Acyclic Nucleotide Analogs Derived from 8-Azapurines: Synthesis and Antiviral Activity

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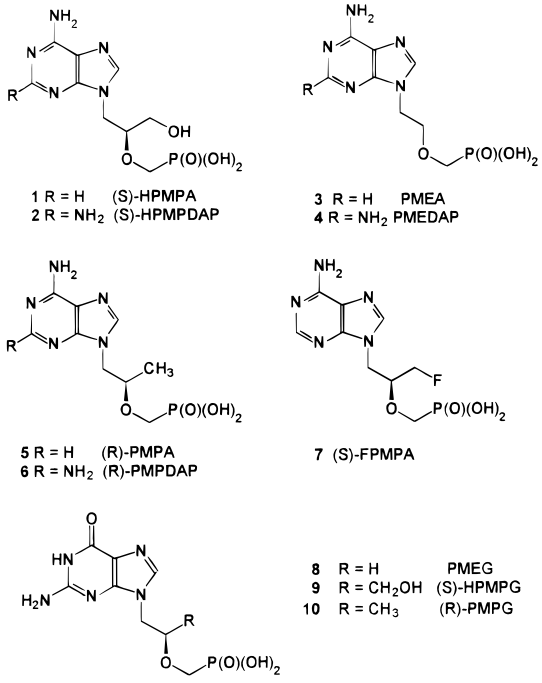
Reaction of phosphoroorganic synthons with 8-azaadenine, 8-aza-2,6-diaminopurine, and 8-azaguanine using cesium carbonate yielded regioisomeric 8-azapurine N^7-, N^8-, and N^9-(2-(phosphonomethoxy)alkyl) derivatives. This reaction followed by deprotection afforded isomeric 2-(phosphonomethoxy)ethyl (PME), (*S*)-(3-hydroxy-2-(phosphonomethoxy)propyl) [(*S*)-HPMP], (*S*)-(3-fluoro-2-(phosphonomethoxy)propyl) [(*S*)-FPMP], (*S*)-(2-(phosphonomethoxy)propyl) [(*S*)-PMP], and (*R*)-(2-(phosphonomethoxy)propyl) [(*R*)-PMP] derivatives. ^{13}C NMR spectra were used for structural assignment of the regioisomers. None of the 8-isomers exhibited any antiviral activity against herpesviruses, Moloney murine sarcoma virus (MSV), and/or HIV. 9-(*S*)-HPMP-8-azaadenine (**23**) and PME-8-azaguanine (**65**) were active against HSV-1, HSV-2, and CMV at 0.2−7 μg/mL, VZV at 0.04−0.4 μg/mL, and MSV (at 0.3−0.6 μg/mL). PME-8-azaguanine (**65**) and (*R*)-PMP-8-azaguanine (**71a**) protected MT-4 and CEM cells against HIV-1- and HIV-2-induced cytopathicity at a concentration of ∼2 μg/mL.

Introduction

In the series of acyclic nucleotide analogues that contain a phosphonomethyl ether function replacing the phosphoric acid ester group four structural types of these analogues possessing high activity against DNA viruses and retroviruses have emerged: *N*-(2-(phosphonomethoxy)ethyl) (PME) derivatives and their 2-substituted analogues, HPMP [*N*-(3-hydroxy-2-(phosphonomethoxy)propyl)] derivatives, FPMP [*N*-(3-fluoro-2-(phosphonomethoxy)propyl)] derivatives, and PMP [*N*-(2-(phosphonomethoxy)propyl)] derivatives. The antiviral activity is mostly connected with the presence of certain purine heterocyclic bases: in the above cases, the activity was always encountered in adenine, 2,6-diaminopurine and guanine derivatives.[1]

Adenine and 2,6-diaminopurine derivatives of the (*S*)-HPMP series, (*S*)-HPMPA (**1**) and (*S*)-HPMPDAP (**2**), exert a marked antiviral effect against DNA viruses.[2] The guanine derivatives (*S*)-HPMPG (**9**) and its racemate are also active;[3] however, they exhibit higher cytotoxicity to the host cells. PME derivatives PMEA (**3**) and PMEDAP (**4**) show potent *in vitro* and *in vivo* antiviral activity against DNA viruses and retroviruses.[4] The latter effect was verified in animal models for retroviral infection (i.e., FIV, MLV, Visna virus).[5] PMEA (Adefovir, **3**)[6] and its oral diester prodrug [bis-(POM)-PMEA][7] are pursued in clinical trials against AIDS and hepatitis B.[8] The guanine derivative PMEG (**8**) is also toxic to most cell lines *in vitro*; however, it has a remarkable activity against papillomaviruses.[9] The anticancer activity of purine PME derivatives was reported in the literature.[10] Similar activity against animal retroviruses *in vivo* and HIV *in vitro* was found for the fluoromethyl derivative of PMEA (FPMPA, **7**).[11]

Retroviruses are the exclusive target for compounds of the PMP group,[12] and the (*R*)-enantiomers of adenine (PMPA, **5**) and 2,6-diaminopurine (PMPDAP, **6**) display the highest known antiretroviral effect among nucleotide analogues.[13] (*R*)-PMPA is the first compound reported to completely protect macaques against SIV infection.[14]



Detailed studies have further shown that the biological effect has an enantiospecific character in all adenine derivatives, while no absolute distinction between the enantiomers was observed in the 2,6-diaminopurine and guanine series.[13] This effect can be interpreted by the involvement of different nucleotide kinases in the activation of these compounds.[15] Guanine derivatives are always the most active and simultaneously the most

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toxic of the triad, the toxicity of the adenine derivatives being the weakest. The inhibition of purine nucleoside phosphorylase by the guanine phosphonates and their metabolites (mono- and diphosphates) that perturbs the general purine nucleotide pools in the cell could explain the cellular toxicity in these cases.[16]

In numerous structure−activity studies we have demonstrated that no activity in any of the usual targets ever occurs with the phosphonate derivatives derived from bases that are devoid of amino groups (hypoxanthine, xanthine) and that an additional substitution of the purine ring at the position 2, 6, and/or 8 typically suppresses the biological activity.[1] However, in some cases the nitrogen atom of the pyrimidine ring in purine moiety can be replaced by the −CH═ grouping without substantial loss of antiviral activity. In particular, the 3-deazaadenine analogue of 9-(*S*)-(3-hydroxy-2-(phosphonomethoxy)propyl)adenine (**1**)[17] showed not only a potent antiviral effect[18] but also a notable activity against *Plasmodium* sp. surpassing that of the parent adenine derivative **1**.[19] 8-Azapurine (*v*-triazolo[4,5-*d*]pyrimidine) derivatives are subject of continuous interest in medicinal chemistry. Thus, 9-isomers of diverse 8-azaadenine and 8-azahypoxanthine nucleosides exhibit antiviral or cytostatic activity.[20] An antileishmanial or antitrypanosomal activity was also reported for 8-azainosine.[21]

As a continuation of our studies on base modification in the series of acyclic nucleotide analogues, we have already investigated the effects of the replacement of −N═ for −CH═ grouping at position 2 of the purine ring.[22] In this communication, we report the synthesis of several types of nucleotide analogues derived from 8-azapurines. Our results were published in preliminary form.[23] Several (phosphonylmethoxy)alkyl derivatives of 8-azapurines were also reported by other authors.[24]

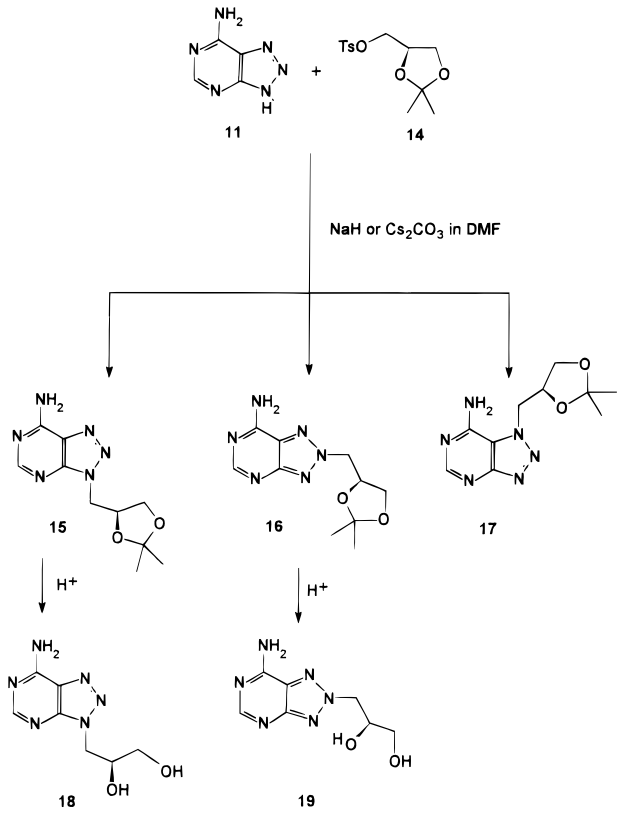
Chemistry

Although the formation of 9-isomers predominates in the glycosylation reactions of 8-azapurines whether by acid fusion, by reaction of halogenoses with chloromercuri or silyl derivative of the bases,[25] or by the phase transfer glycosylation,[26] other regioisomers are regularly formed during such reactions. In order to avoid the low regiospecificity of 8-azapurine alkylation, the acyclic analogues of nucleosides derived from 8-azapurines [9-(2,3-dihydroxypropyl) derivatives of 8-azahypoxanthine and 8-azaadenine] were prepared by ring closure reactions from 4-(azidomethyl)oxirane derivatives.[24,27,28]

We have been interested in evaluating the biological activity of all regioisomers in the 8-azapurine series. Therefore, in our synthetic approach, we have employed alkylations of alkali metal salts of the commercially available 8-azapurine bases. As alkylating agents, we have mostly used synthons and procedures described earlier for the preparation of other nucleotide analogues. As the base, cesium carbonate[29] was frequently found to be useful for such alkylations. The individual major regioisomers were in most cases isolated as phosphonate diesters and characterized by NMR and UV spectra before bromotrimethylsilane deprotection to the free phosphonic acids.

8-Azaadenine Derivatives. In order to make unequivocal assignment of individual regioisomers in the 8-azaadenine series, we have examined the alkylation of 8-azaadenine (**11**) by (*R*)-2,2-dimethyl-4-[[(*p*-tolylsulfonyl)oxy]methyl]-1,3-dioxolane (**14**) (Scheme 1) in the presence of cesium carbonate. The protected 1,3-dioxolane derivatives **15**−**17** were separated by silica gel chromatography, and their structure was unequivocally assigned by ^{13}C NMR and IR spectra. Contrary to the N^9 (**15**) and N^8 (**16**) isomers that are in IR spectra characterized by the frequencies of free and associated 6-amino function [$\nu(NH_2)_{free}$, 3523, 3410 cm^{-1}; ν_{assoc}, 3474, 3327 cm^{-1}] the amino group in the N^7-isomer **17** participates in the hydrogen bond formation with the adjacent oxygen atom of the 1,3-dioxolane ring that is reflected by the presence of $\nu(NH_2)_{intramol.bridge}$, 3467, 3347 cm^{-1}. The ratio of regioisomers (estimated by HPLC) was in this case N^9:N^8:N^7 = 47:46:7. This structural assignment was used for other cases. 9-(*S*)-(2,3-Dihydroxypropyl)-8-azaadenine (**18**) and its N^8-isomer **19** were prepared from these intermediates by acid treatment. These compounds are 8-aza analogues of 9-(*S*)-(2,3-dihydroxypropyl)adenine—an established methylation inhibitor and antiviral agent[30]—and were synthesized in the optically active form for the first time.

Scheme 1

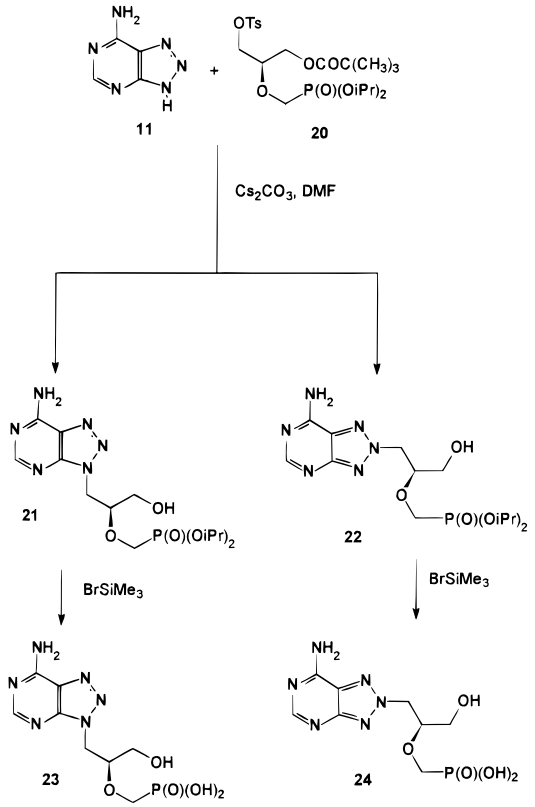


11 R = H
12 R = NH2

13

The synthesis of the 8-aza analogue of the antiviral HPMPA (**23**) (Scheme 2) made use of the cesium carbonate-mediated alkylation of 8-azaadenine (**11**) with 2(*R*)-[(diisopropylphosphono)methoxy]-3-(trimethylacetoxy)propyl *p*-toluenesulfonate (**20**).[31] This reaction

Scheme 2



afforded, after methanolysis, an equimolar mixture of the protected isomeric intermediates **21** and **22**. Their treatment with bromotrimethylsilane followed by hydrolysis gave ultimately (*S*)-9-(3-hydroxy-2-(phosphonomethoxy)propyl)-8-azaadenine (**23**) and its 8-isomer (**24**). The formation of 7-isomer was not detected in this case.

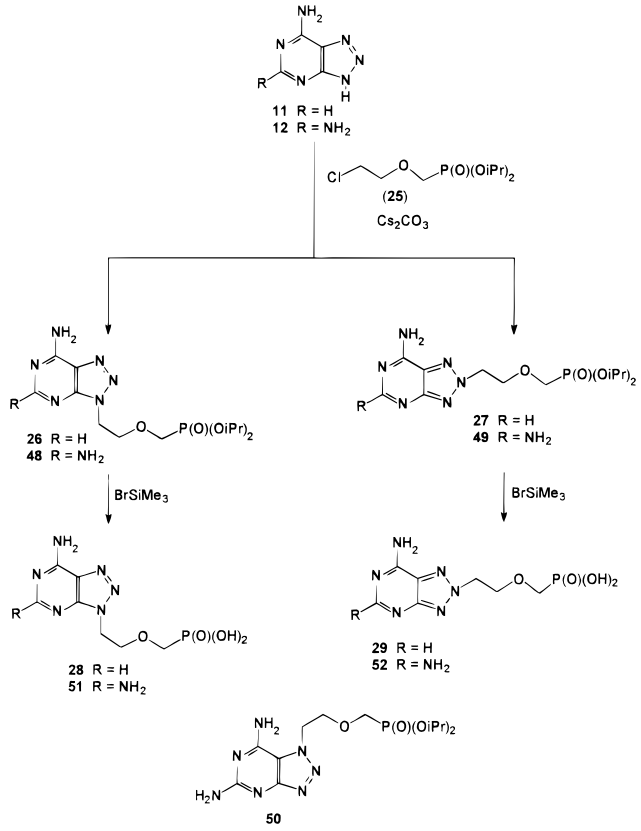
A similar procedure consisting of the alkylation of 8-azaadenine (**11**) by a synthon bearing structural features of the required side chain was applied to the synthesis of 9-(2-(phosphonomethoxy)ethyl)-8-azaadenine (8-aza analogue of PMEA) (**28**) and its regioisomers. 8-Azaadenine (**11**) afforded on treatment with bis(2-propyl) [(2-chloroethoxy)methyl]phosphonate (**25**)[32] protected intermediate diesters: the 9-isomer **26** slightly prevailed over the 8-isomer **27** (ratio, 1.5:1). Additional regioisomers were not detected. Free 8-aza analogues of PMEA (**28**) and its 8-isomer **29** were obtained by transsilylation reaction of individual diesters and subsequent hydrolysis (Scheme 3).

An approximately equimolar ratio in the formation of the N^9- and N^8-isomers was observed also in the cesium carbonate-promoted alkylation of 8-azaadenine with synthon **30**.[33] The intermediary diesters **31** and **32** were separated by silica gel chromatography, and their structures were proven by ^{13}C NMR. Treatment with bromotrimethylsilane afforded the isomers of *N*-(*S*)-(3-fluoro-2-(phosphonomethoxy)propyl)-8-azaadenine (**33, 34**) related to the antiretroviral compound FPMPA [9-(*S*)-(3-fluoro-2-(phosphonomethoxy)propyl)-adenine (**7**) (Scheme 4).

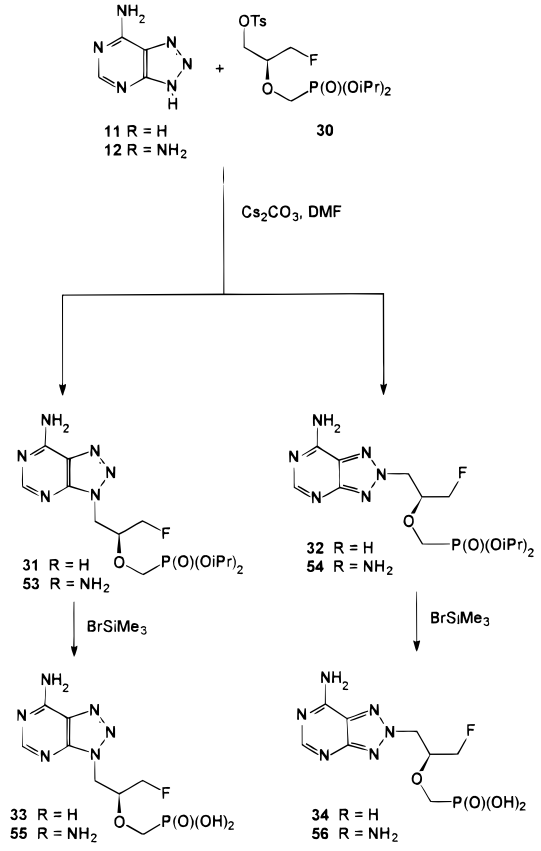
The synthon **35** was applied for the preparation of (*R*)-PMP derivatives.[11] Alkylation of 8-azaadenine in a cesium carbonate-promoted reaction afforded the usual mixture of regioisomeric diesters **36** and **37** with the 8-isomer **37** prevailing. This observation is in agreement with recently published[21] results of other authors who made use of our previously described procedure.[34]

The 9- and 8-isomeric PMP-derivatives **38** and **39** were obtained from the protected intermediates (sepa-

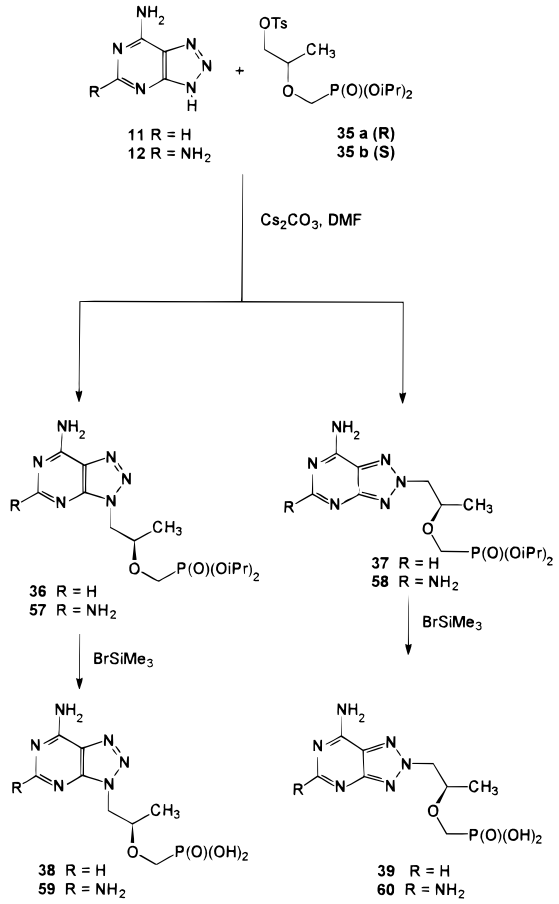
Scheme 3



Scheme 4



Scheme 5

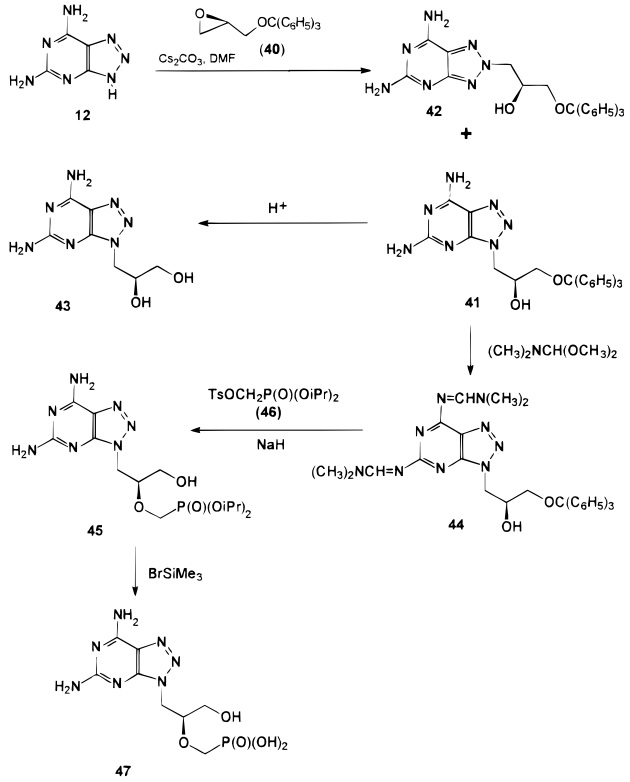


rated by silica gel chromatography) by standard deprotection method (*vide supra*). No significant formation of any additional regioisomer was observed under the reaction conditions (Scheme 5).

8-Aza-2,6-diaminopurine Derivatives. The synthesis of (*S*)-9-(3-hydroxy-2-(phosphonomethoxy)propyl)-8-aza-2,6-diaminopurine (**47**) makes use of a general procedure for stepwise synthesis of HPMP compounds:[35] 8-Aza-2,6-diaminopurine (**12**) was treated with [(trityloxy)methyl]-(*R*)-oxirane (**40**), and the resulting tritylated intermediates **41** and **42** that were formed in an approximately equimolar ratio were separated by silica gel chromatography. The trityl derivative **41** was transformed to the *N*2,*N*6-bis((dimethylamino)methylene) derivative **44** by the reaction with dimethylformamide dimethyl acetal.[36] Condensation with bis(2-propyl) [[(*p*-tolylsulfonyl)oxy]methyl]phosphonate (**46**)[31] in the presence of excess sodium hydride followed by acid hydrolysis gave the diester **45** that was cleaved to the ultimate (*S*)-HPMP derivative **47** by transsilylation reaction. The structure of the 9-isomer was also proven by detritylation of compound **41** to 9-(*S*)-(2,3-dihydroxypropyl)-8-aza-2,6-diaminopurine (**43**) (Scheme 6).

Cesium carbonate mediated reaction of 8-aza-2,6-diaminopurine (**12**) with bis(2-propyl) [(2-chloroethoxy)-methyl]phosphonate (**25**) gave 9-, 7-, and 8-isomeric diesters **48**−**50** in the ratio 10:1:8. We have not succeeded in the complete separation of the 7- (**50**) and 8-isomer (**49**); however, the ^{1}H-NMR signals of compound **50** in the 1:10 mixture with compound **49** were unequivocally separated and assigned. The diesters **48** and **49** were transformed into the free phosphonates **51** and **52** (Scheme 3).

Scheme 6

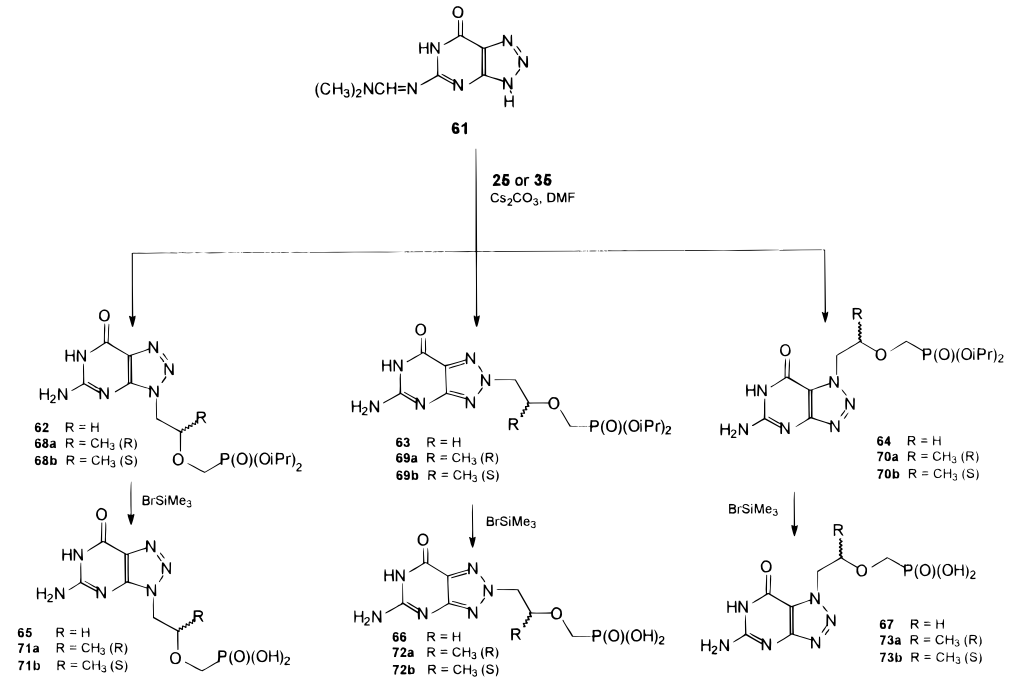


The synthesis of 9-(*S*)-(3-fluoro-2-(phosphonomethoxy)-propyl)-8-aza-2,6-diaminopurine (**55**) followed the route described for the 8-azaadenine analogue **31,** i.e., treatment of the base **12** with the synthon **30**. The isomeric diester intermediates **53** and **54** were separated on silica gel and deprotected to afford the 9-isomer **55** and its 8-congener **56** (Scheme 4).

Contrary to the reaction with compound **25**, 8-aza-2,6-diaminopurine (**12**) gave on treatment with the tosylate **35a** in the presence of cesium carbonate the (*R*)-9- (**57**) and (*R*)-8-isomer (**58**) only, in an approximately equimolar (1.25:1) ratio. The deprotection of the phosphonate diester grouping gave the regioisomers of the 8-aza analogues of (*R*)-PMP-series **59** and **60.** (Scheme 5).

8-Azaguanine Derivatives. Extremely low solubility of 8-azaguanine in the reaction medium unfavorably influences the process of its alkylation. To circumvent the problem of low solubility, 8-azaguanine (**13**) was transformed by treatment with dimethylformamide dineopentylacetal to its N^2-[(dimethylamino)methylene] derivative **61** which is well soluble in dimethylformamide (the use of dimethylformamide dimethyl acetal led to undesirable formation of *N*-methyl derivatives as side products). Compound **61** gave on treatment with synthon **25** and following methanolysis a mixture of 9- (**62**), 8- (**63**), and 7-isomers (**64**) in the ratio of 2:2:1. Their chromatographic separation on silica gel is difficult. However, the 9-isomer **62** could successfully be separated from the other isomers by ion exchange chromatography on Dowex 50 × 8 in the acid form. Elution with water afforded chromatographically pure 9-isomer **62** while the mixture of the remaining 7- and 8-isomers was eluted subsequently with diluted ammonia in aqueous methanol. The diesters were deprotected by transsilylation: compound **62** gave the pure 9-isomer **65** while the mixture of isomers **66** and **67** obtained by

Scheme 7



the deprotection of their diesters was separated by chromatography on Sephadex A-25 in triethylammonium hydrogen carbonate (Scheme 7).

A similar procedure was applied in the synthesis of the enantiomeric PMP derivatives of 8-azaguanine: reaction of N^2-[(dimethylamino)methylene] derivative **61** with the synthons **35a** or **35b** followed by ammonolysis gave the mixture of 9-, 7-, and 8-isomer in the 9:1:8 ratio. This mixture was deprotected by transsilylation, and the resulting free phosphonates were separated on Dowex 50 column (*vide supra*) to afford pure 9-isomers **71a** and **71b** by elution with water. The mixtures of the corresponding 8- (**72a, 72b**) and 7-isomers (**73a,73b**), obtained by elution of the column with aqueous ammonia, were separated on Sephadex A-25 in triethylammonium hydrogen carbonate (Scheme 7).

NMR Spectra. The structure of acyclic nucleosides and nucleotides has been determined from their NMR spectra. The acyclic moieties were unequivocally characterized by ^{1}H NMR (see Experimental Section) and ^{13}C NMR spectra (Tables 1−3). The presence of a phosphonate group is manifested in ^{1}H and ^{13}C NMR spectra by additional splitting of signals of the corresponding hydrogen and carbon atoms in the vicinity of phosphorus due to J(H,P) and J(C,P), respectively.

Determination of the position of the acyclic substituent at the heterocyclic base is more complicated. In the 8-azapurine series, solely 8-azaadenine contains hydrogen atom at C-2 while both 8-azaguanine and 8-aza-2,6-diaminopurine possess exchangeable amine or amide protons only. Therefore, their ^{1}H NMR spectra are useless for the purpose and the required structural evidence has to be deduced mainly from the ^{13}C NMR spectra. Correct structural assignment of carbon signals is thus essential for precise determination of the position of the acyclic side chain. Structural modifications of the substituent have little influence on the chemical shifts of the carbon atoms of the heteroaromatic base. Hence, similar substitution effects of the regioisomers (N^7, N^8, or N^9) can be expected in all three classes of 8-azapurine derivatives under study. Although the ^{13}C NMR data of some 8-azapurine nucleosides were described by Seela[25] and of 8-azapurine acyclic phosphonates by Franchetti et al.,[27] there is some doubt left on the assignment of signals.[25,27,37] Therefore, we have examined this assignment and determined experimentally the effects of substitution at the position N^7, N^8, or N^9.

Detailed NMR study was performed with three isomeric nucleosides derived from 8-azaadenine (**15, 16**, and **17**). Proton-decoupled "attached proton test" ^{13}C NMR spectra of 8-azaadenine derivatives allow a signal of the methine carbon C-2 to be distinguished from signals of the quaternary carbon atoms C-4, C-5, and C-6. Proton-coupled ^{13}C NMR spectra confirm the assignment of C-2 (^1J(C,H) ∼ 200 Hz) and distinguish the signals of carbons C-4 and C-6 (with large *trans*-coupling constants ^3J(C4,H2) respectively ^3J(C6,H2) ∼ 12 Hz) from those of carbon C-5 which is not coupled to proton H-2. The tentative assignment of signals belonging to C-4 and C-6 has been verified as follows: (a) In a proton-coupled ^{13}C NMR spectrum of compound **17** the signal at δ 151.91 (with J(C,H) = 11.7 Hz) shows additional splitting to triplet of doublets by interaction with the NH_2 protons (this splitting disappears after addition of D_2O) and therefore belongs to carbon C-6. (b) Another evidence is based on the deuterium isotopic effects on carbon chemical shifts: The presence of small amount of D_2O in a DMSO solution results in a partial exchange of labile NH_2 protons with deuterium that is indicated by known isotopic upfield shifts of carbon signals in the vicinity of the deuterated amino group. Slow exchange between molecules containing ND_2 and NH_2 in DMSO solution results in "doubling" of the corresponding signals of two isotopomers. The observed isotopic shifts in **17** (N^7-isomer) (−0.066 ppm for signal at δ 151.91, −0.031 ppm for signal at δ 114.23, and ∼ 0 ppm for signals at δ 160.35 and 154.57) together with the expected decrease of the isotopic shifts in the β, γ, and δ position led to the assignment of carbon signals

Table 1. Carbon-13 NMR Spectral Data of 8-Azaadenine Derivatives

comp	solvent	C-2	C-4	C-5	C-6	C-1′ (*J*(C,F))	C-2′ (*J*(C,P); *J*(C,F))	C-3′ (*J*(C,F))	OCH_2P (*J*(P,CH_2))
11	DMSO	156.20	151.35	123.28	156.23				
						N-9 Isomers			
15[a]	DMSO	156.97	149.44	123.83	156.41	49.01	73.53 (−)	66.48	
18	DMSO	156.97	149.87	124.37	156.72	50.49	70.64 (−)	64.09	
23	D_2O	157.84	147.50	125.50	158.10	47.89	81.30 (10.3)	61.74	69.20 (150.2)
28	D_2O	156.77	148.29	124.41	156.57	47.01	69.85 (10.0)		69.19 (150.5)
31[b]	DMSO	156.89	149.47	123.86	156.39	45.64 (8.8)	77.45 (12.5; 19.0)	81.90 (169.2)	63.55 (164.1)
36[c]	DMSO	156.84	149.50	123.84	156.43	50.25	75.16 (13.7)	17.22	62.60 (164.8)
						N-8 Isomers			
16[d]	DMSO	157.01	158.01	125.73	156.77	59.17	73.98 (−)	66.37	
19	DMSO	156.87	158.14	125.73	156.83	60.57	71.11 (−)	63.75	
24	D_2O	157.53	156.30	126.69	157.78	57.86	81.43 (12.4)	61.57	67.30 (156.8)
29	D_2O	156.82	157.18	125.87	156.69	56.85	70.23 (10.0)		69.21 (150.2)
32[e]	DMSO	157.00	158.07	125.77	156.78	56.21 (8.1)	78.03 (13.2; 19.1)	81.80 (169.9)	63.75 (164.8)
37[f]	DMSO	156.86	158.01	125.63	156.80	60.70	75.71 (12.2)	16.91	62.67 (164.8)
39	D_2O	157.52	157.66	126.47	157.42	61.60	77.01 (12.2)	17.54	66.52 (155.7)
						N-7 Isomer			
17[g]	DMSO	154.57	160.35	114.43	151.91	52.35	74.25 (−)	65.92	

Other carbons are as follows: Di-*O*-isopropylidene: [a]109.30, 26.76, 25.73; [d]109.49, 26.84, 25.40; [g]109.36, 26.13, 25.08. $P(OCH(CH_3)_2)_2$: [b]70.42 d and 70.03 d (*J*(C,P) = 6.6), 23.84 d and 23.79 d (*J*(C,P) = 3.7), 23.63 d and 23.57 d (*J*(C,P) = 4.4); [c]70.34 d and 70.21 d (*J*(C,P) = 6.1), 23.88 d and 23.86 d (*J*(C,P) = 3.1), 23.69 d and 23.65 d (*J*(C,P) = 4.6); [e]70.48 d and 70.37 d (*J*(C,P) = 5.9), 24.23 d and 23.82 d (*J*(C,P) = 3.7), 23.66 d and 23.58 d (*J*(C,P) = 3.6); [f]70.38 d and 70.26 d (*J*(C,P) = 6.1), 23.87 d and 23.85 d (*J*(C,P) = 3.1), 23.68 d and 23.64 d (*J*(C,P) = 4.6).

in the order of C-6, C-5 and C-2, C-4 which agrees with the assignment derived above. Similarly, the isotopic shifts for compound **16** (N^8-isomer) are −0.074 ppm for signal at δ 156.77 and −0.037 ppm at δ 125.73, and for compound **15** (N^9-isomer) −0.070 ppm at δ 156.41 and −0.039 ppm at δ 123.83 always for carbon atoms C-6 and C-5, respectively. Analogous isotopic effects, induced by deuteration of hydroxyl protons, were described in ^{13}C NMR spectra of saccharides.[38] The above results are in contrast with the earlier described assignment of C-4 and C-6 signals in the N^7- isomer of 8-aza-2′-deoxyadenosine[36] and in the N^8-isomers of 8-aza-2′-deoxyguanosine[25] and 2-amino-8-aza-2′-deoxyadenosine,[25b] respectively.

On the basis of the structural assignment of the carbon signals, the experimental NMR evidence for the assignment of different regioisomers was made possible by the analysis of vicinal *J*(C,H) couplings between the α-hydrogens of the *N*-substituent and the corresponding carbon atom of the base in proton-coupled ^{13}C NMR spectra. In our previous paper[39] we have used this method for providing evidence for the N^9- and N^2-isomers in 2-azaadenine derivatives. Different vicinal couplings *J*(C,H) for the N^9-, N^8-, and N^7-isomers of 8-aza-2′-deoxyadenosine were described in the literature.[36] This method is applicable for distinguishing the N^9-, N^8-, and N^7-isomers in compounds **15, 16**, and **17**. We have found that in proton-coupled ^{13}C NMR spectra of the N^9- and/or N^7-isomer a characteristic splitting or at least significant line broadening of the C-4 and/or C-5 signal by vicinal coupling with hydrogens H-1′ of the substituent can be observed which is absent in the N^8-isomer.

To determine the effect of substitution in individual positions of 8-azapurines we have recorded also ^{13}C NMR spectra of the free bases in DMSO (Tables 1−3). Average values of the substituent effects of 8-azaadenine, 8-azaguanine, and 8-aza-2,6-diaminopurine and their N^9-, N^8-, and N^7-substituted derivatives are presented in Table 4. It is evident that the substituent effects for regioisomeric derivatives of all three 8-azapurine bases are similar and can be used to distinguish between N^9-, N^8-, and N^7-isomers. In fact, each isomer should be referred to the appropriate tautomeric form of the unsubstituted base. Unfortunately, neither the ^{13}C chemical shifts of individual tautomers nor their population in solution have been published. Significantly larger substituent effects on carbon atoms C-4 and C-5 which were observed in the N^8-isomers, and particularly the N^7-isomers compared to the N^9-isomers (Table 4) may be due to a lower population of the corresponding N^8- and N^7-tautomers of the free bases in solution. Due to the limited solubility of the free phosphonates in DMSO, their NMR spectra were measured in D_2O. The data in Tables 1−3 show that the ^{13}C chemical shifts of the bases in water are rather similar to those in DMSO. However, a different situation is encountered in an alkaline aqueous solution. The 8-azaguanine derivatives **72a** (N-8) and **73a** (N-7) measured in D_2O + NaOD show significant downfield

Table 2. Carbon-13 NMR Spectral Data of 8-Azaguanine Derivatives

compd	solvent	C-2	C-4	C-5	C-6	C-1′	C-2′ (J(P,2′))	C-3′	OCH_2P (J(P,CH_2))
13	DMSO	155.45	153.45	123.76	155.88				
					N-9 Isomers				
62[b]	DMSO	155.63	151.77	124.34	155.98	45.43	69.94 (12.2)		64.81 (164.8)
65	D_2O	157.15	152.17	125.26	159.54	47.30	70.68 (10.7)		69.75 (151.1)
68a[a]	DMSO	155.64	151.91	124.22	156.00	49.86	75.30 (12.7)	17.25	62.79 (165.0)
					N-8 Isomers				
63[d]	DMSO	154.01	159.83	126.66	156.71	55.52	70.18 (12.2)		64.74 (163.3)
66	D_2O	155.54	159.51	126.84	156.51	56.95	71.13 (10.7)		68.75 (154.1)
69a[c]	DMSO	154.34	159.77	126.71	156.75	59.74	75.59 (12.7)	17.02	62.70 (165.0)
72a	NaOD	165.35	161.08	128.74	169.73	60.82	77.02 (10.7)	17.16	68.25 (151.1)
					N-7 Isomers				
64[d]	DMSO	153.82	161.14	113.78	154.28	49.62	70.51 (12.2)		64.82 (164.8)
67	D_2O	155.35	160.82	114.81	155.54	51.43	71.26 (10.7)		68.86 (152.6)
70a[e]	DMSO	153.96	160.99	113.91	154.16	53.87	75.75 (12.7)	17.00	62.70 (165.0)
73a	NaOD	165.71	162.63	116.74	164.54	55.25	77.32 (11.4)	17.63	68.48 (151.1)

Other carbons are as follows. $P(OCH(CH_3)_2)_2$: [a]70.44 d and 70.32 d (J(C,P) = 5.9), 23.92 d and 23.90 d (J(C,P) = 2.9), 23.76 d and 23.71 d (J(C,P) = 4.9); [b]70.46 d (J(C,P) = 6.1), 23.93 d (J(C,P) = 3.1), 23.74 d (J(C,P) = 4.6); [c]70.42 d and 70.32 d (J(C,P) = 5.9), 23.92 d (J(C,P) = 2.9), 23.76 d (J(C,P) = 4.9); [d]70.49 d and 70.47 d (J(C,P) = 6.1), 23.95 d and 23.92 d (J(C,P) = 3.1), 23.75 d and 23.74 d (J(C,P) = 4.6); [e]70.42 d and 70.26 d (J(C,P) = 5.9), 23.92 d (J(C,P) = 2.9), 23.73 d (J(C,P) = 4.9).

shifts of carbons C-2 and C-6 (10−13 ppm) and much smaller downfield shifts of carbons C-4 and C-5 (1−3 ppm). Similar effects in the alkaline aqueous solutions were described by Seela[25b] and explained by the occurence of 8-aza-2′-deoxyguanosine monoanion.

Biological Activity. Several 8-azapurine derivatives within the PME, (*S*)-HPMP, (*S*)- and (*R*)-PMP, and (*S*)-FPMP series were evaluated for their inhibitory effect on the DNA viruses herpes simplex virus type 1 (HSV-1), HSV-2, cytomegalovirus (CMV), varicella-zoster virus (VZV), and vaccinia virus, and against the retroviruses Moloney murine sarcoma virus (MSV) and human immunodeficiency virus type 1 (HIV-1) and HIV-2. Among the PME derivatives, only PME-8-azaguanine (**65**) was inhibitory to HSV-1, the thymidine kinase (TK^-) deficient HSV-1 strains B2006 and VMW1837, HSV-2, CMV, both TK^+ and TK^- VZV strains and vaccinia virus at concentrations varying between 0.04 and 0.4 μg/mL for VZV to 2 and 7 μg/mL for HSV-1, HSV-2, and CMV. (*S*)-HPMP-8-azaadenine (**23**) proved markedly inhibitory to all DNA viruses tested. In particular, **23** was exquisitely inhibitory to VZV (MIC: 0.2−2 μg/mL) (Table 5). In fact, compound **23** was endowed with an antiviral potency that was comparable to that of the prototype compound (*S*)-HPMPA (data not shown). (*S*)-HPMP-8-aza-2,6-diaminopurine (**47**) was approximately 2 orders of magnitude less potent an inhibitor of these viruses than compound **23**. None of the 8-aza-PMP or 8-aza-(*S*)-FPMP derivatives proved markedly inhibitory to any of the DNA viruses tested, except for compounds **38** and **39** that showed a moderate antiviral activity against VZV (MIC: 5−10 μg/mL).

With respect to the antiretroviral activity of the test compounds, PME-8-azaguanine (**65**) and (*R*)-PMP-azaguanine (**71a**) had a substantial inhibitory effect against MSV-induced transformation (IC_{50}: ~2 μg/mL). Interestingly, several other 8-azapurine derivatives proved markedly more active against MSV than HIV. For example, compounds **51**, **47**, **39**, and **59** were almost 2 orders of magnitude less inhibitory to HIV than to MSV (Table 6). The reason for this phenomenon is unclear so far, but 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. Further experiments are needed to resolve these issues.

Experimental Section

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a 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). HPLC was performed on Separon SGX RPS columns (200 × 4 mm) in 0.05 M triethylammonium hydrogen carbonate, pH 7.5 containing 1% acetonitrile (S5), 2% acetonitrile (S6), 4% acetonitrile (S7), 16% acetonitrile (S8), and 20% acetonitrile (S9). Preparative TLC was carried out on 40 × 17 × 0.4 cm loose layer plates of silica gel containing UV indicator. 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 are 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. Carbon-13 NMR spectra were measured on a Varian UNITY-500 (at 125.7 MHz) instrument and

Table 3. Carbon-13 NMR Spectral Data of 8-Aza-2,6-diaminopurine Derivatives

compd	solvent	C-2	C-4	C-5	C-6	C-1′ (J(1′,F))	C-2′ (J(2′,P); J(2′,F))	C-3′ (J(3′,F))	OCH_2P (J(P,CH_2))
12	DMSO	162.58	153.22	119.85	156.31				
						N-9 Isomers			
43	DMSO	162.83	151.95	120.40	156.42	49.22	69.85 (−)	63.78	
47	D_2O	163.71	152.20	121.61	157.74	47.27	80.90 (10.0)	61.82	69.33 (150.3)
48[c]	DMSO	162.92	151.90	120.40	156.36	45.05	69.91 (11.4)		64.74 (163.3)
51	D_2O	163.69	151.90	121.84	157.83	47.22	70.87 (12.2)		70.22 (150.0)
53[a]	DMSO	163.00	152.13	120.32	156.38	44.75 (8.4)	77.60 (13.0;18.3)	82.09 (169.4)	63.68 (164.0)
57[b]	DMSO	162.99	152.05	120.26	156.37	49.57	75.15 (12.7)	17.31	62.72 (165.0)
59	D_2O	163.51	151.85	121.49	157.62	51.26	76.29 (10.8)	17.68	68.10 (150.4)
						N-8 Isomers			
41[d]	DMSO	162.52	160.54	122.40	156.57	56.23	68.95 (−)	65.85	
49[g]	DMSO	162.49	160.51	122.43	156.56	55.45	70.37 (11.4)		64.86 (164.0)
54[e]	DMSO	162.55	160.51	122.62	156.58	55.12 (7.6)	78.07 (13.0;19.1)	82.01 (169.4)	63.78 (164.0)
58[f]	DMSO	162.54	160.57	122.44	156.58	59.83	75.75 (12.7)	17.11	62.77 (164.1)
60	D_2O	163.25	159.70	123.58	158.41	60.94	76.77 (10.8)	17.62	68.12 (150.4)
						N-7 Isomer			
50[h]	DMSO	161.43	163.73	109.78	151.79	49.86	71.35 (10.0)		64.98 (162.5)

Other carbons are as follows: **$P(OCH(CH_3)_2)_2$**: [a]70.54 d and 70.40 d (J(C,P) = 6.1), 23.89 d and 23.85 d (J(C,P) = 3.8), 23.69 d and 23.62 d (J(C,P) = 4.6); [b]70.37 d and 70.23 d (J(C,P) = 5.9), 23.91 d and 23.88 d (J(C,P) = 3.9), 23.73 d and 23.68 d (J(C,P) = 4.9); [c]70.38 d (J(C,P) = 6.9), 23.92 d (J(C,P) = 3.8), 23.72 d (J(C,P) = 4.6); [e]70.59 d and 70.50 d (J(C,P) = 6.1), 23.87 d and 23.84 d (J(C,P) = 3.8), 23.67 d and 23.62 d (J(C,P) = 4.6); [f]70.36 d and 70.26 d (J(C,P) = 5.9), 23.92 d and 23.89 d (J(C,P) = 3.9), 23.73 d and 23.70 d (J(C,P) = 3.9); [g]70.39 d (J(C,P) = 6.1), 23.91 d (J(C,P) = 3.8), 23.71 d (J(C,P) = 4.6); [h]70.39 d (J(C,P) = 6.1), 23.88 d (J(C,P) = 3.8), 23.71 d (J(C,P) = 4.6). $OC(C_6H_5)_3$: [d]86.20, 143.84 (3 × C), 128.45 (6 × C), 128.05 (6 × C), 127.19 (3 × C).

Table 4. Substituent Effects on Carbon-13 Chemical Shifts in 8-Azaadenine, 8-Azaguanine and 8-Aza-2,6-diaminopurine Derivatives

base	substituent effect (ppm) C-2	C-4	C-5	C-6	compounds examined
	N-9 Isomer				
8-azaadenine	0.72	−1.78	0.70	0.26	4
8-azaguanine	0.21	−1.50	0.62	0.15	4
8-aza-2,6-diaminopurine	0.36	−1.21	0.50	0.07	1
	N-8 Isomer				
8-azaadenine	0.74	6.71	2.44	0.55	4
8-azaguanine	−1.21	6.37	3.02	0.82	3
8-aza-2,6-diaminopurine	−0.06	7.31	2.62	0.26	2
	N-7 Isomer				
8-azaadenine	−1.63	9.00	−8.85	−4.32	4
8-azaguanine	−1.56	7.62	−9.92	−1.66	4
8-aza-2,6-diaminopurine	−1.24	10.42	−10.06	−4.50	2

referenced either internally with the solvent signal (δ (CD_3SOCD_3) = 39.7 ppm) or externally (using chemical shift of dioxane in D_2O: δ (dioxane) = 66.86 ppm). Carbon chemical shifts and $^{13}C-^{31}P$ coupling constants were obtained from "normal" proton-decoupled spectra or "attached proton test" spectra.[40] Proton-coupled ^{13}C NMR spectra with NOE enhancement were used to obtain J(C,H) coupling constants in some compounds. Deuterium isotopic shifts of ^{13}C signals were extracted from partially exchanged proton-decoupled ^{13}C NMR spectra in CD_3SOCD_3 solution with a small amount (ca. 3 drops) of D_2O added.

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 and cesium carbonate were purchased from Fluka (Switzerland); 8-azaadenine, 8-aza-2,6-diaminopurine hemisulfate, and 8-azaguanine were obtained from Sigma (Germany). Dimethylformamide dimethyl acetal was purchased from BASF (Germany), dimethylformamide dineopentyl acetal was prepared according to ref 41. 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).

9-(*S*)-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl]-8-azaadenine(15), 8-(*S*)-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl]-8-azaadenine (16), and 7-(*S*)-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl]-8-azaadenine (17). A mixture of 8-azaadenine (**11**) (3.0 g, 22 mmol), cesium carbonate (3.6 g, 11 mmol), and (*R*)-2,2-dimethyl-4-[(tosyloxy)methyl]-1,3-dioxolane[42] (**14**) (6.9 g, 24 mmol) in dimethylformamide (100 mL) was heated at 100 °C under a calcium chloride protecting tube for 20 h until the starting base disappeared (monitored by TLC in S2). According to HPLC analysis (S8) the mixture contains three regioisomers in the ratio of 47:46:7, $k_1 = 5.73$, $k_2 = 4.66$, $k_3 = 3.97$. The mixture was taken down *in vacuo*, and codistilled twice with toluene (50 mL portions), and the residue was extracted with boiling chloroform (total, 250 mL). The extract was evaporated to dryness and the residue adsorbed from methanol on silica gel (30 g). This material was applied onto a column of silica gel (120 g) and chromatographed with chloroform−ethanol mixture (98:2) to afford, after crystallization from methanol, 1.1 g (20%) of the N^9-isomer (**15**). Mp: 192−193 °C. $R_f = 0.4$ (S2). $k = 5.73$ (S8). Anal. ($C_{10}H_{14}N_6O_2$) C, H, N. ^1H NMR ($(CD_3)_2SO$): δ 8.30 s, 1H (H-2 arom); 8.40 and 8.09 2 × br, 2H (NH_2); 4.62 br s, 3H (H-1′ and H-2′); 4.08 dd, 1H (H-3′, J(3′,2′) = 6.3, J(gem) = 8.8); 3.93 dd, 1H (H-3″, J(3″,2′) = 4.4, J(gem) = 8.8); 1.22 s, 6H (2 × CH_3). UV Spectrum (λ_{max} (ϵ_{max})): (pH 2) 265.0 nm (11 600); (pH 7) 278.0 nm (12 200); (pH 12) 278.0 nm (11 700). IR spectrum (chloroform, cm^{-1}): ν(NH_2) free, 3523 w, 3410 m; assoc, 3474 w, 3327 w; sciss(NH_2) free,1638 vs; assoc,1655 m, sh; (ring) 1585 m; s(CH_3) 1384 w, 1374 w; (CH_3) 2992 m, 2890 w; (C−O) 1083 w, sh, 1064 w.

Table 5. Anti-DNA-viral Activity (IC_{50})[a] of Acyclic 8-Azapurine Nucleotide Analogues

compd	HSV-1 KOS	HSV-1 TK⁻ B2006	HSV-1 TK⁻ VMW1837	HSV-2 G	HSV-2 196	HSV-2 Lyons	CMV AD169	CMV Davis	VZV TK⁺ OKA	VZV TK⁺ YS	VZV TK⁻ 07/1	VZV TK⁻ YS/R	Vaccinia virus
						PME Derivatives							
28	>200	>200	>200	>200	>200	>200	>100	>100	>100	>100	>100	>100	>400
29	>400	>400	>400	>400	>400	>400	>100	>100	>100	>100	>100	>100	>400
51	70	150	150	20	150	150	>40	>40	>40	>40	>40	>40	400
65	2	7	2	2	7	2	2.3	2.3	0.4	0.04	0.1	0.4	0.700
66	>400	>400	300	>400	>400	>300	>100	>40	40	40	>40	40	150
						(*S*)-HPMP Derivatives							
23	0.4	0.9	2	40	0.2	2	2	3.5	0.06	0.04	0.02	0.01	0.7−2
24	>400	150	>400	>400	>400	>400	>200	>200	>100	>100	>100	>100	>400
47	20	20	40	20	20	40	>100	>100	9	6	6	7	20
						PMP Derivatives							
22	>400	>400	>400	>400	>400	>400	>50	>50	>50	>50		>50	>400
38	70	70	100	200	70	100	>40	>40	8	8	5	8	>400
39	150	150	100	>400	150	100	>40	>40	10	5	5	5	>400
59	300	300	300	>400	300	300	>50	>50	>50	>50	>50	>50	>400
60	>100	200	>200	100	200	>200	>50	>50	>50	>50	>50	>50	>100
71a	20	>100	20	70	>100	20	35	50	40	28	20	26	>100
71b	300	>400	300	>400	400	300	>40	>40	10	4	>40	>40	300
72a	>100	>400	>400	>400	>400	>400	>50	>50	>20	>20		>20	>400
						(*S*)-FPMP Derivatives							
33	>400	>400	>400			>400	>40	>40	>40	>40	>40	>40	>400
34	>40	>40			>40		>400	>400	>10	>10	>10	>10	>40
55	>400	>400	>400	>400			>50	>50	>50	>50	>50	>50	>400
56	>400	>400	>400	>400			>50	>50	>50	>50	>50	>50	>400
						Other Compounds							
18	>200			>200			>100		>40	>40	>40	37	
19	>200			>200			>100		>40				
43	>100			>100			>50		>50	>50	>50	>50	
ACV[b]	0.015			0.02			13		0.18	0.35	11	22	

[a] 50% inhibitory concentration (μg/mL), or concentration required to reduce virus plaque formation by 50% under experimental conditions (see Methods). [b] ACV = acyclovir.

Table 6. Antiretroviral Activity (IC_{50})[a] of Acyclic 8-Azapurine Nucleotide Analogues

compd	MSV	HIV-1 MT-4	HIV-1 CEM	HIV-2 MT-4	HIV-2 CEM
			PME Derivatives		
28	12.1 ± 1.72	>100		>100	
29	>100	>100		>100	
51	0.750	45.8		68	
65	0.32 ± 0.1	2	2	2	2
66[b]	>40	>100	>100	>100	>100
			(*S*)-HPMP Derivatives		
23	0.57 ± 0.03	>0.8		>0.8	
24	>100	>100		>100	
47	1.0 ± 0.22	>100	>100	>100	>100
			PMP Derivatives		
38	0.85 ± 0.14	5.200	7	6.700	10
39	2.90 ± 1.68	>100	>100	>100	>100
59	1.58 ± 0.73	44.4 ± 10	30 ± 14	45 ± 5	40
60	28.4 ± 0.81	>100	>100	>100	>100
71a	0.43 ± 0.24	2.42 ± 0.91	2	1.75 ± 0.2	2
72a	>40	20	20	20	20
73a	>40	>100	>100	>100	>100
71b	27 ± 0.7	52.7 ± 43.4	40 ± 28	62 ± 34	85 ± 21
72b	>40	>100	>100	>100	>100
			(*S*)-FPMP Derivatives		
33	>200		>100		>100
34	13.1+7.5	15		16	
55	14.9 ± 5.9		50		50
56	>200		50		55
			Other Compounds		
18	>100		>100		>100
19	>100		>100		>100
43	>40		>100		>100
AZT[c]	0.020		0.005		0.008

[a] IC_{50} is the minimum concentration (μg/mL) of the test compound which protects 50% of the cells against virus-induced transformation. [b] Contains 9% of compound **67**. [c] AZT = azidothymidine.

The following fraction contained a mixture of two compounds that were separated by preparative HPLC (water−methanol gradient, 1%/min, up to 50% methanol). The first fraction afforded by crystallization from methanol 1.2 g (22%)

of the N^8-isomer **16**. Mp: 122−124°C. $R_f = 0.3$ (S2). $k = 4.66$ (S8). Anal. ($C_{10}H_{14}N_6O_2$) C, H, N. ^{1}H NMR ($(CD_3)_2SO$): δ 8.28 s, 1H (H-2 arom); 8.25 and 8.07 2 × br, 2H (NH_2); 4.66−4.88 m, 3H (H-1′ and H-2′); 4.15 dd, 1H (H-3′, J(3′,2′) = 5.8, J(gem) = 8.8); 3.93 dd, 1H (H-3″, J(3″,2′) = 4.6, J(gem) = 8.8); 1.29 s and 1.24 s, 6H (2 × CH_3). UV spectrum (λ_{max} (ϵ_{max}): (pH 2) 284.0 nm (12 300); (pH 7) 293.0 nm (11 000), 262.0 nm (4800); (pH 12) 292.0 nm (10 800), 263.0 nm (4800). IR spectrum (chloroform): $\nu(NH_2)$ free, 3523 w, 3409 m; assoc, 3472 w, 3338 m, br; $\nu_{sciss}(NH_2)$ free, 1635 vs; assoc., 1650 s, sh; (ring) 1596 m, 1563 s; s(CH_3) 1385 mw, 1374 sw; (CH_3) 2992 s, 2891 w; (C−O) 1073 m, 1054 m, sh.

The next (minor) fraction from the HPLC gave (after crystallization from methanol 0.22 g (4%) N^7-isomer **17**. Mp: 182 −183 °C. $R_f = 0.3$ (S2). $k = 3.97$ (S8). Anal. ($C_{10}H_{14}N_4O_2$) C, H, N. ^{1}H NMR ($(CD_3)_2SO$): δ 8.30 s, 1H (H-2 arom); 7.70 brs, 2H (NH_2); 5.09 dd, 1H (H-1′, J(1′,2′) = 3.4, J(gem) = 14.7); 4.92 dd, 1H (H-1″, J(1″,2′) = 6.3, J(gem) = 14.7); 4.47 qd, 1H (H-2′, $\sum J$ = 22.5); 4.12 dd, 1H (H-3′, J(3′,2′) = 6.8, J(gem) = 8.8); 3.78 dd, 1H (H-3″, J(3″,2′) = 5.9, J(gem) = 8.8); 1.19 s and 1.08 s, 6H (2 × CH_3). UV spectrum (λ_{max} (ϵ_{max}): (pH 2) 288.0 nm (9000); (pH 7) 290.0 nm (8000); (pH 12) 289.5 nm (7500). IR spectrum (chloroform): $\nu(NH_2)_{intramol.bridge}$ 3467 m, 3347 m; sciss(NH_2) 1635 vs; (ring) 1592 m, 1566 s; s(CH_3) 1385 m, 1376 m; (CH_3) 2994 m, 2899 w; (C−O) 1057 m, 1042 m, sh.

9-(*S*)-(2,3-Dihydroxypropyl)-8-azaadenine (18) and 8-(*S*)-(2,3-dihydroxypropyl)-8-azaadenine (19). The solution of compound **15** or **16** (4 mmol) in 0.25 M sulfuric acid (50 mL) was left to stand overnight at the ambient temperature. The mixtures were diluted with water (50 mL) and neutralized with saturated barium hydroxide solution. The resulting suspensions were heated to boil and filtered through Celite, and the filter was washed with boiling water (500 mL). The filtrates were taken down *in vacuo*, and the residues were codistilled with ethanol (100 mL) and crystallized from 80% aqueous ethanol (ether added to turbidity). Yield: 0.76 g (84%) N^9-isomer **18**. Mp: 228−230 °C. k = 4.02 (S7). Anal. ($C_7H_{10}N_6O_2 \cdot H_2O$) C, H, N. ^{1}H NMR ($(CD_3)_2SO$): δ 8.28 s, 1H (H-2 arom); 8.35 and 8.05 2 × br, 2H (NH_2); 5.02 d, 1H (OH-2′, J = 5.6); 4.84 t, 1H (OH-3′, J = 5.6); 4.59 dd, 1H (H-1′, J(1′,2′) = 4.1, J(gem) = 13.9); 4.43 dd, 1H (H-1′, J(1″,2′) = 8.1, J(gem) = 13.9); 4.07 m, 1H (H-2′, $\sum J$ = 29.0); 3.44 m, 2H (H-3′). UV spectrum (λ_{max} (ϵ_{max}): (pH 2) 265.0 nm (11 700); (pH 7) 278.0 nm (12 000); (pH 12) 278.0 nm (11 700). **N^8-isomer 19**, yield 0.72 g (86%). Mp: 225−227 °C. k = 3.37 (S7). Anal. ($C_7H_{10}N_6O_2$) C, H, N. ^{1}H NMR ($(CD_3)_2SO$): δ 8.27 s, 1H (H-2 arom); 8.19 and 8.02 2 × br, 2H (NH_2); 5.13 d, 1H (OH-2′, J = 5.6); 4.89 t, 1H (OH-3′, J = 5.6); 4.79 dd, 1H (H-1′, J(1′,2′) = 3.4, J(gem) = 13.4); 4.50 dd, 1H (H-1′, J(1″,2′) = 8.6, J(gem) = 13.4); 4.14 m, 1H (H-2′, $\sum J$ = 29.0); 4.46 2 × dt, 2H (H-3′). UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 284.0 nm (12 400); (pH 7) 293.0 nm (11 500), 262.0 nm (4900); (pH 12) 292.0 nm (10 800), 263.0 nm (4700).

9-(*S*)-(2-(Phosphonomethoxy)-3-hydroxypropyl)-8-azaadenine (23) and 8-(*S*)-(2-(Phosphonomethoxy)-3-hydroxypropyl)-8-azaadenine (24). The mixture of 8-azaadenine (**11**) (0.95 g, 7.0 mmol) (2*R*)-2-[bis(2-propyl)phosphonylmethoxy]-3-(trimethylacetoxy)propyl *p*-toluenesulfonate (**20**) (4.1 g, 8.0 mmol) and cesium carbonate (1.1 g, 3.5 mmol) in 45 mL of dimethylformamide was heated at 100 °C for 8 h until the starting compound disappeared (TLC S3). The mixture was evaporated *in vacuo* and the residue codistilled with toluene (3 × 50 mL). The residue was extracted by boiling chloroform (250 mL) and filtered, the filtrate was evaporated, and the residue was left to stand overnight at ambient temperature with 0.1 M sodium methoxide in methanol (100 mL). The resulting mixture was neutralized by addition of Dowex 50 × 8 (H^+-form), the suspension was alkalized with triethylamine and filtered, the resin was washed with methanol (200 mL), and the filtrate was evaporated. The residue was separated by chromatography on the column of silica gel (100 g) in chloroform; the N^9-isomer **21** was eluted by system S2. There was obtained 0.8 g (2.1 mmol) of 9-(*S*)-(2-((diisopropylphosphono)methoxy)-3-hydroxypropyl)-8-azaadenine (**21**) or 0.76 g (0.19 mmol) respectively of the N^8-isomer (**22**) (that was eluted by the system S3) as thick oils. These compounds were treated with bromotrimethylsilane (2.5 mL) in acetonitrile (25 mL) at room temperature overnight and evaporated *in vacuo* and the residues dissolved in water (50 mL). The solutions were alkalized by ammonia and after 20 min evaporated *in vacuo*. The residues were deionized on columns (100 mL) of Dowex 50 × 8 (H^+-form) and the crude residues purified by chromatography on Dowex 1 × 2 column in acetate form. The products were eluted by linear gradient of acetic acid (0−0.5 M, 1 L each). The UV-absorbing fraction of the product was pooled and evaporated *in vacuo*, and the residue was codistilled with water (3 × 20 mL) and finally crystallized from 80% aqueous ethanol (ether added to turbidity). Yield, 0.21 g (34%) of compound **23**. Mp: 125−128 °C. k = 2.5 (S5). E_{Up} = 0.85. Anal. ($C_8H_{13}N_6O_5P \cdot H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.31 s, 1H (H-2 arom); 4.49 d, 2H (H-1′, J(1′,2′) = 5.6); 4.13 m, 1H (H-2′, $\sum J$ = 20.0); 3.84 dd, 1H (H-3′, J(3′,2′) = 3.4, J(gem) = 12.45); 3.57 dd, 1H (H-3″, J(3″,2′) = 5.4, J(gem) = 12.4); 3.95 dd, 1H (PCH_2, J(P−CH) = 8.5, J(gem) = 12.2); 3.49 dd, 1H (PCH_2, J(P−CH) = 10.0, J(gem) = 12.2). UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 265 nm (11 700); (pH 7) 279 nm (12 000); (pH 13) 279 nm (11 700).

8-(*S*)-(3-Hydroxy-2-(phosphonomethoxy)propyl)-8-azaadenine (24) was obtained in a similar manner from the bis(2-propyl) ester **22** (0.76 g, 0.19 mmol) in the yield of 0.16 g (27%). Mp: >250 °C. k = 2.4 (S5). E_{Up} = 0.85. Anal. ($C_8H_{13}N_6O_5P \cdot H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.24 s, 1H (H-2 arom); 4.98 dd, 1H (H-1′, J(1′,2′) = 4.6, J(gem) = 14.2); 4.91 dd, 1H (H-1″, J(1″,2′) = 6.6, J(gem) = 14.2); 4.19 m, 1H (H-2′, $\sum J$ = 20.5); 3.88 dd, 1H (H-3′, J(3′,2′) = 3.9, J(gem) = 12.45); 3.68 dd, 1H (H-3″, J(3″,2′) = 5.4, J(gem) = 12.45); 3.70 dd, 1H (PCH_2, J(P−CH) = 9.8, J(gem) = 12.9); 3.65 dd, 1H (PCH_2, J(P−CH) = 9.5, J(gem) = 12.9). UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 284 nm (12 000); (pH 7) 292 nm (11 100), 256 nm (5100); (pH 13) 294 nm (10 500), 264 nm (5200).

9-(2-((Diisopropylphosphono)methoxy)ethyl)-8-azaadenine (26) and 8-(2-((Diisopropylphosphono)methoxy)-ethyl)-8-azaadenine (27). A mixture of 8-azaadenine (**11**) (0.95 g,7.0 mmol), dimethylformamide (40 mL), cesium carbonate (1.3 g, 4.0 mmol), and bis(2-propyl) [(2-chloroethoxy)-methyl]phosphonate (**25**) (2.1 g, 8.0 mmol) was heated at 100 °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 and the residue of the extract chromatographed on the column (50 g) of silica gel in chloroform. The N^9-isomer was eluted by the system S1; yield 0.65 g (1.8 mmol, 26%) of an amorphous compound **26**.

Further elution by system S2 gave 0.96 g (2.7 mmol) (38%) of the N^8-isomer **27** (amorphous foam). The products were directly used for the deprotection.

9-(2-(Phosphonomethoxy)ethyl)-8-azaadenine (28) and 8-(2-(Phosphonomethoxy)ethyl)-8-azaadenine (29). Bromotrimethylsilane (1.8 or 2.7 mL, respectively) was added to the above residue of the bis(2-propyl) ester **26** (1.8 mmol) in acetonitrile (20 or 25 mL, respectively), and the mixtures were stirred in stoppered flasks for 24 h at room temperature. The mixtures were evaporated *in vacuo*, water (20 mL) was added, and after 30 min of standing at room temperature, the mixture was alkalized by aqueous ammonia and evaporated. The residue in water (20 mL) was applied onto a column (100 mL) of Dowex 50 × 8 (H^+-form). The column was washed with water to the drop of UV absorption, and the column was then eluted with diluted (1:10) ammonia. The UV-absorbing fractions were chromatographed on the column (100 mL) of Dowex 1 × 2 (acetate form). The product was eluted with a linear gradient of acetic acid (0.05−1 M, 1 L each). After crystallisation from water−ethanol mixture (1:4) (ether added to turbidity), there was obtained 0.31 g (63%) of the N^9-isomer **28**. Mp: >250 °C. k = 2.3 (S5). E_{Up} = 0.89. Anal. ($C_7H_{11}N_6O_4P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.22 s, 1H (H-2 arom); 4.79 t, 2H (H-1′, J(1′,2′) = 5.4); 4.10 t, 2H (H-2′, J(1′,2′) = 5.4); 3.50 d, 2H (PCH_2, J(P−CH) = 8.5). UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 264.0 nm (8700); (pH 13) 277.5 nm (8700).

The N^8-isomer **29** was obtained in a similar manner from compound **27** (2.7 mmol) in the yield of 70%. Mp: >250 °C. k = 1.9 (S5). E_{Up} = 0.89. Anal. (for $C_7H_{11}N_6O_4P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 8.20 s, 1H (H-2 arom); 4.95 t, 2H (H-1′, J(1′,2′) = 5.2); 4.20 t, 2H (H-2′, J(1′,2′) = 5.1); 3.50 d, 2H (PCH_2, J(P−CH) = 8.3). UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 288.0 nm (10 500); (pH 13) 290.5 nm (10 900).

(*S*)-9-[2-((Diisopropylphosphono)methoxy)-3-fluoropropyl]-8-azaadenine (31) and (*S*)-8-[2-((diisopropylphosphono)methoxy)-3-fluoropropyl]-8-azaadenine (32). A mixture of 8-azaadenine (**11**) (531 mg, 3.9 mmol), cesium carbonate (611 mg, 1.9 mmol), and compound **30** (1.3 g, 3.7 mmol) in dimethylformamide (15 mL) was stirred for 24 h at 100 °C under exclusion of moisture and taken to dryness. The residue was extracted with boiling chloroform and filtered and the filtrate evaporated. The residue was chromatographed on a column of silica gel (100 mL) in chloroform−methanol mixture, 20:1. Compound **31** (320 mg, 22%) and **32** (510 mg, 35%) were obtained as thick oils.

Compound 31. Anal. ($C_{14}H_{24}FN_6O_4P$) C, H, N, F, P. ^{1}H-NMR (($CD_3)_2SO$): δ 1.04 d + 1.08 d + 1.10 d + 1.14 d, 4 × 3 H (CH_3); 3.75 dd, 1 H (PCH, J(H,P) = 9.5, J(gem) = 13.7); 3.89 dd, 1 H (PCH, J(H,P) = 9.5, J(gem) = 13.7); 4.41 m, 2 H (OCH); 4.33 dm, 1 H (H-2′, J(2′,F) = 23); 4.54 ddd, 1 H (H-3′, J(3′,2′) = 4.2, J(gem) = 10.5, J(3′,F) = 46.2); 4.78 ddd, 1 H (H-3″, J(3″,2′) = 3.2, J(gem) = 10.5, J(3″,F) = 47.6); 4.68 dd, 1 H (H-1′, J(1′,2′) = 7.1, J(gem) = 14.6); 4.78 dd, 1 H (H-1″, J(1″,2′) = 5.1, J(gem) = 14.6); 8.30 s, 1 H (H-2); 8.10 + 8.43, 2 × bs, 2 H (NH_2).

Compound 32. Anal. ($C_{14}H_{24}FN_6O_4P$) C, H, N, F, P. ^{1}H-NMR(($CD_3)_2SO$): δ 1.04 d + 1.08 d + 1.10 d + 1.14 d, 4 × 3 H (CH_3); 3.75 dd, 1 H (PCH, J(H,P) = 9.5, J(gem) = 13.7); 3.89 dd, 1 H (PCH, J(H,P) = 9.5, J(gem) = 13.7); 4.42 ddd, 1 H (H-3′, J(3′,2′) = 4.2, J(gem) = 10.5, J(3′,F) = 46.2); 4.78 ddd, 1 H (H-3″, J(3″,2′) = 3.2, J(gem) = 10.5, J(3″,F) = 47.6); 4.81 dd, 1 H (H-1′, J(1′,2′) = 7.6, J(gem) = 14.2); 4.99 dd, 1 H (H-1″, J(1″,2′) = 4.4, J(gem) = 14.6); 8.29 s, 1 H (H-2); 8.05 + 8.25, 2 × bs, 2 H (NH_2).

(*S*)-9-(3-Fluoro-2-(phosphonomethoxy)propyl)-8-azaadenine (33). Bromotrimethylsilane (437 μL, 3.31 mmol) was added to a solution of compound **21** (323 mg, 0.83 mmol) in acetonitrile (1.6 mL), and the reaction mixture was stirred at ambient temperature for 24 h. The solution was concentrated *in vacuo* and codistilled with toluene. Aqueous ammonia (2,5%, 5 mL) was added and the solution taken to dryness. The residue in water (5 mL) was applied onto a Dowex 50 × 8 column (20 mL, H^+-form) and the column washed with water. Subsequent elution of the column with diluted (2.5%) aqueous ammonia afforded a UV-absorbing eluate that was evaporated *in vacuo*, the residue in water (5 mL) was applied on a column (20 mL) of Dowex 1 × 2 (acetate), and the column was washed with water and then with 1 M acetic acid. The product was then obtained by elution with 4 M acetic acid. The eluate was taken to dryness and the residue codistilled with water to afford compound **33** (200 mg, 79%). Anal. ($C_8H_{12}FN_6O_4P$) C, H, N, F, P. UV spectrum (λ_{max} (ϵ_{max}): (pH 2) 264.0 nm (10 700); (pH 7) 278.0 nm (10 000); (pH 13) 278.0 nm (11 600).

(*S*)-8-(3-Fluoro-2-(phosphonomethoxy)propyl)-8-azaadenine (34). A solution of compound **32** (390 mg, 1.0 mmol) in acetonitrile (2.0 mL) was treated with bromotrimethylsilane (515 μL, 3.9 mmol), and the reaction mixture was stirred for 24 h at ambient temperature. The further workup was performed as described for compound **33**. The ultimate purification was performed by chromatography on Dowex 1 × 2 column and the product eluted with 2 M acetic acid. Yield: 230 mg (75%) of compound **34**. Anal. (For $C_8H_{12}FN_6O_4P$) C, H, N, F, P. UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 284.0 nm (10 100); (pH 7) 278.0 nm (11 600), 256.0 nm (4200); (pH 13) 293 nm (9000), 255 nm (4100). MS (FAB): 307 (M + H), 171, 157.

9-(*R*)-(2-(Phosphonomethoxy)propyl)-8-azaadenine (38) and 8-(*R*)-(2-(Phosphonomethoxy)propyl)-8-azaadenine (39). A mixture of 8-azaadenine (**11**) (1.45 g, 10.8 mmol), cesium carbonate (1.75 g, 5.4 mmol), and dimethylformamide (25 mL) was preheated to 100 °C, and a solution of (*R*)-2-[(bis-(2-propyl)phosphono)methoxy]propyl *p*-toluenesulfonate (**35a**) (3.67 g, 9 mmol) in dimethylformamide (10 mL) was added in one portion. The mixture was then heated for 6 h at 110 °C under exclusion of moisture and taken to dryness. The residue was extracted with boiling chloroform (total 300 mL), filtered and the filtrate evaporated. The residue was chromatographed on a column of silica gel (300 mL). Elution with system S1 afforded 9-(*R*)-(2-((diisopropylphosphono)methoxy)propyl)-8-azaadenine (**36**) as a thick oil. Yield: 0.90 g (37%). UV spectrum (pH 2): λ_{max} 265.5 (ϵ_{max} 14 000). R_f = 0.50 (S2). Further elution with the same solvent afforded 8-(*R*)-(2-((diisopropylphosphono)methoxy)propyl)-8-azaadenine (**37**) as semisolid material in the yield of 0.75 g (22%). UV spectrum (pH 2): λ_{max} 284 nm. R_f 0.40 (S2).

Each fraction was separately treated with acetonitrile (25 mL) and bromotrimethylsilane (2.5 mL) overnight, and the mixtures were worked up as described for the compound **26**. Chromatography on Dowex 1 × 2 column (50 mL) afforded 9-(*R*)-(2-(phosphonomethoxy)propyl)-8-azaadenine (**38**) on elution with 2 M acetic acid. Yield (after crystallization from water−ethanol): 79%. UV spectrum (λ_{max} (ϵ_{max})): (pH 2): 265 nm (14 000). Anal. ($C_8H_{14}N_6O_4P$) C, H, N, P.

8-(*R*)-(2-(Phosphonomethoxy)propyl)-8-azaadenine (**39**) was obtained similarly (final elution with 1 M acetic acid). Yield: 72%. UV spectrum (pH 2): λ_{max} 284 nm. Anal. ($C_8H_{14}N_6O_4P$) C, H, N, P.

8-Aza-2,6-diaminopurine (12) was obtained by stirring a suspension of its hemisulfate (25 mmol) in water (100 mL) under addition of Dowex 50 × 8 (H^+-form) until dissolution, the suspension was poured onto a column of the same cation exchanger (100 mL), and the column was washed with water until neutral. The resin was then treated in aqueous suspension under stirring with concentrated aqueous ammonia until alkaline and filtered, and the resin was washed with boiling water (total, 1 L). The filtrate and washings were evaporated to dryness, the residue was codistilled with ethanol (2 × 50 mL), and the product was filtered from ether, washed with the same solvent, and dried over phosphorus pentoxide *in vacuo*.

9-(*S*)-(2-Hydroxy-3-(triphenylmethoxy)propyl)-8-aza-2,6-diaminopurine (41) and 8-(*S*)-(2-Hydroxy-3-(triphenylmethoxy)propyl)-8-aza-2,6-diaminopurine (42). A mixture of 8-aza-2,6-diaminopurine (**12**) (3 g, 20 mmol), (*R*)-tritylglycidol (**40**) (7.6 g, 24 mmol), and cesium carbonate (0.2 g, 0.6 mmol) in dimethylformamide (100 mL) was heated for 20 h at 120 °C under stirring with exclusion of air moisture until the starting compound disappeared (TLC in S3). The solvent was taken to dryness *in vacuo* and the residue codistilled with toluene (three 100-mL portions). The chromatography of the residue on silica gel column (300 g) in chloroform followed by elution with S1 mixture gave (on crystallization from ether, petroleum ether added to turbidity) 3.2 g (15%) of the 9-isomer **41**. Mp: 225 °C. R_f = 0.80 (S3). Anal. ($C_{26}H_{25}N_7O_2$) C, H, N. ^{1}H NMR (($CD_3)_2SO$): δ 7.70 br (1H, NH), 6.35 br s (2H, NH_2); 7.20−7.45 m (16 H arom + NH); 5.42 δ (OH), J = 5.6; 4.35 dd (H-1′), J(1′,2′) = 5.1, J(gem) = 13.2; 4.29 dd, (H-1″), J(1″,2′) = 7.1, J(gem) = 13.2; 4.25 m, (H-2′), $\sum J$ = 27.9; 3.03 dd (H-3′), J(3′,2′) = 5.1, J(gem) = 9.5; 2.95 dd, (H-3″), J(3″,2′) = 5.1, J(gem) = 9.5. MS (M + H): 468.2.

Further elution afforded 1.3 g (14%, ethanol−ether) of the 8-isomer **42**. Mp: 139 °C. R_f = 0.76 (S3). Anal. ($C_{26}H_{25}N_7O_2$) C, H, N. ^{1}H NMR (($CD_3)_2SO$): δ 7.50 br (1H, NH_2), 6.08 br s (2H, NH_2); 7.20−7.40 m (15 H arom + NH); 5.36 δ (OH), J = 6.1; 4.61 dd (H-1′), J(1′,2′) = 4.6, J(gem) = 13.4; 4.41 dd, (H-1″), J(1″,2′) = 8.1, J(gem) = 13.4; 4.31 m, (H-2′), $\sum J$ = 29.1; 3.04 dd (H-3′), J(3′,2′) = 4.9, J(gem) = 9.5; 3.00 dd, (H-3″), J(3″,2′) = 5.4, J(gem) = 9.5.

9-(*S*)-(2,3-Dihydroxypropyl)-8-aza-2,6-diaminopurine (43). A solution of the trityl derivative **41** (0.75 g, 1.6 mmol) in the mixture of 0.25 M sulfuric acid and dioxane (1:1, 50 mL) was set aside overnight at ambient temperature. The mixture was alkalized with aqueous ammonia to pH 7−8 and taken to dryness. The residue was treated with water and the mixture extracted with ether (three 100-mL portions). The aqueous phase afforded by evaporation a crude product that gave upon recrystallization from water−ethanol mixture (1:1) compound **43** in 0.35 g (97%) yield. Mp: >250 °C. R_f =

0.64 (S4). $k = 1.66$ (S7). Anal. ($C_7H_{11}N_7O_2$) C, H, N. ^{1}H NMR ($(CD_3)_2SO$): δ 7.70 and 7.40 2 × br, 2H (NH_2); 6.40 br s, 2H (NH_2); 5.05 d, 1H (OH-2′), $J = 4.9$; 4.82 t, 1H (OH-3′), $J = 5.6$; 4.35 dd, 1H (H-1′), $J(1',2') = 4.4$, J(gem) = 13.9; 4.32 dd, 1H (H-1′), $J(1'',2') = 8.0$, J(gem) = 13.9; 4.00 m, 1H (H-2′), $\sum J = 28.2$; 3.41 pent and 3.35 pent, 2H (H-3′).

9-(*S*)-(2-(Phosphonomethoxy)-3-hydroxypropyl)-8-aza-2,6-diaminopurine (47). A solution of trityl derivative **41** (2.3 g, 5.0 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (5 mL, 38 mmol) in dimethylformamide (25 mL) was left to stand overnight at room temperature in a closed flask. The reaction mixture was then evaporated at 40 °C/13 Pa and the residue codistilled with dimethylformamide (three 50-mL portions). The residue was then treated with water−pyridine mixture (1:1) in the presence of solid carbon dioxide. The mixture was stirred to reach the room temperature (ca. 30 min) and evaporated *in vacuo* under the above conditions. The residue of compound **44** was then dried by repeated codistillation with pyridine (3 × 50 mL) and dimethylformamide (3 × 50 mL). Bis(2-propyl) [[(*p*-tolylsulfonyl)oxy]methyl]phosphonate (**46**) (2.1 g, 6 mmol) was added to the residue followed by dimethylformamide (25 mL), the solution was cooled down to −20 °C and 60% sodium hydride dispersion in paraffin (0.6 g, 15 mmol) was added under stirring. The mixture was stirred at ambient temperature under exclusion of moisture for 24 h. Methanol−concentrated aqueous ammonia mixture (100 mL, 1:1) was added and the mixture set aside overnight. The reaction mixture was taken to dryness *in vacuo* and the residue refluxed for 2 h with 80% acetic acid (100 mL). The resulting solution was evaporated, the residue codistilled with water (3 × 50 mL) and the residue was deionized on a column of Dowex 50 × 8 (H^+-form, 100 mL); the ammonia eluate containing crude compound **45** was dried over phosphorus pentoxide *in vacuo*. Acetonitrile (30 mL) was added to the residue followed by bromotrimethylsilane (3 mL) and the mixture stirred in a closed flask overnight. The residue was evaporated *in vacuo* and codistilled with acetonitrile (3 × 50 mL), and the residue was dissolved in water (50 mL). Triethylamine was added to pH 8, and after 1 h of standing, the mixture was evaporated *in vacuo*. The residue was deionized on a column (100 mL) of Dowex 50 × 8 (H^+-form, 100 mL), the ammonia eluate was evaporated *in vacuo*, and the crude product was purified by chromatography on a column of Dowex 1 × 2 (acetate form, 100 mL). The column was eluted with linear gradient of acetic acid (0−0.5M, 1 L each) to afford, after evaporation of the relevant fractions, codistillation with water, and crystallization from ethanol (ether added to turbidity) 0.83 g (50%) of compound **47**. Mp: 160 °C. $k = 3.5$ (S6). $E_{Up} = 0.75$. Anal. ($C_8H_{14}N_7O_5P$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 4.57 dd, 1H (H-1′), $J(1',2') = 6.4$, J(gem) = 14.6; 4.53 dd, 1H (H-1″), $J(1'',2') = 5.6$, J(gem) = 14.6; 3.97 qd, 1H (H-2′), $\sum J = 20.8$; 3.78 dd, 1H (H-3′), $J(3',2') = 3.4$, J(gem) = 12.4; 3.54 dd, 1H (H-3″), $J(3'',2') = 5.4$, J(gem) = 12.4; 3.58 dd, 1H (P−CH_2), J(P−CH) = 9.5, J(gem) = 12.4; 3.47 dd, 1H (P−CH, J(P−CH) = 9.5, J(gem) = 12.4).

9-(2-((Diisopropylphosphono)methoxy)ethyl)-8-aza-2,6-diaminopurine (48), 8-(2-((Diisopropylphosphono)methoxy)ethyl)-8-aza-2,6-diaminopurine (49), and 7-(2-((Diisopropylphosphono)methoxy)ethyl)-8-aza-2,6-diaminopurine (50). 8-Aza-2,6-diaminopurine (**12**) (2.26 g, 15.0 mmol), cesium carbonate (2.4 g,7.5 mmol), and bis(2-propyl) [(2-chloroethoxy)methyl]phosphonate (**25**) (4.5 g, 17.4 mmol) in dimethylformamide (75 mL) were heated for 20 h at 100 °C under stirring and exclusion of moisture until the starting base disappeared (TLC in S3). After evaporation of the solvent *in vacuo* and codistillation of the residue with toluene (3 × 50 mL) the residue was adsorbed from methanol on silica gel (50 mL) and the material transferred to a column of the same sorbent (150 g) in chloroform. The N^9-isomer **48** was eluted with the system S1, and the pertinent fractions were pooled and evaporated *in vacuo*. Yield: 0.85 g (15%) (methanol−ether) of the 9-isomer **48**. Mp: 185−187 °C. R_f = 0.64 (S3). $k = 2.7$ (S9). Anal. ($C_{13}H_{24}N_7O_4P$) C, H, N, P. ^{1}H NMR ($(CD_3)_2SO$): δ 7.55 br and 6.40 br s, 2H (2 × NH_2); 4.46 dsept (POCH), J(CH−CH_3) = 6.1 and 6.3, J(P−OCH) = 7.6; 4.45 t (NCH_2), J(CH_2−CH_2) = 5.2; 3.98 t, 2H (OCH_2), J(CH_2−CH_2) = 5.2; 3.74 d (PCH_2), J(P−CH) = 8.5; 1.15 and 1.09 2 × d, (2 × CH_3). UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 253.0 nm (11 600); (pH 7, 13) 259.5 nm (5700), 287.0 nm (9500).

Continued elution of the column with a chloroform−ethanol mixture (96:5) gave 1.0 g (18%) of the N^8- (**49**) and N^7-isomer (**50**) in the ratio 10:1, respectively; R_f = 0.52 and 0.43, respectively (S3). $k = 2.2$ and 1.6 (S9). **N^8-isomer (49)**. ^{1}H NMR ($(CD_3)_2SO$): δ 7.90 br, 2H and 6.35 br s, 2H (2 × NH_2); 4.67 t (NCH_2), J(CH_2−CH_2) = 5.1; 4.46 dsept (POCH), J(CH−CH_3) = 6.1 and 6.3, J(P−OCH) = 7.9; 4.06 t (OCH_2), J(CH_2−CH_2) = 5.1; 3.74 d (PCH_2), J(P−CH) = 8.2; 1.15 and 1.08 2 × d, (2 × CH_3). **N^7-isomer (50)**. ^{1}H NMR ($(CD_3)_2SO$): d 7.20 br and 6.05 br s (2 × NH_2); 4.89 t (NCH_2), J(CH_2−CH_2) = 4.9; 4.46 dsept (POCH), J(CH−CH_3) = 6.1 and 6.3, J(P−OCH) = 4.9; 3.91 t (OCH_2), J(CH_2−CH_2) = 4.9; 3.72 d (PCH_2), J(P−CH) = 7.9; 1.15 and 1.08 2 × d, (2 × CH_3).

9-(2-(Phosphonomethoxy)ethyl)-8-aza-2,6-diaminopurine (51). Bromotrimethylsilane (3 mL) was added to a suspension of the bis(2-propyl) ester **48** (0.8 g, 2.1 mmol) in acetonitrile (30 mL), and the mixture was stirred in a stoppered flask at ambient temperature overnight. The mixture was further worked up as described above for compound **28** and deionized on a column (100 mL) of Dowex 50 × 8 (H^+-form). The crude product was purified by chromatography on the column (100 mL) of Dowex 1 × 2 (acetate). The product was eluted with a linear gradient of acetic acid (0.5−1 M, 1 L each). The crystallization of the residue from 80% aqueous ethanol (ether added to turbidity) gave 0.2 g (31%) of compound **51**. Mp: >250 °C. $k = 1.45$ (S6). $E_{Up} = 0.70$. Anal. ($C_7H_{12}N_7O_4P{\cdot}H_2O$) C, H, N, P. ^{1}H NMR (D_2O + NaOD): δ 4.59 t (NCH_2, J(CH_2−CH_2) = 5.1); 4.03 t, 2H (OCH_2, J(CH_2-CH_2) = 5.4); 3.48 d J(P−CH) = 8.3 (PCH_2).

(*S*)-2,6-Diamino-8-aza-9-[2-((diisopropylphosphono)-methoxy)-3-fluoropropyl]purine (53) and (*S*)-2,6-Diamino-8-aza-8-[2-((diisopropylphosphono)methoxy)-3-fluoropropyl]purine (54). A mixture of 2,6-diamino-8-azapurine (**12**) (581 mg, 3.85 mmol), cesium carbonate (576 mg, 1.8 mmol), and compound **30** (1.225 g, 3.5 mmol) in dimethylformamide (18 mL) was stirred at 120 °C for 6 h under exclusion of moisture and taken to dryness. The residue was extracted with boiling chloroform and filtered and the filtrate evaporated. The residue was chromatographed on a column of silica gel (100 mL) in chloroform−methanol mixtures, 30:1 and 20:1, respectively. Compound **53** (350 mg, 24%) and **54** (100 mg, 7%) were obtained as thick oils.

Compound 53. Anal. ($C_{14}H_{25}FN_7O_4P$) C, H, N, F, P. ^{1}H-NMR ($(CD_3)_2SO$): δ 1.04 d + 1.08 d + 1.10 d + 1.14 d, 4 × 3 H (CH_3); 3.75 dd, 1 H (PCH, J(P,CH) = 9.5, J(gem) = 13.7); 3.89 dd, 1 H (PCH, J(P,CH) = 9.5, J(gem) = 13.7); 4.41 m, 2 H (OCH); 4.33 dm, 1 H (H-2′, J(2′,F) = 23); 4.54 ddd, 1 H (H-3′, J(3′,2′) = 4.2, J(gem) = 10.5, J(3′,F) = 46.2); 4.78 ddd, 1 H (H-3″, J(3″,2′) = 3.2, J(gem) = 10.5, J(3″,F) = 47.6); 4.68 dd, 1 H (H-1′, J(1′,2′) = 7.1, J(gem) = 14.6); 4.78 dd, 1 H (H-1″, J(1″,2′) = 5.1, J(gem) = 14.6); 8.30 s, 1 H (H-2); 8.10 + 8.43, 2 × bs, 2 H (NH_2). **Compound 54.** Anal. ($C_{14}H_{25}FN_7O_4P$) C, H, N, F, P. ^{1}H-NMR (D_2O + NaOD): δ 1.04 d + 1.08 d + 1.10 d + 1.14 d, 4 × 3 H (CH_3); 3.75 dd, 1 H (PCH, J(P,CH) = 9.5, J(gem) = 13.7); 3.89 dd, 1H (PCH, J(H,P) = 9.5, J(gem) = 13.7); 4.42 m, 1 H (H-3′, J(3′,2′) = 4.2, J(gem) = 10.5, J(3′,F) = 46.2); 4.78 ddd, 1 H (H-3″, J(3″,2′) = 3.2, J(gem) = 10.5, J(3″,F) = 47.6); 4.81 dd, 1 H (H-1′, J(1′,2′) = 7.6, J(gem) = 14.2); 4.99 dd, 1 H (H-1″, J(1″,2′) = 4.4, J(gem) = 14.6); 8.29 s, 1 H (H-2); 8.05 + 8.25, 2 × bs, 2 H (NH_2).

9-(*S*)-(3-Fluoro-2-(phosphonomethoxy)propyl)-8-aza-2,6-diaminopurine (55) and 8-(*S*)-(3-Fluoro-2-(phosphonomethoxy)propyl)-8-aza-2,6-diaminopurine (56). A suspension of compound **53** (80 mg) in acetonitrile (1 mL) was treated with bromotrimethylsilane (26 μL) overnight and worked up as described for compound **51**. Yield: 21 mg (33%) of compound **55**, not melting below 260 °C. UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 253.0 nm (8700), 275.0 nm (6700); (pH 7,13) 259.0 nm (4400), 287.0 nm (8000). For $C_8H_{13}FN_7O_4P$ (321.2):

mass spectrum (CAD) 322 $[(M + H)]^+$, 298 (MH − 2N), 276 (MH − 2N − H_2O).

Compound **54** (80 mg) was treated with bromotrimethylsilane in a similar manner to afford compound **56** (36 mg, 57%) not melting below 260 °C. UV spectrum (λ_{max} (ϵ_{max})): (pH 2) 259.0 nm (10 200), 286.0 nm (9500); (pH 7) 259.0 nm (7900), 289.0 nm (6700); (pH 13) 258.0 nm (4400), 310 nm (6200). For $C_8H_{13}FN_7O_4P$ (321.2): mass spectrum (CAD) 322 $[(M + H)]^+$, 304 (MH − H_2O).

9-(*R*)-(2-((Diisopropylphosphono)methoxy)propyl)-8-aza-2,6-diaminopurine (57) and 8-(*R*)-(2-((Diisopropylphosphono)methoxy)propyl)-8-aza-2,6-diaminopurine (58). A suspension of 8-aza-2,6-diaminopurine (**12**) (3.02 g, 20 mmol) and cesium carbonate (3.3 g, 10 mmol) in dimethylformamide (60 mL) was heated at 100 °C for 1 h, and a solution of compound **35a** (8.6 g, 21 mmol) in dimethylformamide (30 mL) was added over 15 min under stirring. The heating and stirring was then continued for an additional 16 h, the mixture stripped of the solvent *in vacuo*, and the residue extracted with boiling chloroform (total, 300 mL). The extract was chromatographed on a column (250 mL) of silica gel in chloroform and the column eluted with system S1 to afford 1.75 g (22.5%) of crystalline compound **57** (R_f 0.70, S3). Mp: 120−122 °C (ethyl acetate−petroleum ether). $[\alpha]_D$ = −4.7° (c = 0.5, 0.1 M HCl). Anal. ($C_{14}H_{26}N_7O_4P$) C, H, N, P. ^{1}H-NMR spectrum (D_2O + NaOD): δ 7.35 + 7.70, 2 × br, 2 × 1H (NH_2); 6.37 br s, 2H (NH_2); 4.34 dd, 1H, $J(1'_a,2')$ = 7.1, J(gem) = 14.4 (H-1′$_a$); 4.28 2 × dd, 1H, $J(1'_a,2')$ = 4.90, J(gem) = 14.4 (H-1′$_a$); 4.47 dq, 1H, J = 6.1, J(P,OCH″$_a$) = (P−OCH″$_a$); 4.43, dq, 1H, J = 6.3, J(P,OCH″$_b$) = 7.8 (POCH″$_b$); 4.11 pent d, 1H, J = 29.5 (H-2′); 3.74 dd, 1H, J(P,CH$_a$) = 9.5, J(gem) = 12.7 (P−CH$_a$); 3.65 dd, 1H, J(P,CH$_b$) = 9.0, J(gem) = 12.7 (PCH$_b$); 1.10 + 1.12 + 1.14 + 1.16 + 1.17, 5 × d (5 × 3H), J = 6.1 (CH_3).

Further elution of the column gave 1.40 g (18%) of 8-(*R*)-(2-((Diisopropylphosphono)methoxy)propyl)-8-aza-2,6-diaminopurine (**58**) (R_f 0.50, S3). Mp: 148−150 °C (ethyl acetate−petroleum ether). $[\alpha]_D$ = −43.7° (c = 0.5, 0.1 M HCl). Anal. ($C_{14}H_{26}N_7O_4P$) C, H, N, P. ^{1}H-NMR spectrum (D_2O + NaOD): δ 7.50 br, 2H + 6.08 br, 2H (NH_2); 4.53 dd, 1H $J(1'_a,2')$ = 5.4, J(gem) = 13.9 (H-1′$_a$); 4.47 dq, 1H and 4.43 dq, 1H, J(P,OCH) = 7.6, J(CH,CH$_3$) = 6.1 (POCH); 4.50 dd, 1H, $J(1'_b,2')$ = 6.60, J(gem) = 13.9 (H-1′$_b$); 4.17 pent d, J = 31.0 (H-2′); 3.75 dd, 1H, J(P,CH$_a$) = 9.5, J(gem) = 12.7 (P,CHb); 3.63 dd, 1H, J(P,-CHb) = 9.0, J(gem) = 12.7 (PCH$_b$); 1.10 + 1.11 + 1.14 + 1.16 + 1.18, 5 × d (5 × 3H), J = 6.1 (CH_3).

9-(*R*)-(2-(Phosphonomethoxy)propyl)-8-aza-2,6-diaminopurine (59) and 8-(*R*)-(2-(Phosphonomethoxy)propyl)-8-aza-2,6-diaminopurine (60). Compound **57** (1.75 g, 4.5 mmol) was treated with acetonitrile (25 mL) and bromotrimethylsilane (2.5 mL) overnight at room temperature and the solution evaporated *in vacuo*. Water (50 mL) was added, the mixture was alkalized by addition of concentrated aqueous ammonia, and the solution was evaporated. Further workup and purification was performed essentially as described for compound **28**. Yield: 0.90 g (65.5%) of 9-(*R*)-(2-(phosphonomethoxy)propyl)-2,6-diamino-8-azaadenine (**59**). Mp: 238−242 °C. $[\alpha]_D$ = +5.6° (c = 0.5, 0.1 M HCl). Anal. ($C_8H_{14}N_7O_4P$) C, H, N, P. ^{1}H-NMR spectrum (D_2O + NaOD): δ 4.49 dd, 1H, $J(1'a,2')$ = 5.6, J(gem) = 14.9 (H-1′a); 4.45 dd, 1H, $J(1'b,2')$ = 5.4, J(gem) = 14.9 (H-1′b); 4.08 m, 1H, J = 30.0 (H-2′); 3.59 dd, 1H, J(P,CHa) = 9.3, J(gem) = 12.2 (PCHa); 3.50 dd, 1H, J(P,CHb) = 9.1, J(gem) = 12.2 (PCHb); 1.17 d, 3H, $J(3',2')$ = 6.3 (H-3′). E_{Up} (pH 7.5) = 0.85.

The reaction of compound **58** (1.40 g, 3.6 mmol) with bromotrimethylsilane was performed essentially as described for compound **59**. Yield: 0.80 g (72.5%) of 8-(*R*)-(2-(phosphonomethoxy)propyl)-8-aza-2,6-diaminopurine (**60**). Mp: 238−240 °C. $[\alpha]_D$ = −23.5° (c = 0.5, 0.1 M HCl). Anal. ($C_8H_{14}N_7O_4P$) C, H, N, P. ^{1}H-NMR spectrum (D_2O + NaOD): δ 4.70 d, 2H, $J(1',2')$ = 5.2; (H-1′); 4.17 m, $\sum J$ = 29.3 (H-2′); 3.58 dd, 1H, J(P,CHa) = 9.4, J(gem) = 12.2 (PCHa); 3.50 dd, 1H, J(P,CHb) = 9.2, J(gem) = 12.2 (PCHb); 1.21 d, 3H, $J(3',2')$ = 6.3 (CH_3). E_{Up} (pH 7.5) = 0.85.

***N*^{2}-[(Dimethylamino)methylene]-8-azaguanine (61).** A mixture of 8-azaguanine (**13**) (3.0 g, 20 mmol), dimethylformamide (30 mL), and dimethylformamide dineopentyl acetal (15 mL) was stirred under exclusion of moisture overnight at ambient temperature. The mixture was taken to dryness at 40 °C/13 Pa, and the residue was codistilled with dimethylformamide (30 mL) under the same conditions and stirred with 70% aqueous ethanol (70 mL) under simultaneous additions of dry ice to neutralize alkaline reaction. Acetone (70 mL) was added, and the precipitate was filtered, washed with acetone and ether, and dried to afford 2.0 g of the HPLC-homogeneous product; k = 3.9 (S7). The mother liquor gave on evaporation and trituration with acetone an additional 2.0 g (yield, total 4.0 g, 98%) of compound **61**, slightly contaminated with 8-azaguanine; k = 0.8 (4% acetonitrile in 0.05 M TEAB). Anal. ($C_7H_9N_7O$) C, H, N.

9-(2-((Diisopropylphosphono)methoxy)ethyl)-8-azaguanine (62), 8-(2-((Diisopropylphosphono)methoxy)-ethyl)-8-azaguanine (63), and 7-(2-((Diisopropylphosphono)methoxy)ethyl)-8-azaguanine (64). A mixture of *N*^{2}-[(dimethylamino)methylene]-8-azaguanine (**61**) (4.2 g, 20 mmol), compound **25** (6.5 g, 25 mmol), and cesium carbonate (3.5 g, 10 mmol) in dimethylformamide (60 mL) was stirred at 100 °C for 8 h under exclusion of moisture. The mixture was taken to dryness at 40 °C/13 Pa and the residue codistilled with toluene (30 mL) under the same conditions. The residue was dissolved in methanol (70 mL), concentrated aqueous ammonia (70 mL) was added, and the mixture was left to stand overnight at room temperature. The solution was evaporated *in vacuo*, and the residue was dissolved in water (100 mL) by addition of Dowex 50 × 8 (H^+-form, ca. 60 mL). This suspension was applied onto a column (200 mL) of the same ion exchanger and the column eluted with water. The elution afforded first the salts followed by an UV-absorbing eluate. This material was taken to dryness *in vacuo* to afford 1.42 g (19%) of the compound **62**. R_f = 0.30 (S2). Anal. ($C_{13}H_{23}N_6O_5P$) C, H, N, P. ^{1}H NMR ($(CD_3)_2SO$): δ 10.93 br, 1H (NH); 6.89 br s, 2H (NH_2); 4.46 m, 2H (POCH), J(P−OCH) = 7.8, J(CH−CH$_3$) = 6.1; 4.45 t, 2H (NCH_2), J = 5.2; 3.97 t, 1H (OCH_2), J = 5.2; 3.73 d, 2H (PCH_2, J(P−CH) = 8.6); 1.15 and 1.10 2 × d (2 × CH_3), J = 6.1.

Further elution of the Dowex 50 column with 2.5% aqueous ammonia gave a UV-absorbing eluate that afforded after evaporation and chromatography of the residue on a silica gel column (200 mL) with methanol−chloroform mixture (5:95) 5.77 g (15.4 mmol, 77%) of an unseparable mixture of the 7-(**64**) and 8-isomer (**63**). R_f = 0.30 (S2), in the ratio 1:10, respectively (as estimated from the relative area of H-1 signals). ^{1}H-NMR (D_2O + NaOD) (**63**): δ 10.93 br, 1H (NH); 6.51 br s, 2H (NH_2); 4.64 t, 2H (1′-CH_2), J = 5.0; 4.46 sept, 1H (POCH), J(P−OCH) = 7.6, J(CH,CH$_3$) = 6.1; 4.04 t, 2H (2′-CH_2), J = 5.0; 3.73 d, 2H (PCH_2), J(P,CH) = 8.3; 1.10 and 1.105, 2 × d, 2 × 3H (2 × CH_3), J = 6.1. (**64**): δ 11.25 br, 1H (NH); 6.46 br s, 2H (NH_2); 4.75 t, 2H (1′-CH_2), J = 5.1; 4.46 sept, 1H (POCH), J(P−OCH) = 7.6, J(CH,CH$_3$) = 6.1; 4.01 t, 2H (2′-CH_2), J = 5.1; 3.75 d, 2H (PCH_2), J(P,CH) = 8.3; 1.15 and 1.145, 2 × d, 2 × 3H (2 × CH_3), J = 6.1.

9-(2-(Phosphonomethoxy)ethyl)-8-azaguanine (65). Compound **62** (1.42 g, 3.8 mmol) in acetonitrile (25 mL) was treated with bromotrimethylsilane (2.5 mL) overnight, and the volatiles were taken to dryness *in vacuo*. The residue in water (50 mL) was alkalized by ammonia and evaporated. The residue was applied on a column (100 mL) of Dowex 50 × 8 (H^+-form) and eluted with water. UV-absorbing fraction was alkalized by ammonia and evaporated *in vacuo*. The residue was applied on a column (150 mL) of Dowex 1 × 2 (acetate) and washed with water till the drop of the absorption. The resin was then stirred batchwise with 1 M formic acid (400 mL) for 30 min, filtered by suction and washed with boiling water (1 L). The filtrates were evaporated, and the residue was codistilled with water (5 × 50 mL) and dissolved in boiling water. Ethanol (2 volumes) was added to the solution and the mixture left to crystallize at 0 °C. Yield: 0.70 g (63%) of compound **65,** not melting below 250 °C. Anal. ($C_7H_{11}N_6O_5P$) C, H, N, P. ^{1}H-NMR spectrum (D_2O + NaOD): δ 4.57 t, 2H, (H-1′) $J(1',2')$ = 5.1; 4.09 t, 2H, (H-2′) J = 5.1; 3.60 d, 2H, (PCH_2) J(P,CH) = 8.8. E_{Up}(pH 7.5) = 0.87.

8-(2-(Phosphonomethoxy)ethyl)-8-azaguanine (66) and 7-(2-(Phosphonomethoxy)ethyl)-8-azaguanine (67). Suspension of crude mixture of compounds **63** and **64** (5.77 g, 15.4 mmol) in acetonitrile (40 mL) was treated overnight with bromotrimethylsilane (4 mL), and the volatiles were evaporated *in vacuo*. The residue in water (50 mL) was alkalized with ammonia and reevaporated and the residue applied on a column of Dowex 50 × 8 (150 mL). The product which is sparingly soluble in water eluted with water with a considerable retention. The residue was filtered from ethanol, washed with ether, and dried. Yield: 3.2 g (71.4%) of the mixture of **66** and **67**. Anal. ($C_7H_{11}N_6O_5P$) C, H, N, P. E_{Up}(pH 7.5) = 0.87 (fluorescent).

9-(*S*)-(2-(Phosphonomethoxy)propyl)-8-azaguanine (71b), 8-(*S*)-(2-(Phosphonomethoxy)propyl)-8-azaguanine (72b), and 7-(*S*)-(2-(Phosphonomethoxy)propyl)-8-azaguanine (73b). A mixture of N^2-[(dimethylamino)-methylene]-8-azaguanine (**61**) (2.15 g, 10 mmol), cesium carbonate (1.75 g, 5.4 mmol), and dimethylformamide (40 mL) was treated under stirring with 2(*S*)-[(bis(2-propyl)phosphonyl)methoxy]propyl *p*-toluenesulfonate (**35b**) (5 g, 12.2 mmol) in dimethylformamide (10 mL) and heated at 100 °C for 4 h. The mixture was filtered while hot, filtrate was evaporated to dryness *in vacuo*, and the residue was left to stand overnight at ambient temperature in a mixture of methanol and concentrated aqueous ammonia (1:1, 200 mL). The mixture was evaporated to dryness and the residue adsorbed from methanolic solution on silica gel (50 g). The sorbent was applied onto the column (200 mL) of silica gel in chloroform and the column eluted with chloroform followed by mixture S1. The minor fractions were discarded, and the main fraction (1.82 g, 47%) of the mixture of isomeric (*S*)-(2-((diisopropylphosphono)methoxy)propyl)-8-azaguanines was dried overnight *in vacuo*. Acetonitrile (25 mL) and bromotrimethylsilane (2.5 mL) were added, and the solution was left to stand overnight at room temperature. The mixture was then evaporated to dryness *in vacuo*, dissolved in water (20 mL), and alkalized with concentrated aqueous ammonia. After 15 min the solution was again evaporated and the residue applied in water to Dowex 50 × 8 (H^+-form) (100 mL). The elution of the column with water afforded (after removal of salts) with retention the UV-absorbing fraction that gave on evaporation and trituration of the residue with ethanol 0.50 g of the 9-isomer **71b**. Anal. ($C_7H_{11}N_6O_5P$) C, H, N, P.

Further elution of the column with 2.5% aqueous ammonia gave in the same manner 0.45 g of a mixture of the 7- (**73b**) and 8-isomer (**72b**) in the ratio of 1:4 (estimated by ^{13}C NMR spectra).

9-(*R*)-(2-(Phosphonomethoxy)propyl)-8-azaguanine (71a),8-(*R*)-(2-(Phosphonomethoxy)propyl)-8-azaguanine (72a), and 7-(*R*)-(2-(Phosphonomethoxy)propyl)-8-azaguanine (73a). The reaction was performed essentially as described for the (*S*)-enantiomers. After the workup of the condensation mixture with aqueous methanolic ammonia the crude mixture of bis(2-propyl) esters was applied onto a column of Dowex 50 × 8 (H^+-form) (150 mL) and the column eluted with 20% aqueous methanol. The UV-absorbing fraction was taken to dryness *in vacuo* and dried, affording the 9-isomer as an amorphous foam. The residue was treated with acetonitrile (30 mL) and bromotrimethylsilane (3 mL) overnight and evaporated *in vacuo*, and the residue was dissolved in 2.5% ammonia and reevaporated *in vacuo*. This residue was applied onto a column (100 mL) of Dowex 1 × 2 (acetate form) and washed with water (1 L) and with 1 M acetic acid (500 mL). The eluates were discarded, and the resin was extracted on filter with boiling water (500 mL). This eluate was evaporated *in vacuo* and the residue crystallized from water (poorly soluble) to afford the 9-(*R*)-isomer **71a** (0.50 g). Anal. ($C_7H_{11}N_6O_5P$) C, H, N, P.

Futher elution of Dowex 50 column with 2.5% aqueous ammonia gave a UV-absorbing fraction that was evaporated *in vacuo*, dried, and treated with acetonitrile (20 mL) and bromotrimethylsilane (2 mL). The reaction mixture formed a thick gel that was dissolved by the addition of 5% aqueous ammonia (100 mL), and the mixture was evaporated and deionized on a column (100 mL) of Dowex 50 × 8 (H^+-form). The ammonia eluate afforded a gel-forming mixture that was dissolved in water by addition of ammonia and applied onto a column of Sephadex A-25 (150 mL) in 0.02 M triethylammonium hydrogen carbonate. The column was eluted with a linear gradient of the same buffer (0.02−0.20 M, 1 L each) to give the main fraction consisting of the mixture of the 7- and 8-isomer (E_{Up} = 0.92, fluorescent spot). The residue after codistillation with methanol was applied onto a column (20 mL) of Dowex 1 × 2 (acetate), the column was washed with water (100 mL), and the product was eluted with 1 M acetic acid. After evaporation *in vacuo*, codistillation with water, and trituration with ethanol, the product was filtered, washed with ethanol and ether, and dried to afford 0.50 g of the mixture of 7-isomer (**73a**) and 8-isomer (**72a**) in the ratio 1:4 (by ^{13}C NMR). Anal. ($C_7H_{11}N_6O_5P$) C, H, N, P. ^1H-NMR (D_2O + NaOD): (**8-isomer**, **72a**): δ 4.65 2 × dd, 2H, $J(1',2')$ = 5.4, $J(1'',2')$ = 5.1, J(gem) = 14.0 (1′-CH_2); 4.17 br sext, 1H, $\sum J$ = 29.5 (2′-CH); 3.52 dd, 1H, J(P−CH) = 9.7, J(gem) = 12.2 + 3.47 dd, 1H, J(P−CH) = 9.0, J(gem) = 12.2 (PCH_2); 1.18 d, 3H ($J(3',2')$ = 6.3 (CH_3); (**7-isomer**, **73a**): δ 4.76 2 × dd, 2H, $J(1',2')$ = $J(1'',2')$ = 5.2, J(gem) = 14.0 (1′-CH_2); 4.13 br sext, 1H, $\sum J$ = 29.5 (2′-CH); 3.50 m, 2H (PCH_2); 1.16 d, 3H, $J(3',2')$ = 6.3 (CH_3).

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 previously established procedure.[2a−c,43,44] Briefly, confluent cell cultures in microtiter trays 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 Cells. The methodology of the anti-HIV assays has been described previously.[2b] Briefly, human MT-4 (~4 × 10^5cells·mL^{-1}) or CEM (~3 × 10^5 cells·mL^{-1}) cells were infected with 100 $CCID_{50}$ HIV-1(III_B) μL^{-1} and seeded in 200 μL wells of a microtiter plate, containing appropriate dilutions of the test compounds. After 5 days (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.[4a,5b] 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 tumours induced following intramuscular inoculation of 3-day-old NMRI mice with MSV) 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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