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Acyclic nucleoside phosphonates with a branched 2-(2-phosphonoethoxy)ethyl chain: Efficient synthesis and antiviral activity

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ABSTRACT

Series of novel acyclic nucleoside phosphonates (ANPs) with various nucleobases and 2-(2-phosphonoethoxy)ethyl (PEE) chain bearing various substituents in β-position to the phosphonate moiety were prepared. The influence of structural alternations on antiviral activity was studied. Several derivatives exhibit antiviral activity against HIV and vaccinia virus (middle micromolar range), HSV-1 and HSV-2 (lower micromolar range) and VZV and CMV (nanomolar range), although the parent unbranched PEE–ANPs are inactive. Adenine as a nucleobase and the methyl group attached to the PEE chain proved to be a prerequisite to afford pronounced antiviral activity.

1. Introduction

Acyclic nucleoside phosphonates (ANPs) possess significant antiviral and cytostatic activity.[1] These nucleotide analogs contain an isopolar phosphonomethyl moiety instead of the nucleotide phosphate ester group which excludes their enzymatic degradation and/or eliminates problems linked to intracellular phosphorylation necessary for nucleoside activation. They are excellent templates for drug design because of the absence of the labile glycosidic bond and the flexibility of the acyclic chain which enables the compounds to adopt a suitable conformation in their target enzyme. Among ANPs, 2-(phosphonomethoxy)alkyl derivatives of purine and pyrimidine bases are particularly potent. 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA, Fig. 1) is active against DNA viruses and retroviruses[2]; its prodrug, adefovir dipivoxil[3] has been approved for hepatitis B virus therapy (Hepsera™).[4] Attachment of the hydroxymethyl group to the 2-(phosphonomethoxy)ethyl chain leads to another active compound, (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl] adenine ((*S*)-HPMPA, Fig. 1), with potent anti-DNA-viral activity.[5] Substitution of the aliphatic PMEA side chain by the methyl group results in (*R*)-9-[2-(phosphonomethoxy)propyl] adenine (PMPA, Fig. 1) with high potency and selectivity against HIV-1 and HIV-2[6]; its oral prodrug tenofovir disoproxil fumarate has been approved for treatment of HIV infection (Viread™) and recently also HBV infection.[6c]

Further structure-activity relationship studies in the series of PMEA congeners included modification of the parent molecule both in the side chain (e.g., unsaturated or aryl ANPs)[7] and in the heterocyclic moiety. They demonstrated that the margins of structural alteration are very narrow. Except for the antiviral activity of the cytosine derivative (*S*)-HPMPC (Vistide™),[6] the choice of the base is limited mostly to adenine, guanine and 2,6-diaminopurine, and to their 8-aza and 3-deaza congeners. The specificity of antiviral action is predominantly determined by the structure of the side chain.

ANPs with the chain elongated by CH_2 group – 2-(phosphonoethoxy)ethyl (PEE) compounds[8] – do not exhibit any significant antiviral activity, but their 6-oxopurine derivatives (PEEG and PEEHx, Fig. 1) posses anti-malarial activity. They inhibit hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRT), the

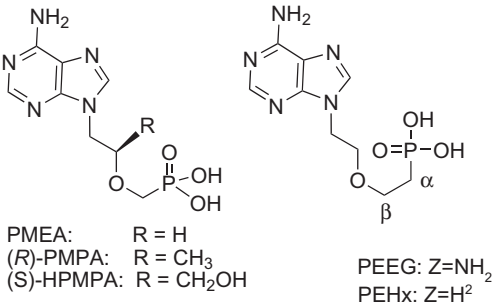

Figure 1.

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key enzyme of the purine salvage pathway essential for the replication and survival of the parasite *Plasmodium falciparum*, with K_i values of 0.1 (PEEG) and 0.3 (PEEHx) μM, respectively.[9] To investigate the effect of branching of the phosphonoethoxyethyl chain on selectivity and ability to inhibit *Pf*HGXPRT or human HGPRT, substituents were added either to the carbon attached directly to the phosphorus atom (α-branched derivatives) or to the adjacent carbon (β-branched derivatives). While the α-branched PEE-6-oxopurines were only very weak inhibitors, some of the β-branched compounds bind tightly to the enzymes.[10,11]

Herein, we report on the synthesis and antiviral activity of a series of ANPs with a β-branched PEE moiety. To further investigate the structure–activity relationship in this group of compounds, the influence of the nucleobase and of various substituents at the PEE-chain was studied. The prepared ANPs can be divided into three groups: (A) derivatives **9a**–**9e** with adenine as a base and variant β-substituents; (B) derivatives **12a**,[10] **13a**,[10] **14**, **15a**, **16a** and **18a** with 2-(1-phosphonopropan-2-yloxy)ethyl chain and various purine or pyrimidine bases as analogs of PMP-compounds; (C) derivatives **12e**, **13e**, **15e** and **16e** (and their benzyl-protected congeners **12d**, **13d**, **15d** and **16d**) with various nucleobases and 2-(3-hydroxy-1-phosphonopropan-2-yloxy)ethyl chain as analogs with a structure motif derived from HPMP-ANPs.

2. Chemistry

Previously published syntheses of branched phosphonates suffered mainly from the elimination reactions. For this extensive series of β-branched PEE–ANPs we decided to improve our previously published method[10] for the preparation of diethyl 2-(2-hydroxyethoxy)alkylphosphonates **3a**–**3c**. The procedure was significantly simplified and can provide convenient and rapid access to a broad spectrum of phosphonate derivatives. The main improvement compared to our previously published step-vise procedure[10] was the one-pot sequence without the isolation of the branched alcohol after the first step. Starting from commercially available diethyl methylphosphonate (Scheme 1), diethyl lithiomethylphosphonate preformed in situ in dry THF under argon atmosphere was reacted with the corresponding aldehydes. The resulting intermediate **1** was directly alkylated by ethyl bromoacetate under mild conditions to avoid elimination reaction. The ethyl (alkoxy)acetate moiety in compound **2** can serve as a latent hydroxyethyl group. These esters **2** were reduced by borane in THF to obtain desired hydroxy derivatives **3** with the already built-in ether bond. The overall yield of the three step sequence was 69% for **3a** and 58% for **3d**.

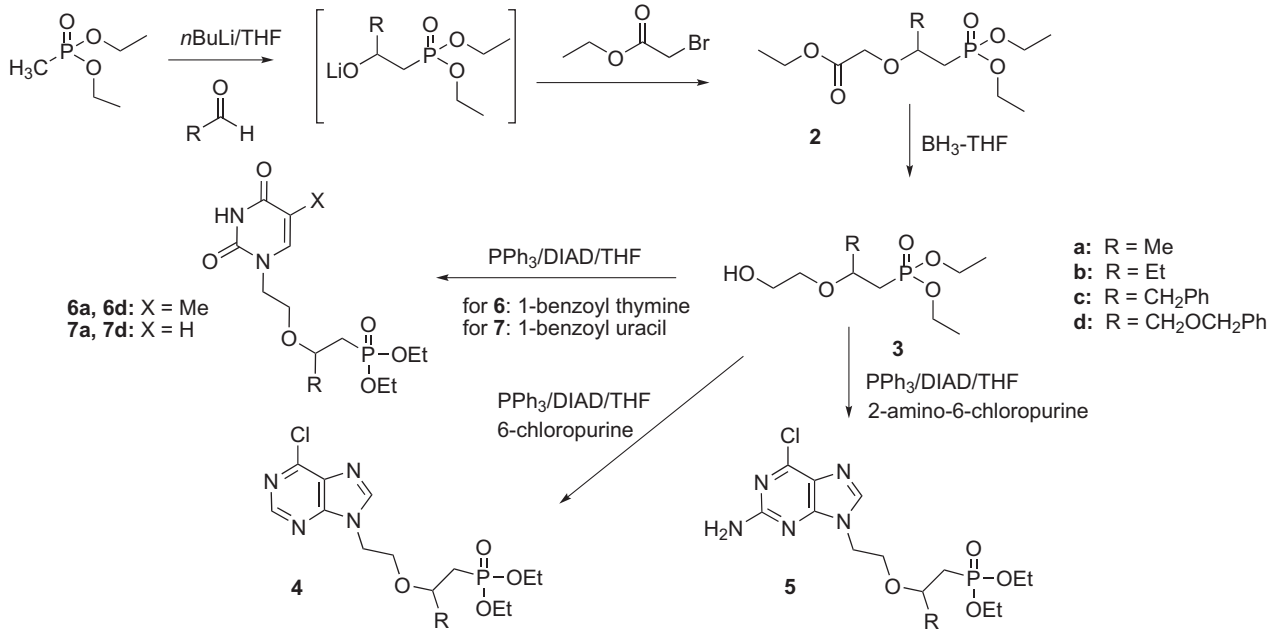
The resulting alcohols **3a**–**3c**[10] and **3d** were introduced under Mitsunobu conditions into the 9-position of the purine or the 1-position of pyrimidine bases (Scheme 1). Thus 6-chloropurine was alkylated by alcohols **3a**–**3d** and 2-amino-6-chloropurine by **3a** and **3d** to form ANP diethyl esters **4a**–**4d** and **5a**,**d**, respectively (details of the synthesis of **4a**–**4c** and **5a** were given in our previous paper[9]). The same procedure followed by base-deprotection was used for alkylation of 3-benzoylthymine and 3-benzoyluracil to form the pyrimidine derivatives **6a**,**d** and **7a**,**d**, respectively. As expected from our previous experience, the yields of the Mitsunobu reaction were moderate (45–70%).

Further procedures in the purine series (Scheme 2) were based on a modification of the 6-position in heterocyclic moiety followed by cleavage of the esters groups under the standard conditions (1. Me_3SiBr/acetonitrile, 2. hydrolysis).

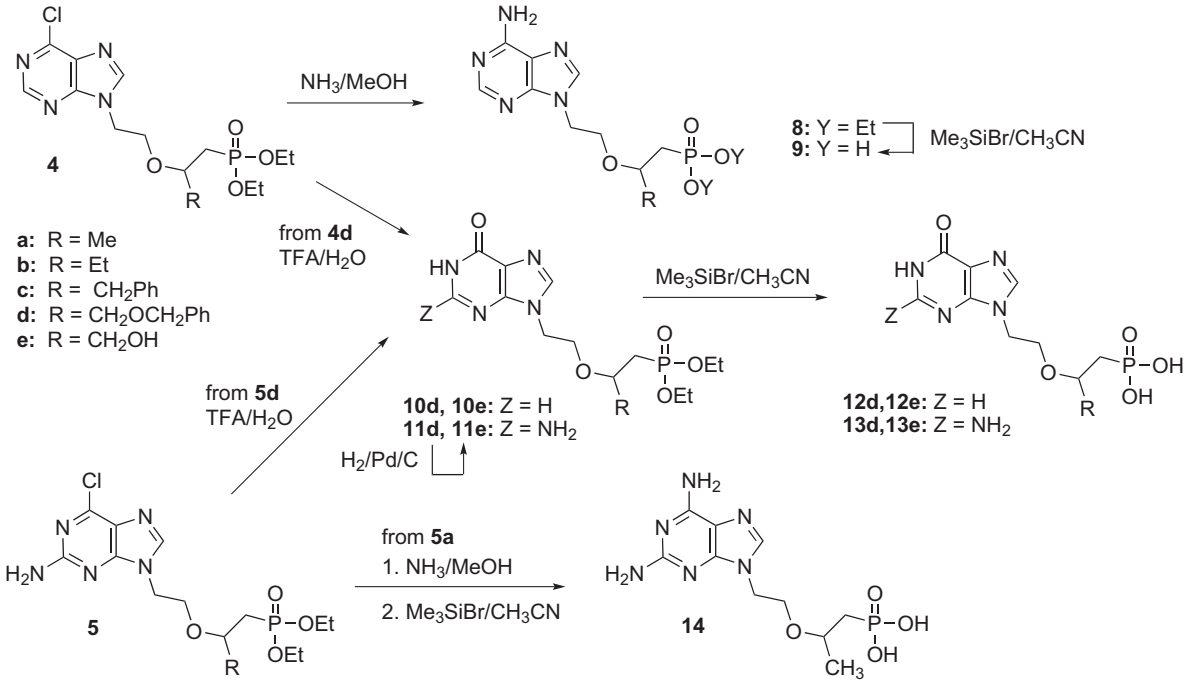
Thus heating of 6-chloropurine derivatives **4a**–**4d** in methanolic ammonia in autoclave afforded intermediates **8a**–**8d**. After subsequent cleavage of the ethyl groups, adenine β-branched PEE-compounds **9a**–**9d** were obtained. During this reaction the benzyl group was partially cleaved from **8d** and, in addition to **9d**, hydroxy derivative **9e** was isolated as the second product.

A routine procedure was applied to hydrolyze halogen in the 6-position of the purine derivatives **4d** and **5d** to obtain hypoxanthine and guanine ANPs. Reaction with 75% aqueous trifluoroacetic acid gave 6-oxopurine compounds **10d** and **11d**, respectively, and sufficient amounts of these products were debenzylated by hydrogenolysis (Pd/C) to form hydroxymethyl PEE-derivatives **10e** and **11e**. Finally, diethyl esters **10d**,**e** and **11d**,**e** were transformed to the target free phosphonic acids **12d**,**e** and **13d**,**e**.

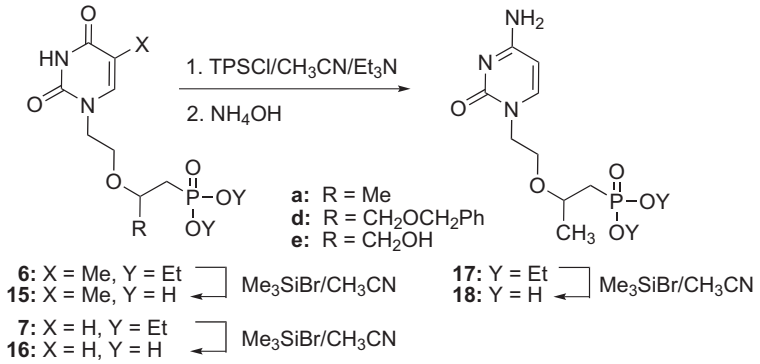
The same procedure as for the above mentioned adenine ANPs was applied for the synthesis of diaminopurine compound **14**, in this case starting from 2-amino-6-chloro derivative **5a**.



Scheme 1.



Scheme 2.



Scheme 3.

In the pyrimidine series (Scheme 3) the benzyl derivatives **6d** and **7d**, prepared by the Mitsunobu approach, were debenzylated by hydrogenolysis (Pd/C) to form hydroxymethyl compounds **6e** and **7e**, respectively. The uracil methyl-PEE diester **7a** was converted into the cytosine derivative **17** by the treatment with 2,4,6-triisopropylbenzenesulfonyl chloride followed by amination with ammonium hydroxide.[12] The diethyl esters derived from cytosine (**17**), thymine (**6a**,**e**) and uracil (**7a**,**e**) were treated with bromotrimethylsilane and after subsequent hydrolysis the free phosphonic acids **18**, **15a**,**e** and **16a**,**e** were isolated, respectively.

3. Biological activity

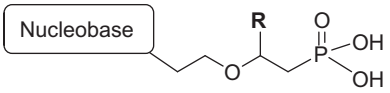
The entitled novel β-branched 9-(phosphonoethoxyethyl) ANPs **9a**–**9e**, **12d**,**e**, **13d**,**e**, **14**, **15a**,**e**, **16a**,**e** and **18**, as well as previously published[10] guanine (**13a**–**13c**) and hypoxanthine (**12a**–**12c**) derivatives, were evaluated against various DNA viruses, including poxviruses [i.e., vaccinia virus (VACV)], herpesviruses [i.e., herpes simplex virus type 1 (HSV-1)] and type 2 (HSV-2), thymidine kinase-deficient HSV-1 (acyclovir-resistant, ACV^r), varicella-zoster virus (VZV), and human cytomegalovirus (HCMV) (Table 1). The compounds were also evaluated against retroviruses [(i.e., human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2)]. All compounds were also examined against several RNA viruses, including vesicular stomatitis virus (VSV), Coxsackie B4 virus, respiratory syncytial virus (RSV), parainfluenza virus type 3, reovirus-1, Sindbis virus and Punta Toro virus. None of the compounds showed activity against the different RNA viruses, except for HIV-1 and HIV-2.

Compounds **9d**,**e**, **12a**–**12d**, **13a**–**13c**, **14**, **15e** and **16e** proved to be inactive against herpes- and poxviruses. Compounds, that in contrast to the parent unbranched PEE–ANPs,[8] exhibited at least moderate anti-herpes and antipoxvirus activities are summarized in Table 1. The antiviral activity was observed mainly for an adenine as a nucleobase and for a methyl group attached to the PEE chain. Thus, compound **9a** that possesses both features emerged as the most active β-branched 9-(phosphonoethoxyethyl) ANPs with EC_{50} values in the range of 0.07–2.3 μM against different human herpesviruses and of 27 μM against vaccinia virus. Although this compound only altered cell morphology at a concentration of 322 μM, it appeared to be cystostatic for HEL (human embryonic lung) fibroblasts with a CC_{50} (concentration inhibiting cell growth

Table 1
Antiviral and cytotoxic/static activity of the compounds against herpes- and poxviruses in cell culture

Compounds	Base	R	Antiviral activity: EC_{50} (μM)[a]										Cytotoxic/static activity	
			VZV				HSV-1 (KOS)	HSV-2 (G)	HSV-1 TK^- (KOS ACV)	Cytomegalovirus		Vaccinia virus	Cell Morphology	Cell growth
			TK^+ strains		TK^- strains					AD-169 strain	Davis strain	Lederle strain	MCC (μM)[b]	CC_{50} (μM)[c]
			YS	OKA	07/1	YS/R								
9a	A	Me	0.10 ± 0.13	0.07 ± 0.04	0.11 ± 0.06	0.07 ± 0.09	1.3 ± 0	2.3 ± 1.40	1.7 ± 0	0.93 ± 0.23	0.90 ± 0.6	27 ± 4.7	322	13 ± 10
9b	A	Et	42	19 ± 12	22 ± 18	43	238 ± 111	286 ± 111	251 ± 95	159	243	>317	>317	>317
9c	A	CH_2Ph	2.9 ± 3.4	2.9 ± 2.6	4.7 ± 3.2	2.5 ± 3.1	53 ± 0	86 ± 47	53 ± 0	59 ± 50	68 ± 22	>265	>265	⩾247 ± 27
12e	Hx	CH_2OH	N.D.	88	197	N.D.	208	315	>315	>315	>315	>315	>315	>315
13d	G	CH_2OCH_2Ph	N.D.	109	94	N.D.	>240	>240	>240	>240	>240	>240	>240	>240
13e	G	CH_2OH	N.D.	>300	>300	N.D.	156	156	261	165	165	>300	>300	300
15a	T	Me	N.D.	47	48	N.D.	171	154	>342	188	248	>342	>342	>342
16a	U	Me	9.9	6.3 ± 2.7	13 ± 4.6	12	43 ± 0	34 ± 13	97 ± 36	36 ± 4.1	34 ± 1.8	>360	>360	291 ± 76
18	C	Me	N.D.	130	168	N.D.	>361	>361	>361	>361	>361	>361	>361	307
Cidofovir			N.D.	0.29 ± 0.25	0.10 ± 0.06	N.D.	1.3 ± 0.6	0.9 ± 0.1	1.0 ± 0.1	0.79 ± 0.51	0.70 ± 0.29	7.3 ± 4.6	>317	254 ± 200
Acyclovir			3.64 ± 1.24	1.56 ± 0.84	71.6 ± 31.6	76.9 ± 41.3	0.30 ± 0.17	0.23 ± 0.15	7.3 ± 4.6	N.D.	N.D.	>250	>444	1,338 ± 484
Ganciclovir			N.D.	N.D.	N.D.	N.D.	0.017 ± 0.01	0.023 ± 0.01	0.12 ± 0.07	5.9 ± 3.1	5.1 ± 2.0	>100	>394	543 ± 492

N.D.: not determined.
[a] Concentration required to reduce virus-induced cytopathicity or viral plaque formation by 50%.
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology (human embryonic lung (HEL) fibroblasts; for details see Section 5).
[c] Cytotoxic concentration required to reduce cell growth by 50% (human embryonic lung (HEL) fibroblasts; for details see Section 5).



by 50%) value of 13 μM. Hence, selectivity indices (ratio CC_{50}:EC_{50}) for compound **9a** were of 120–190 for different VZV strains, 14–15 for HCMV, 6–10 for HSV, and <1 for vaccinia virus compared to, respectively, 880–2540, 195–282, 322–363, and 35 for cidofovir.

Replacement of the Me group by an Et (**9b**) or CH_2Ph (**9c**) group in **9a** resulted in decreased cytostatic activities (CC_{50} values of, respectively, >317 and 247 μM) but also in diminished antiviral activities against herpes- and poxviruses. The benzyl-substituted derivative showed antiviral activity in between the methyl (most active) and ethyl (least active) derivatives. Also, replacement of the methyl by a hydroxymethyl (**9e**) annihilated the anti-DNA virus activity. Substitution of the adenine nucleobase in **9a** by uracil (**16a**), thymine (**15a**) or cytosine (**18**) resulted in lower cystostatic effects but also in a virtually complete loss of antiviral potency.

Among the different β-branched 9-(phosphonoethoxyethyl) ANPs, only **9a** was able to inhibit the replication of HIV-1 (EC_{50} = 11 ± 4.0 μM) and HIV-2 (EC_{50} = 9.0 ± 6.0 μM) in CEM cell cultures. Its antiretroviral activity was in the same concentration range as PMEA (adefovir) (EC_{50}: 7.4 ± 1.7 μM for HIV-1 and 7.0 ± 1.1 μM for HIV-2). However, the cystostatic activity of **9a** in CEM cells (CC_{50} = 20 ± 6.3 μM) may account for the anti-HIV activity observed and thus, the observed anti-HIV activity might not be due to a direct inhibition of virus replication, but rather an indirect cellular antimetabolic activity. Compound **9a** also demonstrated a higher cystostatic activity against the cell cultures compared to compounds **9b** and **9c**. As observed for their cytostatic activity against human fibroblast HEL cell proliferation, when evaluated against murine leukemia cells (L1210), murine mammary carcinoma cells (FM3A) and human T-lymphocyte cells (CEM), **9a** was far more cytostatic than **9b** and **9c** (Table 2).

Table 2
Inhibitory effect of the adenine ANP derivatives on the proliferation of murine leukemia cells (L1210), murine mammary carcinoma cells (FM3A) and human T-lymphocyte cells (CEM)

Compound	R	CC_{50} (μM)		
		L1210	FM3A	CEM
9a	Me	10 ± 0.0	2.7 ± 1.3	20 ± 6.3
9b	Et	>660	432 ± 0	>660
9c	CH_2Ph	273 ± 88	90 ± 37	530

4. Conclusions

The methodology for the synthesis of β-branched acyclic nucleoside phosphonates with PEE moiety was significantly improved and a series of new purine and pyrimidine derivatives were synthesized. Although the parent unsubstituted PEE–ANPs were antivirally inactive,[8] attachment of a methyl, ethyl or benzyl substituent to the acyclic aliphatic chain lead to antivirally active compounds, especially in the adenine series. These new results contribute to the extensive SAR project aimed at the development of new selective antiviral drugs.

5. Experimental

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and the compounds were dried over P_2O_5 at 2 kPa. NMR spectra were recorded on Bruker Avance 400 (^1H at 400 MHz, ^{13}C at 100.6 MHz) spectrometers with TMS as internal standard or referenced to the residual solvent signal. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer. The chemicals were obtained from commercial sources (Sigma–Aldrich) or prepared according to the published procedures. Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å). THF was distilled from sodium benzophenone ketyl under argon. Deionisation was performed on Dowex 50 × 8 (H^+-form) columns by the following procedure: after application of crude product the column was washed with water until the UV absorption dropped. Thereafter, the column was eluted with 2.5% aqueous NH_3. Chromatography on Dowex 1 × 2 (acetate form) was as follows: after application of the

aqueous solution of the crude product onto the column, it was washed with water until the UV absorption dropped. The column was then eluted with a gradient of dilute acetic or formic acid (0–1 M). All tested ANPs were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry. The purity of the target compounds was determined by combustion elemental analysis (C, H, N).

5.1. Diethyl 3-(benzyloxy)-2-(2-hydroxyethoxy) propylphosphonate (3d)

A solution of *n*-butyllithium in hexane (2.5 M, 16 ml) was added slowly to a stirred solution of diethyl methanephosphonate (5 g, 33.3 mmol) in dry THF (50 ml) cooled to −78 °C under argon atmosphere. After 15 min of vigorous stirring benzyloxyacetaldehyde (5 g, 33.3 mmol) was added. The reaction mixture was allowed to rise to −30 °C and stirred for 5 h. Then ethyl bromoacetate (4.5 ml, 40 mmol) was added and the temperature of the reaction mixture was allowed to rise slowly to room temperature. After overnight stirring diethyl ether (250 ml) was added and the mixture was washed with saturated NaCl solution. The organic layer was dried over anhydrous magnesium sulfate and solvent evaporated. The crude intermediate **2d** (ethyl 2-(1-(benzyloxy)-3-(diethoxyphosphoryl)propan-2-yloxy)acetate) was purified by chromatography on silica gel (hexane-$CHCl_3$–MeOH) and used in the following step.

^{1}H NMR (DMSO-d_6): 7.33 m, 5H (Ar); 4.49 s, 2H (CH_2Ph); 4.22 s, 2H (CH_2COOEt); 4.08 dd, 2H, *J* = 14.2 and 7.0 (Et); 3.98 m, 4H (Et); 3.84 m, 1H (CH); 3.61 dd, 1H, *J* = 10.5 and 3.3 (CH_2O-a); 3.54 dd, 1H, *J* = 10.5 and 6.0 (CH_2O-b); 2.06 m, 2H (CH_2P); 1.17 m, 9H (CH_3). ^{13}C NMR (DMSO-d_6): 169.82 (CO); 138.16 (Ar); 128.07, 2C (Ar); 127.26 (Ar); 127.24, 2C (Ar); 74.33 (CH); 72.16 (CH_2); 71.96 d, *J*(P,C) = 8.7 (CH_2); 66.85 (CH_2); 71.96 dd, *J*(P,C) = 6.2 and 13.58 (CH_2OP); 59.98 (CH_2); 27.57 d, *J*(P,C) = 137.8 (CH_2P); 16.04 d, *J*(P,C) = 6.0 (CH_3); 13.89 (CH_3).

Intermediate **2d** was cooled to −20 °C and a solution of borane in THF (1.0 M, 55 ml) was added under argon atmosphere. The reaction mixture was stirred at room temperature for 3 days. Methanol (20 ml) was added at −20 °C and the solution was concentrated after the evolution of hydrogen had stopped. The residue was purified by chromatography on silica gel (hexane-$CHCl_3$–MeOH) and product **3d** (6.7 g) was obtained in 58% yield in three steps.

^{1}H NMR (DMSO-d_6): 7.34 m, 5H (Ar); 4.50 s, 2H (CH_2Ph); 43.97 m, 4H (Et); 3.84 m, 1H (CH); 3.58 m, 2H; 3.51 m, 2H and 3.47 m, 2H (CH_2); 2.00 m, 2H (CH_2P); 1.31 t, 6H (CH_3). MS (ESI): *m/z* = 347 $[M+H]^+$.

5.2. Synthesis of compounds4d, 5d, 6a,d, and 7a,d by Mitsunobu reaction – general procedure

To a solution of triphenylphosphine (3.5 g, 13.4 mmol) in dry THF (45 ml) cooled to −30 °C under argon atmosphere diisopropylazadicarboxylate (DIAD, 2.4 ml, 12.6 mmol) was added slowly. The mixture was stirred for 30 min and this preformed complex was added to the reaction mixture containing corresponding heterocyclic base (6-chloropurine, 2-amino-6-chloropurine, 3-benzoylthymine or 3-benzoyluracil; 6.5 mmol), dry THF (30 ml) and diethyl phosphonate **3a**[10] or **3d** (4.3 mmol) at −30 °C under argon. The resulting mixture was slowly warmed to room temperature and stirred for 48 h. Further procedure differs for corresponding derivatives:

5.3. Diethyl 9-[2-(3-benzyloxy-1-phosphonopropan-2-yloxy) ethyl]-6-chloropurine (4d)

The solvent was evaporated and the crude mixture was purified by chromatography on silica gel (MeOH–$CHCl_3$), yield 53% (starting from **3d** and 6-chloropurine). ^{1}H NMR (DMSO-d_6): 8.77 s, 1H and 8.68 s, 1H (H-2 and H-8); 7.29 m, 3H and 7.18 m, 2H (Ar); 4.45 dd, 2H, *J*(1′,2′) = 5.1, J_g = 10.0 (H-1′); 4.35 d, 2H, *J* = 3.5 (CH_2Ph); 3.96 t, 2H, *J*(2′,1′) = 5.3 (H-2′); 3.90 m, 4H (Et); 3.76 m, 1H (H-3′); 3.47 dd, 1H, *J*(5′a,3′) = 3.5, J_g = 10.6 (H-5′a); 3.39 dd, H, *J*(5′b,3′) = 6.1, J_g = 10.6 (H-5′b); 1.95 td, H, *J*(4′,3′) = 6.1, *J*(4′,P) = 18.1 (H-4′); 1.15 t, H, *J* = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 151.83 (C-4); 151.22 (C-2); 148.73 (C-6); 147.70 (C-8); 138.01 (Ar); 130.59 (C-5); 128.02, 2C (Ar); 127.22 (Ar); 127.10, 2C (Ar); 74.05 (C-3′); 72.03 (CH_2Ph); 71.76 d, *J*(P,C) = 10.9 (C-5′); 66.69 (C-2′); 60.79 m, 2C (Et); 43.95 (C-1′); 27.50 d, *J*(P,C) = 138.3 (C-4′); 16.01 m (Et). MS (ESI): *m/z* = 483 $[M+H]^+$.

5.4. Diethyl 9-[2-(3-benzyloxy-1-phosphonopropan-2-yloxy) ethyl]-2-amino-6-chloropurine (5d)

Water (10 ml) was added and the reaction mixture was heated at 70 °C for 24 h. Diethyl ether (250 ml) was added and the mixture was washed with saturated NaCl solution. The organic layer was dried over anhydrous magnesium sulfate and solvent evaporated. The pure product was obtained after chromatography on silica gel (chloroform–MeOH), yield 59% (starting from **3d** and 2-amino-6-chloropurine). ^{1}H NMR (DMSO-d_6): 8.10 s, H (H-8); 7.30 m, H and 7.23 m, H (Ar); 6.91 s, H (NH_2); 4.38 d, H, *J* = 2.7 (CH_2Ph); 4.18 dd, H, *J*(1′,2′) = 4.8, J_g = 8.3 (H-1′); 3.86 t, 2H, *J*(2′,1′) = 5.3 (H-2′); 3.90 m, 4H (Et); 3.75 m, 1H (H-3′); 3.50 dd, 1H, *J*(5′a,3′) = 3.3, J_g = 10.5 (H-5′a); 3.41 dd, 1H, *J*(5′b,3′) = 6.1, J_g = 10.5 (H-5′b); 1.96 m, 2H (H-4′); 1.16 t, 6H, *J* = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 159.58 (C-2); 153.94 (C-4); 149.09 (C-6); 143.45 (C-8); 138.06 (Ar); 128.05, 2C (Ar); 127.24 (Ar); 127.19, 2C (Ar); 123.11 (C-5); 70.06 (C-3′); 72.14 (CH_2Ph); 71.76 d, *J*(P,C) = 10.3 (C-5′); 66.73 (C-2′); 60.77 m, 2C (Et); 43.18 (C-1′); 27.55 d, *J*(P,C) = 138.55 (C-4′); 16.02 d, *J*(P,C) = 5.7 (Et). MS (ESI): *m/z* = 498 $[M+H]^+$.

5.5. Diethyl 1-[2-(1-phosphonopropan-2-yloxy)ethyl]thymine (6a)

The solvent was evaporated and methanol (30 ml) and 1 M NaOMe in methanol (6 ml) were added. The solution was stirred overnight, neutralized by 0.5 M aqueous HCl and solvent evaporated. The crude mixture was purified by chromatography on silica gel (chloroform–MeOH), yield 65% (starting from **3a** and 3-benzoylthymine). ^{1}H NMR (DMSO-d_6): 11.23 br s, 1H (NH); 7.48 s, 1H (H-6); 3.96 m, 4H (Et); 3.75 m, 2H (H-1′); 3.66 m, 1H (H-3′); 3.56 t, 2H, *J*(2′,1′) = 5.4 (H-2′); 2.04 m, 1H (H-4′a); 1.87 m, 1H (H-4′b); 1.74 s, 3H (CH_3); 1.21 t, 6H, *J* = 7.0 (Et); 1.16d, 3H, *J*(3′,5′) = 6.1 (H-5′). 13C NMR (DMSO-d_6): 164.12 (C-4); 150.72 (C-2); 141.95 (C-6); 107.68 (C-5); 70.66 (C-3′); 65.06 (C-2′); 60.73 dd, *J*(P,C) = 6.3 and 11.9 (Et); 47.05 (C-1′); 32.22 d, *J*(P,C) = 136.0 (C-4′); 20.58 d, *J*(P,C) = 7.9 (C-5′); 16.08 d, *J*(P,C) = 5.9 (Et); 11.68 (CH_3-5). MS (ESI): *m/z* = 349 $[M+H]^+$.

5.6. Diethyl 1-[2-(3-benzyloxy-1-phosphonopropan-2-yloxy) ethyl]thymine (6d)

The same procedure as for thymine derivative **6a** was applied, yield 70% (starting from **3d** and 3-benzoylthymine). ^{1}H NMR (DMSO-d_6): 11.24 br s, 1H (NH); 7.46 s, 1H (H-6); 7.30 m, 5H (Ar); 4.47 s, 2H (CH_2Ph); 3.95 m, 4H (Et); 3.76 m, 3H (H-1′ and H-3′); 3.69 t, 2H, *J*(2′,1′) = 5.0 (H-2′); 3.53 dd, 1H, *J*(5′a,3′) = 3.5, J_g = 10.5 (H-5′a); 3.45 dd, 1H, *J*(5′b,3′) = 6.0, J_g = 10.5 (H-5′b); 1.98 m, 2H (H-4′); 1.20 t, 6H, *J* = 7.1 (Et).^{13}C NMR (DMSO-d_6): 164.12 (C-2); 150.74 (C-4); 141.95 (C-6); 138.15 (Ar); 128.09, 2C (Ar); 127.26 (Ar); 127.19, 2C (Ar); 107.64 (C-5); 74.07 (C-3′); 72.20

(CH_2Ph); 71.75 d, J(P,C) = 10.1 (C-5′); 66.72 (C-2′); 60.82 m, 2C (Et); 47.15 (C-1′); 27.55 d, 2C, J(P,C) = 138.2 (C-4′); 16.05 d, J(P,C) = 5.8 (Et); 11.68 (CH_3). MS (ESI): m/z = 455 $[M+H]^+$.

5.7. Diethyl 1-[2-(1-phosphonopropan-2-yloxy)ethyl]uracil (7a)

The same procedure as for thymine derivative **6a** was applied, yield 45% (starting from **3a** and 3-benzoyluracil). ^{1}H NMR (DMSO-d_6): 11.24 br s, ^{1}H (NH); 7.57 d, 1H, J(6,5) = 7.8 (H-6); 5.51 d, 1H, J(5,6) = 7.8 (H-5); 3.96 m, 4H (Et); 3.78 dd, 2H, J(1′,2′) = 9.8 and 5.2 (H-1′); 3.67 m, 1H (H-3′); 3.57 dd, 2H, J(2′,1′) = 8.8 and 5.3 (H-2′); 2.04 m, 1H (H-4′a); 1.86 m, 1H (H-4′b); 1.21 t, 6H (Et); 1.16 d, 3H, J(3′,5′) = 6.1 (H-5′).13C NMR (DMSO-d_6): 163.55 (C-4); 150.76 (C-2); 146.19 (C-6); 100.13 (C-5); 70.71 (C-3′); 65.025 (C-2′); 60.75 dd, J(P,C) = 6.3 and 11.1 (Et); 47.36 (C-1′); 32.23 d, J(P,C) = 136.0 (C-4′); 20.59 d, J(P,C) = 7.8 (C-5′); 16.08 d, J(P,C) = 5.8 (Et). MS (ESI): m/z = 335 $[M+H]^+$.

5.8. Diethyl 9-[2-(3-benzyloxy-1-phosphonopropan-2-yloxy) ethyl]uracil (7d)

The same procedure as for thymine derivative **6a** was applied, yield 70% (starting from **3d** and 3-benzoyluracil). ^{1}H NMR (DMSO-d_6): 11.25 br s, 1H (NH); 7.56 d, J = 7.9, 1H (H-6); 7.30 m, 5H (Ar); 5.46 d, H, J = 7.9 (H-5); 4.47 s, H (CH_2Ph); 3.95 m, H (Et); 3.79 m, H (H-1′ and H-3′); 3.69 t, 2H, J(2′,1′) = 5.1 (H-2′); 3.53 dd, 1H, J(5′a,3′) = 3.4, J_g = 10.5 (H-5′a); 3.44 dd, 1H, J(5′b,3′) = 6.0, J_g = 10.5 (H-5′b); 1.99 m, 2H (H-4′); 1.20 t, 6H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 163.55 (C-2); 150.77 (C-4); 146.16 (C-6); 138.13 (Ar); 128.09, 2C (Ar); 126.24, 3C (Ar); 100.08 (C-5); 74.06 (C-3′); 72.19 (CH_2Ph); 71.75 d, J(P,C) = 10.3 (C-5′); 66.68 (C-2′); 60.83 m, 2C (Et); 47.42 (C-1′); 27.58 d, 2C, J(P,C) = 138.1 (C-4′); 16.05 m, J(P,C) = 5.9 (Et). MS (ESI): m/z = 441 $[M+H]^+$.

5.9. Synthesis of 9-[2-(1-phosphonoalkan-2-yloxy)ethyl]-adenines 9a–9e – general procedure

A solution of 6-chloropurine derivatives 4a-4c[10] or 4d(1 mmol) in methanolic ammonia (60 ml) was stirred in an autoclave at 70 °C for 30 h, solvent was evaporated and the residue co-distilled with acetonitrile. A mixture of this crude diethyl ester adenine intermediate **8a**–**8d**, acetonitrile (20 ml) and $BrSiMe_3$ (2 ml) was stirred overnight at room temperature. After evaporation and co-distillation with acetonitrile, the residue was treated with aqueous ammonia (2.5%) and evaporated to dryness. The residue was applied onto a column of Dowex 50 × 8 (H^+ form, 40 ml) and washed with water. Elution with 2.5% aqueous ammonia and evaporation afforded crude product as ammonium salt. This residue was applied onto a column of Dowex 1 × 2 (acetate, 50 ml), washed with water followed by a gradient of acetic acid (0–0.5 M) and formic acid (0.5 M). The main UV-absorbing fraction was evaporated and co-distilled with water to obtain product as a white solid.

5.10. 9-[2-(1-Phosphonopropan-2-yloxy)ethyl]adenine (9a)

Starting from 6-chloropurine derivative **4a**, yield 61% in two steps. ^{1}H NMR (DMSO-d_6): 8.16 s, 1H and 8.13 s, 1H (H-2 and H-8); 7.36 br s, 2H (NH_2); 4.27 t, 2H, J(1′,2′) = 5.3 (H-1′); 3.73 t, 2H, J(2′,1′) = 5.3 (H-2′); 3.65 m, 1H (H-3′); 1.87 ddd, 1H, J(4′a,3′) = 4.6, J_g = 14.7, J(4′a,P) = 19.4 (H-4′a); 1.58 ddd, 1H, J(4′b,3′) = 8.7, J_g = 14.7, J(4′b,P) = 17.4 (H-4′b); 1.12 d, 3H, J(3′,5′) = 6.1 (H-5′). 13C NMR (DMSO-d_6): 155.31 (C-6); 151.53 (C-2); 149.24 (C-4); 141.34 (C-8); 118.37 (C-5); 71.31 (C-3′); 65.30 (C-2′); 43.08 (C-1′); 35.22 d, J(P,C) = 132.0 (C-4′); 20.74 d, J(P,C) = 5.0 (C-5′). Anal. Calcd for $C_{10}H_{16}N_5O_4P.1/2H_2O$: C, 38.71; H, 5.52; N, 22.57. Found: C, 38.67; H, 5.53; N, 22.45. MS (ESI): m/z = 302 $[M+H]^+$.

5.11. 9-[2-(1-Phosphonobutan-2-yloxy)ethyl]adenine (9b)

Starting from 6-chloropurine derivative **4b**, yield 54% in two steps. ^{1}H NMR (DMSO-d_6): 8.14 s, 1H and 8.11 s, 1H (H-2 and H-8); 7.28 br s, 2H (NH_2); 4.27 t, 2H, J(1′,2′) = 5.2 (H-1′); 3.82 dt, 1H, J(2′a,1′) = 4.8, J_g = 9.9 (H-2′a); 3.68 dt, 1H, J(2′b,1′) = 5.7, J_g = 10.7 (H-2′b); 3.46 m, 1H (H-3′); 1.80 ddd, 1H, J(4′a,3′) = 4.6, J_g = 14.9, J(4′a,P) = 19.6 (H-4′a); 1.61 m, 2H (H-4′b and H-5′a); 1.36 td, 1H, J(5′b,3′) = J(5′b,6′) = 7.1, J_g = 14.2 (H-5′b); 0.63 t, 3H, J(6′,5′) = 7.3 (H-6′). 13C NMR (DMSO-d_6): 155.59 (C-6); 151.88 (C-2); 149.32 (C-4); 141.24 (C-8); 118.44 (C-5); 76.12 (C-3′); 65.75 (C-2′); 43.17 (C-1′); 32.45 d, J(P,C) = 134.1 (C-4′); 26.96 d, J(P,C) = 5.7 (C-5′); 8.71 (C-6′). Anal. Calcd for $C_{11}H_{18}N_5O_4P.1/2H_2O$: C, 40.74; H, 5.91; N, 21.60. Found: C, 40.44; H, 5.85; N, 21.31. MS (ESI^-): m/z = 314 $[M-H]^-$.

5.12. 9-[2-(3-Phenyl-1-phosphonopropan-2-yloxy)ethyl] adenine (9c)

Starting from 6-chloropurine derivative **4c**, yield 48% in two steps. ^{1}H NMR (DMSO-d_6): 8.12 s, 1H and 7.95 s, 1H (H-2 and H-8); 7.31 br s, 2H (NH_2); 7.10 m, 3H and 7.00 m, 2H (Ar); 4.20 m, 2H (H-1′); 3.78 m, 2H and 3.63 ddd, 1H, J = 4.5, 6.9 and 10.6. (H-2′ and H-3′); 2.91 dd, 1H, J(5′a,3′) = 3.8, J_g = 13.5 (H-5′a); 2.65 dd, 1H, J(5′b,3′) = 7.0, J_g = 13.9 (H-5′b); 1.79 ddd, 1H, J(4′a,3′) = 5.2, J_g = 15.0, J(4′a,P) = 19.3 (H-4′a); 1.66 ddd, 1H, J(4′b,3′) = 7.4, J_g = 15.0, J(4′b,P) = 17.4 (H-4′b). 13C NMR (DMSO-d_6): 155.60 (C-6); 151.80 (C-2); 149.24 (C-4); 141.13 (C-8); 138.23 (Ar); 129.20, 2C (Ar); 127.70, 2C (Ar); 125.70 (Ar); 118.49 (C-5); 76.24 (C-3′); 66.22 (C-2′); 43.12 (C-1′); 40.31 d, J(P,C) = 7.0 (C-5′); 32.53 d, J(P,C) = 132.0 (C-4′). Anal. Calcd for $C_{16}H_{20}N_5O_4P.4/5H_2O$: C, 49.05; H, 5.56; N, 17.88. Found: C, 48.98; H, 5.65; N, 17.87. MS (ESI^-): m/z = 376 $[M-H]^-$.

5.13. Diethyl 9-[2-(3-benzyloxy-1-phosphonopropan-2-yloxy) ethyl]adenine (8d)

Starting from 6-chloropurine derivative **4d**, intermediate, yield 67%. ^{1}H NMR (DMSO-d_6): 8.13 s, 1H and 8.11 s, 1H (H-2 and H-8); 7.28 m, 5H, 2H (Ar); 7.19 s, 2H (NH_2); 4.39 d, 2H, J = 3.7 (CH_2Ph); 4.27 dd, 2H, J(1′,2′) = 5.1, J_g = 8.3 (H-1′); 3.97 m, 2H (H-2′); 3.88 m, 6H (H-2′ and Et); 3.76 m, 1H (H-3′); 3.49 dd, 1H, J(5′a,3′) = 3.4, J_g = 10.5 (H-5′a); 3.41 dd, 1H, J(5′b,3′) = 6.0, J_g = 10.5 (H-5′b); 1.96 m, 2H (H-4′); 1.16 t, 6H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 155.78 (C-6); 152.14 (C-2); 149.37 (C-4); 141.02 (C-8); 138.10 (Ar); 128.05, 2C (Ar); 127.20, 3C (Ar); 118.48 (C-5); 74.05 (C-3′); 72.13 (CH_2Ph); 71.73 d, J(P,C) = 10.7 (C-5′); 67.08 (C-2′); 60.80 m, 2C (Et); 43.07 (C-1′); 27.57 d, J(P,C) = 138.2 (C-4′); 16.02 m (Et). MS (ESI): m/z = 464 $[M+H]^+$.

5.14. 9-[2-(3-Benzyloxy-1-phosphonopropan-2-yloxy)ethyl] adenine ($_{9d}$) and 9-[2-(3-Hydroxy-1-phosphonopropan-2-yloxy)ethyl]adenine (*9e*)

Starting from intermediate **8d**, yield 45% of **9d** and 27% of debenzylated product **9e**.

9d: ^{1}H NMR (DMSO-d_6): 8.14 s, 1H and 8.13 s, 1H (H-2 and H-8); 7.28 m, 5H, 2H (Ar); 7.22 s, 2H (NH_2); 4.37 d, 2H, J = 3.7 (CH_2Ph); 4.28 t, 2H, J(1′,2′) = 5.2 (H-1′); 3.86 m, 2H (H-2′); 3.79 m, 1H (H-3′); 3.57 dd, 1H, J(5′a,3′) = 3.0, J_g = 10.6 (H-5′a); 3.40 dd, 1H, J(5′b,3′) = 6.0, J_g = 10.6 (H-5′b); 1.77 m, 2H (H-4′). ^{13}C NMR (DMSO-d_6): 155.58 (C-6); 151.82 (C-2); 149.27 (C-4); 141.22 (C-8); 138.26 (Ar); 128.03, 2C (Ar); 127.11, 3C (Ar); 118.41 (C-5); 74.82 (C-3′); 72.32 (CH_2Ph); 72.15 d, J(P,C) = 10.6 (C-5′); 66.96 (C-2′); 43.14 (C-1′); 30.22 d, J(P,C) = 134.1 (C-4′). Anal. Calcd for

$C_{17}H_{22}N_5O_5P.3/2H_2O$: C, 47.00; H, 5.80; N, 16.12. Found: C, 47.20; H, 5.86; N, 16.26. MS (ESI^-): m/z = 406 $[M-H]^-$.

9e: ^1H NMR (DMSO-d_6): 8.19 s, 1H and 8.13 s, 1H (H-2 and H-8); 7.28 s, 2H (NH_2); 4.27 t, 2H, $J(1',2')$ = 5.2 (H-1′); 3.83 dd, 2H, $J(2',1')$ = 5.4, J_g = 8.7 (H-2′); 3.61 m, 1H (H-3′); 3.49 dd, 1H, $J(5'a,3')$ = 3.8, J_g = 11.5 (H-5′a); 3.33 dd, 1H, $J(5'b,3')$ = 6.1, J_g = 11.5 (H-5′b); 1.71 m, 2H (H-4′). ^{13}C NMR (DMSO-d_6): 155.61 (C-6); 151.84 (C-2); 149.26 (C-4); 141.32 (C-8); 118.38 (C-5); 76.80 (C-3′); 67.05 (C-2′); 63.60 d, J(P,C) = 8.2 (C-5′); 43.17 (C-1′); 30.44 d, J(P,C) = 133.9 (C-4′). Anal. Calcd for $C_{10}H_{16}N_5O_5P.3/4H_2O$: C, 35.20; H, 5.51; N, 20.52. Found: C, 35.49; H, 5.35; N, 20.39. MS (ESI^-): m/z = 316 $[M-H]^-$.

5.15. 9-[2-(1-Phosphonopropan-2-yloxy)ethyl]diaminopurine (14)

Starting from 2-amino-6-chloropurine derivative **5a**[10] the same procedure was used as above; yield 54% in two steps. ^1H NMR (DMSO-d_6): 7.76 s, 1H and 7.19 s, 1H (H-2 and H-8); 6.32 br s, 2H (NH_2); 4.10 t, 2H, $J(1',2')$ = 5.1 (H-1′); 3.66 t, 2H, $J(2',1')$ = 5.1 (H-2′); 3.65 m, 1H (H-3′); 1.86 ddd, 1H, $J(4'a,3')$ = 4.5, J_g = 14.8, $J(4'a,P)$ = 19.2 (H-4′a); 1.57 ddd, 1H, $J(4'b,3')$ = 8.7, J_g = 14.8, $J(4'b,P)$ = 17.4 (H-4′b); 1.14 d, 3H, $J(3',5')$ = 6.0 (H-5′). 13C NMR (DMSO-d_6): 159.01 (C-2); 155.63 (C-6); 150.70 (C-4); 138.03 (C-8); 112.71 (C-5); 71.60 (C-3′); 65.67 (C-2′); 42.76 (C-1′); 35.56 d, J(P,C) = 131.7 (C-4′); 20.85 d, J(P,C) = 5.8 (C-5′). Anal. Calcd for $C_{10}H_{17}N_6O_4P{\cdot}H_2O$: C, 35.93; H, 5.73; N, 25.14. Found: C, 35.89; H, 5.55; N, 24.96. MS (ESI^-): m/z = 315 $[M-H]^-$.

5.16. Transformation of 6-chloropurine and 2-amino-6-chloropurine derivatives 4d and 5d to hypoxanthine and guanine derivatives 10d and 11d – general procedure

The 6-chloro derivative **4d** or **5d** (2 mmol) was dissolved in trifluoroacetic acid (75%, 20 ml) and stirred overnight. The solvent was evaporated and the residue co-distilled with water (3×) and ethanol. The crude mixture was purified by chromatography on silica gel (chloroform–MeOH).

5.17. Diethyl 9-[2-(3-benzyloxy-1-phosphonopropan-2-yloxy) ethyl]hypoxanthine (10d)

Starting from **4d**, yield 67%. ^1H NMR (DMSO-d_6): 12.30 br s, 1H (NH); 8.11 s, 1H and 8.04 s, 1H (H-2 and H-8); 7.28 m, 5H (Ar); 4.40 d, 2H, J = 3.5 (CH_2Ph); 4.28 dd, 2H, $J(1',2')$ = 4.9, J_g = 8.8 (H-1′); 3.91 m, 6H (H-2′ and Et); 3.76 m, 1H (H-3′); 3.49 dd, 1H, $J(5'a,3')$ = 3.4, J_g = 10.5 (H-5′a); 3.41 dd, 1H, $J(5'b,3')$ = 5.9, J_g = 10.5 (H-5′b); 1.96 m, 2H (H-4′); 1.17 t, 6H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 156.36 (C-6); 148.14 (C-4); 145.30 (C-2); 140.46 (C-8); 138.08 (Ar); 128.05, 2C (Ar); 127.23 (Ar); 127.19, 2C (Ar); 123.40 (C-5); 74.07 (C-3′); 72.13 (CH_2Ph); 71.78 d, J(P,C) = 10.5 (C-5′); 67.16 (C-2′); 60.80 m, 2C (Et); 43.53 (C-1′); 27.55 d, J(P,C) = 138.3 (C-4′); 16.00 m (Et). MS (ESI): m/z = 465 $[M+H]^+$.

5.18. Diethyl 9-[2-(3-benzyloxy-1-phosphonopropan-2-yloxy) ethyl]guanine (11d)

Starting from **5d**, yield 61%. ^1H NMR (DMSO-d_6): 10.70 br s, 1H (NH); 7.88 s, 1H (H-8); 7.26 m, 5H (Ar); 6.54 s, 2H (NH_2); 4.42 d, 2H, J = 2.8 (CH_2Ph); 4.08 dd, 2H, $J(1',2')$ = 5.0, J_g = 8.0 (H-1′); 3.93 m, 4H (Et); 3.82 t, 2H, $J(2',1')$ = 5.2 (H-2′); 3.75 m, 1H (H-3′); 3.51 dd, 1H, $J(5'a,3')$ = 3.4, J_g = 10.5 (H-5′a); 3.42 dd, 1H, $J(5'b,3')$ = 6.0, J_g = 10.5 (H-5′b); 1.97 m, 2H (H-4′); 1.18 t, 6H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 156.18 (C-6); 153.60 (C-2); 150.77 (C-4); 138.09 (Ar); 137.62 (C-8); 128.07, 2C (Ar); 127.24, 3C (Ar); 118.29 (C-5); 74.07 (C-3′); 72.17 (CH_2Ph); 71.76 d, J(P,C) = 10.6 (C-5′); 67.01 (C-2′); 60.83 m, 2C (Et); 43.07 (C-1′); 27.55 d, 2C, J(P,C) = 137.9 (C-4′); 16.40 m, J(P,C) = 5.9 (Et). MS (ESI): m/z = 480 $[M+H]^+$.

5.19. Debenzylation of 6d, 7d, 10d and 11d – general procedure

Benzyl derivative (1.5 mmol) was hydrogenated at atmospheric pressure in methanol (20 ml, 0.1 ml of 4.5 M HCl in DMF added) over 10% palladium on charcoal (0.2 g) under stirring at room temperature for 20 h. The mixture was filtered through a pad of Celite, the catalyst was washed with hot water and hot methanol (100 ml each) and the solvent was evaporated to obtain the crude product in quantitative yield.

5.20. Diethyl 9-[2-(3-hydroxy-1-phosphonopropan-2-yloxy) ethyl]thymine (6e)

Starting from **6d**. ^1H NMR (DMSO-d_6): 11.22 br s, 1H (NH); 7.52 s, 1H (H-6); 3.95 m, 4H (Et); 3.76 t, 2H, $J(1',2')$ = 5.2 (H-1′); 3.66 t, 2H, $J(2',1')$ = 5.2 (H-2′); 3.55 m, 1H (H-3′); 3.44 dd, 1H, $J(5'a,3')$ = 4.3, J_g = 11.4 (H-5′a); 3.37 dd, 1H, $J(5'b,3')$ = 5.6, J_g = 11.4 (H-5′b); 1.92 m, 2H (H-4′); 1.21 t, 6H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 164.15 (C-2); 150.77 (C-4); 142.03 (C-6); 107.65 (C-5); 75.94 (C-3′); 66.69 (C-2′); 63.02 d, J(P,C) = 11.3 (C-5′); 60.76 m, 2C (Et); 47.15 (C-1′); 27.50 d, 2C, J(P,C) = 138.3 (C-4′); 16.07 d, J(P,C) = 5.7 (Et); 11.70 (CH_3). MS (ESI): m/z = 365 $[M+H]^+$.

5.21. Diethyl 9-[2-(3-hydroxy-1-phosphonopropan-2-yloxy) ethyl]uracil (7e)

Starting from **7d**. ^1H NMR (DMSO-d_6): 11.23 br s, 1H (NH); 7.60 d, J = 7.9, 1H (H-6); 5.50 d, 1H, J = 7.9 (H-5); 4.75 t, 1H, J = 5.6 (OH); 3.96 m, 4H (Et); 3.79 t, 2H, $J(1',2')$ = 5.1 (H-1′); 3.67 t, 2H, $J(2',1')$ = 5.1 (H-2′); 3.55 m, 1H (H-5′a); 3.44 m, 1H (H-5′b); 1.92 m, 2H (H-4′); 1.21 t, 6H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 163.60 (C-2); 150.81 (C-4); 146.26 (C-6); 100.12 (C-5); 76.04 (C-3′); 66.69 (C-2′); 63.06 d, J(P,C) = 11.5 (C-5′); 60.78 m, 2C (Et); 47.50 (C-1′); 27.52 d, 2C, J(P,C) = 138.4 (C-4′); 16.08 m, J(P,C) = 5.9 (Et). MS (ESI): m/z = 351 $[M+H]^+$.

5.22. Diethyl 9-[2-(3-hydroxy-1-phosphonopropan-2-yloxy) ethyl]hypoxanthine (10e)

Starting from **10d**. ^1H NMR (DMSO-d_6): 8.11 s, 1H and 8.03 s, 1H (H-2 and H-8); 4.83 t, 1H, J = 5.2 (OH); 4.26 dd, 2H, $J(1',2')$ = 5.2, J_g = 8.8 (H-1′); 3.91 m, 4H (Et); 3.86 t, 2H, J = 4.5 (H-2v); 3.58 m, 1H (H-3v); 3.40 m, 2H (H-5); 1.89 m, 2H (H-4′); 1.17 t, 6H, J = 7.0 (Et).^{13}C NMR (DMSO-d_6): 156.46 (C-6); 148.17 (C-4); 145.21 (C-2); 140.56 (C-8); 123.62 (C-5); 75.98 (C-3′); 67.14 (C-2′); 63.00 d, J(P,C) = 11.6 (C-5′); 60.75 m, 2C (Et); 43.50 (C-1′); 27.48 d, J(P,C) = 138.4 (C-4′); 16.02 m (Et). MS (ESI): m/z = 375 $[M+H]^+$.

5.23. Diethyl 9-[2-(3-hydroxy-1-phosphonopropan-2-yloxy) ethyl]guanine (11e)

Starting from **11d**. ^1H NMR (DMSO-d_6): 11.69 br s, 1H (NH); 8.97 s, 1H (H-8); 7.28 s, 2H (NH_2); 4.21 t, 2H, $J(1',2')$ = 3.5 (H-1′); 3.91 m, 6H (H-2′ and Et); 3.59 m, 1H (H-3′); 3.45 dd, 1H, $J(5'a,3')$ = 3.6, J_g = 11.5 (H-5va); 3.35 dd, 1H, $J(5'b,3')$ = 5.6, J_g = 11.5 (H-5′b); 1.93 m, 2H (H-4′); 1.18 t, 6H, J = 7.0 (Et).^{13}C NMR (DMSO-d_6): 155.13 (C-6); 153.64 (C-2); 149.67 (C-4); 137.62 (C-8); 116.29 (C-5); 75.99 (C-3′); 65.83 (C-2′); 62.93 d, 2C, J(P,C) = 12.3 (Et); 60.77 d, J(P,C) = 13.4 (C-5′); 44.30 (C-1′); 27.32 d, J(P,C) = 138.8 (C-4′); 16.02 d, 2C, J(P,C) = 5.8 (Et). MS (ESI^-): m/z = 390 $[M-H]^-$.

5.24. Diethyl 1-[2-(1-phosphonopropan-2-yloxy)ethyl]cytosine (17)

Compound **7a** (470 mg, 1.4 mmol), Et_3N (0.6 mL), and 2,4,6-triisopropylbenzenesulfonyl chloride (1.56 g, 5.08 mmol) were stirred in CH_3CN (20 mL) at room temp. for 3 days. NH_4OH (25%, 5 mL) was added, the mixture was stirred for 24 h, and the solvents were evaporated. The residue in ethyl acetate was washed with brine, the aqueous fraction was than washed with five portions of $CHCl_3$, and the organic fractions were dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography to give 350 mg (75%). ^{1}H NMR (DMSO-d_6): 7.50 d, 1H, J(6,5) = 7.2 (H-6); 5.61 d, 1H, J(5,6) = 7.2 (H-5); 3.96 m, 4H (Et); 3.74 dd, 2H, J(1′,2′) = 11.7 and 5.7 (H-1′); 3.63 m, 1H (H-3′); 3.54 t, 2H, J(2′,1′) = 5.2 (H-2′); 2.01, 1H (H-4′a); 1.85 m, 1H (H-4′b); 1.20 t, 6H (Et); 1.15 d, 3H, J(3′,5′) = 6.1 (H-5′). 13C NMR (DMSO-d_6): 165.76 (C-4); 155.48 (C-2); 146.99 (C-6); 92.53 (C-5); 70.63 (C-3′); 65.29 (C-2′); 60.75 dd, J(P,C) = 6.3 and 12.3 (Et); 48.63 (C-1′); 32.25 d, J(P,C) = 135.7 (C-4′); 20.64 d, J(P,C) = 7.3 (C-5′); 16.09 d, J(P,C) = 5.4 (Et). MS (ESI): m/z = 334 $[M+H]^+$.

5.25. Synthesis of phosphonic acids 12d,e, 13d,e, 15a,e, 16a,e and 18 – general procedure

A mixture of diethyl ester (1 mmol), acetonitrile (20 ml) and $BrSiMe_3$ (1 ml) was stirred overnight at room temperature. After evaporation and co-distillation with acetonitrile, the residue was treated with aqueous ammonia (2.5%) and evaporated to dryness. This residue was applied onto a column of Dowex 1 × 2 (acetate, 50 ml), washed with water followed by a gradient of acetic acid (0–1 M) and formic acid (0.25–1 M). The main UV-absorbing fraction was evaporated and co-distilled with water to obtain product as a white solid.

5.26. 9-[2-(3-Benzyloxy-1-phosphonopropan-2-yloxy)ethyl] hypoxanthine (12d)

Starting from **10d**, yield 55%. ^{1}H NMR (DMSO-d_6): 12.56 br s, 1H (NH); 8.50 s, 1H and 8.12 s, 1H (H-2 and H-8); 7.27 m, 5H (Ar); 4.39 d, 2H, J = 3.6 (CH_2Ph); 4.32 t, 2H, J = 5.1 (H-1′); 3.87 m, 2H (H-2′); 3.78 m, 1H (H-3′); 3.56 dd, 1H, J(5′a,3′) = 3.0, J_g = 10.5 (H-5′a); 3.41 dd, 1H, J(5′b,3′) = 6.4, J_g = 10.5 (H-5′b); 1.76 m, 2H (H-4′). 13C NMR (DMSO-d_6): 155.59 (C-6); 147.80 (C-4); 146.11 (C-2); 140.46 (C-8); 138.21 (Ar); 128.03, 2C (Ar); 127.17 (Ar); 127.11, 2C (Ar); 121.53 (C-5); 74.73 (C-3′); 71.30 d, J(P,C) = 10.4 (C-5′); 72.10 (CH_2Ph); 66.72 (C-2′); 43.97 (C-1′); 30.33 d, J(P,C) = 137.8 (C-4′). Anal. Calcd for $C_{17}H_{21}N_4O_6P.1/4H_2O$: C, 49.46; H, 5.25; N, 13.57. Found: C, 49.12; H, 5.07; N, 13.91. MS (ESI^-): m/z = 407 $[M-H]^-$ As a side product 30% of **12e** was isolated.

5.27. 9-[2-(3-Hydroxy-1-phosphonopropan-2-yloxy)ethyl] hypoxanthine (12e)

Starting from **10e**, yield 76%. ^{1}H NMR (DMSO-d_6): 12.46 br s, 1H (NH); 8.40 s, 1H and 8.09 s, 1H (H-2 and H-8); 4.30t, 2H, J = 5.1 (H-1′); 3.84 t, 2H, J = 4.3 (H-2′); 3.59 m, 1H (H-3′); 3.49 dd, 1H, J(5′a,3′) = 3.9, J_g = 11.5 (H-5′a); 3.33 dd, 1H, J(5′b,3′) = 6.1, J_g = 11.5 (H-5′b); 1.71 m, 2H (H-4′). 13C NMR (DMSO-d_6): 155.89 (C-6); 147.90 (C-4); 145.81 (C-2); 140.58 (C-8); 122.17 (C-5); 76.62 (C-3′); 66.71 (C-2′); 63.54 d, J(P,C) = 8.7 (C-5′); 43.81 (C-1′); 30.31 d, J(P,C) = 134.4 (C-4′). Anal. Calcd for $C_{10}H_{15}N_4O_6P.1/2MeOH$: C, 37.73; H, 5.13; N, 16.76. Found: C, 37.42; H, 5.41; N, 16.68. MS (ESI^-): m/z = 317 $[M-H]^-$.

5.28. 9-[2-(3-Benzyloxy-1-phosphonopropan-2-yloxy)ethyl] guanine (13d)

Starting from **11d**, yield 92%. ^{1}H NMR (DMSO-d_6): 10.55 br s, 1H (NH); 7.71 s, 1H (H-8); 7.30 m, 5H (Ar); 6.45 s, 2H (NH_2); 4.41 q, 2H, J = 12.10 (CH_2Ph); 4.06 t, 2H, J(1′,2′) = 5.1 (H-1′); 3.78 m, 3H (H-2′ and H-3′); 3.59 dd, 1H, J(5′a,3′) = 2.7, J_g = 10.5 (H-5′a); 3.42 dd, 1H, J(5′b,3′) = 6.5, J_g = 10.5 (H-5′b); 1.78 m, 2H (H-4′). 13C NMR (DMSO-d_6): 156.54 (C-6); 153.35 (C-2); 150.89 (C-4); 138.27 (Ar); 137.68 (C-8); 128.08, 2C (Ar); 127.16, 3C (Ar); 116.00 (C-5); 74.75 (C-3′); 72.29 d, J(P,C) = 7.1 (C-5′); 72.13 (CH_2Ph); 66.97 (C-2′); 42.83 (C-1′); 30.30 d, J(P,C) = 134.5 (C-4′). Anal. Calcd for $C_{17}H_{22}N_5O_6P.1/2H_2O$: C, 47.22; H, 5.36; N, 16.20. Found: C, 47.20; H, 5.16; N, 16.26. MS (ESI^-): MS (ESI^-): m/z = 422 $[M-H]^-$.

5.29. 9-[2-(3-Hydroxy-1-phosphonopropan-2-yloxy)ethyl] guanine (13e)

Starting from **11e**, yield 81%. ^{1}H NMR (DMSO-d_6): 10.76 br s, 1H (NH); 8.02 s, 1H (H-8); 6.59 s, 2H (NH_2); 4.09 t, 2H, J(1′,2′) = 5.0 (H-1′); 3.77 t, 2H, J(2′,1′) = 5.0 (H-2′); 3.59 m, 1H (H-3′); 3.50 dd, 1H, J(5′a,3′) = 3.8, J_g = 11.4 (H-5′a); 3.35 dd, 1H, J(5′b,3′) = 6.0, J_g = 11.4 (H-5′b); 1.73 m, 2H (H-4′). 13C NMR (DMSO-d_6): 156.02 (C-6); 153.72 (C-2); 150.69 (C-4); 137.82 (C-8); 116.09 (C-5); 76.65 (C-3′); 66.69 (C-2′); 63.55 m, (C-5′); 43.19 (C-1′); 30.38 d, J(P,C) = 128.8 (C-4′). Anal. Calcd for $C_{14}H_{23}N_4O_6P{\cdot}H_2O$: C, 34.19; H, 5.17; N, 19.94. Found: C, 34.36; H, 4.96; N, 19.85. MS (ESI^-): m/z = 332 $[M-H]^-$.

5.30. 1-[2-(1-Phosphonopropan-2-yloxy)ethyl]thymine (15a)

Starting from **6a**, yield 90%. ^{1}H NMR (DMSO-d_6): 11.22 br s, 1H (NH); 7.47 s, 1H (H-6); 3.75 m, 2H (H-1′); 3.65 m, 1H (H-3′); 3.53 t, 2H, J(2′,1′) = 3.3 (H-2′); 1.90 m, 1H (H-4′a); 1.74 s, 3H (CH_3); 1.60 m, 1H (H-4′b); 1.16 d, 3H, J(3′,5′) = 6.1 (H-5′).13C NMR (DMSO-d_6): 164.16 (C-4); 150.74 (C-2); 142.08 (C-6); 107.62 (C-5); 71.36 (C-3′); 64.89 (C-2′); 47.11 (C-1′); 35.25 d, J(P,C) = 132.4 (C-4′); 20.77 d, J(P,C) = 4.9 (C-5′); 11.69 (CH_3-5). Anal. Calcd for $C_{10}H_{17}N_2O_6P$: C, 41.10; H, 5.86; N, 9.59. Found: C, 39.85; H, 5.93; N, 9.69. MS (ESI^-): m/z = 291 $[M-H]^-$.

5.31. 9-[2-(3-Hydroxy-1-phosphonopropan-2-yloxy)ethyl] thymine (15e)

Starting from **6e**, yield 73%. ^{1}H NMR (DMSO-d_6): 11.20 br s, 1H (NH); 7.54 s, 1H (H-6); 3.75 t, 2H, J(1′,2′) = 5.2 (H-1′); 3.64 t, 2H, J(2′,1′) = 5.2 (H-2′); 3.57 m, 1H (H-3′); 3.50 dd, 1H, J(5′a,3′) = 3.9, J_g = 11.4 (H-5′a); 3.35 dd, 1H, J(5′b,3′) = 5.9, J_g = 11.4 (H-5′b); 1.71 m, 2H (H-4′). 13C NMR (DMSO-d_6): 164.17 (C-2); 150.80 (C-4); 142.17 (C-6); 107.60 (C-5); 76.61 (C-3′); 66.52 (C-2′); 63.52 d, J(P,C) = 10.0 (C-5′); 47.15 (C-1′); 30.37 d, 2C, J(P,C) = 134.3 (C-4′); 11.70 (CH_3). Anal. Calcd for $C_{10}H_{17}N_2O_7P.1/2H_2O$: C, 37.86; H, 5.72; N, 8.83. Found: C, 37.62; H, 5.61; N, 8.57. MS (ESI^-): m/z = 307 $[M-H]^-$.

5.32. 1-[2-(1-Phosphonopropan-2-yloxy)ethyl]uracil (16a)

Starting from **7a**, yield 87%. ^{1}H NMR (DMSO-d_6): 7.60 d, 1H, J(6,5) = 7.8 (H-6); 5.50 d, 1H, J(5,6) = 7.8 (H-5); 3.78 dd, 2H, J(1′,2′) = 8.9 and 4.8 (H-1′); 3.60 m, 1H (H-3′); 3.51 dd, 2H, J(2′,1′) = 8.9 and 4.8 (H-2′); 1.75 m, 1H (H-4′a); 1.37 m, 1H (H-4′b); 1.15 d, 3H, J(3′,5′) = 6.0 (H-5′). 13C NMR (DMSO-d_6): 163.60 (C-4); 150.77 (C-2); 146.42 (C-6); 99.99 (C-5); 72.42 (C-3′); 64.45 (C-2′); 47.41 (C-1′); 36.68 d, J(P,C) = 129.5 (C-4′); 20.96 d, J(P,C) = 3.1 (C-5′). Anal. Calcd for $C_9H_{15}N_2O_6P{\cdot}NH_3$: C, 36.61; H, 6.15; N, 14.23. Found: C, 36.47; H, 6.08; N, 14.03. MS (ESI^-): m/z = 277 $[M-H]^-$.

5.33. 9-[2-(3-Hydroxy-1-phosphonopropan-2-yloxy)ethyl]uracil (16e)

Starting from **7e**, yield 66%. ^{1}H NMR (DMSO-d_6): 11.33 br s, 1H (NH); 7.74 d, J = 7.9, 1H (H-6); 5.59 d, 1H, J = 7.8 (H-5); 3.90 t, 2H, J(1′,2′) = 5.1 (H-1′); 3.75 t, 2H, J(2′,1′) = 5.1 (H-2′); 3.61 dd, 1H, J(5′a,3′) = 3.9, J_g = 11.4 (H-5′a); 3.46 dd, 1H, J(5′b,3′) = 5.9, J_g = 11.4 (H-5′b); 1.83 m, 2H (H-4′). 13C NMR (DMSO-d_6): 163.60 (C-2); 150.81 (C-4); 146.26 (C-6); 100.12 (C-5); 76.04 (C-3′); 66.69 (C-2′); 63.06 d, J(P,C) = 11.5 (C-5′); 60.78 m, 2C (Et); 47.50 (C-1′); 27.52 d, 2C, J(P,C) = 138.4 (C-4′); 16.08 m, J(P,C) = 5.9 (Et). Anal. Calcd for $C_9H_{15}N_2O_7P.1/2H_2O$: C, 35.65; H, 5.32; N, 9.24. Found: C, 35.24; H, 5.20; N, 8.97. MS (ESI): m/z = 351 $[M+H]^+$.

5.34. 1-[2-(1-Phosphonopropan-2-yloxy)ethyl]cytosine (18)

Starting from **17**, yield 38%. ^{1}H NMR (DMSO-d_6): 7.58 d, 1H, J(6,5) = 7.2 (H-6); 7.48 br s, 2H (NH_2); 5.67 d, 1H, J(5,6) = 7.2 (H-5); 3.76 dd, 2H, J(1′,2′) = 10.8 and 5.3 (H-1′); 3.62 m, 1H (H-3′); 3.52 t, 2H, J(2′,1′) = 5.2 (H-2′); 1.86 m, 1H (H-4′a); 1.55 m, 1H (H-4′b); 1.15 d, 3H, J(3′,5′) = 6.1 (H-5′). 13C NMR (DMSO-d_6): 164.84 (C-4); 154.39 (C-2); 147.28 (C-6); 92.55 (C-5); 71.47 (C-3′); 64.91 (C-2′); 48.62 (C-1′); 35.52 d, J(P,C) = 132.1 (C-4′); 20.81 d, J(P,C) = 4.5 (C-5′). Anal. Calcd for $C_9H_{16}N_3O_5P.1/2H_2O.1/2MeOH$: C, 38.35; H, 6.09; N, 14.50. Found: C, 38.20; H, 5.88; N, 14.22. MS (ESI^-): m/z = 276 $[M-H]^-$.

5.35. Antiviral activity assays

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strains Lyons and G, varicella-zoster virus (VZV) strain Oka, TK-VZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, human immunodeficiency virus (HIV) type 1 (III_B) and type 2 (ROD), respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, Parainfluenza 3, Reovirus-1, Sindbis, Punta Toro, feline coronavirus (FIPV), influenza A virus subtypes H1N1 and H3N2, and influenza B virus. The antiviral assays, other than HIV, were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey kidney cells (Vero), human epithelial cervix carcinoma cells (HeLa), Crandell-Rees feline kidney cells (CRFK), or Madin Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathicity or viral plaque formation by 50%. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/ml) were infected with 100 $CCID_{50}$ of HIV-1(IIIB) or HIV-2(ROD)/ml and seeded in 200-μL-wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

5.36. Cytotoxicity assays

Cytostatic activity measurements were based on the inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Alternatively, cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of HEL cell morphology.

Cytostatic activities against L1210 (murine leukemia), FM3A (murine mammary carcinoma) and CEM (human T-lymphoblast) cell lines were measured essentially as originally described for the mouse leukemia L1210 cell assays.[13]

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