SYNTHESIS AND CYTOSTATIC ACTIVITY OF *N*-[2-(PHOSPHONOMETHOXY)ALKYL] DERIVATIVES OF N^6-SUBSTITUTED ADENINES, 2,6-DIAMINOPURINES AND RELATED COMPOUNDS

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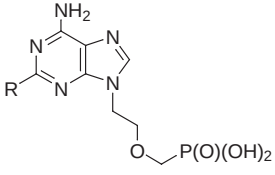
N^6-Substituted adenine and 2,6-diaminopurine derivatives of 9-[2-(phosphonomethoxy)-ethyl] (PME), 9-[(*R*)-2-(phosphonomethoxy)propyl] [(*R*)-PMP] and enantiomeric (*S*)-PMP series were synthesized by reactions of primary or secondary amines with 6-chloro-9-{[2-(diisopropoxyphosphoryl)methoxy]alkyl}purines (**26**–**28**) or 2-amino-6-chloro-9-{[2-(diisopropoxyphosphoryl)methoxy]alkyl}purines (**29**–**31**) followed by treatment of the diester intermediates **32** with bromo(trimethyl)silane and hydrolysis. Diesters **32** were also obtained by reaction of N^6-substituted purines with synthons **23**–**25** bearing diisopropoxyphosphoryl group. Alkylation of 2-amino-6-chloropurine (**9**) with diethyl [2-(2-chloroethoxy)ethyl]phosphonate (**148**) gave the diester **149** which was analogously converted to N^6-substituted 2,6-diamino-9-[2-(2-phosphonoethoxy)ethyl]purines **151**–**153**. Alkylation of N^6-substituted 2,6-diaminopurines with (*R*)-[(trityloxy)methyl]oxirane (**155**) followed by reaction of thus-obtained intermediates **156** with dimethylformamide dimethylacetal and condensation with diisopropyl [(tosyloxy)methyl]phosphonate (**158**) followed by deprotection of the intermediates **159** gave N^6-substituted 2,6-diamino-9-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]purines **160**–**163**. The highest cytostatic activity *in vitro* was exhibited by the following N^6-derivatives of 2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purine (PMEDAP): 2,2,2-trifluoroethyl (**53**), allyl (**54**), [(2-dimethylamino)ethyl] (**68**), cyclopropyl (**75**) and dimethyl (**91**). In CCRF-CEM cells, the cyclopropyl derivative **75** is deaminated to the guanine derivative PMEG (**3**) which is then converted to its diphosphate.

Keywords: Purines; Nucleotide analogs; Phosphonates; Acyclic nucleoside phosphonates; ANP; Cytostatic activity; Antineoplastic; Anticancer.

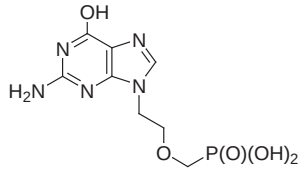
Acyclic nucleoside phosphonates (ANP), isopolar analogues of nucleotides, which contain phosphonomethoxy function linked to an acyclic side chain instead of phosphate group bound to the nucleoside sugar moiety, attract considerable attention. An important representative of this class is 1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (CAS 113852-37-2, HPMPC, Cidofovir)[1] showing general anti-DNA-viral activity. Its parenteral form

Vistide™ was approved for treatment of cytomegalovirus retinitis in AIDS patients[2]. Cidofovir is also used in the therapy of acyclovir- and foscarnet-resistant HSV-1 lesions and in other viral diseases, such as papillomavirus induced warts, nasopharyngeal carcinoma, *etc.* (for a review, see ref.[3]).

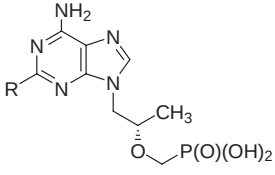
Another important ANP, 9-[2-(phosphonomethoxy)ethyl]adenine (**1**) (PMEA, Adefovir, ADV, CAS 106941-25-7) exhibits antiviral activity *in vitro* directed against some DNA viruses[4] and against retroviruses[5,6]. Also diseases caused by other mammalian retroviruses – MAIDS virus[7], FLV (ref.[8]), Visna virus[9] and simian immunodeficiency virus (SIV)[10] were successfully treated with this drug[11]. Its bis[(trimethylacetoxy)methyl] ester (Bis-POM-PMEA, Preveon) shows an increased oral resorption and enhanced transport into the cell[12]. It is active *in vitro* against HIV-1 and HIV-2. It was scrutinized for AIDS therapy[13] but later withdrawn due to certain nephrotoxicity at a therapeutic dosage. Presently, it is in phase III clinical trials for chronic hepatitis B virus (HBV) infections, in particular in patients with lamivudine-resistant HBV strains[14].



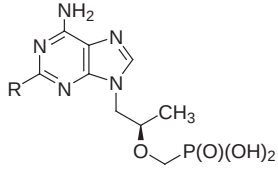
1 R=H, PMEA
2 R=NH_2, PMEDAP



3 PMEG



4 R=H, (*R*)-PMPA
5 R=NH_2, (*R*)-PMPDAP



6 R=H, (*S*)-PMPA
7 R=NH_2, (*S*)-PMPDAP

The antiviral potency of the 2,6-diaminopurine derivative (PMEDAP, **2**) and guanine derivative (PMEG, **3**) is higher compared to PMEA. PMEDAP is extremely active *in vivo* against mammalian retroviruses[15]. However, PMEG is highly cytotoxic and the interest in antiviral properties of this compound thus focused chiefly on its effect on papillomaviruses[16].

The structure of another ANP group – PMP-derivatives[17] – is characterized by substitution with methyl group in position 2 of the side-chain: 9-[(*R*)-2-

(phosphonomethoxy)propyl]adenine [(*R*)-PMPA, **4**] and 9-[(*R*)-2-(phosphonomethoxy)propyl]-2,6-diaminopurine [(*R*)-PMPDAP, **5**] exhibit high potency against HIV-1 and HIV-2 *in vitro*[18] and (*R*)-PMPA (Tenofovir) shows therapeutic and prophylactic activity in simian model of immunodeficiency disease[19]. Tenofovir or its oral prodrug Tenofovir disoproxil (Viread™) reached presently the final clinical phase for treatment of AIDS in both NRTI-naïve and NRTI-resistent (**n**ucleoside **r**everse **t**ranscriptase **i**nhibitors) patients. The activity in the PMP series is strictly enantiospecific. Both (*S*)-PMPA (**6**) and (*S*)-PMPDAP (**7**) are antivirally inactive. Replacement of the methyl group at the side chain by other alkyl or cycloalkyl groups results in complete loss of antiviral activity[20].

We have systematically investigated the relation between the structure of the heterocyclic base and antiviral activity of PME derivatives[21]. Most of our attention concerned the *C*-substitution of the heterocyclic base[22]. These observations led us to devise the general structure of the heterocyclic part of the pharmacophore. It is characterised by cumulation of amino groups in the pyrimidine part of the purine system. In order to decide whether the role of the amino groups consists solely in their basicity or their participation in hydrogen bond formation, we have synthesized the 2-(aminomethyl) analogues of PMEG and PMPG (ref.[23]), as well as the 6-(aminomethyl) analogues of PME-, PMP- and HPMP-derivatives of adenine and 2,6-diaminopurine[24]. None of them exerted any antiviral activity. Nor was any activity observed with 6-(*C*-hetaryl)[25], or 6-[1-(*N*-alkylamino)-ethyl[26] derivatives. The quaternary PME derivatives of 2,4-diaminopyrimidine and 4,6-diaminopyrimidine[27] which are also relevant to the hypothetical pharmacophore, were devoid of any activity as well.

We have also studied the influence of *N*-substitution of the 6-amino group in the PME and PMP derivatives of adenine and 2,6-diaminopurine on their antiviral activity. In the original contributions, we reported on extremely high antiviral activity of numerous PME derivatives of these groups against DNA viruses, in particular CMV and VZV (ref.[28]). Later, other papers described their significant *in vitro* activity against Epstein–Barr virus[29]. Already in the first phase of the SAR study, several compounds (*e.g.* N^6-cyclopropyl, N^6-allyl and N^6-dimethyl derivatives of PMEDAP), showed markedly higher antiviral activities compared to the other members of the group.

Adefovir exhibits also cytostatic activity *in vivo* in rat and mouse carcinomas and sarcomas[30]. The therapeutic effect is still more pronounced in PMEDAP[31], both in monotherapy and in combination with other anticancer drugs (taxotere)[32]. Cytostatic activity was reported also for ANPs in

N^6-substituted-purine series: we have reported on an antiproliferative effect of these compounds on murine lymphocytes. This study revealed a parallel between antiviral and antiproliferative activities, at least in some of the PME and PMP series derived from adenine and/or 2,6-diaminopurine. Some of the compounds showed extremely high *in vitro* activities[33]. Cytostatic activity of the N^6-cyclopropyl derivative of PMEDAP (cypr-PMEDAP) was further investigated by other authors[34]; this compound is believed to act as a prodrug of PMEG (**3**) forming this highly cytotoxic anticancer compound[35] by the action of cellular AMP deaminase[36]. We have investigated antitumour activity of cypr-PMEDAP and several of its congeners selected on the basis of their *in vitro* cytostatic activity (*vide infra*), in an *in vivo* model of haematological malignancy of inbred Sprague–Dawley rats. However, in this model, they were less potent or exhibited the same antineoplastic effect as PMEDAP (ref.[37]).

Biochemical studies[38] demonstrated phosphorylation of ANP to their mono- and diphosphates. Thus, PMEA is converted to its mono- and diphosphate (dADP and dATP analogues) under catalysis of nucleotide kinases[39]. The diphosphate (PMEApp) selectively inhibits cellular DNA polymerases[40], acting as a substrate/inhibitor for reverse transcriptases[41], and its incorporation in the growing DNA chain leads to chain termination. PMEDAP and PMEG are also phosphorylated by cellular nucleotide kinases[39,42]. In addition to the action of their diphosphates on DNA polymerases and reverse transcriptases, PMEG and its anabolites efficiently inhibit purine nucleoside phosphorylases[43]; this activity might explain high toxicity of guanine derivatives in all investigated ANP series.

As already mentioned, our opening SAR studies on antiviral and antiproliferative effects provided three lead ANP structures with cyclopropylamino, allylamino and dimethylamino substituents in position 6 of the purine ring. Variation of substituents in compounds of this study thus leads mainly to (a) alkyl, alkenyl and alkynylamino derivatives, (b) cycloalkylamino derivatives, (c) dialkylamino, dialkenylamino, dicycloalkylamino analogues and (d) *N*-hetaryl (pyrrolidin-1-yl, piperidin-1-yl, morpholin-4-yl, *etc.*) congeners. Substituents bearing one or two hydroxyalkyl, alkoxyalkyl, aminoalkyl or dialkylaminoalkyl groups were also introduced.

In this paper we describe (i) the synthesis of N^6-substituted purine ANP derivatives, (ii) detailed SAR investigation of the effect of substituents at the 6-amino group in the adenine and 2,6-diaminopurine derivatives on the cytostatic activity *in vitro* and (iii) introductory biochemical studies with special attention to the metabolism of N^6-substituted ANPs, their phosphorylation and deamination to the 6-oxopurine compounds. The ul-

timate objective was the selection of optimum candidates for investigation of their antineoplastic activity *in vivo*.

The SAR study was performed in several series of ANP type including PME, (*R*)-PMP, (*S*)-PMP derivatives and was complemented by related N^6-substituted (*S*)-HPMPDAP derivatives, N^6-substituted 9-[2-(2-phosphonoethoxy)ethyl] (PEE) analogues[44] (homologs of PMEDAP) and by regioisomeric N^6-substituted 7-PMEDAP derivatives. The limited choice of N^6-substituents in two last mentioned series followed the structure of those substituents which gave optimum effects in the basic series of ANPs.

Synthesis

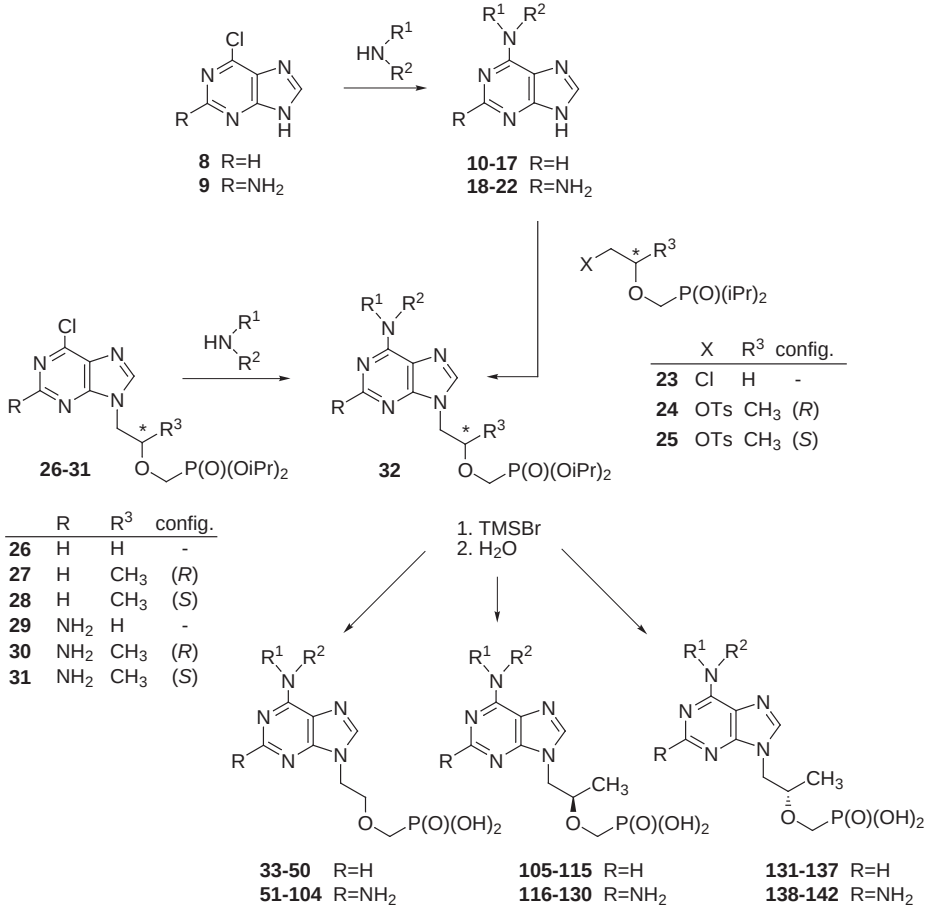
The preparation of 9-PME or 9-PMP derivatives substituted at the 6-amino function of adenine or 2,6-diaminopurine employed two routes (Scheme 1): Transformation of 6-chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-purine (**26**) and 2-amino-6-chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]-ethyl}purine (**29**) to the intermediate *N*-substituted 6-amino derivatives **32** by the action of excess primary or secondary amine in ethanol solution. The crude reaction product was desalted and cleaved with bromo(trimethyl)silane (Me_3SiBr) to afford, after deionization and ion exchange chromatography the free acids of PME derivatives of the adenine series **35**–**50** or the 2,6-diaminopurine series (**51**–**104**). The required starting materials **26** and **29** are easily available by alkylation of 6-chloropurine (**8**) or 2-amino-6-chloropurine (**9**) with diisopropyl [(2-chloroethoxy)methyl]-phosphonate (**23**).

Enantiomeric (*R*)- and (*S*)-PMP-derivatives were prepared analogously starting from the 6-chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]propyl}purines (**27**, **28**) and -2-amino-6-chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]propyl}-purines (**30**, **31**). The deprotection of the corresponding intermediate diesters **32** and purification of the free ANPs was performed analogously to afford N^6-substituted (*R*)-PMP derivatives of adenine **105**–**115** or 2,6-diaminopurine **116**–**130**, or, the enantiomeric N^6-substituted derivatives of (*S*)-PMPA **131**–**137** or (*S*)-PMPDAP **138**–**142**. Also in this case, the starting 6-chloro derivatives are accessible by alkylation of 6-chloropurines **8**, **9** with 2-[(diisopropoxyphosphoryl)methoxy]propyl tosylates **24**, **25** (ref.[17b]).

Reactions with amines were usually performed in refluxing ethanolic solution under exclusion of CO_2, except for low-boiling amines (methylamine, dimethylamine, 2,2,2-trifluoroethylamine, *etc.*); where the reactions were performed in an autoclave. (In alternative preparation of

6-(dimethylamino)purine derivatives **32** (R^1 = R^2 = CH_3), dimethylamine was replaced by dimethylammonium *N*,*N*-dimethylcarbamate; in this case, the reaction of compounds **26**, **29** proceeded smoothly in boiling acetonitrile.)

The other general route for the synthesis of N^6-substituted ANPs makes use of the alkylation of N^6-substituted adenines (**10**–**17**) or 2,6-diaminopurines **18**–**22** with appropriate synthons **23**–**25** under the earlier described conditions[17b,22]. The thus-obtained intermediary phosphonate diesters **32** were subsequently converted to the free phosphonic acids by the above

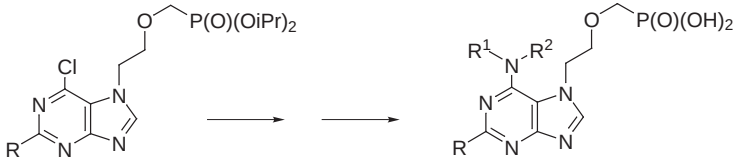


For list of substituents R^1 and R^2 see Table I.

SCHEME 1

procedure. The starting heterocyclic bases **10**–**22** were obtained from 6-chloropurine (**8**) or its 2-amino congener **9** by the action of an appropriate amine followed by deionisation. The alkylation was performed in DMF in the presence of either Cs_2CO_3 or, preferably, DBU (1,8-diazabicyclo-[5.4.0]undec-7-ene). This route was used particularly in those cases where a larger quantity of the phosphonate was required.

7-[2-(Phosphonomethoxy)ethyl] derivatives **144**–**147** were prepared by reacting 6-chloropurine (**143a**) or 2-amino-6-chloropurine intermediate **143b** with excess primary or secondary amine in ethanol followed by deionisation, deprotection with Me_3SiBr and purification (Scheme 2). The starting materials are accessible as by-products of the alkylation of 6-chloropurines **8**, **9** with compound **23** (ref.[22]).



143 a, R=H
b, R=NH_2

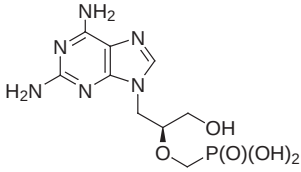
144-147

For list of substituents R^1 and R^2 see Table I.

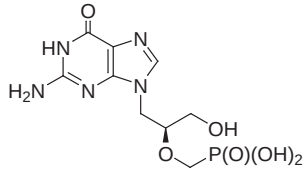
SCHEME 2

Homologues of PMEDAP derivatives, N^6-substituted 2-amino-9-[2-(2-phosphonoethoxy)ethyl]purines (PEE derivatives) were synthesized by the procedure described in Scheme 3: Reaction of 2-amino-6-chloropurine (**9**) with diethyl [2-(2-chloroethoxy)ethyl]phosphonate (**148**) gave the diester intermediate **149**; this compound was transformed by reaction with amines to compounds **150** which in turn gave the free phosphonates **151**–**153** by transsilylation with Me_3SiBr followed by hydrolysis. Alkaline hydrolysis of compound **149** followed by ester cleavage afforded the guanine derivative **154** (PEEG) (Scheme 3).

The last type of ANP derivatives included in this study, is the 9-[3-hydroxy-2-(phosphonomethoxy)propyl] derivatives (HPMP derivatives). With respect to our previous knowledge of their biological activity[21], we have synthesized solely the (*S*)-enantiomers related to (*S*)-HPMPDAP (**164**) or (*S*)-HPMPG (**165**). Owing to the laborious multistep synthesis, the choice of 6-substituents was limited to those groups which generally show the most promising effects (dimethylamino, allylamino, cyclopropylamino, [(2-dimethylamino)ethyl]amino group). Treatment of N^6-substituted

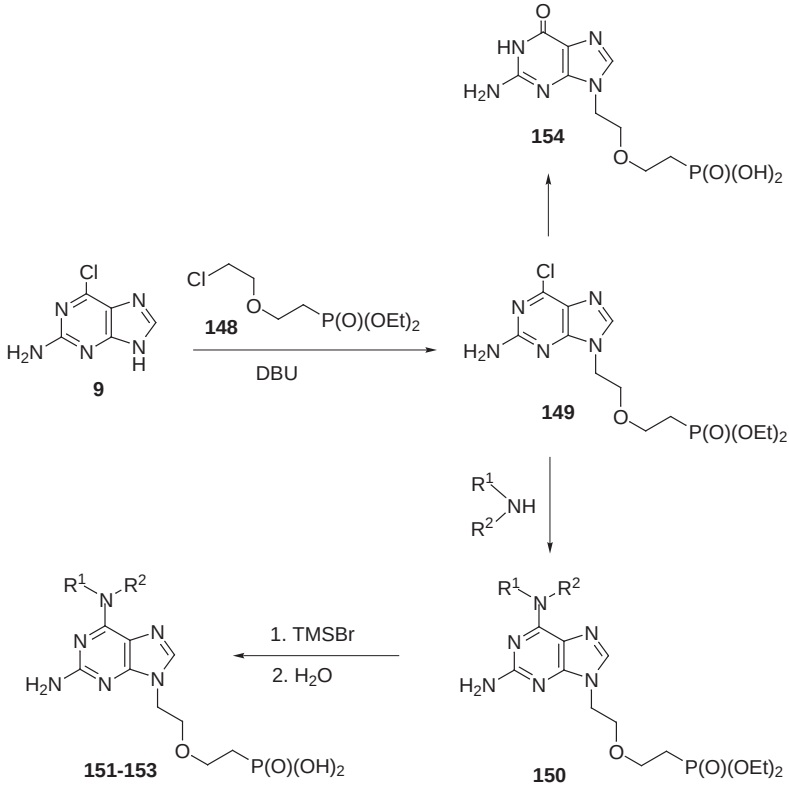


164 (*S*)-HPMPDAP



165 (*S*)-HPMPG

2,6-diaminopurines **18**–**21** with (*S*)-tritylglycidol (**155**) gave 9-[(*S*)-2-hydroxy-3-(trityloxy)propyl] derivatives **156**. The 2-amino group in the purine base was protected by reaction with (dimethoxymethyl)dimethylamine (dimethylformamide dimethylacetal) and the resulting formamidine derivatives **157** were directly reacted with diisopropyl [(tosyloxy)methyl]phosphonate (**158**)[1c] and excess NaH. After alkaline work-up, the resulting crude

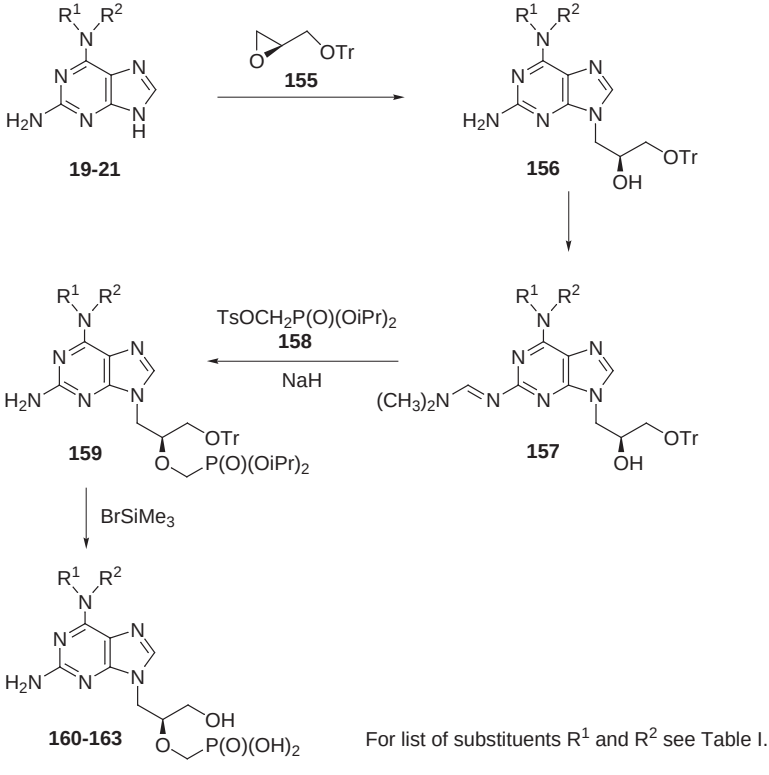


For list of substituents R^1 and R^2 see Table I.

SCHEME 3

trityl-containing diester **159** was treated with Me_3SiBr to deprotect simultaneously trityl and phosphonate ester groups. This procedure afforded N^6-substituted (*S*)-HPMPDAP derivatives **160–163**. Ion exchange chromatography of the deionised crude materials was used for the ultimate purification (Scheme 4).

Free acyclic nucleoside phosphonic acids prepared in this study were usually crystallized from water or aqueous ethanol giving frequently mono- or dihydrates. Their structure was routinely confirmed by ^1H NMR which showed, in addition to characteristic signals of side-chain protons (and methyl group in the PMP-series), C-8 (and C-2) protons, also the protons corresponding to N^6-substituent(s). Their electrophoretic mobility was generally slightly lower compared to the parent phosphonates, except for the N^6-(ω-aminoalkyl)amino derivatives which showed a substantially decreased mobility.



SCHEME 4

Cytostatic Activity

The evaluation of cytostatic activity of N^6-substituted adenine and 2,6-diaminopurine ANPs *in vitro* was performed with four cell lines: two of them (L1210, L929) were mouse cell lines, two (HeLaS3 and CCRF-CEM) were of human origin. The procedure consisted of two steps: in the first approximation, the cells were grown in the presence of tested compounds at a constant drug concentration and, after a certain period of time, the cells were counted and the inhibition expressed as percentage of cell count in a control culture grown in the culture medium alone. Essentially all ANPs prepared in this study were included in this assay (Table I). Compounds which passed the first screen showing significant inhibitory activity (usually 50–70% of the control, at least in two cell lines) were selected for the second evaluation step, and their IC_{50} values for individual cell cultures were estimated using standard methodology (Table II). The results lead to the following conclusions:

a) The human lymphoblastoid cell line CCRF-CEM is generally most sensitive to the action of ANPs, followed by mouse leukemia L929, while L1210 and HeLaS3 are generally less sensitive to the drug action *in vitro*.

b) The *N*-substitution of 6-amino group in PMEA with alkyl, alkenyl or cycloalkyl group as well as with alkyl groups ω-substituted by amino, dimethylamino or hydroxy function (**33**–**46**) results in inactive compounds. Only some *N*,*N*-disubstituted derivatives, *e.g.* *N*,*N*-diethylamino- (**48**) and *N*-(piperidin-1-yl)purine (**50**) derivatives show indication of slight growth inhibition which, however, was not confirmed in the second scrutiny.

c) The *N*-substitution of 6-amino group in PMEDAP led to numerous compounds with cytostatic activity: (i) in the *N*-monoalkyl group, some effect was observed in all compounds; N^6-methyl (**51**), butyl (**56**) and *sec*-butyl (**57**) derivatives were modestly active, but the strongest activity was observed in N^6-propyl (**52**) and isobutyl (**58**) derivatives. (ii) The most active compound in the N^6-alkenyl-PMEDAP series is the allyl derivative **54**; it is closely followed by the structurally related but-2-en-1-yl, but-3-en-2-yl and 2-methylallyl derivatives (**59**, **60** and **62**, respectively) all of which contain the structural element of the *N*-allyl group. The isomeric but-3-en-1-yl (homoallyl) derivative **61** is much less active. Cytostatic activity was observed also for N^6-propargyl-PMEDAP (**55**).

d) Substitution in the N^6-alkyl chain in the PMEDAP series with hydroxy (**63**–**66**, **71**) or methoxy group (**67**) as well as the presence of an amino group (**69**) inactivates the compounds. On the contrary, the introduction of

TABLE I
Cytostatic activity[a] of N^6-substituted 6-amino-9-[2-(phosphonomethoxy)alkyl]purines and 2,6-diamino-9-[2-(phosphonomethoxy)alkyl]purines

Compound	R^1	R^2	% Control			
			L-1210	L-929	HeLaS3	CCRF-CEM
	N^6-Substituted 6-amino-9-[2-(phosphonomethoxy)ethyl]purines					
33	Allyl	H	85	68	85	88
34	Cyclopropyl	H	100	90	97	91
35	Isopropyl	H	100	93	103	97
36	Isobutyl	H	96	81	100	95
37	2-Hydroxyethyl	H	91	83	101	94
38	2-Methoxyethyl	H	101	83	94	93
39	3-Hydroxypropyl	H	98	86	96	97
40	2-Hydroxypropyl	H	94	92	96	96
41	1-Hydroxypropan-2-yl	H	–	–	–	90
42	2-Aminoethyl	H	97	86	96	79
43	2-(Dimethylamino)ethyl	H	91	92	92	–
44	3-Aminopropyl	H	91	74	104	92
45	4-Aminobutyl	H	89	85	101	108
46	Cyclohexyl	H	87	85	94	89
47	Methyl	methyl	92	78	92	101
48	Ethyl	ethyl	70	69	96	87
49	$-(CH_2)_4-$		94	75	97	106
50	$-(CH_2)_5-$		95	68	100	100
	N^6-Substituted 2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purines					
51	Methyl	H	89	73	94	62
52	Propyl	H	57	46	72	58
53	2,2,2-Trifluoroethyl	H	63	26	50	43
54	Allyl	H	58	15	48	41
55	Propargyl	H	83	69	83	42
56	Butyl	H	72	67	78	63
57	*sec*-Butyl	H	72	84	91	59
58	Isobutyl	H	70	25	65	55
59	But-2-en-1-yl	H	83	38	71	82
60	But-3-en-2-yl	H	90	85	59	48
61	But-3-en-1-yl	H	70	70	92	86
62	2-Methylallyl	H	66	40	81	41
63	2-Hydroxyethyl	H	89	84	100	93
64	3-Hydroxypropyl	H	77	105	105	81
65	2-Hydroxypropyl	H	78	94	105	82

TABLE I
(*Continued*)

Compound	R^1	R^2	% Control			
			L-1210	L-929	HeLaS3	CCRF-CEM
66	1-Hydroxypropan-2-yl	H	93	81	100	71
67	2-Methoxyethyl	H	94	73	105	72
68	2-(Dimethylamino)ethyl	H	60	28	75	70
69	3-Aminopropyl	H	89	98	88	81
70	3-(Dimethylamino)propyl	H	68	28	75	66
71	3-Amino-2-hydroxypropyl	H	83	95	92	86
72	6-Aminohexyl	H	87	100	108	94
73	Carboxymethyl	H	87	86	108	89
74	5-Carboxypentyl	H	85	95	83	97
75	Cyclopropyl	H	50	39	67	36
76	Cyclobutyl	H	66	53	68	42
77	Cyclopentyl	H	72	55	106	55
78	Cyclohexyl	H	89	63	105	87
79	Cycloheptyl	H	78	80	78	81
80	Cyclooctyl	H	97	87	82	93
81	Cyclopropylmethyl	H	67	43	64	47
82	Cyclohexylmethyl	H	100	81	100	101
83	Benzyl	H	96	82	93	93
84	4-Aminobenzyl	H	73	68	97	73
85	Phenyl	H	85	60	85	84
86	1-Naphtyl	H	85	69	101	80
87	2-Pyridylmethyl	H	82	72	67	99
88	3-Pyridylmethyl	H	73	63	81	100
89	4-Pyridylmethyl	H	97	61	81	101
90	Diphenylmethyl	H				93
91	Methyl	methyl	70	54	67	40
92	Methyl	ethyl	58	31	63	46
93	Allyl	allyl	88	84	100	84
94	2-Hydroxyethyl	2-hydroxyethyl	102	93	91	80
95	2-Methoxyethyl	2-methoxyethyl	92	87	98	89
96	$-(CH_2)_4-$		57	90	91	62
97	$-(CH_2)_5-$		65	41	81	81
98	$-(CH_2)_2-O-(CH_2)_2-$		86	56	81	85
99	$-(CH_2)_2-NH-(CH_2)_2-$		75	75	95	96
100	$-(CH_2)_2-N(CH_3)-(CH_2)_2-$		73	77	120	95
101	$-(CH_2)_2-NH-(CH_2)_3-$		92	82	99	96
102	Cyclopropyl	cyclopropyl	81	41	73	89
103	1-(Cyclopropyl)ethyl	H	80	80	63	67
104	Benzyl	methyl	81	80	97	101

TABLE I
(*Continued*)

Compound	R^1	R^2	% Control			
			L-1210	L-929	HeLaS3	CCRF-CEM
	N^6-Substituted 6-amino-9-[(*R*)-2-(phosphonomethoxy)propyl]purines					
105	Isopropyl	H	91	95	87	102
106	Allyl	H	82	57	107	94
107	Cyclopropyl	H	73	57	102	74
108	Isobutyl	H	81	70	92	92
109	Cyclohexyl	H	84	93	86	96
110	2-(Dimethylamino)ethyl	H	73	57	102	74
111	3-(Dimethylamino)propyl	H	98	84	96	
112	Methyl	methyl	85	74	83	78
113	Methyl	ethyl	68	66	112	80
114	Ethyl	ethyl	84	80	83	81
115	$-(CH_2)_4-$		95	73	88	92
	N^6-Substituted 2,6-diamino-9-[(*R*)-2-(phosphonomethoxy)propyl]purines					
116	Methyl	H	92	110	92	112
117	Cyclopropyl	H	74	84	96	89
118	Butyl	H	101	86	116	94
119	*sec*-Butyl	H	105	87	96	90
120	Isobutyl	H	84	75	94	92
121	2,2,2-Trifluoroethyl	H	80	80	105	95
122	Cyclopentyl	H	71	73	96	51
123	Cyclohexyl	H	91	86	89	102
124	Benzyl	H	100	82	95	118
125	Phenethyl	H	82	75	85	90
126	Methyl	methyl	91	75	91	120
127	$-(CH_2)_4-$		99	63	85	82
128	$-(CH_2)_5-$					99
129	$-(CH_2)_2-O-(CH_2)_2-$		96	83	87	103
130	2-(Dimethylamino)ethyl		65	18	65	
	N^6-Substituted 6-amino-9-[(*S*)-2-(phosphonomethoxy)propyl]purines					
131	Allyl	H	100	88	108	91
132	Isobutyl	H	99	82	100	86
133	Isopropyl	H	100	80	105	90
134	Cyclopropyl	H	96	95	98	92
135	2-(Dimethylamino)ethyl	H	96	91	103	95
136	Methyl	methyl	99	93	98	99
137	$-(CH_2)_4-$		100	82	100	105

TABLE I
(*Continued*)

Compound	R^1	R^2	% Control			
			L-1210	L-929	HeLaS3	CCRF-CEM
	N^6-Substituted 2,6-diamino-9-[(*S*)-2-(phosphonomethoxy)propyl]purines					
138	Allyl		90	84	81	100
139	Cyclopropyl		75	61	79	74
140	Isopropyl					90
141	Methyl	methyl	74	69	76	76
142	$-(CH_2)_4-$		89	78	79	95
	N^6-Substituted 6-amino-7-[2-(phosphonomethoxy)ethyl]purines					
144	Cyclopropyl	H				90
145	Methyl	methyl	90	103	99	82
146	$-(CH_2)_4-$		96	89	100	89
	N^6-Substituted 2,6-diamino-7-[2-(phosphonomethoxy)ethyl]purines					
147	Methyl	methyl	89	99	98	
	N^6-Substituted 2,6-diamino-9-[2-(2-phosphonomethoxy)ethyl]purines					
151	Allyl	H	101	89	94	
152	Cyclopropyl	H	103	85	94	
153	$-(CH_2)_4-$		99	95	94	
154	9-[2-(2-phosphonoethoxy)ethyl]guanine		98	83	91	
	N^6-Substituted 2,6-diamino-9-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]purines					
160	Methyl	H	90	100	95	96
161	Cyclopropyl	H	85	92	100	89
162	2-Dimethylaminoethyl	H	93	75	79	95
163	Methyl	methyl	101	98	100	94

[a] Inhibition of the cell growth at c = 10 μmol l^{-1}.

TABLE II
Cytostatic characteristics of selected compounds *in vitro*

Compound	R^1	R^2	IC_{50}, μmol l^{-1}			
			L1210	L929	HeLaS3	CCRF-CEM
	N^6-Substituted 2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purines					
52	Propyl	H	15	15		15
53	222-Trifluoroethyl	H	15	2	25	3.3
54	Allyl	H	11.8	1	20	3
55	Propargyl	H				12
56	Butyl	H				31
57	*sec*-Butyl	H				40
58	Isobutyl	H		2.5	30	4.5
59	But-2-en-1-yl	H		7.5		
60	But-3-en-2-yl	H				6
61	But-3-en-1-yl	H	>50	32.5		
62	2-Methylallyl	H	35	5		6
68	2-(Dimethylamino)ewthyl	H	16.5	5		24
70	3-(Dimethylamino)propyl	H		5		30
75	Cyclopropyl	H	7.5	2	18.5	2
76	Cyclobutyl	H	25	10	>50	7.6
77	Cyclopentyl	H		9.8		12
81	Cyclopropylmethyl	H	10			10
84	4-Aminobenzyl	H	47	50		40
91	Methyl	methyl		2.5	25	4.5
92	Ethyl	methyl	8.8	4.0	26	35
96	$-(CH_2)_4-$		30			20
97	$-(CH_2)_5-$		20	5	20	
98	$-(CH_2)_2-O-(CH_2)_2-$			3.8		
102	Cyclopropyl	cyclopropyl		20		
103	1-(Cyclopropyl)ethyl	H				20
	N^6-Substituted 6-amino-9-[(*R*)-2-(phosphonomethoxy)propyl]purines					
106	Allyl	H		5		
110	2-(Dimethylamino)ethyl	H		4.2		
	N^6-Substituted 2,6-diamino-9-[(*R*)-2-(phosphonomethoxy)propyl]purines					
122	Cyclopentyl	H				22
139	Cyclopropyl	H		5		

the dimethylamino function in the N^6-alkyl moiety substantially enhances the cytostatic effect (**68**, **70**). N^6-(2,2,2-Trifluoroethyl)-PMEDAP (**53**) ranks among the most efficient cytostatics of the ANP series.

e) Introduction of N^6-cycloalkyl substituents into the PMEDAP molecule enhances the activity in the case of cyclopropyl (**75**) and cyclobutyl derivatives (**76**), less so in the cyclopentyl congener **77**. A slight effect is still observable in the cyclohexyl derivative **78**, further ring expansion to seven- or eight-membered cycloalkane leads to inactive compounds **79** and **80**. The order of activity is as follows: cyclopropyl > cyclobutyl >> cyclopentyl >> cyclohexyl. Compound **75** ranks among the most efficient ANP inhibitors of the cell growth. Special features of the cyclopropyl substituents are also illustrated by the rather significant activity of the N^6-(cyclopropylmethyl)-PMEDAP (**81**) and by the N^6-(1-cyclopropylethyl)-PMEDAP (**103**).

f) Substitution of PMEDAP at N^6 with benzyl, phenyl or 1-naphthyl group (**83**–**86**) does not significantly enhance the cytostatic activity *in vitro*. In contrast to *N*-substitution with regioisomeric *C*-pyridylmethyl function is more effective: medium activity was registered for compounds **87**–**89**.

g) Approximately the same pattern of *N*-substitution effects was encountered in the *N*,*N*-disubstituted derivatives of PMEDAP: the *N*,*N*-dimethyl (**91**) and *N*-ethyl-*N*-methyl derivative (**92**) are strongly active, followed by *N*,*N*-dicyclopropyl compound **102**. Substitution of the *N*-alkyl group by hydroxyl or alkoxyl (**94**, **95**) inactivates the system. 6-Pyrrolidin-1-yl (**96**), 6-(piperidin-1-yl) (**97**) and 6-(morpholin-4-yl) (**98**) derivatives are active, while piperazine (**99**) and 4-methylpiperazine (**100**) derivatives are not.

h) In the series of N^6-substituted (*R*)-PMPA analogues, the allyl (**106**) and 2-(dimethylamino)ethyl (**110**) derivatives are active (in the L929 cell line), cyclopropyl congener (**107**) and the N^6,N^6-dialkyl derivatives **112** and**113** also show some activity. Introduction of other substituents also did not lead to active compounds. This observation is very significant in the light of the prodrug hypothesis (*vide supra*). In these cases, deamination would lead to hypoxanthine derivatives, which are in all ANP series devoid of any biological activity.

i) Contrary to the N^6-substituted (*R*)-PMPA or PMEDAP analogues, the N^6-substitution in the series of (*R*)-PMPDAP is much less useful. In this case, the hypothetical deamination would lead to cytostatic (*R*)-PMPG. However, of all alkyl, cycloalkyl or *N*-hetaryl derivatives tested (**116**–**130**) only the cyclopropyl (**117**) and cyclopentyl derivative (**122**) show certain cytostatic effects.

j) The (*S*)-enantiomers of N^6-substituted PMPA (**131**–**137**) or PMPDAP (**138**–**142**) were inactive in the test system.

k) Also inactive were the 7-isomers of N^6-substituted PMEA (**144**–**146**) and N^6,N^6-dimethyl derivative of 7-PMEDAP (**147**), as well as the side-chain homologues of PMEDAP with CH_2CH_2 group inserted between the ether oxygen and phosphorus atoms (**151**–**154**).

l) All four (*S*)-HPMPDAP derivatives substituted at the 6-amino group by selected substituents (**160**–**163**) were devoid of any cytostatic activity in our system.

*Metabolic Transformation of N^6-Cyclopropyl-PMEDAP (**75**)*

As mentioned above, we were interested in verifying the hypothesis that cypr-PMEDAP (**75**) acts as a prodrug for PMEG in which it is converted by cellular AMP deaminase. Therefore, we have exposed CCRF-CEM cells *in vitro* to [^{3}H]-labelled compound **75** and analyzed the cell pool for this compound, PMEDAP (**2**), PMEG (**3**) and their potential metabolites by HPLC. Kinetics of intracellular metabolism of compound **75** is shown in Table III. The concentration of N^6-cyclopropyl-PMEDAP (**75**) and its metabolite PMEGpp in the cellular pool remained approximately the same after 24 h incubation period, but after another 24 h the PMEGpp concentration compared to N^6-cyclopropyl-PMEDAP (**75**) increased twice. During the same period, the amount of primary deamination product – PMEG – increased

TABLE III
Metabolism of N^6-cyclopropyl-[8-^{3}H]PMEDAP (**75**) in T-lymphoblastoids CCRF-CEM

N^6-Cyclopropyl-PMEDAP Metabolite	Intracellular level[a]			
	24 h of treatment		48 h of treatment	
	pmol/10^7 cells	μmol l^{-1}	pmol/10^7 cells	μmol l^{-1}
N^6-Cyclopropyl-PMEDAP (**75**)	3.53	1.045	3.04	0.898
PMEG (**3**)	0.23	0.067	0.65	0.195
PMEGp	1.43	0.423	3.08	0.913
PMEGpp	3.16	0.934	5.97	1.765

[a] Extracellular concentration of N^6-cyclopropyl-[8-^{3}H]PMEDAP (**75**) was 3.83 μM.

three times and that of the PMEGpp precursor – PMEGp – twice. The increment of N^6-cyclopropyl-PMEDAP (**75**) and its metabolites in the cellular pool after 24 h amounted to 8.35 pmol/10^7 cells and after 48 h to 12.7 pmol/10^7 cells (Table III). This observation reflects the saturation character of intracellular uptake of [8-^{3}H]cypr-PMEDAP with respect to time.

Data from the *in vitro* deamination assay (Table IV) unequivocally demonstrate the enzyme-catalyzed conversion of N^6-cyclopropyl-[8-^{3}H]PMEDAP to [8-^{3}H]PMEG. This deamination is most probably catalyzed by AMP deaminase or AMP deaminase-like enzyme. Our study confirms the previously published findings[34,36] that compound **75** behaves in CCRF-CEM cells as a prodrug of PMEG. HPLC analysis of the acid soluble pool of CCRF-CEM cells has shown that the main metabolite is PMEGpp. The intracellular concentration of PMEGpp reached 1.7 μmol l^{-1} after 48 h at 3.83 μM (≈IC_{64}, IC_{50} = 1.7–2 μmol l^{-1}) concentration in the cell culture medium. PMEG is easily phosphorylated by GMP kinase isoenzymes[39,42] and PMEGpp is a very efficient competitive inhibitor of DNA polymerase α and ε (ref.[40b]) with K_i values 0.55 and 0.059 μmol l^{-1}, respectively. Therefore, the strong cytostatic effect of compound **75** is due to the inhibition of DNA synthesis by PMEGpp. The rate-limiting step of this process is the intracellular deamination to PMEG which is most probably catalyzed by AMP deaminase. However, it is not yet clear which isoenzyme of AMP deaminase is responsible for the intracellular formation of PMEG. Hatse *et al.*[34] demonstrated that compound **75** is not deaminated by commercial rabbit muscle enzyme even at extremely high enzyme concentration. *In vitro* only the crude cell-free extracts[34,36] were effective. The *in vitro* N^6-cyclopropyl-PMEDAP (**75**) deamination assay in this study made use of an extract prepared in the presence of a non-ionic detergent (Nonidet NP-40) and treated with streptomycin. It seems to be the most efficient source of enzyme activ-

TABLE IV
N^6-Cyclopropyl-[8-^{3}H]PMEDAP (**75**) deamination *in vitro*[a]

N^6-Cyclopropyl-[8-^{3}H]PMEDAP (**75**) μmol l^{-1}	[8-^{3}H]PMEG (**3**) formed pmol ml^{-1} mg^{-1} of protein
10	14.63
50	58.35

[a] After 120 min at 37 °C.

ity (58.35 pmol ml^{-1} mg^{-1} of protein, Table IV) and indicates membrane localization of AMP deaminase isoenzyme[45].

In conclusion, we have synthesized 123 new N^6-substituted adenine and 2,6-diaminopurine derivatives of the PME-, (*R*)-PMP, (*S*)-PMP, PEE and (*S*)-HPMP series of acyclic nucleoside phosphonates and tested their cytostatic activity *in vitro* on four cell-lines. Based on these data, the following group of PMEDAP derivatives demonstrated the highest activity: N^6-(2,2,2-trifluoroethyl) (**53**), N^6-allyl (**54**), N^6-[2-(dimethylamino)ethyl] (**68**), N^6-cyclopropyl (**75**) and N^6,N^6-dimethyl (**91**). These compounds were selected for further biological evaluation. They are followed by less, but significantly active N^6-isobutyl-PMEDAP (**58**), N^6-(but-2-en-1-yl)-PMEDAP (**59**) and N^6-morpholino-PMEDAP (**98**) and, also, by N^6-allyl-(*R*)-PMPA (**106**), N^6-[2-(dimethylamino)ethyl]-(*R*)-PMPA (**110**) and N^6-cyclopropyl-(*R*)-PMPDAP (**139**).

In CCRF-CEM cells, the cyclopropyl derivative **75** is deaminated to PMEG (**3**) which is then converted to its diphosphate.

EXPERIMENTAL

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 Büchi Melting Point B-545 apparatus. TLC was performed on Silufol UV254 plates (Kavalier Votice, Czech Republic) in systems S1 (chloroform–ethanol 95 : 5) and S2 (chloroform–ethanol 9 : 1). Paper electrophoresis was performed on a Whatman No. 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogencarbonate, pH 7.5.

^{1}H NMR spectra were taken on Varian UNITY-200 (at 200 MHz) 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. ^{1}H chemical shifts (δ, ppm) and coupling constants (*J*, Hz) were obtained by the first-order analysis of the spectra. Numbering system for assignment of NMR signals is outlined in Fig. 1. Optical rotation was determined with a Perkin–Elmer type 241 polarimeter in 0.01 M HCl solutions.

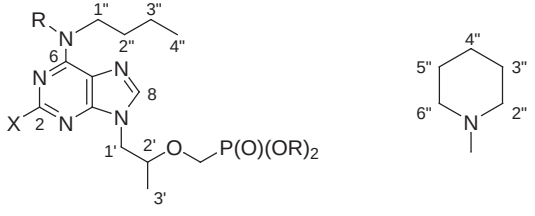


FIG. 1
General numbering scheme for assignment of NMR signals

Materials

Bromo(trimethyl)silane, 6-chloropurine, all amines except for isomeric butenylamines, sodium hydride and cesium carbonate were purchased from Aldrich (Prague, Czech Republic), 2-amino-6-chloropurine from Mack (Germany). Dowex 50 X 8 and Dowex 1 X 2 were obtained from Fluka (Switzerland). Dimethylformamide was distilled from P_2O_5 *in vacuo*. Ethanol was absolutised by standard procedure. Acetonitrile was refluxed with CaH_2 and distilled. All solvents were stored over molecular sieves (4 Å). Butenylamines were prepared using the procedures described in the literature[46]. (*R*)-[(Trityloxy)methyl]oxirane was obtained from Raylo (Canada). Diisopropyl [(2-chloroethoxy)methyl]phosphonate was prepared as described in ref.[22], diisopropyl [(tosyloxy)methyl]phosphonate according to ref.[1c].

Deionisation of the Reaction Mixtures

The solution of reaction products in water (20–25 ml) was applied to a column of Dowex 50 X 8 (H^+ form) (100 ml if not stated otherwise) and the column was washed with water (20% aqueous methanol for deionisation of phosphonate diesters) until the UV-absorption (254 nm) and acid reaction of the eluate dropped (standard elution rate, 3 ml/min). The elution continued with 2.5% ammonia (in water or 20% aqueous methanol) and the UV-absorbing eluate was collected and evaporated *in vacuo*.

Purification of Phosphonates by Dowex 1 X 2 Column Chromatography

Unless stated otherwise, 100 ml columns of Dowex 1 X 2 (100–200 mesh, acetate form) were used. The deionized sample in water (20–25 ml) was alkalinized with concentrated aqueous ammonia to pH 9–10 and applied onto the column. Elution with water (3 ml/min) was continued until the initial UV-absorption (254 nm) of the eluate dropped. The column was then eluted (3ml/min, 30-ml fractions) either with linear gradient of acetic acid or with 1 M acetic acid as indicated.

N^6-Substituted 6-Aminopurines. General Procedure

A mixture of 6-chloropurine (**8**) (2.0 g, 13 mmol) and primary or secondary amine (6 ml) in ethanol (70 ml) was refluxed for 6 h. The course of the reaction was checked with TLC in system S2. The mixture was evaporated to dryness *in vacuo*, deionized on a Dowex 50 column (see above) and the ammonia eluate evaporated to dryness. The residue was crystallized from ethanol (ether added to turbidity). The following compounds were prepared by this procedure:

6-(Isopropylamino)purine (**10**). Yield 1.9 g (82.5%), m.p. 199 °C. For $C_8H_{11}N_5$ (177.2) calculated: 54.22% C, 6.26% H, 39.52% N; found: 53.97% C, 6.13% H, 37.73% N.

6-(Cyclopropylamino)purine (**12**). Yield 2.1 g (100%), m.p. 250 °C. For $C_8H_9N_5$ (175.2) calculated: 54.85% C, 5.18% H, 39.98% N; found: 54.67% C, 5.10% H, 39.96% N. ^{1}H NMR (DMSO-d_6): 11.50 br, 1 H and 8.15 br, 1 H (NH); 8.23 s, 1 H and 8.11 s, 1 H (H-2 + H-8); 3.04 m, 1 H (N-CH); 0.71 m, 2 H and 0.60 m, 2 H (C-CH_2).

6-Isobutylaminopurine (**13**). Yield 2.2 g (88.5%), m.p. 217 °C. For $C_9H_{13}N_5$ (191.2) calculated: 56.53% C, 6.85% H, 36.62% N; found: 56.38% C, 6.77% H, 36.66% N.

6-[(2-Hydroxyethyl)amino]purine (**14**). Crystallized from water; yield 2.3 g (89.5%), m.p. 253 °C. For $C_7H_9N_5O \cdot H_2O$ (197.2) calculated: 42.64% C, 5.62% H, 35.51% N; found:

42.51% C, 5.42% H, 35.62% N. ^{1}H NMR (DMSO-d_6): 12.80 br, 1 H (N-H); 8.18 br s, 1 H (H-8); 8.10 s, 1 H (H-2); 7.45 brs, 1 H (N-H); 4.80 br, 1 H (OH); 3.58 m, 4 H (CH_2).

6-{2-[(Dimethylamino)ethyl]amino}purine (**15**). Precipitated from ethanol with ether; yield 1.7 g (63.5%), m.p. 163 °C. For $C_9H_{14}N_6$ (206.2) calculated: 52.41% C, 6.84% H, 40.75% N; found: 52.50% C, 6.75% H, 40.82% N.

6-(Dimethylamino)purine (**16**) [with dimethylamine solution in ethanol (33%, 70 ml) in an autoclave at 110 °C for 14 h]. Yield 84.5%, m.p. > 280 °C. For $C_7H_9N_5$ (163.2) calculated: 51.52% C, 5.56% H, 42.92% N; found: 51.69% C, 5.47% H, 42.67% N.

6-(Pyrrolidin-1-yl)purine (**17**). Yield 5.25 g (85.5%), m.p. > 300 °C. For $C_9H_{11}N_5$ (189.2) calculated: 57.13% C, 5.86% H, 37.01% N; found: 57.02% C, 5.85% H, 36.93% N.

6-(Allylamino)purine (**11**)

The compound was prepared by the same procedure from compound **8** with allylamine. After evaporating to dryness *in vacuo*, and application of the residue onto a Dowex 50 X 8 (H^+ form) column (150 ml), the column was eluted with water until the acidity of the eluate dropped. The resin was then suspended in water (200 ml), the suspension was alkalinized with ammonia and, after 15 min stirring, filtered. The resin was extracted with boiling water (four 250-ml portions) and the combined eluates were evaporated *in vacuo*. Crystallization from water afforded compound **11** (1.6 g, 70.0%), m.p. 216 °C. For $C_8H_9N_5$ (175.2) calculated: 54.85% C, 5.18% H, 39.98% N; found: 54.53% C, 5.10% H, 39.73% N.

N^6-Substituted 2,6-Diaminopurines. General Procedure

A mixture of 2-amino-6-chloropurine (**9**) (5.09 g, 30 mmol), ethanol (80 ml) and primary amine (10 ml) was refluxed under exclusion of CO_2 for 8 h. The course of the reaction was checked by TLC in system S2. The mixture was filtered off from insoluble material and the filtrate was taken down to dryness *in vacuo*. The residue in water (70 ml) was dissolved on acidification by addition of Dowex 50 X 8 resin (H^+ form). This suspension was applied to a column (150 ml) of the same ion exchange resin and the column was eluted with water until the acidity and UV-absorption of the eluate dropped (the elution was followed by continuous detection of UV-absorption of the eluate). The product was then eluted with dilute (2.5%) aqueous ammonia, the UV-absorbing eluate was evaporated *in vacuo* and the residue was stirred with the mixture of ethanol and ether (1 : 1, 150 ml). The crystalline product was filtered off, washed with the same mixture and dried *in vacuo*. The following compounds were prepared by this procedure:

2-Amino-6-(cyclopropylamino)purine (**19**). Yield 5.0 g (87.6%) of compound **19**, m.p.135 °C. For $C_8H_{10}N_6$ (190.2) calculated: 50.52% C, 5.30% H, 44.18% N; found: 50.26% C, 5.24% H, 43.98% N.

6-(Allylamino)-2-aminopurine (**18**). Yield 4.8 g (84%), m.p. 163–164 °C. For $C_8H_{10}N_6$ (190.2) calculated: 50.52% C, 5.30% H, 44.18% N; found: 50.35% C, 5.24% H, 43.89% N.

2-Amino-6-{[2-(dimethylamino)ethyl]amino}purine (**20**). Yield 5.8 g (86.9%), m.p. 217 °C. For $C_9H_{15}N_7$ (221.3) calculated: 48.86% C, 6.83% H, 44.31% N; found: 48.83% C, 6.68% H, 44.46% N. MS (*m/z*): 222.1 (100) MH^+; 177 (27) (M – Me_2N).

2-Amino-6-(dimethylamino)purine (**21**) [with dimethylamine solution in ethanol (33%, 80 ml) in an autoclaveat 110 °C for 16 h]. Yield 5.2 g (97%), m.p. 263 °C. For $C_7H_{10}N_6$ (178.2) calculated: 47.18% C, 5.66% H, 47.16% N; found: 47.41% C, 5.55% H, 46.90% N. MS (*m/z*): 179.0.

2-Amino-6-(pyrrolidin-1-yl)purine (**22**). Yield 1.82 g (68.6%), m.p. 267 °C. For $C_9H_{12}N_6$ (204.2) calculated: 52.93% C, 5.92% H, 41.15% N; found: 52.97% C, 5.87% H, 40.98% N.

6-Chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}purine (**26**) and
6-Chloro-7-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}purine (**143a**)

The compounds were prepared by alkylation of 6-chloropurine (**8**) with diisopropyl 2-chloroethoxymethylphosphonate (**23**) in the presence of NaH as described in ref.[22].

2-Amino-6-chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}purine (**29**) and
2-Amino-6-chloro-7-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}purine (**143b**)

The compounds were prepared by alkylation of 2-amino-6-chloropurine (**9**) with diisopropyl [(2-chloroethoxy)methyl]phosphonate (**23**) in the presence of DBU as described in ref.[22].

6-Chloro-9-{(*S*)-2-[(diisopropoxyphosphoryl)methoxy]propyl}purine (**28**) and
6-Chloro-9-{(*R*)-2-[(diisopropoxyphosphoryl)methoxy]propyl}purine (**27**)

The compounds were prepared as described in ref.[17]

2-Amino-6-chloro-9-{(*S*)-2-[(diisopropoxyphosphoryl)methoxy]propyl}purine (**31**) and
2-Amino-6-chloro-9-{(*R*)-2-[(diisopropoxyphosphoryl)methoxy]propyl}purine (**30**)

The compounds were prepared as described in ref.[17]

Preparation of N^6-Mono- and Disubstituted
6-Amino-9-[2-(phosphonomethoxy)ethyl]purines **33**–**50**

Method A. A primary or secondary amine (5 ml) was added to a solution of compound **26** (1.9 g, 5 mmol) in ethanol (50 ml) and the solution was refluxed for 6–8 h. The course of the reaction was monitored by TLC in systems S1 and S2. After completion, the mixture was taken down *in vacuo* and the residue codistilled twice with ethanol (20 ml each). The residue was treated with aqueous methanol (1 : 4, 50 ml) and Dowex 50 X 8 (H^+ form) until dissolution and persistent acid reaction. This suspension was applied onto a column (150 ml) of the same ion exchanger equilibrated in aqueous methanol (1 : 4) and the column was washed with the same eluent. The elution was continued until the UV-absorption of the eluate (254 nm) dropped to the original value. The column was then washed with 2.5% ammonia in aqueous methanol (1 : 4) and the UV-absorbing ammonia fraction collected. After evaporation *in vacuo*, the residue of compound **32** was codistilled with ethanol (2 × 25 ml) and dried overnight *in vacuo* over phosphorus pentoxide. Acetonitrile (40 ml) and Me_3SiBr (4 ml) were added and the solution was left to stand overnight at ambient temperature. The mixture was evaporated *in vacuo*, codistilled with acetonitrile (20 ml) and water (50 ml) was added. After 10 min, the acid solution was alkalinized with concentrated aqueous ammonia and evaporated. The residue in water (20–30 ml) was applied onto a column (100 ml) of Dowex 50 X 8 (H^+ form) equilibrated in water and the described desalting procedure was repeated. The residue from the ammonia eluate was dissolved in water (20 ml) by adding concentrated aqueous ammonia to pH 10–10.5 and the solution was applied onto a column (100 ml) of Dowex 1 X 2 (acetate form) washed with 0.02 M acetic acid. The column was washed first with 0.02 M acetic acid until the UV-absorption dropped and then with a linear

gradient of acetic acid (0.02–0.30 M, 1 l each). The main UV-absorbing fraction was taken down to dryness and the residue was codistilled with water (3 × 25 ml). The product was obtained by recrystallization of the residue from water. After standing overnight in a refrigerator, the product was collected, washed with ethanol and ether, and dried. The following compounds were obtained:

6-(Allylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**33**). Yield 1.15 g (69.4%), m.p. 225 °C. For $C_{11}H_{16}N_5O_4P \cdot H_2O$ (331.4) calculated: 39.88% C, 5.48% H, 21.14% N, 9.35% P; found: 40.09% C, 5.22% H, 21.18% N, 9.26% P. ^{1}H NMR (D_2O + NaOD): 8.12 s, 1 H and 8.04 s, 1 H (H-2 + H-8); 6.03 ddt, 1 H, *J*(2",1") = 4.9, *J*(2",3"*cis*) = 10.5, *J*(2",3"*trans*) = 17.3 (H-2"); 5.26 dq, 1 H, *J*(3"*trans*,1") = *J*(gem) = 1.5, *J*(3"*trans*,2") = 17.6 (H-3"*trans*); 5.21 dq, 1 H, *J*(3"*cis*,1") = *J*(gem) = 1.5, *J*(3"*cis*,2") = 10.7 (H-3"*cis*); 4.36 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.11 m, 2 H (H-1"); 3.97 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.65 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2).

6-(Cyclopropylamino)-9-[2-(phosphonomethoxy)ethylpurine (**34**). Yield 1.30 g (83%), m.p. 242 °C. For $C_{11}H_{16}N_5O_4P$ (313.2) calculated: 42.18% C, 5.15% H, 22.36% N, 9.89% P; found: 41.90% C, 5.18% H, 22.19% N, 9.59% P. ^{1}H NMR (D_2O + NaOD): 8.13 s, 2 H (H-2 + H-8); 4.39 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.99 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.69 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 2.79 m, 1 H (H-1"); 0.95 m, 2 H and 0.70 m, 2 H (H-2" + H-3").

6-(Isobutylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**36**). Yield 1.42 g (81.8%), m.p. 137 °C. For $C_{12}H_{20}N_5O_4P \cdot H_2O$ (347.3) calculated: 41.50% C, 6.38% H, 20.16% N, 8.92% P; found: 41.84% C, 6.03% H, 20.10% N, 8.95% P. ^{1}H NMR (D_2O + NaOD): 8.12 s, 1 H and 8.08 s, 1 H (H-2 + H-8); 4.38 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.00 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.72 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.26 m, 2 H (H-1"); 1.92 m, 1 H (H-2"); 1.00 d, 6 H, *J*(2",3") = 6.6 (H-3").

6-[(2-Hydroxyethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**37**). Yield 0.80 g (50.4%), m.p. 208 °C. For $C_{10}H_{16}N_5O_5P$ (317.2) calculated: 37.86% C, 5.08% H, 22.08% N, 9.76% P; found: 37.75% C, 4.97% H, 21.91% N, 10.06% P. ^{1}H NMR (D_2O + NaOD): 8.40 s, 1 H and 8.38 s, 1 H (H-2 + H-8); 4.52 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 3.99 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.89 t, 2 H, *J*(1",2") = 4.9 (H-2"); 3.77 m, 2 H (H-1"); 3.65 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2).

6-[(2-Methoxyethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**38**) (the product was eluted with retention from Dowex 50 column with water). Yield 1.2 g (72.4%), m.p. 182 °C (water). For $C_{11}H_{18}N_5O_5P$ (331.3) calculated: 39.88% C, 5.48% H, 21.14% N, 9.35% P; found: 39.95% C, 5.45% H, 20.81% N, 9.44% P. ^{1}H NMR (D_2O + NaOD): 8.41 s, 1 H and 8.39 s, 1 H (H-2 + H-8); 4.53 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.99 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.80 m, 4 H (H-1" + H-2"); 3.65 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.42 s, 3 H (OCH_3).

6-[(3-Hydroxypropyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**39**). Yield 1.04 g (59.5%), m.p. 165 °C. For $C_{11}H_{18}N_5O_5P \cdot H_2O$ (349.4) calculated: 37.83% C, 5.77% H, 20.05% N, 8.87% P; found: 37.80% C, 5.49% H, 20.21% N, 8.89% P. ^{1}H NMR (D_2O + NaOD): 8.41 s, 1 H and 8.38 s, 1 H (H-2 + H-8); 4.54 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 4.02 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.78 t, 2 H, *J*(3",2") = 6.4 (H-3"); 3.70 brt, 2 H, *J*(1",2") = 6.4 (H-1"); 3.68 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 2.03 brpent, 2 H, *J* = 6.4 (H-2").

6-(2-Hydroxypropyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**40**). Yield 1.20 g (68.7%), m.p. 163 °C. For $C_{11}H_{18}N_5O_5P \cdot H_2O$ (349.4) calculated: 37.83% C, 5.77% H, 20.05% N, 8.87% P; found: 38.08% C, 5.54% H, 20.12% N, 8.97% P. ^{1}H NMR (D_2O + NaOD): 8.00 s, 1 H (H-2); 7.93 s, 1 H (H-8); 4.25 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 4.05 m, 1 H (H-2"); 3.91 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.68 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.50 dd, 1 H, *J* = 4.6 and 13.9; 3.35 dd, 1 H, *J* = 8.0 and 13.9 (H-1"); 1.24 d, 3 H, *J*(3",2") = 6.4 (H-3").

6-[(1-Hydroxypropan-2-yl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**41**). Yield 1.00 g (57.2%), m.p. 142 °C. For $C_{11}H_{18}N_5O_5P \cdot H_2O$ (349.4) calculated: 37.83% C, 5.77% H, 20.05% N, 8.87% P; found: 37.61% C, 5.72% H, 20.05% N, 8.62% P. ^{1}H NMR (D_2O + NaOD): 8.42 s, 1 H and 8.39 s, 1 H (H-2 + H-8); 4.54 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 4.29 m, 1 H (H-1"); 4.01 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.86 dd, 1 H, *J* = 4.3 and 11.9; 3.73 dd, 1 H, *J* = 6.8 and 11.9 (H-2"); 3.68 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 1.38 d, 3 H, *J*(1",CH_3) = 6.7 (CH_3).

6-[(2-Aminoethyl)amino] 9-[2-(phosphonomethoxy)ethyl]purine (**42**). Yield 0.92 g (58%), m.p. 298 °C. For $C_{10}H_{17}N_6O_4P$ (316.3) calculated: 37.98% C, 5.42% H, 26.57% N, 9.79% P; found: 37.78% C, 5.67% H, 26.62% N, 9.50% P. ^{1}H NMR (D_2O + NaOD): 8.20 s, 1 H and 8.15 s, 1 H (H-2 + H-8); 4.40 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.95 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.63 brt, 2 H, *J*(1",2") = 6.1 (H-1"); 3.49 d, 2 H, *J*(P,CH) = 8.1 (P-CH_2); 2.90 t, 2 H, *J*(2",1") = 6.1 (H-2").

6-{[(2-(Dimethylamino)ethyl]amino}-9-[2-(phosphonomethoxy)ethyl]purine (**43**). Yield 1.50 g (79.7%), m.p. 154 °C. For $C_{12}H_{21}N_6O_4P \cdot 2H_2O$ (376.4) calculated: 37.90% C, 6.63% H, 22.10% N, 8.14% P; found: 38.06% C, 6.81% H, 22.30% N, 8.40% P. ^{1}H NMR (D_2O + NaOD): 8.19 s, 2 H (H-2 + H-8); 4.41 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.02 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.95 brt, 2 H, *J*(1",2") = 5.2 (H-1"); 3.69 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.50 t, 2 H, *J*(2",1") = 5.2 (H-2"); 3.01 s, 6 H (N-CH_3).

6-[(3-Aminopropyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**44**). Yield 1.3 g (74.6%) (elution from Dowex 1 column with 0.02 M acetic acid), m.p. 233–234 °C. For $C_{11}H_{19}N_6O_4P \cdot H_2O$ (348.4) calculated: 37.93% C, 6.08% H, 24.13% N, 8.89% P; found: 37.70% C, 5.89% H, 24.34% N, 9.12% P. ^{1}H NMR (D_2O + NaOD): 8.13 s, 1 H and 8.05 s, 1 H (H-2 + H-8); 4.33 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.51 m, 2 H (H-1"); 3.46 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 2.75 t, 2 H, *J*(3",2") = 7.1 (H-3"); 1.82 m, 2 H (H-2").

6-[(4-Aminobutyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**45**). Yield 1.3 g (75.5%) (elution from Dowex 1 column with 0.02 M acetic acid), m.p. 265 °C. For $C_{12}H_{21}N_6O_4P$ (344.3) calculated: 41.86% C, 6.15% H, 24.41% N, 9.00% P; found: 4165% C, 6.23% H, 24.14% N, 8.89% P. ^{1}H NMR (D_2O + NaOD): 8.14 s, 1 H and 8.09 s, 1 H (H-2 + H-8); 4.36 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.48 m, 2 H (H-1"); 3.46 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 2.63 t, 2 H, *J*(4",3") = 7.1 (H-4"); 1.67 m, 2 H and 1.52 m, 2 H (H-2" + H-3").

6-[Cyclohexylamino]-9-[2-(phosphonomethoxy)ethyl]purine (**46**). Yield 1.25 g (70.3%), m.p. 140–142 °C. For $C_{14}H_{22}N_5O_4P$ (355.4) calculated: 47.32% C, 6.24% H, 19.71% N, 8.72% P; found: 47.67% C, 6.23% H, 19.72% N, 8.75% P. ^{1}H NMR (D_2O + NaOD): 8.32 s, 1 H and 8.27 s, 1 H (H-2 + H-8); 4.49 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 4.01 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.82 m, 1 H (N-CH); 3.69 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 2.02 m, 2 H and 1.82 m, 2 H and 1.68 m, 1 H and 1.45 m, 4 H and 1.26 m, 1 H (C-CH_2).

6-(Dimethylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**47**) [prepared with dimethylamine solution in ethanol (30%, 70 ml) in an autoclave at 100 °C for 8 h]. Yield 1.25 g (78.3%), m.p. 205–207 °C (water). For $C_{10}H_{16}N_5O_4P \cdot H_2O$ (319.3) calculated: 37.62% C, 5.68% H, 21.94% N, 9.70% P; found: 37.83% C, 5.59% H, 22.01% N, 10.08% P. ^{1}H NMR (D_2O + NaOD): 8.33 s, 1 H and 8.26 s, 1 H (H-2 + H-8); 4.49 t, 2 H, *J*(1′,2′) = 4.8 (H-1′); 3.99 t, 2 H, *J*(2′,1′) = 4.8 (H-2′); 3.68 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.55 br, 6 H (N-CH_3).

6-(Diethylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**48**). Yield 1.48 g (85.2%), m.p. 189 °C. For $C_{12}H_{20}N_6O_4P \cdot H_2O$ (347.4) calculated: 41.50% C, 6.38% H, 20.16% N, 8.92% P; found: 41.77% C, 6.12% H, 20.33% N, 8.91% P. ^{1}H NMR (D_2O + NaOD): 8.02 s, 1 H and 7.95 s, 1 H (H-2 + H-8); 4.31 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.65 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.65 m, 4 H (N-CH_2); 1.12 t, 6 H, *J*(CH_3,CH_2) = 7.1 (CH_3).

9-[2-(Phosphonomethoxy)ethyl]-6-(pyrrolidin-1-yl)purine (**49**). Yield 1.43 g (82.8%), m.p. 261 °C. For $C_{12}H_{18}N_5O_4P \cdot H_2O$ (345.3) calculated: 41.74% C, 5.84% H, 20.28% N, 8.97% P; found: 41.84% C, 5.56% H, 20.36% N, 9.04% P. ^{1}H NMR (D_2O + NaOD): 8.03 s, 1 H and 7.91 s, 1 H (H-2 + H-8); 4.32 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.93 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.72 m, 2 H (H-2"); 3.56 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 3.42 m, 2 H (H-5"); 2.04 m, 2 H and 1.99 m, 2 H (H-3" + H-4").

9-[2-(Phosphonomethoxy)ethyl]-6-(piperidin-1-yl)purine (**50**). Yield 1.38 g (80.8%), m.p. 175 °C. For $C_{13}H_{20}N_5O_4P$ (341.3) calculated: 45.75% C, 5.91% H, 20.52% N, 9.08% P; found: 46.08% C, 5.91% H, 20.22% N, 8.89% P. ^{1}H NMR (D_2O + NaOD): 8.07 s, 1 H and 7.98 s, 1 H (H-2 + H-8); 4.34 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.95 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.89 m, 4 H (H-2" + H-6"); 3.66 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 1.69 m, 2 H and 1.57 m, 4 H (H-3" + H-4").

Method B. Compound **23** (5.8 ml, 25 mmol) was added to the mixture of the N^6-substituted adenine derivative **10**, **14** or **17** (20 mmol) and cesium carbonate (4 g, 12.3 mmol) in DMF (80 ml), stirred at 100 °C. The stirring and heating was continued for 16 h, the suspension was filtered and the solvent was stripped down *in vacuo*. The residue was extracted with hot chloroform (3 × 100 ml) and, after evaporation of the solvent, purified by chromatography on silica gel in chloroform solution. Oily product **32** was dried *in vacuo* and treated with acetonitrile (150 ml) and Me_3SiBr (15 ml) overnight. The mixture was evaporated to dryness and taken up in water (100 ml). After 10 min, the solution was alkalinized with aqueous NH_3 and evaporated. The desalting was performed on a Dowex 50 X 8 column (200 ml) as described in *Method A*. The crude product was purified on a Dowex 1 X 2 column (200 ml) as described in *Method A*. The product was crystallized from water. The following compounds were prepared:

6-(Isopropylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**35**). Yield 3.80 g (60.2%), m.p. 193 °C. For $C_{11}H_{18}N_5O_4P$ (315.4) calculated: 41.91% C, 5.75% H, 22.21% N, 9.82% P; found: 41.90% C, 5.80% H, 22.05% N, 9.57% P. ^{1}H NMR (D_2O + NaOD): 8.36 s, 1 H and 8.32 s, 1 H (H-2 + H-8); 4.51 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.18 m, 1 H (H-1"); 4.01 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.67 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 1.39 d, 6 H, *J*(1",2") = 6.6 (H-2").

6-[(2-Hydroxyethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**37**). Yield 3.95 g (62%), m.p. 208 °C (water). ^{1}H NMR spectrum is identical with that of the compound described under *Method A*.

9-[2-(Phosphonomethoxy)ethyl]-6-(pyrrolidin-1-yl)purine (**49**). Yield 5.30 g (76.8%), m.p. 259 °C. ^{1}H NMR spectrum is identical with that of the compound prepared by *Method A*.

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2,6-Diamino-9-[2-(phosphonomethoxy)ethyl]purines **51**–**104**

Method C. A primary or secondary amine (5 ml) was added to a stirred solution of compound **29** (1.95 g, 5 mmol) in ethanol (70 ml), and the mixture was refluxed under stirring for 6–8 h. The reaction course was monitored by TLC in systems S1 and S2. After completion, the mixture was worked up as described in *Method A* and the desalted product purified by Dowex 1 X 2 chromatography with linear gradient of acetic acid (0.02–0.3 M, 1 l each) and finally crystallized from water. The following compounds were synthesized:

2-*Amino-6-(propylamino)-9-[2-(phosphonomethoxy)ethyl]purine* (**52**). Yield 1.2 g (74%), m.p. 239 °C. For $C_{11}H_{19}N_6O_4P$ (330.3) calculated: 40.00% C, 5.80% H, 25.45% N, 9.38% P; found: 40.32% C, 5.74% H, 25.78% N, 9.45% P. ^{1}H NMR (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.24 t,

2 H, *J*(1′,2′) = 5.2 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.50 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 3.44 m, 2 H (H-1"); 1.66 sext, 2 H, *J* = 7.3 (H-2"); 0.98 t, 3 H, *J*(CH_3,CH_2) = 7.4 (H-3").

2-Amino-6-[(2,2,2-trifluoroethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**53**) [prepared with 2,2,2-trifluoroethylamine in an autoclave at 100 °C for 16 h]. Yield 1.6 g (84%), m.p. 235 °C. For $C_{10}H_{14}F_3N_6O_4P \cdot H_2O$ (388.2) calculated: 30.94% C, 4.15% H, 14.68% F, 21.65% N, 7.98% P; found: 30.89% C, 3.98% H, 14.72% F, 21.87% N, 7.57% P. ^{1}H NMR (D_2O + NaOD): 7.91 s, 1 H (H-8); 4.34 br q, 2 H, *J*(H,F) = 8.8 (H-1"); 4.24 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2).

6-(Allylamino)-2-amino-9-[2-(phosphonomethoxy)ethyl]purine (**54**). Yield 1.35 g (82.2%), m.p. 239 °C. For $C_{11}H_{17}N_6O_4P$ (328.3) calculated: 40.25% C, 5.22% H, 25.60% N, 9.44% P; found: 40.02% C, 5.20% H, 25.43% N, 9.72% P. ^{1}H NMR (D_2O + NaOD): 7.83 s, 1 H (H-8); 6.00 m, 1 H (H-2"); 5.26 dt, 1 H, *J*(3"*trans*,1") = 1.0, *J*(gem) = 1.5, *J*(3"*trans*,2") = 17.3 (H-3"*trans*); 5.19 dt, 1 H, *J*(3"*cis*,1") = 1.0, *J*(gem) = 1.5, *J*(3"*cis*,2") = 10.5 (H-3"*cis*); 4.23 brt, 2 H (H-1′); 4.13 m, 2 H (H-1"); 3.97 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.64 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2).

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-(propargylamino)purine (**55**). Yield 1.3 g (78%), m.p. 262 °C. For $C_{11}H_{15}N_6O_6P$ (326.3) calculated: 40.50% C, 4.63% H, 26.76% N, 9.49% P; found: 40.41% C, 4.74% H, 26.61% N, 9.53% P. ^{1}H NMR (DMSO-d_6): 7.74 s, 1 H (H-8); 7.63 br, 1 H (NH); 6.05 br, 2 H (NH_2); 4.22 m, 2 H (H-1"); 4.14 t, 2 H, *J*(1′,2′) = 5.4 (H-1′); 3.80 t, 2 H, *J*(2′,1′) = 5.4 (H-2′); 3.58 d, 2 H, *J*(P,CH) = 8.7 (P-CH_2); 3.02 t, 1 H, *J* = 2.4 (H-3").

2-Amino-6-(butylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**56**). Yield 1.55 g (90%), m.p. 217–220 °C. For $C_{12}H_{21}N_6O_4P$ (344.3) calculated: 41.86% C, 6.15% H, 24.41% N, 9.00% P; found: 41.61% C, 6.34% H, 24.52% N, 8.84% P. ^{1}H NMR (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.50 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.46 m, 2 H (H-1"); 1.62 brpent, 2 H (H-2"); 1.40 sext, 2 H (H-3"); 0.93 s, 3 H, *J* = 7.3 (H-4").

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-(propylamino)purine (**57**). Yield 1.17 g (68%), m.p. 262 °C. For $C_{12}H_{21}N_6O_4P$ (344.3) calculated: 41.86% C, 6.15% H, 24.41% N, 9.00% P; found: 41.68% C, 5.94% H, 24.18% N, 9.24% P. ^{1}H NMR (D_2O + NaOD): 7.92 s, 1 H (H-8); 4.28 brt, 2 H (H-1′); 3.97 brt, 2 H, *J*(1′,2′) = 5.0 (H-2′); 3.73 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.27 m, 2 H (H-1"); 1.92 m, 1 H (H-2"); 0.97 d, 6 H, *J*(2",3") = 6.6 (H-3").

2-Amino-6-(isobutylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**58**). Yield 1.30 g (57.2%), m.p. 262 °C. For ($C_{12}H_{21}N_6O_4P$) (344.3) calculated: 41.86% C, 6.15% H, 24.41% N, 9.00% P; found: 42.07% C, 5.94% H, 24.31% N, 8.83% P. ^{1}H NMR (D_2O + NaOD): 7.90 s, 1 H (H-8); 4.26 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 4.16 m, 1 H (H-1"); 3.50 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 1.62 brpent, 2 H, *J* = 7.3 (H-2"); 1.25 d, 3 H, *J*(1",CH_3) = 6.6 (1"-CH_3); 0.94 t, 3 H, *J*(2",3") = 7.3 (H-3").

2-Amino-6-(but-2-en-1-ylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**59**). Yield 1.4 g (81%), m.p. 244 °C. For $C_{12}H_{19}N_7O_4P$ (342.3) calculated: 42.11% C, 5.59% H, 24.55% N, 9.05% P; found: 42.20% C, 5.70% H, 24.70% N, 9.04% P. ^{1}H NMR (D_2O + NaOD): 7.65 s, 1 H (H-8); 5.52 brdq, 1 H, *J*(3",1") = 1.0, *J*(3",4") = 6.5, *J*(3",2") = 15.4 (H-3"); 5.40 dtq, 1 H, *J*(2",4") = 1.2, *J*(2",1") = 5.6, *J*(2",3") = 15.4 (H-2"); 4.02 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.81 m, 2 H (H-1"); 3.68 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.29 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 1.47 d, 3 H, *J*(4",3") = 6.5 (H-4").

2-Amino-6-(but-3-en-2-ylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**60**). Yield 1.16 g (68%), m.p. 265 °C. For $C_{12}H_{19}N_7O_4P$ (342.3) calculated: 42.11% C, 5.59% H, 24.55% N, 9.05% P; found: 42.02% C, 5.60% H, 24.40% N, 9.16% P. ^{1}H NMR (D_2O + NaOD): 8.00 s, 1 H (H-8); 6.15 ddd, 1 H, *J*(3",2") = 4.6, *J*(3",4"a) = 17.3, *J*(3",4"b) = 10.5 (H-3"); 5.34 ddt, 1 H,

J(4"a,2") = 1.5, *J*(gem) = 1.5, *J*(4"a,3") = 17.3 (H-4"a); 5.26 dt. 1 H, *J*(4"b,2") = 1.5, *J*(gem) = 1.5, *J*(4"b,3") = 10.5 (H-4"b); 4.92 m, 1 H (H-2"); 4.36 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 4.03 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.65 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 1.50 d, 3 H, *J*(1",2") = 6.8 (H-1").

2-Amino-6-(but-3-en-1-ylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**61**). Yield 1.32 g (77%), m.p. 216 °C. For $C_{12}H_{19}N_6O_4P$ (342.3) calculated: 42.11% C, 5.59% H, 24.55% N, 9.05% P; found: 41.98% C, 5.67% H, 24.30% N, 9.21% P. ^{1}H NMR (D_2O + NaOD): 7.85 s, 1 H (H-8); 5.92 ddt, 1 H, *J*(3",2") = 6.8, *J*(3",4"a) = 17.2, *J*(3",4"b) = 10.3 (H-3"); 5.18 ddt, 1 H, *J*(4"a,2") = 1.6, *J*(gem) = 2.0, *J*(4"a,3") = 17.2 (H-4"a); 5.14 ddt, 1 H, *J*(4"b,2") = 1.1, *J*(gem) = 2.0, *J*(4"b,3") = 10.3 (H-4"b); 4.23 t, 2 H, *J*(1′,2′) = 5.4 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.4 (H-2′); 3.53 m, 2 H (H-1"); 3.52 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 2.41 qt, 2 H, *J*(2",4") = 1.3, *J*(2",1") = *J*(2",3") = 6.8 (H-2").

2-Amino-6-(2-methylallylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**62**). Yield 1.33 g (78%), m.p. 233 °C. For $C_{12}H_{19}N_6O_4P$ (342.3) calculated: 42.11% C, 5.59% H, 24.55% N, 9.05% P; found: 41.96% C, 5.77% H, 24.37% N, 9.17% P. ^{1}H NMR (D_2O + NaOD): 8.92 s, 1 H (H-8); 4.91 m, 2 H (C=CH_2); 4.29 t, 2 H, *J*(1′,2′) = 5.4 (H-1′); 4.05 m, 2 H (N-CH_2); 3.98 t, 2 H, *J*(2′,1′) = 5.4 (H-2′); 3.62 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 1.83 s, 3 H (CH_3).

2-Amino-6-[(2-hydroxyethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**63**). Yield 1.25 g (75.2%), m.p. 234 °C. For $C_{10}H_{17}N_6O_5P$ (332.3) calculated: 36.13% C, 5.16% H, 25.29% N, 9.34% P; found: 36.38% C, 5.08% H, 25.11% N, 9.31% P. ^{1}H NMR (D_2O + NaOD): 7.83 s, 1 H (H-8); 4.21 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.94 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.83 t, 2 H, *J*(2",1") = 5.4 (H-2"); 3.68 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.65 m, 2 H (H-1").

2-Amino-6-[(3-hydroxypropyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**64**). Yield 1.38 g (79.7%), m.p. 219 °C. For $C_{11}H_{19}N_6O_5P$ (346.3) calculated: 38.15% C, 5.53% H, 24.27% N, 8.94% P; found: 38.04% C, 5.47% H, 23.97% N, 8.67% P. ^{1}H NMR (D_2O + NaOD): 7.85 s, 1 H (H-8); 4.23 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.71 t, 2 H, *J* = 6.3 (H-3"); 3.58 m, 2 H (H-1"); 3.54 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 1.89 m, 2 H (H-2").

2-Amino-6-[(2-hydroxypropyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**65**). Yield 1.40 g (38.4%), m.p. 246 °C. For $C_{11}H_{19}N_6O_5P{\cdot}H_2O$ (364.3) calculated: 36.27% C, 5.81% H, 23.07% N, 8.50% P; found: 36.65% C, 5.46% H, 23.32% N, 8.49% P. ^{1}H NMR (D_2O + NaOD): 7.91 s, 1 H (H-8); 4.26 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 4.10 m, 1 H (H-2"); 3.90 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.63 m, 1 H; 3.51 m, 1 H (H-2"); 3.49 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 1.25 d, 3 H, *J*(3",2") = 6.4 (H-3").

2-Amino-6-[(1-hydroxypropan-2-yl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**66**). Yield 1.45 g (83.7%), m.p. 200 °C. For $C_{11}H_{19}N_6O_5P$ (346.3) calculated: 38.15% C, 5.53% H, 24.27% N, 8.94% P; found: 38.34% C, 5.59% H, 24.48% N, 8.07% P. ^{1}H NMR (D_2O + NaOD): 7.83 s, 1 H (H-8); 4.40 m, 1 H (H-2"); 4.22 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 3.93 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.76 dd, 1 H, *J* = 4.9 and 11.6 and 3.68 dd, 1 H, *J* = 7.0 and 11.6 (H-1"); 3.65 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 1.29 d, 3 H, *J* = 6.7 (H-3").

2-Amino-6-[(2-methoxyethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**67**). Yield 1.39 g (80%), m.p. 192 °C. For $C_{11}H_{19}N_6O_5P$ (346.3) calculated: 38.15% C, 5.53% H, 24.27% N, 8.94% P; found: 38.27% C, 5.48% H, 23.96% N, 8.85% P. ^{1}H NMR (D_2O + NaOD): 7.95 s, 1 H (H-8); 4.33 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.96 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.75 m, 4 H (H-1" + H-2"); 3.71 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.42 s, 3 H (OCH_3).

2-Amino-6-{[2-(dimethylamino)ethyl])amino}-9-[2-(phosphonomethoxy)ethyl]purine (**68**). Yield 1.55 g (75%), m.p. 237 °C. For $C_{12}H_{22}N_7O_4P{\cdot}3H_2O$ (413.3) calculated: 34.87% C, 6.83% H, 23.72% N, 7.49% P; found: 35.06% C, 6.52% H, 23.43% N, 7.44% P. ^{1}H NMR (D_2O + NaOD): 7.86 s, 1 H (H-8); 4.25 t, 2 H, *J*(1′,2′) = 4.8 (H-1′); 3.98 t, 2 H, *J*(2′,1′) = 4.8 (H-2′);

3.91 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.90 brt, 2 H, *J*(1",2") = 5.7 (H-1"); 3.46 t, 2 H, *J*(1",2") = 5.7 (H-2"); 3.00 s, 6 H (N-CH_3).

2-Amino-6-[(3-aminopropyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**69**). Yield 1.30 g (75.3%). Amorphous precipitate. For $C_{11}H_{20}N_7O_4P$ (345.3) calculated: 38.26% C, 5.84% H, 28.40% N, 8.97% P; found: 38.11% C, 5.95% H, 28.21% N, 9.14% P. ^{1}H NMR (D_2O + NaOD): 7.88 s, 1 H (H-8); 4.25 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.56 t, 2 H, *J*(1",2") = 7.0 (H-1"); 3.48 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 2.71 t, 2 H, *J*(3",2") = 7.1 (H-3"); 1.80 pent, 2 H, *J* = 7.0 (H-2").

2-Amino-6-{[3-(dimethylamino)propyl]amino}-9-[2-(phosphonomethoxy)ethyl]purine (**70**). Yield 1.50 g (70.2%), m.p. 115 °C. For $C_{13}H_{24}N_7O_4P\cdot 3H_2O$ (427.4) calculated: 36.53% C, 7.07% H, 22.94% N, 7.25% P; found: 36.66% C, 7.08% H, 22.68% N, 7.25% P. ^{1}H NMR (D_2O + NaOD): 7.85 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 4.4 (H-1′); 3.98 t, 2 H, *J*(2′,1′) = 4.4 (H-2′); 3.73 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.72 brt, 2 H, *J* = 7.0 (H-1"); 3.226 t, 2 H, *J* = 7.6 (H-3"); 2.96 s, 6 H (N-CH_3); 2.14 m, 2 H (H-2").

2-Amino-6-[(3-amino-2-hydroxypropyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**71**). Yield 1.63 g (90%), m.p. 275 °C. For $C_{11}H_{20}N_7O_5P$ (361.3) calculated: 36.57% C, 5.58% H, 27.14% N, 8.57% P; found: 36.91% C, 5.71% H, 27.25% N, 8.75% P. ^{1}H NMR (D_2O + NaOD): 7.95 s, 1 H (H-8); 4.30 t, 2 H, *J*(1′,2′) = 5.4 (H-1′); 3.95 t, 2 H, *J*(2′,1′) = 5.4 (H-2′); 3.92 tt, 1 H, *J*(2",1") = *J*(2",3") = 4.5 and 7.0 (H-2"); 3.70 m, 1 H and 3.61 m, 1 H (H-1"); 3.54 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 2.80 dd, 1 H, *J*(3"a,2") = 4.5, *J*(gem) = 13.4 (H-3"a); 2.70 dd, 1 H, *J*(3"b,2") = 7.0, *J*(gem) = 13.4 (H-3"b).

2-Amino-6-[(6-aminohexyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**72**). Yield 1.70 g (84%), m.p. 153 °C. For $C_{14}H_{26}N_7O_4P\cdot H_2O$ (405.4) calculated: 41.48% C, 6.96% H, 24.19% N, 7.64% P; found: 41.64% C, 7.05% H, 24.10% N, 7.59% P. ^{1}H NMR (D_2O + NaOD): 7.88 s, 1 H (H-8); 4.28 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.98 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.72 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 3.51 m, 2 H and 3.02 m, 2 H (N-CH_2); 1.68 m, 4 H and 1.43 m, 4 H (C-CH_2).

2-Amino-6-[(carboxymethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**73**). Yield 1.19 g (69%), m.p. 273 °C. For $C_{10}H_{15}N_6O_6P$ (346.2) calculated: 34.69% C, 4.37% H, 24.27% N, 8.95% P; found: 34.49% C, 4.49% H, 24.09% N, 9.13% P. ^{1}H NMR (D_2O + NaOD): 7.86 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.3 (H-1′); 4.02 brs, 2 H (N-CH_2); 3.86 t, 2 H, *J*(2′,1′) = 5.3 (H-2′); 3.48 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2).

2-Amino-6-[(5-carboxypentyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**74**). Yield 1.64 g (81%), m.p. 168 °C. For $C_{14}H_{23}N_6O_6P\cdot H_2O$ (405.4) calculated: 40.00% C, 5.99% H, 19.99% N, 7.37% P; found: 40.14% C, 6.08% H, 20.11% N, 7.53% P. ^{1}H NMR (D_2O + NaOD): 7.83 s, 1 H (H-8); 4.19 t, 2 H, *J*(1′,2′) = 5.3 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.3 (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 3.46 m, 2 H (H-1"); 2.22 t, 2 H, *J*(5",4") = 7.5 (H-5"); 1.67 m, 2 H and 1.62 m, 2 H and 1.41 m, 2 H (H-3" + H-2" + H-4").

2-Amino-6-(cyclopropylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**75**). Yield 1.40 g (85.3%), m.p. 263 °C. For $C_{11}H_{17}N_6O_4P$ (328.3) calculated: 40.25% C, 5.22% H, 25.60% N, 9.44% P; found: 40.41% C, 5.33% H, 25.70% N, 9.18% P. ^{1}H NMR (D_2O + NaOD): 7.86 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 4.6 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 4.6 (H-2′); 3.54 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 2.82 m, 1 H (H-1"); 0.89 m, 2 H; 0.67 m, 2 H (H-2" + H-3").

2-Amino-6-(cyclobutylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**76**). Yield 1.59 g (88%), m.p. 262 °C. For $C_{12}H_{19}N_6O_4P\cdot H_2O$ (360.3) calculated: 40.00% C, 5.87% H, 23.32% N, 8.60% P; found: 40.19% C, 5.84% H, 23.46% N, 8.36% P. ^{1}H NMR (D_2O + NaOD): 7.86 s, 1 H (H-8); 4.52 m, 1 H (N-CH); 4.22 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.0

(H-2′); 3.53 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 2.42 m, 2 H and 2.04 m, 2 H and 1.78 m, 2 H (C-CH_2).

2-Amino-6-(cyclopentylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**77**). Yield 1.55 g (82.8%), m.p. 267 °C. For $C_{13}H_{21}N_6O_4P \cdot H_2O$ (374.3) calculated: 41.71% C, 6.19% H, 22.45% N, 8.27% P; found: 42.03% C, 6.02% H, 22.39% N, 8.32% P. ^{1}H NMR (D_2O + NaOD): 7.89 s, 1 H (H-8); 4.29 t, 2 H, *J*(1′,2′) = 5.5 (H-1′); 4.32 m, 1 H (H-1"); 3.97 t, 2 H, *J*(2′,1′) = 5.5 (H-2′); 3.62 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 2.01 m, 2 H and 1.71 m, 2 H and 1.62 m, 2 H and 1.55 m, 2 H (H-2" + H-3" + H-4" + H-5"); 1.17 d, 3 H, *J*(3′,2′) = 6.3 (H-3′).

2-Amino-6-(cyclohexylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**78**). Yield 1.60 g (86.4%), m.p. 264 °C. For $C_{14}H_{23}N_6O_4P$ (370.4) calculated: 45.40% C, 6.26% H, 22.69% N, 8.36% P; found: 44.99% C, 6.09% H, 22.77% N, 8.09% P. ^{1}H NMR (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.25 brt, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.95 m, 1 H (H-1"); 3.90 t, 2 H, *J*(1′,2′) = 5.2 (H-2′); 3.52 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 1.98 m, 2 H and 1.75 m, 2 H and 1.62 m, 1 H and 1.35 m, 4 H and 1.22 m, 1 H (C-CH_2).

2-Amino-6-(cycloheptylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**79**). Yield 1.63 g (85%), m.p. 257 °C. For $C_{15}H_{25}N_6O_4P$ (384.4) calculated: 46.87% C, 6.56% H, 21.86% N, 8.06% P; found: 46.64% C, 6.58% H, 21.77% N, 8.11% P. ^{1}H NMR (D_2O + NaOD): 7.85 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 4.10 m, 1 H (N-CH); 3.91 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.58 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 1.93 m, 2 H and 1.64–1.40 m, 10 H (C-CH_2).

2-Amino-6-(cyclooctylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**80**). Yield 1.53 g (77%), m.p. 250 °C. For $C_{16}H_{27}N_6O_4P$ (398.4) calculated: 48.24% C, 6.83% H, 21.09% N, 7.77% P; found: 48.04% C, 6.88% H, 20.87% N, 7.80% P. ^{1}H NMR (D_2O + NaOD): 7.89 s, 1 H (H-8); 4.14 m, 1 H (N-CH); 4.25 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 1.83 m, 2 H and 1.62 m, 4 H and 1.52–1.43 m, 8 H (C-CH_2).

2-Amino-6-[(cyclopropylmethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**81**). Yield 1.62 g (90%), m.p. 234–235 °C. For $C_{12}H_{19}N_6O_4P \cdot H_2O$ (360.3) calculated: 40.00% C, 5.87% H, 23.32% N, 8.60% P; found: 39.98% C, 5.92% H, 23.11% N, 8.71% P. ^{1}H NMR (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.50 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 3.35 m, 2 H (N-CH_2); 1.15 m, 1 H (C-CH); 0.57 m, 2 H and 0.31 m, 2 H (cyclopropyl-CH_2).

2-Amino-6-[(cyclohexylmethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**82**). Yield 1.39 g (69%), m.p. 219 °C. For $C_{15}H_{25}N_6O_4P \cdot H_2O$ (402.4) calculated: 44.77% C, 6.76% H, 20.89% N, 7.70% P; found: 44.54% C, 6.74% H, 20.60% N, 7.80% P. ^{1}H NMR (D_2O + NaOD): 7.89 s, 1 H (H-8); 4.26 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.52 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 3.26 m, 2 H (N-CH_2); 1.65 m, 6 H and 1.12 m, 3 H and 0.90 m, 2 H (C-CH).

2-Amino-6-(benzylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**83**). Yield 1.55 g (82%), m.p. 149–150 °C. For $C_{15}H_{19}N_6O_4P$ (378.3) calculated: 47.62% C, 5.06% H, 22.21% N, 8.19% P; found: 47.81% C, 5.05% H, 22.23% N, 8.32% P. ^{1}H NMR (D_2O + NaOD): 7.72 s, 1 H (H-8); 7.03 m, 5 H (arom.); 4.41 s, 2 H (CH_2-Ph); 4.08 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.80 t, 2 H, *J*(1′,2′) = 5.0 (H-2′); 3.65 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2).

2-Amino-6-[(4-aminobenzyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**84**). Yield 1.73 g (88%), m.p. 177 °C. For $C_{15}H_{20}N_7O_4P$ (393.3) calculated: 45.80% C, 5.12% H, 24.93% N, 7.87% P; found: 45.58% C, 5.07% H, 24.72% N, 7.67% P. ^{1}H NMR (D_2O + NaOD): 7.79 s, 1 H (H-8); 7.07 d, 2 H and 6.69 d, 2 H (arom.); 4.48 m, 2 H (Ph-CH_2); 4.18 t, 2 H, *J*(1′,2′) = 5.4 (H-1′); 3.87 t, 2 H, *J*(2′,1′) = 5.4 (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2).

2-Amino-6-(phenylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**85**). Yield 1.60 g (87.8%), m.p. 240–245 °C. For $C_{14}H_{17}N_6O_4P$ (364.3) calculated: 46.16% C, 4.70% H, 17.57% N, 8.50% P; found: 46.29% C, 4.76% H, 17.82% N, 8.38% P. ^{1}H NMR (D_2O + NaOD): 7.77 s, 1 H (H-8); 7.48 d, 2 H and 7.32 t, 2 H and 7.12 t, 1 H (arom.); 4.11 t, 2 H, *J*(1′,2′) = 4.5 (H-1′); 3.86 t, 2 H, *J*(1′,2′) = (H-2′); 3.64 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2).

2-Amino-6-(1-naphthylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**86**). Yield 1.25 g (58%), m.p. 209 °C. For $C_{19}H_{23}N_6O_4P$ (430.3) calculated: 53.02% C, 5.39% H, 19.53% N, 7.20% P; found: 53.17% C, 5.51% H, 19.81% N, 6.97% P. ^{1}H NMR (D_2O + NaOD): 7.77 s, 1 H (H-8); 7.88 + 7.87 + 7.74 + 7.65 4 × d, 4 × 1 H; 7.50 + 7.45 + 7.44 3 × t, 3 × 1 H (arom.); 4.13 t, 2 H, *J*(1′,2′) = 5.5 (H-1′); 3.86 t, 2 H, *J*(1′,2′) = 5.5 (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2).

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-[(2-pyridylmethyl)amino]purine (**87**). Yield 1.59 g (84%), m.p. 256 °C. For $C_{14}H_{18}N_7O_4P$ (379.3) calculated: 44.33% C, 4.78% H, 25.85% N, 8.17% P; found: 44.57% C, 5.00% H, 25.82% N, 8.21% P. ^{1}H NMR (D_2O + NaOD): 8.39 m, 2 H and 7.36 m, 2 H (Py); 7.93 s, 1 H (H-8); 4.77 m, 2 H (N-CH_2-Py); 4.26 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.50 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2).

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-[(3-pyridylmethyl)amino]purine (**88**). Yield 1.40 g (74%), m.p. 133 °C. For $C_{14}H_{18}N_7O_4P$ (379.3) calculated: 44.33% C, 4.78% H, 25.85% N, 8.17% P; found: 44.45% C, 4.90% H, 25.71% N, 8.26% P. ^{1}H NMR (D_2O + NaOD): 8.60 brs, 1 H and 8.48 brd, 1 H, *J* = 5.6 and 8.26 brd, 1 H, *J* = 8.0 and 7.72 dd, 1 H, *J* = 5.6 and 8.0 (Py); 7.77 s, 1 H (H-8); 4.75 m, 2 H (N-CH_2-Py); 4.16 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.85 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.62 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2).

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-[(4-pyridylmethyl)amino]purine (**89**). Yield 1.52 g (80%), m.p. 259 °C. For $C_{14}H_{18}N_7O_4P$ (379.3) calculated: 44.33% C, 4.78% H, 25.85% N, 8.17% P; found: 44.62% C, 4.98% H, 26.06% N, 8.06% P. ^{1}H NMR (D_2O + NaOD): 8.37 d, 2 H and 7.34 d, 2 H (Py); 4.75 m, 2 H (N-CH_2-Py); 4.25 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2).

2-Amino-6-[(diphenylmethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**90**). Yield 1.35 g (57%), m.p. 167 °C. For $C_{21}H_{23}N_6O_4P{\cdot}H_2O$ (472.4) calculated: 53.39% C, 5.33% H, 17.79% N, 6.56% P; found: 53.10% C, 5.18% H, 17.41% N, 6.38% P. ^{1}H NMR (D_2O + NaOD): 7.50 s, 1 H (H-8); 7.04 m, 4 H and 6.90–6.75 m, 6 H (arom.); 6.49 brs, 1 H (H-1"); 3.99 m, 2 H (H-1′); 3.70 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.49 m, 2 H (P-CH_2).

2-Amino-6-[ethyl(methyl)amino]-9-[2-(phosphonomethoxy)ethyl purine (**92**). Yield 1.44 g (83%), m.p. 142 °C. For $C_{11}H_{19}N_6O_4P{\cdot}H_2O$ (348.4) calculated: 37.93% C, 6.08% H, 24.13% N, 8.89% P; found: 38.20% C, 5.89% H, 24.09% N, 9.09% P. ^{1}H NMR (D_2O + NaOD): 7.81 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.80 brq, 2 H, *J*(1",2") = 7.1 (H-1"); 3.53 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.17 brs, 3 H (N-CH_3); 1.17 t, 3 H, *J*(2",1") = 7.1 (H-2").

2-Amino-6-(diallylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**93**). Yield 1.30 g (70.5%), m.p. 177 °C. For $C_{14}H_{21}N_6O_6P$ (368.3) calculated: 45.65% C, 5.75% H, 22.82% N, 8.41% P; found: 45.77% C, 5.80% H, 22.97% N, 8.26% P. ^{1}H NMR (D_2O + NaOD): 7.83 s, 1 H (H-8); 5.90 ddt, 2 H, *J*(2",1") = 5.1, *J*(2",3"*cis*) = 10.5, *J*(2",3"*trans*) = 17.1 (H-2"); 5.18 dq, 2 H, *J*(3",1") = *J*(gem) = 1.5, *J*(3",2") = 10.5 (H-3"*cis*); 5.15 dq, 2 H, *J*(3",1") = *J*(gem) = 1.5, *J*(3",2") = 17.1 (H-3"*trans*); 4.39 m, 4 H (H-1"); 4.25 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.60 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2).

2-Amino-6-[bis(2-hydroxyethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**94**). Yield 1.50 g (79.7%), m.p. 211 °C. For $C_{11}H_{21}N_6O_6P{\cdot}H_2O$ (394.3) calculated: 36.55% C, 5.88% H, 21.31% N, 7.85% P; found: 36.34% C, 5.57% H, 21.48% N, 7.91% P. ^{1}H NMR (D_2O +

NaOD): 7.83 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.02 m, 4 H (H-1"); 3.91 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.88 t, 4 H, *J*(2",1") = 5.6 (H-2"); 3.64 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2).

2-Amino-6-[bis(2-methoxyethyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**95**). Yield 1.52 g (75%), m.p. 136 °C. For $C_{14}H_{25}N_6O_6P$ (404.4) calculated: 41.59% C, 6.23% H, 20.78% N, 7.66% P; found: 41.39% C, 6.19% H, 20.62% N, 7.59% P. ^{1}H NMR (D_2O + NaOD): 7.89 s, 1 H (H-8); 4.37 t, 2 H, *J*(1′,2′) = 4.8 (H-1′); 4.20 m, 4 H (H-1"); 3.95 t, 2 H, *J*(2′,1′) = 4.8 (H-2′); 3.79 t, 4 H, *J*(2",1") = 5.5 (H-2"); 3.70 d, 2 H, *J*(P,CH) = 8.9 (P-CH_2); 3.38 s, 6 H (OCH_3).

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-(pyrrolidin-1-yl)purine (**96**). Yield 1.45 g (84.7%), m.p. 254–256 °C. For $C_{12}H_{19}N_6O_4P$ (342.3) calculated: 42.11% C, 5.59% H, 24.55% N, 9.05% P; found: 41.89% C, 5.39% H, 24.38% N, 8.76% P. ^{1}H NMR (D_2O + NaOD): 7.74 s, 1 H (H-8); 4.21 t, 2 H, *J*(1′,2′) = 5.3 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.3 (H-2′); 3.68 br, 2 H (H-2"); 3.54 dd, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.39 br, 2 H (H-5"); 1.97 br, 2 H (H-3"); 1.92 br, 2 H (H-4").

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-(piperidin-1-yl)purine (**97**). Yield 1.50 g (84%), m.p. 155–156 °C. For $C_{13}H_{21}N_6O_4P$ (356.3) calculated: 43.82% C, 5.94% H, 23.59% N, 8.69% P; found: 44.01% C, 5.86% H, 23.80% N, 8.91% P. ^{1}H NMR (D_2O + NaOD): 7.85 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.92 m, 4 H (H-2" + H-6"); 3.90 t, 2 H, *J* = 5.2 (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 1.66 m, 2 H (H-3"); 1.58 m, 4 H (H-4" + H-5").

2-Amino-6-morpholino-9-[2-(phosphonomethoxy)ethyl]purine (**98**). Yield 1.20 g (67%), m.p. 170 °C. For $C_{12}H_{19}N_6O_5P$ (358.3) calculated: 40.23% C, 5.35% H, 23.46% N, 8.64% P; found: 40.13% C, 5.47% H, 23.29% N, 8.55% P. ^{1}H NMR (D_2O + NaOD): 7.80 s, 1 H (H-8); 4.22 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.00 brt, 4 H (H-2" + H-6"); 3.90 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.84 brt, 4 H, *J*(3",2") = 4.5 (H-3" + H-5"); 3.67 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2).

2-Amino-9-[2-(phosphonomethoxy)ethyl]-6-(piperazin-1-yl)purine (**99**). Yield 1.30 g (69%), m.p. 347 °C. For $C_{12}H_{20}N_7O_4P{\cdot}H_2O$ (375.4) calculated: 38.40% C, 5.91% H, 26.12% N, 8.25% P; found: 38.37% C, 6.12% H, 26.04% N, 8.08% P. ^{1}H NMR (D_2O + NaOD): 7.86 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.98 brt, 4 H, *J* = 5.0 (N-CH_2); 3.91 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.53 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 2.90 brt, 2 H, *J* = 5.0 (N-CH_2).

2-Amino-6-(4-methylpiperazin-1-yl)-9-[2-(phosphonomethoxy)ethyl]purine (**100**). Yield 1.77 g (91%), m.p. 226 °C. For $C_{13}H_{22}N_7O_4P{\cdot}H_2O$ (389.4) calculated: 40.10% C, 6.21% H, 25.18% N, 7.96% P; found: 40.07% C, 6.35% H, 24.95% N, 7.85% P. ^{1}H NMR (D_2O + NaOD): 7.89 s, 1 H (H-8); 4.26 t, 2 H, *J*(1′,2′) = 5.4 (H-1′); 4.05 brt, 4 H, *J* = 5.0 (N-CH_2); 3.92 t, 2 H, *J*(2′,1′) = 5.4 (H-2′); 3.52 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 2.56 m, 4 H (N-CH_2); 2.31 s, 3 H (N-CH_3).

2-Amino-6-(1,4-diazacycloheptan-1-yl)-9-[2-(phosphonomethoxy)ethyl]purine (**101**). Yield 1.69 g (87%), m.p. 308 °C. For $C_{13}H_{22}N_7O_4P{\cdot}H_2O$ (389.4) calculated: 40.10% C, 6.21% H, 25.18% N, 7.96% P; found: 40.19% C, 6.24% H, 25.22% N, 8.09% P. ^{1}H NMR (D_2O + NaOD): 7.78 s, 1 H (H-8); 4.23 t, 2 H, *J*(1′,2′) = 5.3 (H-1′); 3.90 m, 4 H (N-CH_2); 3.89 t, 2 H, *J*(2′,1′) = 5.3 (H-2′); 3.53 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 2.91 m, 2 H and 2.72 m, 2 H and 1.84 m, 2 H (N-CH_2 + 2 × C-CH_2).

2-Amino-6-(dicyclopropylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**102**). Yield 1.47 g (76%), not melting below 280 °C. For $C_{14}H_{21}N_6O_4P{\cdot}H_2O$ (386.3) calculated: 43.52% C, 6.00% H, 21.75% N, 8.02% P; found: 43.37% C, 6.23% H, 21.56% N, 7.96% P. ^{1}H NMR (D_2O + NaOD): 7.92 s, 1 H (H-8); 4.26 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.49 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 1.14 m, 2 H and 0.54 m, 2 H and 0.45 m, 2 H and 0.37 m, 4 H (2 × cyclopropyl).

2-Amino-6-{[(1-(cyclopropyl)ethyl]amino}-9-[2-(phosphonomethoxy)ethyl]purine (**103**). Yield 1.65 g (88%), m.p. 159 °C. For $C_{13}H_{21}N_6O_4P \cdot H_2O$ (374.3) calculated: 41.71% C, 6.19% H, 22.45% N, 8.27% P; found: 41.87% C, 6.24% H, 22.57% N, 8.27% P. ^{1}H NMR (D_2O + NaOD): 7.88 s, 1 H (H-8); 4.29 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.96 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.80 m, 1 H (N-CH); 3.59 d, 2 H, *J*(P,CH) = 8.4 (P-CH_2); 1.29 s, 3 H, *J*(CH_3,CH) = 6.6 (CH_3); 1.05 m, 1 H and 0.54 m, 1 H and 0.48 m, 1 H and 0.37 m, 1 H and 0.29 m, 1 H (cyclopropyl).

2-Amino-6-[benzyl(methyl)amino]-9-[2-(phosphonomethoxy)ethyl]purine (**104**). Yield 1.64 g (80%), m.p.126 °C. For $C_{16}H_{21}N_6O_4P \cdot H_2O$ (410.4) calculated: 46.83% C, 5.65% H, 20.48% N, 7.55% P; found: 46.68% C, 5.58% H, 20.59% N, 7.60% P. ^{1}H NMR (D_2O + NaOD): 7.79 s, 1 H (H-8); 7.05 d, 2 H and 6.69 t, 2 H and 6.95 t, 1 H (arom.); 4.82 m, 2 H (Ph-CH_2); 4.21 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 3.91 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 3.65 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 3.10 brs, 3 H (N-CH_3).

Method D. Compound **29** (7.8 g, 20 mmol) in 30% solution of a primary or secondary amine in ethanol (150 ml) was stirred at 100 °C for 8 h in an autoclave and evaporated to dryness. The residue was codistilled with toluene (2 × 25 ml) and treated with acetonitrile (100 ml) and TMSBr (15 ml) overnight. Further work-up was made as described in *Method A*, final crystallization from water. The following compounds were prepared by this procedure:

2-Amino-6-(methylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**51**). Yield 73%, not melting below 300 °C. For $C_9H_{15}N_6O_4P \cdot H_2O$ (320.2) calculated: 33.76% C, 5.35% H, 26.24% N, 9.67% P; found: 33.80% C, 5.19% H, 26.49% N, 9.48% P. ^{1}H NMR (D_2O + NaOD): 7.79 s, 1 H (H-8); 4.19 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.92 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.60 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 3.00 s, 3 H (N-CH_3).

2-Amino-6-(dimethylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**91**). Yield 79%, m.p. > 280 °C. For $C_{10}H_{17}N_6O_4P$ (316.3) calculated: 37.98% C, 5.42% H, 26.57% N, 9.79% P; found: 38.13% C, 5.40% H, 26.38% N, 9.99% P. ^{1}H NMR (D_2O + NaOD): 7.76 s, 1 H (H-8); 4.20 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.90 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.57 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 3.23 s, 6 H (N-CH_3).

2-Amino-6-(dimethylamino)-7-[2-(phosphonomethoxy)ethyl]purine (**147**) [prepared from the chloro derivative **143b** and 30% solution of dimethylamine in ethanol]. Yield 77%, m.p. 145 °C. For $C_{10}H_{17}N_6O_4P \cdot H_2O$ (334.3) calculated: 35.93% C, 5.73% H, 25.14% N, 9.27% P; found: 36.10% C, 5.93% H, 24.78% N, 9.25% P. ^{1}H NMR (D_2O + NaOD): 8.23 s, 1 H (H-8); 4.58 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 3.94 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.62 d, 2 H, *J*(P,CH) = 8.8 (P-CH_2); 3.25 s, 6 H (N-CH_3).

Method E. Compound **23** (6 ml, 43 mmol) was added to a mixture of the N^6-substituted 2,6-diaminopurine (**18**, **19**, **20**) (20 mmol) and cesium carbonate (4 g, 12.3 mmol) in DMF (80 ml) and the mixture was stirred at 100 °C for 16 h. The suspension was filtered off and evaporated *in vacuo*. The residue was extracted with hot chloroform (4 × 100 ml) and the residue after evaporation of the solvent was purified by chromatography on silica gel, in chloroform solution. The resulting foam was dried *in vacuo* and treated with acetonitrile (50 ml) and TMSBr (5 ml) overnight. The mixture was evaporated to dryness and taken up in water (100 ml), the solution was alkalinized with aqueous NH_3 and evaporated. Desalting on Dowex 50 X 8 column (200 ml) and final purification on a Dowex 1 X 2 column (200 ml) were performed as described in *Method A*. The product was crystallized from water. The following compounds were prepared:

2-Amino-6-(cyclopropylamino)-9-[2-(phosphonomethoxy)ethyl]purine (**51**). Yield 3.8 g (57.9%), m.p. 264 °C. ^{1}H NMR spectrum is identical with compound described under *Method A*.

6-(Allylamino)-2-amino-9-[2-(phosphonomethoxy)ethyl]purine (**54**). Yield 5.14 g (78.3%), m.p. 239 °C. ^{1}H NMR spectrum is identical with compound described under *Method A*.

2-Amino-6-{[2-(dimethylamino)ethyl]amino}-9-[2-(phosphonomethoxy)ethyl]purine (**68**). Yield 3.50 g (48.7%), m.p. 237 °C. ^{1}H NMR spectrum is identical with compound described under *Method A*.

Preparation of N^6-Mono- and Disubstituted
6-Amino-9-[(*R* or *S*)-2-(phosphonomethoxy)propyl]purines **105**–**115**, **131**–**137**

Method F. A primary or secondary amine (4 ml) was added to a stirred solution of compound **27** or **28** (1.95 g, 5 mmol) in ethanol (40 ml). The mixture was refluxed under stirring for 6–8 h. The course of the reaction was monitored by TLC in systems S1 and S2. After completion, the mixture was worked up as described in Method A and the desalted product purified by Dowex 1 X 2 chromatography with linear gradient of acetic acid (0.02–0.3 M, 1 l each) and finally crystallized from water. The following compounds were prepared by this method:

6-(Isopropylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**105**). Yield 1.29 g (74%), m.p. 126 °C. For $C_{12}H_{20}N_5O_4P\cdot H_2O$ (347.3) calculated: 41.50% C, 6.38% H, 20.16% N, 8.92% P; found: 41.47% C, 6.18% H, 20.38% N, 8.64% P. ^{1}H NMR (D_2O + NaOD): identical with that of compound **133**. $[\alpha]_D^{20}$ –14.0.

6-(Allylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**106**). Yield 1.30 g (79.4%), m.p. 127 °C. For $C_{12}H_{18}N_5O_4P$ (327.3) calculated: 44.04% C, 5.54% H, 21.40% N, 9.46% P; found: 43.87% C, 5.83% H, 21.53% N, 9.32% P. ^{1}H NMR (D_2O + NaOD): 8.38 s, 1 H and 8.33 s, 1 H (H-2 + H-8); 6.01 ddt, 1 H, *J*(2",1") = 5.0, *J*(2",3"*cis*) = 10.7, *J*(2",3"*trans*) = 17.6 (H-2"); 5.30 brd, 1 H, *J*(3"*trans*,2") = 17.6 (H-3"*trans*); 5.27 brd, 1 H, *J*(3"*cis*,2") = 10.7 (H-3"*cis*); 4.47 dd, 1 H, *J*(1′a,2′) = 2.4, *J*(gem) = 14.6 (H-1′a); 4.29 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.22 m, 2 H (H-1"); 4.01 m, 1 H (H-2′); 3.73 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.2 (P-CHa); 3.53 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.2 (P-CHb); 1.19 d, 3 H, *J*(3′,2′) = 6.1 (H-3′). $[\alpha]_D^{20}$ –14.5.

6-(Cyclopropylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**107**). Yield 1.40 g (85.5%), m.p. 156 °C. For $C_{12}H_{18}N_5O_4P$ (327.3) calculated: 44.04% C, 5.54% H, 21.40% N, 9.46% P; found: 43.76% C, 5.64% H, 21.64% N, 9.79% P. ^{1}H NMR (D_2O + NaOD): 8.48 s, 1 H and 8.41 s, 1 H (H-2 + H-8); 4.52 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.6 (H-1′a); 4.34 dd, 1 H, *J*(1′b,2′) = 7.1, *J*(gem) = 14.6 (H-1′b); 4.03 m, 1 H (H-2′); 3.72 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.2 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 13.2 (P-CHb); 2.92 m, 1 H (H-1"); 1.22 d, 3 H, *J*(3′,2′) = 6.3 (H-3′); 1.12 m, 2 H; 0.90 m, 2 H (H-2" + H-3"). $[\alpha]_D^{20}$ –12.3.

6-(Isobutylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**108**). Yield 1.35 g (78.7%), m.p. 174 °C. For $C_{13}H_{22}N_5O_4P$ (343.2) calculated: 45.48% C, 6.46% H, 20.40% N, 9.02% P; found: 45.47% C, 6.41% H, 20.26% N, 9.30% P. ^{1}H NMR (D_2O + NaOD): 8.34 s, 1 H and 8.30 s, 1 H (H-2 + H-8); 4.46 dd, 1 H, *J*(1′a,2′) = 2.9, *J*(gem) = 14.6 (H-1′a); 4.28 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.6 (H-1′b); 4.00 m, 1 H (H-2′); 3.70 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.2 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.2 (P-CHb); 3.38 brd, 2 H, *J*(1",2") = 5.9 (H-1"); 2.03 m, 1 H (H-2"); 1.19 d, 3 H, *J*(2′,3′) = 6.3 (H-3′); 1.04 brd, 6 H, *J*(2",3") = 5.9 (H-3"). $[\alpha]_D^{20}$ –12.7.

6-(Cyclohexylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**109**). Yield 1.20 g (65%), amorphous precipitate. For $C_{15}H_{24}N_5O_4P$ (369.4) calculated: 48.78% C, 6.55% H, 18.96% N, 8.39% P; found: 48.70% C, 6.35% H, 19.13% N, 8.49% P. ^{1}H NMR (D_2O + NaOD): 8.35 s, 1 H and 8.33 s, 1 H (H-2 + H-8); 4.46 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.6 (H-1′a); 4.28 dd, 1 H, *J*(1′b,2′) = 7.6, *J*(gem) = 14.6 (H-1′b); 3.99 m, 1 H (H-2′); 3.80 m, 1 H (N-CH); 3.78 brdd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 13.7 (P-CHa); 3.55 dd, 1 H, *J*(P,CHb) = 9.0, *J*(gem) = 13.7 (P-CHb); 2.04 m, 2 H and 1.78 m, 2 H and 1.66 m, 1 H and 1.52–1.36 m, 4 H and 1.28 m, 1 H (C-CH_2); 1.10 d, 3 H, *J*(2′,3′) = 6.9 (H-3′). $[\alpha]_D^{20}$ –11.7.

6-{[2-(Dimethylamino)ethyl]amino}-9-[(R)-2-(phosphonomethoxy)propyl]purine (**110**). Yield 1.10 g (58.5%), amorphous precipitate. For $C_{13}H_{23}N_6O_4P\cdot H_2O$ (376.4) calculated: 41.49% C, 6.70% H, 22.33% N, 8.23% P; found: 41.19% C, 6.57% H, 22.38% N, 8.48% P. ^{1}H NMR (D_2O + NaOD): 8.24 s, 1 H and 8.20 s, 1 H (H-2 + H-8); 4.36 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.6 (H-1′a); 4.22 dd, 1 H, *J*(1′b,2′) = 7.3, *J*(gem) = 14.6 (H-1′b); 4.01 m, 1 H (H-2′); 3.99 brt, 2 H, *J*(1",2") = 5.6 (H-1"); 3.70 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 13.2 (P-CHa); 3.52 t, 2 H, *J*(2",1") = 5.2 (H-2"); 3.49 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.2 (P-CHb); 3.01 s, 6 H (N-CH_3); 1.21 d, 3 H, *J*(3′,2′) = 6.1 (H-3′). $[\alpha]_D^{20}$ –10.6.

6-{[3-(Dimethylamino)propyl]amino}-9-[(R)-2-(phosphonomethoxy)propyl]purine (**111**). Yield 1.30 g (65.6%), amorphous precipitate. For $C_{14}H_{25}N_6O_4P\cdot H_2O$ (390.3) calculated: 43.07% C, 6.97 H, 21.53% N, 7.93% P; found: 43.13% C, 6.70% H, 21.69% N, 7.73% P. ^{1}H NMR (D_2O + NaOD): 8.16 s, 2 H (H-2 + H-8); 4.34 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.6 (H-1′a); 4.19 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 3.98 m, 1 H (H-2′); 3.69 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.67 brt, 2 H, *J*(1",2") = 7.0 (H-1"); 3.49 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 3.28 brt, 2 H, *J*(3",2") = 7.5 (H-3"); 2.94 s, 6 H (N-CH_3); 2.15 m, 2 H (H-2"); 1.19 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –11.9.

6-(Dimethylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**112**). Yield 1.05 g (63%), m.p. 184 °C. For $C_{11}H_{18}N_5O_4P\cdot H_2O$ (333.4) calculated: 39.64% C, 6.05% H, 21.01% N, 9.29% P; found: 39.44% C, 6.03% H, 20.81% N, 9.82% P. ^{1}H NMR (D_2O + NaOD): 8.14 s, 1 H and 8.04 s, 1 H (H-2 + H-8); 4.32 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.4 (H-1′a); 4.20 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.4 (H-1′b); 3.97 m, 1 H (H-2′); 3.71 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.9 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 3.35 brs, 6 H (N-CH_3); 1.21 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –14.0.

6-[Ethyl(methyl)amino]-9-[(R)-2-(phosphonomethoxy)propyl]purine (**113**). Yield 1.40 g (80.6%), hygroscopic precipitate. For $C_{12}H_{20}N_5O_4P\cdot H_2O$ (347.4) calculated: 41.50% C, 6.38% H, 20.16% N, 8.92% P; found: 41.60% C, 6.27% H, 20.24% N, 9.09% P. ^{1}H NMR (D_2O + NaOD): 8.34 s, 1 H and 8.30 s, 1 H (H-2 + H-8); 4.48 dd, 1 H, *J*(1′a,2′) = 3.4, *J*(gem) = 14.6 (H-1′a); 4.31 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.15 m, 2 H (H-1"); 4.02 m, 1 H (H-2′); 3.72 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.2 (P-CHa); 3.53 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.2 (P-CHb); 3.50 brs, 3 H (N-CH_3); 1.35 t, 3 H, *J*(2",1") = 7.1 (H-2"); 1.22 d, 3 H, *J*(3′,2′) = 6.1 (H-3′). $[\alpha]_D^{20}$ –9.7.

6-(Diethylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**114**). Yield 1.00 g (55.3%), hygroscopic precipitate. For $C_{13}H_{22}N_5O_4P\cdot H_2O$ (361.4) calculated: 43.21% C, 6.69% H, 19.38% N, 8.57% P; found: 43.60% C, 6.52% H, 19.16% N, 8.31% P. ^{1}H NMR (D_2O + NaOD): 8.19 s, 1 H and 8.17 s, 1 H (H-2 + H-8); 4.27 dd, 1 H, *J*(1′a,2′) = 3.4, *J*(gem) = 14.4 (H-1′a); 4.16 dd, 1 H, *J*(1′b,2′) = 5.1, *J*(gem) = 14.4 (H-1′b); 3.95 m, 4 H (H-1"); 3.88 m, 1 H (H-2′); 3.61 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.9 (P-CHa); 3.57 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.9 (P-CHb); 1.18 t, 6 H, *J*(2",1") = 7.0 (H-2"); 1.00 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –10.3.

9-[(R)-2-(Phosphonomethoxy)propyl]-6-(pyrrolidin-1-yl)purine (**115**). Yield 1.30 g (72.3%), m.p. 135 °C. For $C_{13}H_{20}N_5O_4P \cdot H_2O$ (359.4) calculated: 43.46% C, 6.17% H, 19.49% N, 8.62% P; found: 43.62% C, 5.78% H, 19.47% N, 8.52% P. ^{1}H NMR (D_2O + NaOD): 8.35 s, 1 H and 8.28 s, 1 H (H-2 + H-8); 4.47 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.6 (H-1′a); 4.30 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.18 brt, 2 H, *J*(2",3") = 6.0 (H-2"); 4.01 m, 1 H (H-2′); 3.72 brt, 2 H, *J*(5",4") = 6.0 (H-5"); 3.70 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.50 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 2.17 m, 4 H (H-3" + H-4"); 1.23 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –13.1.

Method G. Cesium carbonate (1.0 g, 2.5 mmol) and N^6-substituted adenine or 2,6-diaminopurine derivative (**10**–**13**, **15**–**17**) (5 mmol) in DMF (15 ml) was stirred at 100 °C for 30 min and a solution of compound **25** (2.5 g, 6.12 mmol) in DMF (10 ml) was added. The mixture was heated at 110 °C for 16 h and evaporated to dryness. The residue was extracted with boiling chloroform, the extract was taken down to dryness and the residue was purified on a silica gel column (150 ml) in chloroform–methanol system. Diester **32** was dried *in vacuo* over P_2O_5 and treated with bromo(trimethyl)silane (4 ml) in acetonitrile (40 ml) overnight. Further work-up was performed as described in *Method A*. The purified product after chromatography on Dowex 1 was dried and precipitated from ethanol with ether. The following compounds were prepared:

6-(Allylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**131**). Yield, from compound **11**, 1.40 g (81%), m.p. 173 °C. For $C_{12}H_{18}N_5O_4P \cdot H_2O$ (345.3) calculated: 41.74% C, 5.84% H, 20.28% N, 8.97% P; found: 42.04% C, 5.57% H, 20.40% N, 8.77% P. ^{1}H NMR (D_2O + NaOD): 8.38 s, 1 H and 8.35 s, 1 H (H-2 + H-8); 6.00 ddt, 1 H, *J*(2",1") = 5.0, *J*(2",3"*cis*) = 11.0, *J*(2",3"*trans*) = 17.5 (H-2"); 5.30 brd, 1 H *J*(3"*trans*,2") = 17.5 (H-3"*trans*); 5.27 brd, 1 H, *J*(3"*cis*,2") = 11.0 (H-3"*cis*); 4.47 dd, 1 H, *J*(1′a,2′) = 2.7, *J*(gem) = 14.6 (H-1′a); 4.29 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.23 m, 2 H (H-1"); 4.00 m, 1 H (H-2′); 3.71 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.51 dd, 1 H, *J*(P,CHb) = 10.0, *J*(gem) = 12.9 (P-CHb); 1.18 d, 3 H, *J*(3′,2′) = 6.1 (H-3′). $[\alpha]_D^{20}$ +15.5.

6-(Isobutylamino)-9-[(S)-2-(phosphonomethoxy)propyl purine (**132**). Yield, from compound **13**, 1.24 g (72.6%), m.p. 114 °C. For $C_{13}H_{22}N_5O_4P$ (343.3) calculated: 45.48% C, 6.46% H, 20.40% N, 9.02% P; found: 45.65% C, 6.45% H, 20.14% N, 8.72% P. ^{1}H NMR (D_2O + NaOD): 8.38 s, 1 H and 8.35 s, 1 H (H-2 + H-8); 4.50 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.6 (H-1′a); 4.32 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.03 m, 1 H (H-2′); 3.74 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.2 (P-CHa); 3.55 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.2 (P-CHb); 3.42 brd, 2 H, *J*(1",2") = 6.1 (H-1"); 2.06 m, 1 H (H-2"); 1.22 d, 3 H, *J*(2′,3′) = 6.3 (H-3′); 1.04 brd, 6 H, *J*(2",3") = 6.1 (H-3"). $[\alpha]_D^{20}$ +11.4.

6-(Isopropylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**133**). Yield, from compound **10**, 1.18 g (74%), m.p. 124 °C. For $C_{12}H_{20}N_5O_4P \cdot H_2O$ (347.3) calculated: 41.50% C, 6.38% H, 20.16% N, 8.92% P; found: 41.59% C, 6.13% H, 20.18% N, 8.57% P. ^{1}H NMR (D_2O + NaOD): 8.39 s, 2 H (H-2 + H-8); 4.50 dd, 1 H, *J*(1′a,2′) = 2.7, *J*(gem) = 14.6 (H-1′a); 4.32 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.19 m, 1 H (H-1"); 4.03 m, 1 H (H-2′); 3.73 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.53 dd, 1 H, *J*(P,CHb) = 10.0, *J*(gem) = 12.9 (P-CHb); 1.42 d, 6 H, *J*(1",2") = 6.1 (H-2"); 1.22 d, 3 H, *J*(2′,3′) = 6.1 (H-3′). $[\alpha]_D^{20}$ +14.8.

6-(Cyclopropylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**134**). Yield, from compound **12**, 1.50 g (82.5%), m.p. 126 °C (decomp.). For $C_{12}H_{18}N_5O_4P \cdot 2H_2O$ (363.3) calculated: 39.67% C, 6.10% H, 19.28% N, 8.53% P; found: 39.49% C, 6.07% H, 19.51% N, 8.23% P. ^{1}H NMR (D_2O + NaOD): 8.45 s, 1 H and 8.38 s, 1 H (H-2 + H-8); 4.50 dd, 1 H, *J*(1′a,2′) = 3.2,

J(gem) = 14.6 (H-1′a); 4.32 dd, 1 H, *J*(1′b,2′) = 7.1, *J*(gem) = 14.6 (H-1′b); 4.01 m, 1 H (H-2′); 3.70 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 13.2 (P-CHa); 3.50 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 13.2 (P-CHb); 2.90 m, 1 H (H-1"); 1.21 d, 3 H, *J*(3′,2′) = 6.3 (H-3′); 1.10 m, 2 H and 0.88 m, 2 H (H-2" + H-3"). $[\alpha]_D^{20}$ +17.9.

6-{[2-(Dimethylamino)ethyl]amino}-9-[(S)-2-(phosphonomethoxy)propyl]purine (**135**). Yield, from compound **15**, 1.25 g (66.4%), m.p. 165 °C. For $C_{13}H_{23}N_6O_4P \cdot H_2O$ (376.3) calculated: 41.49% C, 6.70% H, 22.33% N, 8.23% P; found: 41.72% C, 6.27% H, 22.05% N, 8.10% P. ^{1}H NMR (D_2O + NaOD): 8.23 s, 1 H and 8.22 s, 1 H (H-2 + H-8); 4.36 dd, 1 H, *J*(1′a,2′) = 2.7, *J*(gem) = 14.6 (H-1′a); 4.23 dd, 1 H, *J*(1′b,2′) = 7.1, *J*(gem) = 14.6 (H-1′b); 4.06 m, 1 H (H-2′); 4.01 brt, 2 H (H-1"); 3.76 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 13.2 (P-CHa); 3.56 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 13.2 (P-CHb); 3.55 t, 2 H, *J*(2",1") = 5.6 (H-2"); 3.06 s, 6 H (N-CH_3); 1.25 d, 3 H, *J*(3′,2′) = 6.1 (H-3′). $[\alpha]_D^{20}$ +17.6.

6-(Dimethylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**136**). Yield, from compound **16**, 1.30 g (78%), m.p. 178 °C. For $C_{11}H_{18}N_5O_4P \cdot H_2O$ (333.3) calculated: 39.64% C, 6.05% H, 21.01% N, 9.29% P; found: 39.59% C, 5.88% H, 20.68% N, 9.15% P. ^{1}H NMR (D_2O + NaOD): 8.38 s, 1 H and 8.34 s, 1 H (H-2 + H-8); 4.49 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.6 (H-1′a); 4.32 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.02 m, 1 H (H-2′); 3.78 brs, 3 H (N-CH_3); 3.71 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.2 (P-CHa); 3.51 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 13.2 (P-CHb); 3.46 brs, 3 H (N-CH_3); 1.21 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ +14.4.

9-[(S)-2-(Phosphonomethoxy)propyl]-6-(pyrrolidin-1-yl)purine (**137**). Yield, from compound **17**, 0.90 g (52.7%), m.p. 147–148 °C. For $C_{13}H_{20}N_5O_4P \cdot H_2O$ (359.3) calculated: 43.46% C, 6.17% H, 19.49% N, 8.62% P; found: 43.76% C, 5.97% H, 19.67% N, 8.78% P. ^{1}H NMR (D_2O + NaOD): 8.37 s, 1 H and 8.32 s, 1 H (H-2 + H-8); 4.49 dd, 1 H, *J*(1′a,2′) = 2.9, *J*(gem) = 14.6 (H-1′a); 4.31 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 4.22 brt, 2 H (H-2"); 4.01 m, 1 H (H-2′); 3.75 br, 2 H (H-5"); 3.69 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.50 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 2.17 m, 4 H (H-3" + H-4"); 1.21 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ + 12.5.

Preparation of N^6-Mono- and Disubstituted
2,6-Diamino-9-[(*R* or *S*)-2-(phosphonomethoxy)propyl]purines **116**–**130**, **138**–**142**

Method H. A primary or secondary amine (4 ml) was added to a stirred solution of compound **30** or **31** (2.03 g, 5 mmol) in ethanol (40 ml). The mixture was refluxed under stirring for 6–8 h. The course of the reaction was monitored by TLC in systems S1 and S2. After completion, the mixture was worked up as described in *Method A*; the desalted product was purified by Dowex 1 X 2 chromatography with linear gradient of acetic acid (0.02–0.3 M, 1 l each) and finally crystallized from water. The following compounds were prepared:

2-Amino-6-(methylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**116**) [prepared with 30% solution of methylamine in ethanol (70 ml) in an autoclave at 110 °C for 10 h]. Yield 1.27 g (76%), does not melt below 250 °C. For $C_{10}H_{17}N_6O_4P \cdot H_2O$ (334.3) calculated: 35.93% C, 5.73% H, 25.14% N, 9.27% P; found: 35.70% C, 5.91% H, 25.45% N, 9.41% P. ^{1}H NMR (D_2O + NaOD): 7.82 s, 1 H (H-8); 4.15 dd, 1 H, *J*(1′a,2′) = 2.0, *J*(gem) = 14.6 (H-1′a); 4.03 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.6 (H-1′b); 3.97 m, 1 H (H-2′); 3.75 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.58 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 3.01 brs, 3 H (N-CH_3); 1.21 d, 3 H, *J*(3′,2′) = 6.1 (H-3′). $[\alpha]_D^{20}$ –11.3.

2-Amino-6-(cyclopropylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**117**). Yield 1.20 g (70.1%), m.p. 178–179 °C. For $C_{12}H_{19}N_6O_4P$ (342.3) calculated: 42.11% C, 5.59% H,

24.55% N, 9.05% P; found: 41.86% C, 5.84% H, 24.77% N, 8.87% P. ^{1}H NMR (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.18 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.4 (H-1′a); 4.06 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.4 (H-1′b); 3.96 m, 1 H (H-2′); 3.73 dd, 1 H, *J*(P,CHa) = 9.8, *J*(gem) = 12.9 (P-CHa); 3.56 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 2.84 m, 1 H (H-1"); 1.17 d, 3 H, *J*(3′,2′) = 6.3 (H-3′); 0.94 m, 2 H and 0.70 m, 2 H (H-2"). $[\alpha]_D^{20}$ –10.6.

2-Amino-6-(butylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**118**). Yield 1.50 g (83.7%), m.p. 142–144 °C. For $C_{13}H_{23}N_6O_4P$ (358.4) calculated: 43.57% C, 6.47% H, 23.45% N, 8.64% P; found: 43.31% C, 6.56% H, 23.57% N, 8.36% P. ^{1}H NMR (D_2O + NaOD): 7.83 s, 1 H (H-8); 4.16 dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 14.6 (H-1′a); 4.05 dd, 1 H, *J*(1′b,2′) = 6.1, *J*(gem) = 14.6 (H-1′b); 3.93 m, 1 H (H-2′); 3.67 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.5 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.5 (P-CHb); 3.43 brm, 2 H (H-1"); 1.59 brpent, 2 H (H-2"); 1.38 brsext, 2 H (H-3"); 1.18 d, 3 H, *J*(3′,2′) = 5.9 (H-3′); 0.92 t, 3 H, *J* = 7.3 (H-4"). $[\alpha]_D^{20}$ –11.9.

2-Amino-6-(sec-butylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**119**). Yield 1.44 g (80.3%), m.p. 148–149 °C. For $C_{13}H_{23}N_6O_4P$ (358.4) calculated: 43.57% C, 6.47% H, 23.45% N, 8.64% P; found: 43.31% C, 6.56% H, 23.57% N, 8.36% P. ^{1}H NMR (D_2O + NaOD): 7.88 s, 1 H (H-8); 4.19 dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 14.6 (H-1′a); 4.15 br, 1 H (H-2"); 4.08 dd, 1 H, *J*(1′b,2′) = 6.1, *J*(gem) = 14.6 (H-1′b); 3.94 m, 1 H (H-2′); 3.63 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.5 (P-CHa); 3.51 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.5 (P-CHb); 1.61 brpent, 2 H (H-3"); 1.25 d, 3 H, *J* = 6.6 (H-1"); 1.17 d, 3 H, *J*(3′,2′) = 6.3 (H-3′); 0.92 t, 3 H, *J* = 7.3 (H-4"). $[\alpha]_D^{20}$ –14.5.

2-Amino-6-(isobutylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**120**). Yield 1.25 g (66.4%), m.p. 244 °C. For $C_{13}H_{23}N_6O_4P \cdot H_2O$ (376.3) calculated: 41.49% C, 6.70% H, 22.33% N, 8.23% P; found: 42.49% C, 6.47% H, 22.45% N, 7.92% P. ^{1}H NMR (D_2O + NaOD): 7.84 s, 1 H (H-8); 4.19 dd, 1 H, *J*(1′a,2′) = 3.7, *J*(gem) = 14.7 (H-1′a); 4.06 dd, 1 H, *J*(1′b,2′) = 6.3, *J*(gem) = 14.7 (H-1′b); 3.93 m, 1 H (H-2′); 3.29 m, 2 H (H-1"); 3.68 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 13.2 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 13.2 (P-CHb); 1.92 sept, 1 H (H-2"); 1.17 d, 3 H, *J*(2′,3′) = 6.3 (H-3′); 0.96 d, 6 H, *J*(2",3") = 6.6 (H-3"). $[\alpha]_D^{20}$ –13.2.

2-Amino-6-[(2,2,2-trifluoroethyl)amino]-9-[(R)-2-(phosphonomethoxy)propyl]purine (**121**) [with 2,2,2-trifluoroethylamine in an autoclave at 100 °C for 16 h]. Yield 1.56 g (81%), m.p. 268 °C. For $C_{11}H_{16}F_3N_6O_4P$ (384.3) calculated: 34.38% C, 4.20% H, 14.83% F, 21.87% N, 8.06% P; found: 34.35% C, 4.17% H, 14.70% F, 21.70% N, 8.22% P. ^{1}H NMR (D_2O + NaOD): 7.92 s, 1 H (H-8); 4.37 brq, 2 H, *J*(H,F) = 9.3 (H-1"); 4.19 dd, 1 H, *J*(1′a,2′) = 4.5, *J*(gem) = 14.4 (H-1′a); 4.12 dd, 1 H, *J*(1′b,2′) = 5.8, *J*(gem) = 14.4 (H-1′b); 3.95 m, 1 H (H-2′); 3.60 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.2 (P-CHa); 3.51 dd, 1 H, *J*(P,CHb) = 9.3, *J*(gem) = 12.2 (P-CHb); 1.17 d, 3 H, *J*(3′,2′) = 6.4 (H-3′).

2-Amino-6-(cyclopentylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**122**). Yield 1.37 g (70.5%), m.p. 258 °C. For $C_{14}H_{23}N_6O_4P \cdot H_2O$ (388.4) calculated: 43.30% C, 6.49% H, 21.64% N, 7.98% P; found: 43.37% C, 6.32% H, 21.43% N, 8.43% P. ^{1}H NMR (D_2O + NaOD): 7.92 s, 1 H (H-8); 4.18 d, 2 H, *J*(1′,2′) = 5.1 (H-1′); 4.33 m, 1 H (H-1"); 3.97 brsext, 1 H (H-2′); 3.64 dd, 1 H, *J*(P,CHa) = 9.8, *J*(gem) = 12.2 (P-CHa); 3.56 dd, 1 H, *J*(P,CHb) = 9.3, *J*(gem) = 12.2 (P-CHb); 2.00 m, 2 H and 1.70 m, 1 H and 1.60 m, 1 H and 1.54 m, 1 H (H-2" + H-3" + H-4" + H-5"); 1.17 d, 3 H, *J*(3′,2′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –15.4.

2-Amino-6-(cyclohexylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**123**). Yield 1.15 g (59.8%), m.p. 163–165 °C. For $C_{15}H_{25}N_6O_4P$ (384.4) calculated: 46.86% C, 6.56% H, 21.87% N, 8.07% P; found: 46.64% C, 6.34% H, 22.08% N, 7.87% P. ^{1}H NMR (D_2O +

NaOD): 7.92 s, 1 H (H-8); 4.18 dd, 1 H, *J*(1′a,2′) = 4.4, *J*(gem) = 14.6 (H-1′a); 4.10 dd, 1 H, *J*(1′b,2′) = 5.6, *J*(gem) = 14.6 (H-1′b); 3.93 m, 1 H (H-2′); 3.90 m, 1 H (N-CH); 3.52 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.4 (P-CHa); 3.43 dd, 1 H, *J*(P,CHb) = 9.3, *J*(gem) = 12.4 (P-CHb); 1.95 m, 2 H; 1.75 m, 2 H; 1.62 m, 1 H; 1.42–1.15 m, 5 H (C-CH_2); 1.12 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –15.9.

2-Amino-6-(benzylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**124**). Yield 1.42 g (72.4%), m.p. 158–160 °C. For $C_{16}H_{21}N_6O_4P$ (392.2) calculated: 48.98% C, 5.39% H, 21.42% N, 7.89% P; found: 49.16% C, 5.37% H, 21.23% N, 7.86% P. ^{1}H NMR (D_2O + NaOD): 7.77 s, 1 H (H-8); 7.22 m, 5 H (arom.); 4.58 brs, 2 H (phenyl-CH_2); 4.09 dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 14.4 (H-1′a); 3.99 dd, 1 H, *J*(1′b,2′) = 5.6, *J*(gem) = 14.4 (H-1′b); 3.86 m, 1 H (H-2′); 3.68 dd, 1 H, *J*(P,CHa) = 10.0, *J*(gem) = 12.0 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 10.3, *J*(gem) = 12.0 (P-CHb); 1.13 d, 3 H, *J*(3′,2′) = 5.9 (H-3′). $[\alpha]_D^{20}$ –4.9.

2-Amino-6-(phenethylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**125**). Yield 1.80 g (91.7%), m.p. 255–257 °C. For $C_{16}H_{21}N_6O_4P$ (392.4) calculated: 48.98% C, 5.39% H, 21.42% N, 7.89% P; found: 48.62% C, 5.35% H, 21.16% N, 8.01% P. ^{1}H NMR (D_2O + NaOD): 7.71 s, 1 H (H-8); 7.20–7.0 m, 5 H (arom.); 4.04 dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 14.4 (H-1′a); 3.96 dd, 1 H, *J*(1′b,2′) = 5.6, *J*(gem) = 14.4 (H-1′b); 3.84 m, 1 H (H-2′); 3.66 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 12.0 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.0, *J*(gem) = 12.0 (P-CHb); 3.50 brt, 2 H (H-1"); 2.73 brt, 2 H, *J*(2",1") = 7.0 (H-2"); 1.10 d, 3 H, *J*(3′,2′) = 5.9 (H-3′).

2-Amino-6-(dimethylamino)-9-[(R)-2-(phosphonomethoxy)propyl]purine (**126**) [prepared with 30% solution of dimethylamine in ethanol (70 ml) in an autoclave at 110 °C for 10 h]. Yield 1.22 g (73.8%), m.p. 154–156 °C. For $C_{11}H_{19}N_6O_4P$ (330.3) calculated: 40.00% C, 5.80% H, 25.44% N, 9.38% P; found: 40.08% C, 5.75% H, 25.43% N, 9.21% P. ^{1}H NMR (D_2O + NaOD): 7.79 s, 1 H (H-8); 4.16 dd, 1 H, *J*(1′a,2′) = 3.7, *J*(gem) = 14.6 (H-1′a); 4.06 dd, 1 H, *J*(1′b,2′) = 6.4, *J*(gem) = 14.6 (H-1′b); 3.91 m, 1 H (H-2′); 3.65 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.47 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.9 (P-CHb); 3.27 s, 6 H (N-CH_3); 1.17 d, 3 H, *J*(3′,2′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –10.6.

2-Amino-9-[(R)-2-(phosphonomethoxy)propyl]-6-(pyrrolidin-1-yl)purine (**127**). Yield 1.18 g (66.2%), m.p. 180–182 °C. For $C_{13}H_{21}N_6O_4P$ (356.4) calculated: 43.81% C, 5.94% H, 23.58% N, 8.69% P; found: 43.69% C, 6.17% H, 25.39% N, 8.76% P. ^{1}H NMR (D_2O + NaOD): 7.85 s, 1 H (H-8); 4.18 d, 2 H, *J*(1′,2′) = 4.9 (H-1′); 3.98 m, 1 H (H-2′); 3.75 br, 2 H (H-2"); 3.65 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.2 (P-CHa); 3.57 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.2 (P-CHb); 3.41 br, 2 H (H-5"); 2.00 br, 2 H (H-3"); 1.93 br, 2 H (H-4"); 1.21 d, 3 H, *J*(2′,3′) = 6.1 (H-3′). $[\alpha]_D^{20}$ –10.6.

2-Amino-9-[(R)-2-(phosphonomethoxy)propyl]-6-piperidinopurine (**128**). Yield 1.25 g (67.5%), m.p. 156–158 °C. For $C_{14}H_{23}N_6O_4P$ (370.3) calculated: 45.40% C, 6.26% H, 22.69% N, 8.36% P; found: 45.61% C, 6.04% H, 22.48% N, 8.41% P. ^{1}H NMR (D_2O + NaOD): 7.88 s, 1 H (H-8); 4.17 dd, 1 H, *J*(1′a,2′) = 4.1, *J*(gem) = 14.4 (H-1′a); 4.09 dd, 1 H, *J*(1′b,2′) = 6.1, *J*(gem) = 14.4 (H-1′b); 3.95 brt, 4 H, *J*(2",3") = 5.4 (H-2" + H-6"); 3.93 m, 1 H (H-2′); 3.55 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.4 (P-CHa); 3.44 dd, 1 H, *J*(P,CHb) = 9.0, *J*(gem) = 12.4 (P-CHb); 1.69 br, 2 H (H-3"); 1.60 br, 4 H (H-4" + H-5"); 1.14 d, 3 H, *J*(2′,3′) = 6.1 (H-3′). $[\alpha]_D^{20}$ –8.0.

2-Amino-6-morpholino-9-[(R)-2-(phosphonomethoxy)propyl]purine (**129**). Yield 1.14 g (61.2%), m.p. 161–163 °C. For $C_{13}H_{21}N_6O_5P$ (372.3) calculated: 41.94% C, 5.69% H, 22.57% N, 8.32% P; found: 42.03% C, 5.66% H, 22.78% N, 8.19% P. ^{1}H NMR (D_2O + NaOD): 7.81 s, 1 H (H-8); 4.18 dd, 1 H, *J*(1′a,2′) = 3.2, *J*(gem) = 14.4 (H-1′a); 4.05 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.4 (H-1′b); 4.03 brt, 4 H, *J*(2",3") = 4.5 (H-2"); 3.90 m, 1 H (H-2′); 3.80 brt, 4 H,

J(3",2") = 4.5 (H-3"); 3.68 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 1.15 d, 3 H, *J*(3′,2′) = 6.1 (H-3′). $[\alpha]_D^{20}$ –7.6.

2-Amino-6-{[(2-(dimethylamino)ethyl]amino}-9-[(R)-2-(phosphonomethoxy)propyl]purine (**130**). Yield 1.60 g (78%), m.p. 168 °C. For $C_{18}H_{24}N_7O_4P \cdot 3H_2O$ (409.4) calculated: 38.14% C, 6.89% H, 23.95% N, 7.57% P; found: 37.97% C, 6.71% H, 23.56% N, 7.38% P. ^{1}H NMR (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.17 dd, 1 H, *J*(1′a,2′) = 3.0, *J*(gem) = 14.4 (H-1′a); 4.05 dd, 1 H, *J*(1′b,2′) = 6.8, *J*(gem) = 14.4 (H-1′b); 3.94 m, 1 H (H-2′); 3.92 m, 2 H (H-1"); 3.69 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.5 (P-CHa); 3.51 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.5 (P-CHb); 3.48 brt, 2 H, *J* = 5.0 (H-2"); 3.01 s, 6 H (N-CH_3); 1.20 d, 3 H, *J*(2′,3′) = 6.3 (H-3′). $[\alpha]_D^{20}$ –8.3.

2-Amino-6-(allylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**138**). Yield 1.39 g (77%), m.p. 166 °C. For $C_{12}H_{19}N_6O_4P \cdot H_2O$ (360.3) calculated: 40.00% C, 5.87 H, 23.32% N, 8.60% P; found: 39.80% C, 5.59% H, 23.24% N, 8.48% P. ^{1}H NMR (D_2O + NaOD): 7.84 s, 1 H (H-8); 6.01 ddt, 1 H, *J*(2",1") = 4.9, *J*(2",3"*cis*) = 10.5 and 17.3 (H-2"); 5.27 brd, 1 H, *J* = 17.3 and 5.20 brd, 1 H, *J* = 10.5 (H-3"); 4.18 dd, 1 H, *J*(1′a,2′) = 2.9, *J*(gem) = 14.7 (H-1′a); 4.14 m, 2 H (H-1"); 4.05 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.7 (H-1′b); 3.93 m, 1 H (H-2′); 3.68 dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9 (P-CHa); 3.52 dd, 1 H, *J*(P,CHb) = 9.8, *J*(gem) = 12.9 (P-CHb); 1.18 d, 3 H, *J*(2′,3′) = 6.1 (H-3′). $[\alpha]_D^{20}$ +9.2.

2-Amino-6-(cyclopropylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**139**). Yield 1.28 g (71%), m.p. 202 °C. For $C_{12}H_{19}N_6O_4P \cdot H_2O$ (360.3) calculated: 40.00% C, 5.87 H, 23.32% N, 8.60% P; found: 39.78% C, 5.79% H, 23.61% N, 8.36% P. ^{1}H NMR spectrum is identical with that of compound **117**. $[\alpha]_D^{20}$ +21.4.

2-Amino-6-(isopropylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**140**). Yield 1.20 g (69%), not melting below 250 °C. For $C_{12}H_{20}N_5O_4P \cdot H_2O$ (347.4) calculated: 41.50% C, 6.38% H, 20.16% N, 8.92% P; found: 41.33% C, 6.10% H, 19.96% N, 8.88% P. ^{1}H NMR (D_2O + NaOD): δ 7.90 s, 1 H (H-8); 4.31 m, 1 H (N-CH); 4.17 dd, 1 H , J(1′a,2′)= 4.5, *J*(gem) = 14.6 (H-1′a); 4.11 dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 14.6 (H-1′b); 3.94 m, 1 H (H-2′); 3.56 dd, 1 H, *J*(P,CHa) = 9.4, *J*(gem) = 12.2 (P-CHa); 3.47 dd, 1 H, *J*(P,CHb) = 9.2, *J*(gem) = 12.2 (P-CHb); 1.30 d, 6 H, *J*(CH_3,H) = 6.5 (CH_3); 1.15d, 3 H, *J*(3′,2′) = 6.4 (H-3′). $[\alpha]_D^{20}$ +14.8.

2-Amino-6-(dimethylamino)-9-[(S)-2-(phosphonomethoxy)propyl]purine (**141**) [prepared with 30% solution of dimethylamine in ethanol (70 ml) in an autoclave at 110 °C for 10 h]. Yield 1.19 g (68.5%), m.p.189 °C. For $C_{11}H_{19}N_6O_4P \cdot H_2O$ (348.4) calculated: 37.93% C, 6.08% H, 24.13% N, 8.89% P; found: 37.66% C, 5.79% H, 24.42% N, 8.69% P. ^{1}H NMR spectrum is identical with that of compound **126**. $[\alpha]_D^{20}$ +10.3.

2-Amino-9-[(S)-2-(phosphonomethoxy)propyl]-6-(pyrrolidin-1-yl)purine (**142**). Yield 1.18 g (66%), m.p. 216 °C. For $C_{13}H_{21}N_6O_4P$ (356.4) calculated: 43.81% C, 5.94% H, 23.58% N, 8.69% P; found: 43.63% C, 5.76% H, 23.58% N, 8.62% P. ^{1}H NMR spectrum is identical with that of compound **127**. $[\alpha]_D^{20}$ +20.1.

Preparation of N^6-Mono- and Disubstituted 6-Amino-7-[2-(phosphonomethoxy)ethyl]purines **144**–**146**

Method I. A primary or secondary amine (5 ml) was added to solution of compound **143** (1.5 g, 3.84 mmol) in ethanol (30 ml) and the mixture was refluxed under stirring for 16 h. The volatiles were evaporated, the residue codistilled with ethanol (2 × 20 ml) and further worked up as described in *Method A*. The following compounds were prepared:

6-(Cyclopropylamino)-7-[2-(phosphonomethoxy)ethyl]purine (**144**). Yield 51.3%, m.p. 251 °C (water). For $C_{11}H_{16}N_5O_4P \cdot H_2O$ (331.4) calculated: 39.88% C, 5.48% H, 21.14% N, 9.35% P; found: 40.19% C, 5.29% H, 21.86% N, 9.14% P. ^{1}H NMR (D_2O + NaOD): 8.49 s, 1 H and 8.45 s, 1 H (H-2 + H-8); 4.67 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 3.99 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.63 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 2.94 m, 1 H (H-1"); 1.06 m, 2 H and 0.86 m, 2 H (H-2" + H-3"). ^{13}C NMR (D_2O + NaOD): 152.71 (C-4); 150.99 (C-6); 148.62 (C-8); 145.62 (C-2); 112.34 (C-5); 71.59 d, *J*(P,C) = 9.8 (C-2′); 67.56 d, *J*(P,C) = 156.3 (P-C); 48.00 (C-1′); 24.25 (N-C); 7.25, 2 C (C-C).

6-(Dimethylamino)-7-[2-(phosphonomethoxy)ethyl]purine (**145**) [prepared with dimethylamine solution in ethanol (30%, 50 ml) in an autoclave at 100 °C for 8 h]. Yield 58.3%, m.p. 230 °C. For $C_{10}H_{16}N_5O_4P \cdot H_2O$ (319.2) calculated: 37.62% C, 5.68% H, 21.94% N, 9.70% P; found: 37.75% C, 5.29% H, 21.91% N, 9.69% P. ^{1}H NMR (D_2O + NaOD): 8.59 s, 1 H and 8.46 s, 1 H (H-2 + H-8); 4.74 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 3.96 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 3.60 d, 2 H, *J*(P,CH) = 8.6 (P-CH_2); 3.43 s, 6 H (N-CH_3). ^{13}C NMR (D_2O + NaOD): 156.61 (C-4); 151.00 (C-6); 149.26 (C-2); 147.48 (C-8); 111.37 (C-5); 70.38 d, *J*(P,C) = 11.7 (C-2′); 66.77 d, *J*(P,C) = 156.3 (P-C); 50.77, 2 C (N-C); 48.86 (C-1′); 24.69, 2 C (C-C).

7-[2-(Phosphonomethoxy)ethyl]-6-(pyrrolidin-1-yl)purine (**146**). Yield 42.8%, m.p. 259 °C. For $C_{12}H_{18}N_5O_4P$ (327.3) calculated: 44.04% C, 5.54% H, 21.40% N, 9.46% P; found: 43.98% C, 5.51% H, 21.25% N, 9.59% P. ^{1}H NMR (D_2O + NaOD): 8.33 s, 1 H and 8.14 s, 1 H (H-2 + H-8); 4.62 t, 2 H, *J*(1′,2′) = 4.5 (H-1′); 3.96 t, 2 H, *J*(2′,1′) = 4.5 (H-2′); 3.66 m, 4 H (H-2" + H-5"); 3.61 d, 2 H, *J*(P,CH) = 8.7 (P-CH_2); 2.06 m, 4 H (H-3" + H-4"). ^{13}C NMR (D_2O + NaOD): 154.74 (C-4); 150.68 (C-6); 148.74 (C-2); 144.60 (C-8); 111.87 (C-5); 70.27 d, *J*(P,C) = 10.7 (C-2′); 66.59 d, *J*(P,C) = 157.3 (P-C); 49.12 (C-1′); 40.75, 2 C (N-CH_3).

2-Amino-6-chloro-9-{2-[2-(diethoxyphosphoryl)ethoxy]ethyl}purine (**149**)

Mixture of bis(2-chlorethyl) ether (180 ml) and triethyl phosphite (54 ml) was heated at 160 °C for 15 h and the volatiles distilled off at 65–70 °C/2 kPa. The residual crude diethyl [2-(2-chloroethoxy)ethyl]phosphonate (**148**) (88 g) was used in further reaction steps.

2-Amino-6-chloropurine (**9**) (15 g, 0.1 mol) and compound **148** in DMF (150 ml) was heated to 100 °C and DBU (15 ml) was added under stirring. The clear reaction mixture was heated at 100 °C for additional 6 h and taken down to dryness. The residue was taken into ethyl acetate (400 ml) and extracted with water (3 × 100 ml). The aqueous phase was saturated with NaCl and extracted with chloroform (50-ml portions) until the product disappeared. The combined fractions were dried with anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The residue was purified on a silica gel column (300 ml) to afford 15.0 g (39.7%) of compound **149** as viscose oil. For $C_{13}H_{21}ClN_5O_4P$ (377.7) calculated: 41.33% C, 5.60% H, 9.38% Cl, 18.54% N, 8.20% P; found: 41.14% C, 5.67% H, 9.14% Cl, 18.56% N, 7.95% P. ^{1}H NMR (DMSO-d_6): 8.10 s, 1 H (H-8); 6.91 brs, 2 H (NH_2); 4.20 t, 2 H, *J*(1′,2′) = 5.3 (H-1′); 3.92 dq, 4 H, *J*(CH_2,CH_3) = 7.1, *J*(P,OCH) = 8.3 (P-OCH_2); 3.74 t, 2 H, *J*(2′,1′) = 5.3 (H-2′); 3.58 dt, 2 H, *J*(4′,5′) = 7.1, *J*(P,4′) = 13.4 (H-4′); 2.00 dt, 2 H, *J*(5′,4′) = 7.1, *J*(P,5′) = 18.3 (H-5′); 1.17 t, 6 H, *J*(CH_2,CH_3) = 7.1 (CH_3).

Preparation of N^6-Substituted
2,6-Diamino-9-[2-(2-phosphonoethoxy)ethyl]purines **151**–**153**

Method J. Compound **149** (3.8 g, 10 mmol) in ethanol (60 ml) was treated with the corresponding amine (6 ml) and the mixture refluxed for 6–8 h. After evaporation, the diester

150 was worked up as described in *Method A*. The product after treatment with Me_3SiBr was purified on a Dowex 1 X 2 column and the product was crystallized from water. The following compounds were prepared:

2-Amino-6-(allylamino)-9-[2-(2-phosphonoethoxy)ethyl]purine (**151**). Yield 1.7 g (49.7%), m.p. 242 °C. For $C_{12}H_{19}N_6O_4P$ (342.3) calculated: 42.11% C, 5.59% H, 24.55% N, 9.05% P; found: 41.82% C, 5.46% H, 24.30% N, 8.78% P. ^{1}H NMR (D_2O + NaOD): 7.68 s, 1 H (H-8); 5.95 ddt, 1 H, *J*(2",1") = 4.9, *J*(2",3"*cis*) = 10.5, *J*(2",3"*trans*) = 17.3 (H-2"); 5.21 dq, 1 H, *J*(3"*trans*,1") = *J*(gem) = 1.5, *J*(3"*trans*,2") = 17.3 (H-3"*trans*); 5.14 dq, 1 H, *J*(3"*cis*,1") = *J*(gem) = 1.5, *J*(3"*cis*,2") = 10.5 (H-3"*cis*); 4.14 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 4.03 m, 2 H (H-1"); 3.80 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.71 m, 2 H (H-4′); 1.81 m, 2 H (H-5′).

2-Amino-6-(cyclopropylamino)-9-[2-(2-phosphonoethoxy)ethyl]purine (**152**). Yield 1.50 g (41.7%), m.p. 253 °C. For $C_{12}H_{19}N_6O_4P{\cdot}H_2O$ (360.3) calculated: 40.00% C, 5.87 H, 23.32% N, 8.60% P; found: 39.79% C, 5.64% H, 22.92% N, 8.73% P. ^{1}H NMR (D_2O + NaOD): 7.78 s, 1 H (H-8); 4.23 t, 2 H *J*(1′,2′) = 5.1 (H-1′); 3.86 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 3.73 m, 2 H (O-CH_2); 2.83 m, 1 H (H-1"); 1.75 m, 2 H (P-CH_2); 0.88 m, 2 H and 0.67 m, 2 H (H-2" + H-3").

2-Amino-9-[2-(2-phosphonoethoxy)ethyl]-6-(pyrrolidin-1-yl)purine (**153**). Yield 1.45 g (40.7%), m.p. 281 °C. For $C_{13}H_{21}N_6O_4P$ (356.3) calculated: 43.82% C, 5.94% H, 23.59% N, 8.69% P; found: 43.52% C, 5.85% H, 23.24% N, 8.40% P. ^{1}H NMR (D_2O + NaOD): 7.60 s, 1 H (H-8); 4.15 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 3.82 t, 2 H, *J*(1′,2′) = 5.1 (H-2′); 3.73 m, 2 H (H-4′); 3.63 m, 2 H and 3.31 m, 2 H (H-2" + H-5"); 1.92 m, 2 H and 1.86 m, 2 H (H-3" + H-4"); 1.77 m, 2 H (H-5′).

9-[2-(2-Phosphonoethoxy)ethyl]guanine (**154**)

Compound **149** (3.8 g, 10 mmol) in 1 M HCl (75 ml) was refluxed for 1 h, neutralized with aqueous ammonia and evaporated. The residue was deionized on a Dowex 50 X 8 (100 ml) column (*cf. Method A*) and dried *in vacuo*. Acetonitrile (50 ml) and Me_3SiBr (5 ml) were added and the mixture stirred overnight at ambient temperature. The mixture was evaporated and worked up as decribed in *Method A*. After desalting on Dowex 50, the ammonia eluate was evaporated and applied, in an aqueous alkaline (pH 10) solution onto a column (150 ml) of Dowex 1 X 2 (acetate form). The column was thoroughly washed with water, the resin was suspended in 1 M formic acid (200 ml) and stirred for 20 min. The resin was filtered, washed with boiling water (total, 1 l), the combined washings were evaporated *in vacuo* and the residue codistilled with water (5 × 25 ml). The residue gave on crystallization from water compound **154**, not melting below 280 °C. Yield 1.35 g (44.5%). For $C_9H_{14}N_5O_5P$ (303.2) calculated: 35.65% C, 4.65% H, 23.10% N, 10.22% P; found: 35.80% C, 4.61% H, 23.30% N, 8.98% P. ^{1}H NMR (D_2O + NaOD): 7.74 s, 1 H (H-8); 4.24 t, 2 H, *J*(1′,2′) = 5.2 (H-1′); 3.87 t, 2 H, *J*(2′,1′) = 5.2 (H-2′); 3.74 m, 2 H (H-4′); 1.76 m, 2 H (H-5′).

Preparation of N^6-Substituted
2,6-Diamino-9-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]purines **160**–**163**

Method K. N^6-Substituted 2,6-diaminopurine (42 mmol) and (*R*)-[(trityloxy)methyl]oxirane (**155**) (40 mmol) in DMF (80 ml) was heated to 100 °C and Cs_2CO_3 (1 g) was added. The mixture was stirred at 100 °C for 2 h and evaporated *in vacuo*. Chromatography on silica gel (500 ml column) afforded compound **156** in 94–96% yield as amorphous foam. Its dry chloroform solution was treated with dimethylformamide dimethylacetal at room tempera-

ture overnight and evaporated *in vacuo*. The residue was codistilled with toluene (3 × 50 ml), ethanol (50 ml) and dried over phosphorus pentoxide *in vacuo*. Diisopropyl [(tosyloxy)methyl]phosphonate (**158**) (14.5 g, 41.4 mmol) in dimethylformamide (100 ml) was added and the solution was cooled to –30 °C. Sodium hydride (5.0 g, 60% dispersion in paraffin oil, 125 mmol) was added under stirring and the mixture was left to warm to 0 °C. Stirring was continued at 0 °C for 5 h under exclusion of moisture and then at room temperature for 48 h. Excess of sodium hydride was neutralized by cautious addition of acetic acid, the mixture was diluted with methanol (200 ml) and concentrated aqueous ammonia (50 ml) was added. After standing overnight at room temperature, the solvents were stripped down *in vacuo* and the residue was refluxed with 80% acetic acid (250 ml) for 40 min. The reaction mixture was evaporated *in vacuo*, water (200 ml) was added and the suspension extracted with ether (3 × 100 ml). The aqueous phase was concentrated *in vacuo* and applied on a Dowex 50 X 8 (H^+ form) column (150 ml). The column was washed with water till the neutral reaction and then with 2.5% aqueous ammonia. The UV-absorbing ammonia eluate was evaporated to dryness, codistilled with ethanol and dried over phosphorus pentoxide *in vacuo*. The crude compound **159** was treated with Me_3SiBr (20 ml) in acetonitrile (100 ml) at room temperature overnight, evaporated and codistilled with acetonitrile (50 ml). Water (100 ml) was added to the residue, neutralized with concentrated aqueous ammonia and evaporated. The mixture was again deionized on a column (150 ml) of Dowex 50 X 8 (H^+ form), the ammonia eluate evaporated and applied, in an aqueous alkaline solution onto a column (150 ml) Dowex 1 X 2 (acetate form). The column was eluted with linear gradient of acetic acid (0–0.5 M, 1.5 l each), the main UV-absorbing fraction was evaporated *in vacuo* and excess acetic acid removed by repeated codistillation with water *in vacuo*. The products were crystallized from 80% ethanol (with ether added until turbidity persisted). The following compounds were obtained:

2-Amino-9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]-6-(methylamino)purine (**160**). Yield 5.1 g (38.4%), m.p. 255 °C. For $C_{10}H_{17}N_6O_5P$ (332.3) calculated: 36.15% C, 5.16% H, 25.29% N, 9.32% P; found: 35.99% C, 5.20% H, 25.12% N, 9.19% P. ^{1}H NMR (D_2O + NaOD): 7.85 s, 1 H (H-8); 4.22 dd, 1 H, *J*(1′a,2′) = 4.8, *J*(gem) = 14.6 (H-1′a); 4.16 dd, 1 H, *J*(1′b,2′) = 6.5, *J*(gem) = 14.6 (H-1′b); 3.77 m, 1 H (H-2′); 3.72 dd, 1 H, *J*(3′a,2′) = 3.5, *J*(gem) = 12.2 (H-3′a); 3.51 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 12.2 (P-CHa); 3.49 dd, 1 H, *J*(P,CHb) = 10.0, *J*(gem) = 12.2 (P-CHb); 3.48 dd, 1 H, *J*(3′b,2′) = 3.4, *J*(gem) = 12.2 (H-3′b); 3.03 s, 3 H (N-CH_3).

2-Amino-6-(cyclopropylamino)-9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]purine (**161**). Yield 3.60 g (25%), m.p. 202 °C. For $C_{14}H_{26}N_7O_4P{\cdot}0.5H_2O$ (367.3) calculated: 39.24% C, 5.49% H, 22.88% N, 8.43% P; found: 39.14% C, 5.49% H, 23.48% N, 8.79% P. ^{1}H NMR (D_2O + NaOD): 7.91 s, 1 H (H-8); 4.30 dd, 1 H, *J*(1′a,2′) = 3.9, *J*(gem) = 14.8 (H-1′a); 4.22 dd, 1 H, *J*(1′b,2′) = 6.7, *J*(gem) = 14.8 (H-1′b); 3.86 m, 1 H (H-2′); 3.76 dd, 1 H, *J*(3′a,2′) = 4.2, *J*(gem) = 12.4 (H-3′a); 3.73 dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.9 (P-CHa); 3.60 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.9 (P-CHb); 3.58 dd, 1 H, *J*(3′b,2′) = 4.6, *J*(gem) = 12.4 (H-3′b); 2.84 m, 1 H and 0.97 m, 2 H and 0.76 m, 2 H (cyclopropyl).

2-Amino-6-{[(2-(dimethylamino)ethyl]amino}-9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]purine (**162**). Yield 6.70 g (41%), m.p. 143–145 °C. For $C_{13}H_{24}N_7O_5P{\cdot}H_2O$ (407.4) calculated: 38.33% C, 6.43% H, 24.07% N, 7.60% P; found: 38.40% C, 6.80% H, 23.98% N, 7.46% P. ^{1}H NMR (D_2O + NaOD): 7.87 s, 1 H (H-8); 4.28 dd, 1 H, *J*(1′a,2′) = 3.9, *J*(gem) = 14.9 (H-1′a); 4.21 dd, 1 H, *J*(1′b,2′) = 7.1, *J*(gem) = 14.9 (H-1′b); 3.95 m, 2 H (N-CH_2); 3.87 m, 1 H (H-2′); 3.78 dd, 1 H, *J*(3′a,2′) = 4.0, *J*(gem) = 12.4 (H-3′a); 3.70 dd, 1 H, *J*(P,CHa) = 9.4, *J*(gem) = 12.9

(P-CHa); 3.58 dd, 1 H, *J*(3′b,2′) = 4.9, *J*(gem) = 12.4 (H-3′b); 3.54 dd, 1 H, *J*(P,CHb) = 9.6, *J*(gem) = 12.9 (P-CHb); 3.48 t, 2 H, *J* = 5.7 (N-CH_2); 3.60 s, 6 H (N-CH_3).

2-Amino-6-(dimethylamino)-9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]purine (**163**). Yield 4.70 g (32%), m.p. 238 °C. For $C_{11}H_{19}N_6O_5P \cdot 2H_2O$ (382.3) calculated: 34.56% C, 6.06% H, 21.98% N, 8.10% P; found: 34.43% C, 6.11% H, 21.80% N, 8.14% P. ^{1}H NMR (D_2O + NaOD): 7.81 s, 1 H (H-8); 4.23 dd, 1 H, *J*(1′a,2′) = 4.9, *J*(gem) = 13.7 (H-1′a); 4.18 dd, 1 H, *J*(1′b,2′) = 6.3, *J*(gem) = 13.7 (H-1′b); 3.78 m, 1 H (H-2′); 3.77 dd, 1 H, *J*(3′a,2′) = 3.3, *J*(gem) = 11.6 (H-3′a); 3.57 dd, 1 H, *J*(P,CHa) = 9.0, *J*(gem) = 12.2 (P-CHa); 3.55 dd, 1 H, *J*(3′b,2′) = 3.6, *J*(gem) = 11.6 (H-3′b); 3.53 dd, 1 H, *J*(P,CHb) = 9.5, *J*(gem) = 12.2 (P-CHb); 3.24 s, 6 H (N-CH_3).

Inhibition of the Cell Growth

Inhibition of the cell growth was estimated in mouse leukemia L1210 cells (ATCC CCL 219), CCRF-CEM T lymphoblastoid cells (ATCC CCL 119), murine L929 cells (ATCC CCL 1) and human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2) as described in ref.[47].

Intracellular Metabolism of [8-^{3}H]cypr-PMEDAP

Cells. Human lymphoblastoid cells CCRF-CEM (ATCC CCL 119) were grown in a synthetic medium RPMI-1640 containing 10% FCS, 2 mM glutamine, 15 mM HEPES and 0.01% penicillin/streptomycin. Cultivation at 37 °C in CO_2 incubator (the doubling time of the cell line was 24 h). [8-^{3}H]cypr-PMEDAP (3.83 μM, 16 MBq/μmol) was added to the exponentially growing cells on reaching a density of $3 \cdot 10^5$ cells/ml and incubation in the presence of the compound continued for additional 24 or 48 h. At indicated time intervals, the cells were pelleted and exhaustively washed with PBS at 4 °C by centrifuging at 5 300 *g* for 1 min. The sediment was resuspended in water (200 μl) at 4 °C and 10% TCA (200 μl) was added. After 10 min of vigorous stirring, the precipitate was sedimented for 5 min at 11 000 *g*. TCA was extracted from the supernatant with 400 μl of a 1,1,2-trichloro-1,2,2-trifluoroethane–trioctylamine mixture (4 : 1, v/v). The aqueous phase was separated by centrifugation at 11 000 *g* for 5 min and an appropriate aliquot was analysed by HPLC.

HPLC analysis of intracellular pool. The acid-soluble extract was analysed in a Waters HPLC system (996 PDA Detector, PDA Software Millenium^{32}, version 3.05, 616 Pump with 600S Controller and Waters Fraction Collector II) equipped with 15 cm × 4 mm Supelcosil™ LC 18T 3 μm reverse-phase column. The linear gradient (curve 6) at a flow rate of 0.75 ml/min was used: 10–100%B, 30 min (solvent A, 50 mM potassium dihydrogenphosphate, 3 mM tetrabutylammonium hydrogensulfate, pH 6.1; solvent B, 50 mM potassium dihydrogenphosphate, 3 mM tetrabutylammonium hydrogensulfate, 50% acetonitrile, pH 6.1). 15-s fractions were collected and radioactivity was counted in Aquasafe-500 scintillator (4 ml per sample). Peaks of PMEG (**3**), PMEGp, PMEGpp, and cypr-PMEDAP (**75**) were identified using external standards. The actual cytoplasmic concentration of metabolites mentioned was calculated from the known specific radioactivity of applied [8-^{3}H]cypr-PMEDAP and intracellular volume of T-lymphoblastoids CCRF-CEM (ref.[48]).

Deamination of [8-^{3}H]cypr-PMEDAP *in vitro*

Preparation of cell-free extract. Frozen CCRF-CEM cells were suspended in the extraction buffer (50 mM Tris-HCl pH 7.4, 20 mM KCl and 2 mM DTT) in the presence of protease in-

hibitor cocktail (Sigma). Nonidet P40 was added to a final concentration of 0.1% and the suspension was sonicated (3 × 10 s), homogenized in Dounce tissue grinder (20 strokes with pestle A). The homogenate was then centrifuged at 30 000 *g* for 35 min and nucleic acids were precipitated from the resulting supernatant with 15% streptomycin sulfate. After additional centrifugation (30 000 *g*, 35 min), the crude extract was gel-filtered on a PD 10 column equilibrated in the extraction buffer, supplemented with KCl, DTT and NaN_3 to the final concentration 150 mM, 3 mM and 3 mM. The crude enzyme preparation (25 mg protein per ml) was stored at 4 °C.

[8-^{3}H]cypr-PMEDAP deamination assay. The reaction mixture (100 μl) contained: [8-^{3}H]cypr-PMEDAP (10 or 50 μM), 50 mM Tris-HCl pH 7.4, 150 mM KCl and 2 mM DTT and cell-free extract (1.75 mg protein). Incubation at 37 °C for 2 h. The reaction was stopped by addition of an equal volume of 10% TCA and further processed to remove trichloroacetic acid (*vide supra*). The resulting aqueous phase was injected into a Waters HPLC column (see above) and chromatography was performed using the three-step gradient: Solvent A, 50 mM potassium dihydrogenphosphate, 3 mM tetrabutylammonium hydrogensulfate, pH 6.8; solvent B, 50 mM potassium dihydrogenphosphate, 3 mM tetrabutylammonium hydrogensulfate, 30% acetonitrile, pH 6.8. Programme: 5–10% B, 10 min (linear, curve 6); 10–60% B, 10 min (convex, curve 4). 10-s fractions were collected and the radioactivity was counted as above. Peaks of PMEG (**3**), and cypr-PMEDAP (**75**) were identified using external standards and quantified from the known specific radioactivity of the used [8-^{3}H]cypr-PMEDAP.

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