled with toluene (3 × 50 mL). The residue was extracted with boiling chloroform (total, 200 mL), and the extract was concentrated in vacuo. Chromatography on a column (80 g) of silica gel in chloroform afforded, on elution with the same solvent, compound **54** (1.46 g, 36%) as an amorphous foam: R_f = 0.52 (chloroform–methanol, 85:15). Anal. ($C_{16}H_{27}N_4O_4PS$) C, H, N, P, S. ^{1}H NMR spectrum ($(CD_3)_2SO$): δ 7.02 d, 1H (H-8) J(7,8) = 3.4; 7.01 br, 2H (NH_2); 6.44 d, 1H (H-7), J(8,7) = 3.4; 4.48 dsept, 2H (P–OCH), J(P,OCH) = 7.6; 4.24 t, 2H (H-1′); 3.84 t, 2H (H-2′), J(1′,2′) = 5.3; 3.76 d, 2H ($P-CH_2$), J(P,CH) = 8.5; 2.45 s, 3H (SCH_3); 1.17 and 1.13 2 × d, 2 × 6H (4 × CH_3), J = 6.1. Mass spectrum: 403.3 (M + H).

Bis(2-propyl)4-Amino-7-[2-(phosphonomethoxy)ethyl]-pyrrolo[2,3-*d*]pyrimidine (55). A mixture of the methylthio derivative **54** (1.46 g, 3.6 mmol) and Raney-nickel catalyst (15 g) in methanol (200 mL) was heated for 4 h until the disappearance of the starting material at reflux temperature (TLC chloroform–methanol, 85:15). The reaction mixture was filtered while hot, and the precipitate was washed with boiling methanol (200 mL). The filtrate was evaporated in vacuo, and the residue was dried to afford amorphous compound **55** (0.82 g, 64%): R_f = 0.30 (chloroform–methanol, 85:15). ^{1}H NMR spectrum ($(CD_3)_2SO$): δ 8.03 s, 1H (H-2); 7.14 brs, 1H (H-8); 6.93 brs, 2H (NH_2); 6.51 brs, 1H (H-7); 4.48 m, 2H (P–OCH); 4.27 brt, 2H (H-1′); 3.83 brt, 2H (H-2′), J(1′,2′) = 5.0; 3.74 d, 2H ($P-CH_2$), J(P,CH) = 8.0; 1.17 and 1.14 2 × d, 2 × 3H (CH_3), J = 6.0. Mass spectrum: 357.2 (M + H).

4-Amino-7-[2-(Phosphonomethoxy)ethyl]pyrrolo[2,3-*d*]pyrimidine (56). A mixture of compound **55** (0.82 g, 2.3 mmol), acetonitrile (25 mL), and TMSBr (2.5 mL) was stirred in a closed flask for 24 h at room temperature and evaporated in vacuo. The residue was codistilled with acetonitrile (3 × 50 mL), water (50 mL) was added to the residue, and the solution was brought to pH 8 by triethylamine. After concentration in vacuo the mixture was deionized on a column (100 mL) of Dowex 50 × 8 under the standard conditions, and the crude material was further purified by chromatography on Dowex 1 × 2 (see above) by linear gradient of acetic acid (0–0.5 M, 1 L each). After evaporation of the relevant fraction the residue was codistilled with water and ethanol (25 mL each), and compound **56** was crystallized from aqueous ethanol (ether added to turbidity): yield 0.33 g (52%), mp >250 °C, k = 4.3 (2% acetonitrile/0.05 M TEAB), E_{Up} = 0.76. Anal. ($C_9H_{13}N_4O_4P\cdot H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 7.94 s, 1H (H-2); 7.24 d, 1H (H-8), J(7,8) = 3.4; 6.43 d, 1H (H-7), J(7,8) = 3.4;

4.29 t, 2H (H-1′); 3.95 t, 2H (H-2′), $J(CH_2,CH_2)$ = 5.1; 3.58 d, 2H (P−CH_2), J(P,CH) = 8.5. Mass spectrum: 273.1 (M + H). UV spectrum (λ_{max} = (ϵ_{max})): pH 2, 275.0 (9900); pH 7, 273.0 (9900); pH 12, 273.0 (10 000).

4-Aminobenzimidazole (57). 5% Ru/C catalyst (1.0 g) was added to the solution of 2,6-dinitroaniline (20 g, 109 mmol) in ethanol (300 mL), and hydrazine hydrate (10.0 mL) was added dropwise during 30 min under stirring at 60 °C. The resulting mixture was refluxed for 3 h, filtered over Celite, and washed with ethanol (200 mL). The filtrate was concentrated to half of the original volume and left to stand overnight. Product which crystallized overnight was collected, washed with ethanol and petroleum ether, and dried in vacuo: yield 15.0 g (90%) of 4-nitro-1,2-phenylenediamine. This product (15.0 g, 90 mmol) in DMF (100 mL) was treated with triethyl orthoformate (150 mL) and HCl/DMF (6 M, 45 mL), and the mixture was stirred for 2 days at room temperature. The yellow product which separated was filtered, washed with DMF, acetone, and ether, and dried in vacuo: yield 13 g (88.5%) 4-nitrobenzimidazole, mp 248 °C (literature[51] gives mp 248−249 °C).

This compound (6.5 g, 40 mmol) in methanol (250 mL) was hydrogenated over 10% Pd/C catalyst until the reaction was complete (3 h). The suspension was filtered over Celite and evaporated. The residue in water (100 mL) was applied on a Dowex 50 × 8 (H^+-form) column (150 mL) and the column was washed with water until the acid reaction of the eluate diappeared. The column was then washed with 2.5% aqueous ammonia, the UV-absorbing eluate was evaporated in vacuo, and the residue was dried by codistillation with ethanol (3 × 50 mL). The residue afforded, by crystallization from ethyl acetate (petroleum ether to turbidity), compound **57** (3.2 g, 60%): mp 142 °C. Anal. ($C_7H_7N_3$) C, H, N.

4-Amino-1-[2-(phosphonomethoxy)ethyl]benzimidazole (59). 4-Aminobenzimidazole (**57**) (1.86 g, 14 mmol) was added to a stirred suspension of sodium hydride (14 mmol) in DMF (30 mL), and after 30 min of stirring synthon **4** (4 g, 15.4 mmol) was added. The reaction mixture was stirred for 8 h at 110 °C and evaporated in vacuo, and the residue was extracted with boiling chloroform (total, 200 mL). The extract was purified by preparative chromatography on a silica gel plate in chloroform−methanol mixture (95:5). The product **58** was eluted with methanol and evaporated, and the resulting oil was dried in vacuo. Acetonitrile (60 mL) and TMSBr (6 mL) were added, and the mixture, after standing overnight in a closed flask, was taken down to dryness in vacuo. The residue in water (20 mL) was alkalified by concentrated aqueous ammonia and evaporated, and the residue was deionized under the standard conditions. The residue was purified on a Dowex 1 × 2 column (100 mL). The column was first washed with water and then eluted with a linear gradient of acetic acid (0−0.5 M, 1 L each) to afford a fraction of product at 0.4−0.5 M. The fractions were evaporated, the residue codistilled with water (3 × 25 mL) and ethanol, and the residue crystallized from ethanol−ether: yield 1.56 g (42%) of compound **59**, mp 203 °C. Anal. ($C_7H_{11}N_6O_4P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 9.04 s, 1H (H-2); 7.27 t, 1H (H-6), J = 8.1; 7.03 d, 1H (H-7), J(7,6) = 8.3; 6.78 d, 1H (H-5), J(5,6) = 7.8; 4.51 t, 2H (H-1′), J(1′,2′) = 5.0; 4.02 t, 2H (H-2′), J(1′,2′) = 5.0; 3.71 d, 2H (P−CH_2), J(P−CH) = 8.8. UV spectrum: (pH 2) λ_{max} = 269 nm (ϵ_{max} = 4400), 274 (ϵ_{max} = 4600).

2,6-Diamino-1-deazapurine (60a). A solution of 7-amino-5-(dibenzylamino)imidazo[4,5-*b*]pyridine[52] (2.3 g, 70 mmol) in methanol containing concentrated hydrochloric acid (1 mL) and palladium chloride solution (40%, 1 mL) was hydrogenated in the presence of 10% Pd/C (1.0 g) for 6 h at 55 °C and overnight at ambient temperature. The suspension was filtered over Celite, neutralized with methanolic ammonia, and evaporated. The residue was extracted with chloroform, and the insoluble residue was deionized on Dowex 50 × 8 (100 mL). The residue of the ammonia eluate (880 mg) was purified by preparative HPLC with water/methanol gradient. The evaporation of the eluate gave a yellow amorphous compound which was dried in vacuo: yield 630 mg (61%) of compound **60a**, not melting below 310 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): δ 11.85 brs, 1H (NH); 7.61 s, 1H (H-8); 5.75 brs, 2H (NH_2); 5.52 s, 1H (H-1); 5.20 brs, 2H (NH_2). Anal. ($C_6H_7N_5$) C, H, N.

2,6-Diamino-3-deazapurine (60b). A mixture of 4(5)-cyano-5(4)-cyanomethylimidazole[53] and methanolic ammonia (370 mL, saturated at −60 °C) was heated in a steel autoclave for 38 h at 110 °C. The mixture was evaporated, and the residue was purified by preparative HPLC with water/methanol gradient: yield 1.6 g (19%) of compound **60b**, not melting at <310 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): δ 12.75 brs, 1H (NH); 7.69 s, 1H (H-8); 5.76 brs, 2H (NH_2); 5.74 s, 1H (H-3); 5.00 br, 2H (NH_2). Anal. ($C_6H_7N_5$) C, H, N.

2,6-Diamino-9-[2-(phosphonomethoxy)ethyl]-1-deazapurine (62a). A mixture of compound **60a** (500 mg, 3.4 mmol) and cesium carbonate (550 mg, 1.7 mmol) in DMF (25 mL) was stirred at 120 °C. Synthon **4** (870 mg, 1.7 mmol) in DMF (5 mL) was added after 1 h, and the stirring and heating were continued for an additional 4 h. The mixture was taken down to dryness and the residue codistilled with toluene (two 25 mL portions). Extraction with boiling chloroform (total, 250 mL) and chromatography on a column of silica gel with chloroform−methanol gradient gave, after evaporation and drying in vacuo, compound **61a** as a yellow foam: yield 670 mg (54%). ^{1}H NMR spectrum (CD_3COCD_3): δ 7.69 s, 1H (H-8); 5.74 s, 1H (H-1); 5.47 brs, 2H (NH_2); 4.90 brs, 2H (NH2); 4.65 m, 2H (P−OCH); 4.27 t, 2H, (H-1′), J(1′,2′) = 5.2; 3.95 t, 2H (H-2′), J(2′,1′) = 5.2; 3.79 d, 2H (P−CH_2), J(P,CH) = 8.6; 1.27 and 1.24 2 × d, 2 × 6H (CH_3), $J(CH_3$,CH) = 6.1.

This compound (0.65 g, 1.75 mmol) was treated with TMSBr (1.5 mL) in acetonitrile (15 mL) at room temperature overnight, and the mixture was worked up as described for compound **65**. Final purification was performed on Dowex 1 × 2 with acetic acid gradient (0.01−0.2 M, 1 L each): yield, 390 mg (78%) of pale yellow amorphous compound **62a**, mp 159−152 °C. Anal. ($C_9H_{14}N_5O_4P\cdot 2H_2O$) C, H, N, P. ^{1}H NMR spectrum (D_2O): δ 7.92 s, 1H (H-8); 5.88 s, 1H (H-1); 4.35 t, 2H (H-1′), J(1′,2′) = 4.9; 3.94 t, 2H (H-2′), J(2′,1′) = 4.9; 3.61 d, 2H (P−CH_2), J(P,CH) = 8.6. UV spectrum: (pH 1) λ_{max} = 263 nm (ϵ_{max} = 12 600), 291 nm (ϵ_{max} = 12 000) (pH 7) λ_{max} = 263 nm (ϵ_{max} = 10 000), 288 nm (ϵ_{max} = 8700); (pH 12) λ_{max} = 264 nm (ϵ_{max} = 9800), 285 nm (ϵ_{max} = 8500).

2,6-Diamino-9-[2-(phosphonomethoxy)ethyl]-3-deazapurine (62b). Compound **60b** (600 mg, 4 mmol) and cesium carbonate (650 mg, 2 mmol) in DMF (25 mL) were stirred at 120 °C. After 1 h, a solution of synthon **4** (1.05 g, 4 mmol) in DMF (25 mL) was added, and the heating was continued for additional 10 h. The mixture was further worked up and purified as described for compound **61a**: yield 310 mg (21%) of compound **61b** as an amorphous foam. ^{1}H NMR ($(CD_3)_2SO$): δ 7.63 s, 1H (H-8); 5.72 brs, 2H (NH_2); 5.69 s, 1H (H-3); 5.01 brs, 2H (NH_2); 4.51 m, 2H (P−OCH); 4.12 t, 2H (H-1′), J(1′,2′) = 5.1; 3.79 t, 2H (H-2′), J(2′,1′) = 5.1; 3.75 d, 2H (P−CH_2), J(P,CH) = 8.3; 1.19 and 1.16 2 × d, 2 × 6H (CH_3), $J(CH_3$,CH) = 6.1.

Compound **61b** (180 mg, 0.8 mmol) was treated with TMSBr (1 mL) in acetonitrile (10 mL) overnight, and the mixture was worked up as described for compound **62a**. Purification by Dowex 50 and Dowex 1 chromatography (the product was eluted by 0.5 M acetic acid) gave 114 mg (82%) of compound **62b**: mp 275−277 °C. Anal. ($C_9H_{14}N_5O_4P\cdot 2H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 7.98 s, 1H (H-8); 6.22 s, 1H (H-3); 4.27 t, 2H (H-1′), J(1′,2′) = 5.1; 3.91 t, 2H (H-2′), J(2′,1′) = 5.1; 3.47 d, 2H (P−CH_2), J(P,CH) = 8.3. UV spectrum: (pH 1) λ_{max} = 270 nm (ϵ_{max} = 9000), 316 nm (ϵ_{max} = 7100); (pH 7) λ_{max} = 271 nm (ϵ_{max} = 8000), sh 292 nm (ϵ_{max} = 5900); (pH 12) λ_{max} = 271 nm (ϵ_{max} = 8600), sh 295 nm (ϵ_{max} = 6100).

4,7-Diamino-1*H*-imidazo[4,5-*d*]pyridazine (63) (cf. ref 54). Hydrazine hydrate (2.4 g, 75 mmol) in ethanol (50 mL) was added at 80 °C to a solution of 4,5-dicyanoimidazole (2.95 g, 25 mmol) in ethanol (200 mL). The mixture was refluxed for 10 h and evaporated in vacuo, and the residue was codistilled with ethanol (two 50 mL portions). The product was dissolved in a mixture of 50% aqueous ethanol and 10% aqueous ammonia (1:1, 200 mL) and left to stand for 70 h at room temperature. TLC revealed the presence of compound **63** only.

The solution was taken down to dryness, and the residue was applied in water (100 mL) on Dowex 50 × 8 (H^+-form). The column was washed with water until neutral, and the resin was suspended in 10% aqueous ammonia (500 mL). The suspension was filtered, and the resin was washed with boiling water (total, 1 L). Evaporation of the eluate afforded compound **63** (2.97 g, 79%), not melting below 300 °C. ^{1}H NMR ($(CD_3)_2SO$): δ 12.20 brs, 1H (NH); 8.02 s, 1H (H-8); 6.64 brs, 4H (NH_2).

Bis(2-propyl) 4,7-Diamino-1-[2-(phosphonomethoxy)-ethyl]-1*H*-imidazo[4,5-*d*]-pyridazine (64) Method A. A mixture of compound **63** (750 mg, 5 mmol) and cesium carbonate in DMF (40 mL) was stirred at 110 °C, and a solution of synthon **4** (1.3 g, 5 mmol) in DMF (10 mL) was added after 1 h. The heating was continued for an additional 6 h, and the mixture was worked up as described for compound **61a**: yield, 610 mg (33%) of the diester **64** as an amorphous foam.

Method B. Sodium hydride (160 mg of 60% dispersion, 4 mmol) was added to the stirred suspension of compound **63** (600 mg) in DMF (40 mL), and the mixture was heated at 100 °C for 1 h. A solution of the synthon **4** (1.05 g, 4 mmol) in DMF (5 mL) was added, and the heating continued for an additional 4 h. The mixture was processed as described in method A: yield 570 mg (38.5%) of compound **64**, mp 138−140 °C. ^{1}H NMR spectrum ($(CD_3)_2SO$): δ 7.86 s, 1H (H-8); 7.60 brs, 2H and 6.00 brs, 2H (NH_2); 4.50 m, 2H (P−OCH); 4.27 t, 2H (H-1′), J(1′,2′) = 5.4; 3.92 t, 2H (H-2′), J(2′,1′) = 5.4; 3.77 d, 2H (P−CH_2), J(P,CH) = 8.1; 1.15 and 1.10 2 × d, 2 × 6H (CH_3), J(CH_3,CH) = 6.1. ^{13}C NMR spectrum ($(CD_3)_2SO$): δ 154.10 (C-8); 148.68 and 147.33 (C-6, C-3); 134.85 and 132.29 (C-4 and C-5); 70.47 d (P−OC), J(P,C) = 5.9; 69.86 d (C-2′) J(P,C) = 10.7; 65.19 d (P−C), J(P,C) = 164.1; 52.09 (C-1′); 23.92 d, J(P,C) = 2.9 and 23.76 d, J(P,C) = 3.9 (CH_3).

4,7-Diamino-1-[2-(phosphonomethoxy)ethyl]-1*H*-imidazo[4,5-*d*]pyridazine (65). The diester **64** (1.18 g, 3 mmol) was treated with TMSBr (3 mL) in acetonitrile (30 mL) overnight at room temperature and worked up as described for compound **62a.** Final purification was performed by preparative HPLC with water/methanol gradient): yield 690 mg (80%), mp 318−320 °C. Anal. ($C_8H_{13}N_6O_4P{\cdot}2H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.00 s, 1H (H-8); 4.34 t, 2H (H-1′), J(1′,2′) = 5.0; 3.99 t, 2H (H-2′), J(2′,1′) = 5.0; 3.51 d, 2H (P−CH_2), J(P,CH) = 8.3. UV spectrum: (pH 1) λ_{max} = 263 nm (ϵ_{max} = 3800); (pH 7) λ_{max} = 265 nm (ϵ_{max} = 4500); (pH 12) λ_{max} = 265 nm (ϵ_{max} = 4500).

2-Amino-3,4-dihydro-4-oxo-3-[2-(2-phosphonomethoxy)-ethyl]pteridine (68). Sodium hydride (80 mg of 60% dispersion, 2 mmol) was added to a suspension of 2-amino-4-hydroxypteridine (**66**)[55] (320 mg, 2 mmol) in DMF (25 mL), and the mixture was stirred at 100 °C. Synthon **4** (520 mg, 2 mmol) in DMF (5 mL) was added, the mixture was stirred for 16 h at 100 °C and taken down in vacuo. The residue was codistilled with toluene (two 25 mL portions) and extracted with boiling chloroform−methanol mixture (4:1; total, 300 mL), and the extract was purified on a column (200 mL) of silica gel in chloroform by chloroform−methanol gradient: yield 330 mg of oily diester **67**. ^{1}H NMR (CD_3COCD_3): δ 8.69 d, 1H (H-7) J(7,6) = 2.0; 8.42 d, 1H (H-6), J(6,7) = 2.0; 7.12 brs, 2H (NH_2); 4.71 m, 2H (P−OCH); 4.39 t, 2H (H-1′), J(1′,2′) = 5.2; 4.04 t, 2H (H-2′), J(2′,1′) = 5.2; 3.91 d, 2H (P−CH_2), J(P,CH) = 8.1; 1.32 d, 3H + 1.31 d, 3H + 1.27 d, 3H + 1.26 d, 3H (CH_3), J(CH_3,CH) = 6.1.

This compound (300 mg, 0.8 mmol) was treated with TMSBr (1.2 mL) in acetonitrile (12 mL) at room temperature overnight and worked up as decribed for compound **62a**. The deionized crude product was purified by preparative HPLC with water/methanol gradient: yield 135 mg (58%) of compound **68**, mp 186−188 °C. Anal. ($C_9H_{12}N_5O_5{\cdot}2H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.72 d, 1H (H-7), J(7,6) = 2.2; 8.47 d, 1H (H-6), J(6,7) = 2.2; 4.34 t, 2H (H-1′), J(1′,2′) = 5.0; 3.96 t, 2H (H-2′), J(2′,1′) = 5.0; 3.69 d, 2H (P−CH_2), J(P,CH) = 8.8. UV spectrum: (pH 1) λ_{max} = 276 nm (ϵ_{max} = 3700), 320 nm (ϵ_{max} = 4300); (pH 7) λ_{max} = 240 nm (ϵ_{max} = 8700), 270 nm (ϵ_{max} = 7200); (pH 12) λ_{max} = 244 nm (ϵ_{max} = 9700), 271 nm (ϵ_{max} = 8000).

Di(isooctyl) 2-Chloroethoxymethylphosphonate (69). A mixture of tri(isooctyl)phosphite (65 g, 0.176 mol) and 2-chloroethyl chloromethyl ether (31.8 g, 0.281 mol) was heated under stirring for 4 h at 120 °C. The volatiles were distilled off at 120 °C/15 kPa, and the residue was distilled in a kugelrohr apparatus at 160 °C/13 Pa: yield 61.1 g (87%) of compound **69**. This product was used for further alkylations.

Di(isooctyl) 9-[2-(Phosphonomethoxy)ethyl]adenine (70a). A mixture of adenine (2.7 g, 20 mmol) and Cs_2CO_3 (1.7 g, 10 mmol) in DMF (50 mL) was heated at 100 °C, and compound **69** (8.8 g) was added. Heating at 100 °C and stirring were continued for 8 h, and the solvent was evaporated in vacuo. The residue afforded, on a silica gel chromatography (200 mL) column in chloroform, waxy product **70a** (5.4 g, 54%). Mass spectrum: (M + 1) 498.4. Anal. ($C_{24}H_{44}N_5O_4P$) C, H, N, P.

Di(isooctyl) 2,6-Diamino-9-[2-(phosphonomethoxy)-ethyl]purine (70b). Compound **70b** was prepared essentially as described for compound **70a** from 20 mmol of 2,6-diaminopurine: yield 4.4 g (44%) of amorphous compound **70b**. Anal. ($C_{24}H_{45}N_6O_4P$) C, H, N, P.

Di(isooctyl) 2-Amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (71) and Di(isooctyl) 2-Amino-6-chloro-7-[2-(phosphonomethoxy)ethyl]purine (73). NaH (1.6 g, 60% dispersion in mineral oil, 40 mmol) was added under stirring and cooling with ice to a suspension of 2-amino-6-chloropurine (6.82 g, 40 mmol) in DMF (70 mL). The mixture was stirred for 1 h prior to the addition of compound **69** (17.6 g, 44 mmol), and the bath temperature was gradually increased to 80 °C. The reaction mixture was stirred for 2 h at this temperature and then for 16 h at 100 °C. The mixture was taken down to dryness in vacuo, and the residue was codistilled with toluene (2 × 25 mL) and extracted with chloroform (100 mL). The extract was chromatographed on a column (400 mL) of silica gel in chloroform. The first fraction (R_f = 0.50 in S1) gave 10.0 g (47%) of compound **71** as a semisolid material. Anal. ($C_{24}H_{43}ClN_5O_4P$) C, H, Cl, N, P.

Further elution with chloroform−methanol mixture (95:5) gave compound **73** (1.9 g, 9%) as a semisolid material (R_f = 0.40 in S1). Anal. ($C_{24}H_{43}ClN_5O_4P$) C, H, Cl, N, P.

Di(isooctyl) 9-[2-(Phosphonomethoxy)ethyl]guanine (72). A solution of compound **71** (3.25 g, 6.1 mmol) in 1 M HCl in 50% aqueous dioxane (60 mL) was refluxed for 12 h, alkalized by aqueous NH_3, and evaporated in vacuo. The residue was chromatographed on a column (100 mL) of silica gel first with chloroform, and then the product was eluted with chloroform−methanol mixture (95:5): yield 2.2 g (70%) compound **72** as an amorphous foam. Anal. ($C_{16}H_{28}N_5O_5P$) C, H, N, P. Mass spectrum: (M + 1) 514.2.

Di(isooctyl) 7-[2-(Phosphonomethoxy)ethyl]guanine (74). A solution of compound **73** (1.9 g, 3.6 mmol) in 1 M HCl in 50% aqueous dioxane (30 mL) was processed analogously as described for compound **72**: yield 1.3 g (70%) of compound **74** as an amorphous foam. Anal. ($C_{16}H_{28}N_5O_5P$) C, H, N, P.

Isopropyl 9-[2-(Phosphonomethoxy)ethyl]guanine (75a). Compound **32** (1.3 g, 3.5 mmol) and lithium azide (1.3 g) in DMF (15 mL) was stirred for 3 h at 100 °C, and the solvent was evaporated in vacuo. The residue in water (20 mL) was applied on the Dowex 50 × 8 (H^+-form) column (100 mL) and washed with water until the drop of UV absorption. The column was then eluted with 1.5% aqueous ammonia, and the UV-absorbing eluate was taken down to dryness. This residue was in water (20 mL, alkalized by ammonia) applied to the Dowex 1 × 2 column (100 mL, acetate form), and the column was eluted with linear gradient of acetic acid (0.02−1M, 1 L each). The UV-absorbing fraction of the product was pooled and evaporated, and the residue was codistilled with water (2 × 25 mL). The product was crystallized from water to afford 0.96 g (83%) of compound **75a**. Anal. ($C_{11}H_{18}N_5O_5P$) C, H, N, P. ^{1}H NMR ($(CH_3)_2SO$): d 10.61 br s, 1H (NH); 7.71 s, 1H (H-8); 6.48 br s, 2H (NH_2); 4.44 m, 1H (P−OCH); 4.11 t, 2H (H-

1′), J(1′,2′) = 5.1; 3.80 t, 2H (H-2′), J(1′,2′) = 5.1; 3.66 d, 2H (P−CH_2), J(P−CH) = 8.6; 1.14 d, 6H (CH_3), J(CH_3,CH) = 6.1.

Isooctyl 9-[2-(Phosphonomethoxy)ethyl]guanine (75b). Compound **75b** was prepared from compound **72** (3.5 mmol) in a manner similar to that described for compound **75a**: amorphous foam, yield 81%. ^{1}H NMR (($CD_3)_2SO$): δ 11.40 br s, 1H (NH); 8.38 s, 1H (H-8); 7.00 br s, 2H (NH_2); 4.42 t, 2H (H-1′), J(1′,2′) = 5.0; 3.87 t, 2H (H-2′), J(1′,2′) = 5.0; 3.80 m, 2H (P−OCH); 3.68 d, 2H (P−CH_2), J(P−CH) = 8.1; 2.40 m, 2H + 1.50 m, 2H + 1.22 m, 4H + 1.10 m, 1H (CH_2); 0.81 br d, 6H (CH_3), J(CH_3,CH) = 5.0.

Antiviral 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) cell cultures, following the previously established procedure.[1a,56] Briefly, confluent cell cultures in microtiter 36-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 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 MT-4 and CEM Cells. The methodology of the anti-HIV assays has been described previously.[3b] Briefly, human MT-4 (~4 × 10^5-cells mL^{-1}) or CEM (~3 × 10^5 cells mL^{-1}) cells were infected with 100 $CCID_{50}$ of HIV-1 (III_B)/mL and seeded in 200 μL wells of a microtiter plate, containing appropriate dilutions of the test compounds. After 5 (MT-4) or 4 days (CEM) of incubation at 37 °C, the number of viable (MT-4) cells was determined in a blood cell counting chamber by trypan blue dye exclusion or 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.[3b] 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. Twenty-four hours 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[2c]) 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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