European Journal of Medicinal Chemistry 44 (2009) 2408–2424

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Acyclic nucleoside bisphosphonates: Synthesis and properties of chiral 2-amino-4,6-bis[(phosphonomethoxy)alkoxy]pyrimidines

Petra Doláková*, Martin Dračínský, Milena Masojídková, Veronika Šolínová, Václav Kašička, Antonín Holý

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i. Flemingovo nám. 2, CZ-166 10 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 28 May 2008
Received in revised form 15 September 2008
Accepted 18 September 2008
Available online 2 October 2008

Keywords:
Acyclic nucleoside phosphonates
Pyrimidine
Bisphosphonates
Alkylation

ABSTRACT

2-Amino-4,6-bis[(phosphonomethoxy)alkoxy]pyrimidines bearing two equal or different achiral or chiral phosphonoalkoxy chains have been prepared either by aromatic nucleophilic substitution of 2-amino-4,6-dichloropyrimidine or by alkylation of 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine with appropriate phosphonate-bearing building block. Alkylation of 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine proved to be the method of choice for efficient preparation of variety of bisphosphonates. The enantiomeric purity of selected compounds was determined by capillary electrophoresis. Antiviral activity of bisphosphonates is discussed.

1. Introduction

Acyclic nucleoside phosphonates (ANPs) [1] represent a key class of nucleotide analogs with a broad spectrum of antiviral and cytostatic activity. Among ANPs, particularly 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir, **1a**, Fig. 1) is active against DNA and retroviruses [2]; its prodrug, adefovir dipivoxil [3], was approved for hepatitis B therapy (Hepsera) [4]. 9-(*R*)-[2-(Phosphonomethoxy)propyl]adenine (PMPA, tenofovir, **1c**) is a promising anti-HIV drug; its prodrug Viread was approved for treatment of AIDS [5]. A third type of antiviral compounds is represented by 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC, cidofovir, Vistide, **2**) which possesses general anti-DNA-viral activity [6]. Cidofovir was approved for treatment of cytomegalovirus retinitis in AIDS patients. The 2,6-diaminopurine derivatives (**1b** and **1d**) and their guanine counterparts are potent antivirals and exhibit powerful antitumor activity [7].

We have recently described a new type of antiviral acyclic nucleoside phosphonates originating from 2-substituted 4-amino-6-hydroxypyrimidines [8]. Alkylation of 6-hydroxypyrimidines by phosphonate-bearing building block afforded a mixture of O^6- and N^1-regioisomer. While none of the isomeric 1-[2-(phosphonomethoxy)ethyl]pyrimidin-6-one derivatives **4** was antivirally active, compounds derived from 2,4-diamino-6-hydroxypyrimidine (**3a–d**) and 2-amino-4,6-dihydroxypyrimidine significantly inhibited replication of retroviruses and herpes viruses in cell culture. Compounds **3** can be considered as analogs of 2,6-diaminopurine with an open imidazole ring of the purine moiety. This structural relation is strongly supported by the finding that the corresponding analog of PMPDAP, i.e. 2,4-diamino-6-[2-(phosphonomethoxy)propoxy]pyrimidine (**3d**), has the same selective antiretroviral activity as (*R*)-PMPDAP (**1d**). This activity is also limited only to the (*R*)-enantiomer, while the (*S*)-enantiomer is similarly devoid of antiviral activity as is (*S*)-PMPDAP. Further SAR studies demonstrated that 5-substituted derivatives of 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine (**3a**) markedly inhibited retrovirus replication in cell culture [9]. The 5-methyl derivative **3b** was exquisitely inhibitory to human immunodeficiency virus and Moloney murine sarcoma virus-induced cytopathicity in cell culture but also cytostatic to CEM cell cultures. The 5-halogen-substituted derivatives (**3c**) showed pronounced antiretroviral activity, comparable to that of the reference drugs adefovir and tenofovir, but were devoid of any measurable toxicity.

The isomeric compounds **6a** and **7a** (Fig. 2) bearing two phosphonoalkoxy chains [8a] may be considered as a second group of "open-ring" ANPs and potential antivirals. Direct alkylation of 2-amino-4,6-dihydroxypyrimidine (**5**) with 2-(diisopropoxyphosphorylmethoxy)ethylchloride gave a mixture of disubstituted regioisomers **6a** and **7a** approximately in the same ratio. While 2-amino-4-[2-(phosphonomethoxy)ethoxy]-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (**7a**) was not antivirally active, 2-amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**6a**)

* Corresponding author. Tel.: +420 220 183 262; fax: +420 220 183 560.
E-mail address: dolakova@uochb.cas.cz (P. Doláková).

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doi:10.1016/j.ejmech.2008.09.031

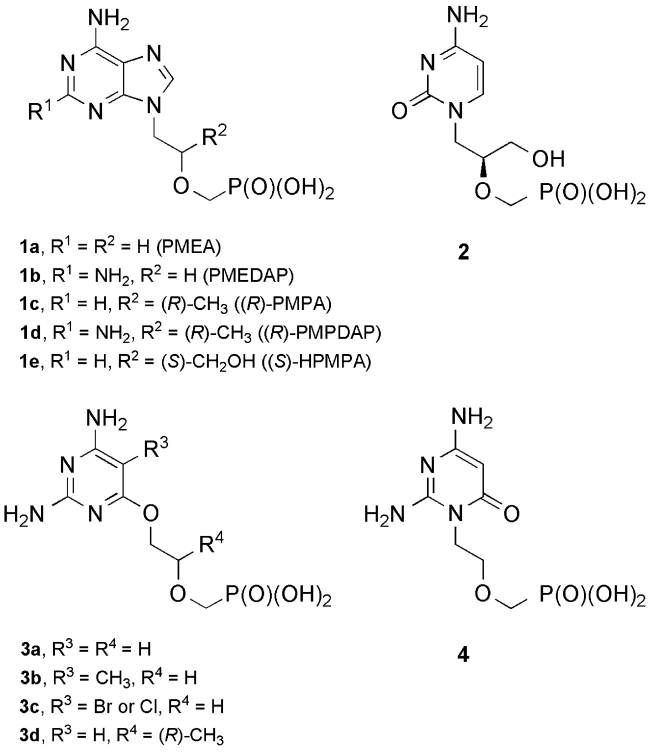


Fig. 1. Acyclic nucleoside phosphonates.

was reported to show antiretroviral activity [8a]. Another study concerning bisphosphonate derivatives **8** and **9** [10] was also performed in our laboratory.

In this paper, we describe efficient synthesis of series of bisphosphonates derived from 4,6-(dihydroxy)pyrimidine. We were interested in regioselective synthesis of *O*-alkylated pyrimidine derivatives bearing two equal or different chiral phosphonoalkoxy chains as potentially biologically active compounds from a large family of phosphonate antivirals.

2. Results and discussion

2.1. Stepwise synthesis of bisphosphonates from 2-amino-4,6-dichloropyrimidine

Synthesis of bisphosphonates bearing two 3-hydroxy-2-(phosphonomethoxy)propoxy (HPMPO) chains at positions 4 and 6 of the pyrimidine moiety started from 2-amino-4,6-dichloropyrimidine (**10**) (Scheme 1). While alkylation of 2-amino-4,6-dihydroxypyrimidine (**5**) afforded a mixture of *O*- and *N*-alkylated products **6a** and **7a** [8a], nucleophilic aromatic substitution of **10** with appropriate alkylating agent can give only *O*-regioisomer. Reaction of **10** with 2 equiv of (*S*)-1,2-isopropylideneglycerol (**11**) was performed in THF in the presence of NaH as a base; subsequent deprotection by diluted hydrochloric acid gave 2,3-dihydroxypropoxy derivative **12** in 64% yield. Primary hydroxyl groups were protected by treatment with 4,4′-dimethoxytrityl chloride and alkylation of secondary hydroxy groups by diisopropoxyphosphorylmethyl bromide followed by deprotection with acetic acid afforded bis-HPMPO derivative **13a**. Diisopropyl esters were cleaved under standard conditions (bromotrimethylsilane in acetonitrile, followed by hydrolysis) to afford free phosphonic acid **13b**. The enantiomer **14b** was prepared by the same procedure as compound **13b** from pyrimidine **10** and (*R*)-1,2-isopropylideneglycerol (**16**). The diastereoisomer **18b** was prepared by reaction of pyrimidine **10** with 1 equiv of **11** and 1 equiv of NaH in THF; the monoalkylated product **15** was treated with **16** under the same conditions to afford dihydroxypropoxy derivative **17**. Further procedure was identical with that described for compound **13b**. Compounds **13b**, **14b** and **18b** were purified by preparative HPLC; triethylammonium salts of phosphonic acids were converted to the free phosphonic acids on a column of Dowex 50 × 8 in H^+ form.

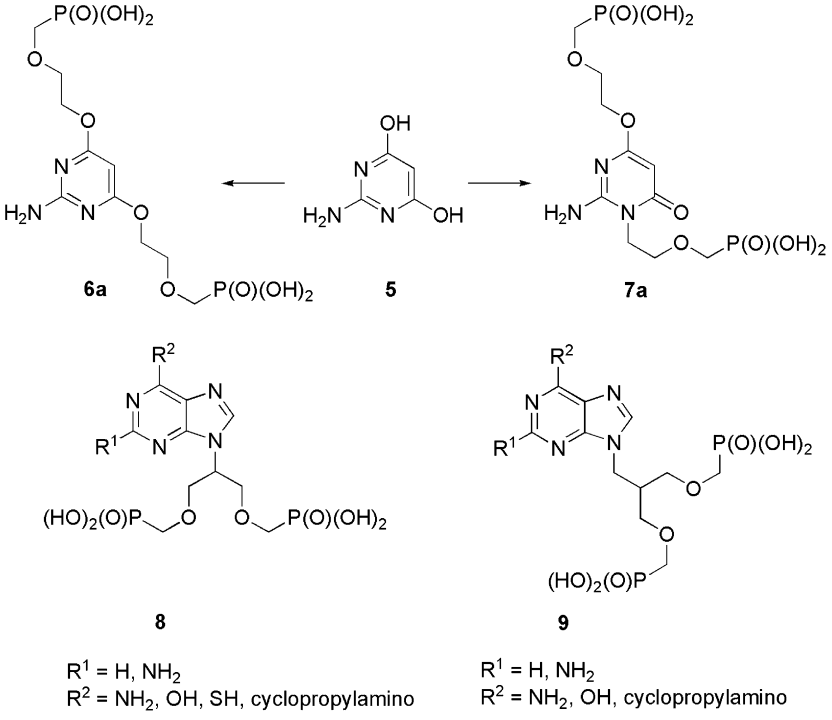
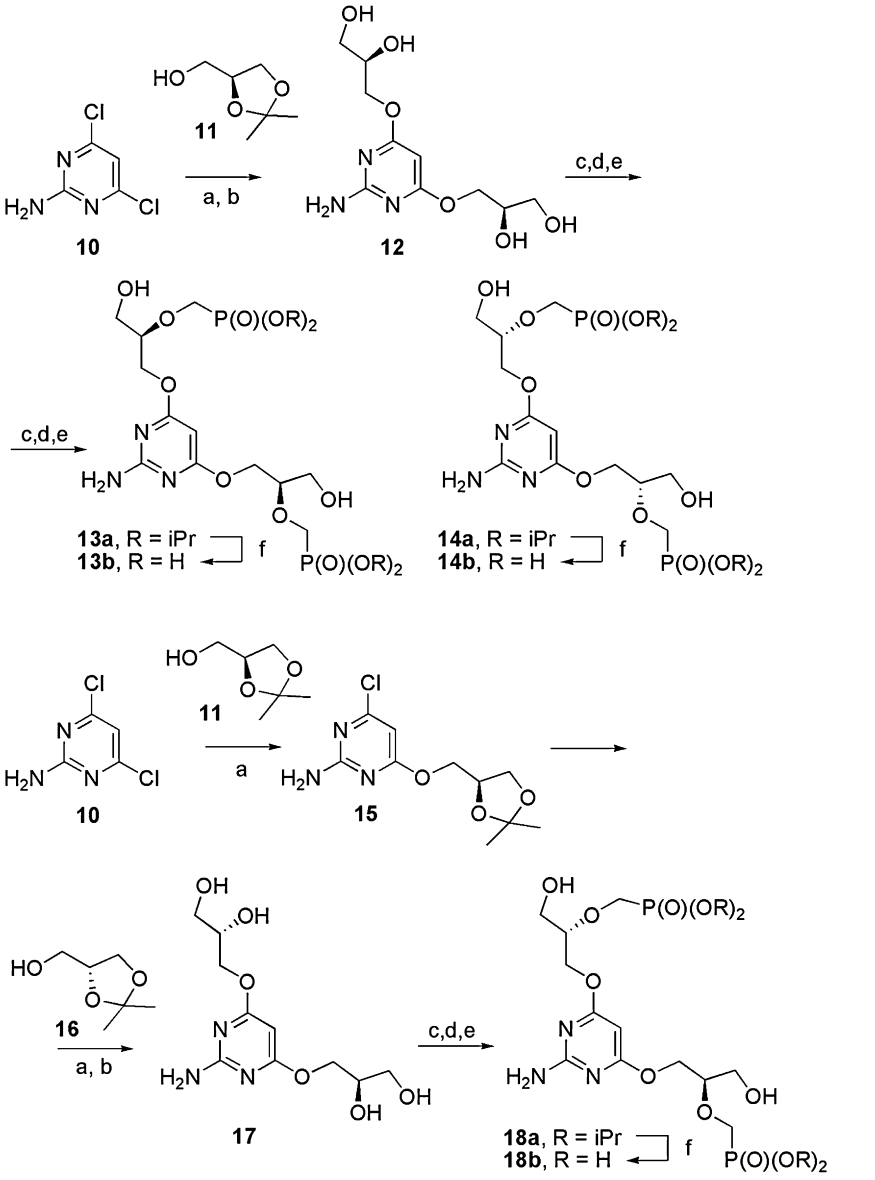


Fig. 2. Acyclic nucleoside bisphosphonates.



Scheme 1. Synthesis of bis-HPMPO derivatives.

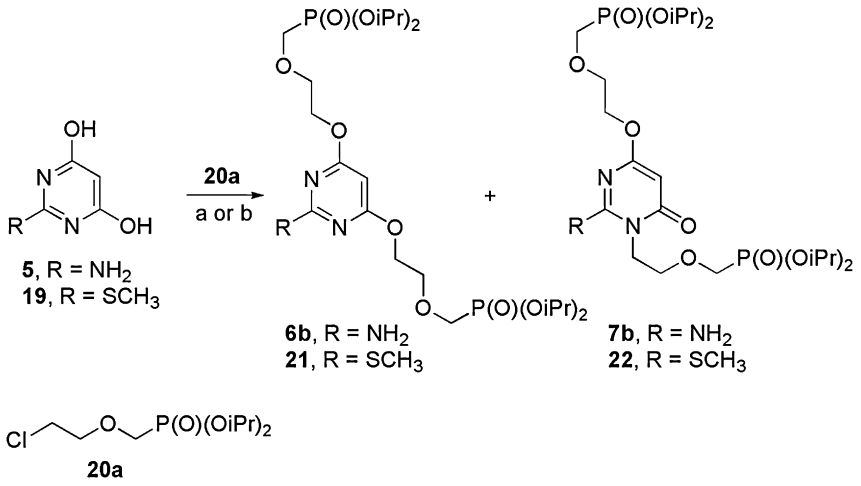
Synthesis of bisphosphonates bearing two 2-(phosphonomethoxy)propoxy (PMPO) chains or two different phosphonoalkoxy chains by this method failed. Reaction of **10** with phosphonoalkoxyalkanol gives only product of monosubstitution. 2-Amino-4-chloro-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine is further unreactive towards nucleophilic aromatic substitution. The ether bond at position 6 of the pyrimidine moiety is relatively labile and under harsh reaction conditions (basic, acidic or high temperature) decomposes. For details see Supporting information.

2.2. Synthesis of bisphosphonates from 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine

Since synthesis of bisphosphonates bearing two different chains from **10** failed, we focused on alkylation of dihydroxypyrimidines **5** and **19** with phosphonate **20a** [11] (Table 1). Previously described [8a] alkylation of 2-amino-4,6-dihydroxypyrimidine (**5**) in the presence of NaH as a base in DMF afforded a mixture of compounds **6b** and **7b** in approximately 1:1 ratio in a very low yield. Alkylation of **5** in DMSO slightly increased the yield. Alkylation of commercially available 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine (**19**) in DMSO gave *O*-alkylated regioisomer **21** as a major product in 54% yield; regioisomer **22** was formed in 12% yield (Scheme 2). Alkylation of **19** in DMF did not proceed at all because of poor solubility of disodium salt of pyrimidine **19** in DMF; this might be also a reason of a low yield in the reaction described in literature [8a]. Thus, the alkylation of pyrimidine **19** was the method of choice for the synthesis of bisphosphonates. This method afforded compound

Table 1
Substitution pattern of compounds **20**, **23** and **24**

Compd. **20**	R^1, R^2	Y	Compd. **23**, **24**	R^1
a	H	Cl	**23a**	H
b	(*S*)-CH_3	OTs	**23b**	(*S*)-CH_3
c	(*R*)-CH_3	OTs	**23c**	(*R*)-CH_3
d	(*S*)-CH_2OH	OTs	**24a**	(*S*)-CH_3
e	(*R*)-CH_2OH	OTs	**24b**	(*R*)-CH_3



Starting mat.	Product	R. conditions	Yield (%)
5	**6b**	a	5
5	**7b**	a	8
5	**6b**	b	12
5	**7b**	b	6
19	**21**	b	54
19	**22**	b	12

a) NaH, DMF; b) NaH, DMSO.

Scheme 2. Alkylation of 4,6-(dihydroxy)pyrimidines.

6a in 18% overall yield compared to the previously reported 3.5% yield; and moreover, formation of *N*-alkylated regioisomers was suppressed. The reaction sequence further allows introduction of modifications at positions 2, 4 and 6 of the 4,6-(dihydroxy)pyrimidine moiety in satisfactory yields.

Alkylation of pyrimidine **19** with 1 equiv of appropriate phosphonate **20** (Table 1) in DMSO afforded a mixture of mono- and dialkylated products **23** and **24** (Scheme 3, Table 1), respectively. Monoalkylated product **23** was subsequently alkylated by **20** in DMF to afford bis derivative bearing two different substituents **25a–i** (Table 2). 2-Methylsulfanyl group of compounds **24** and **25** (Table 2) was oxidized by *m*-CPBA in dichloromethane [12] to give 2-methylsulfonyl derivatives **26**, which were further converted to 2-amino congeners using liquid ammonia in THF at r.t. [13]. Final deprotection of diisopropyl esters by bromotrimethylsilane afforded free phosphonic acids **6a** and **27a–k**.

Compound **26j** upon treatment with primary or secondary amine [12b] and subsequent deprotection with bromotrimethylsilane afforded N^2-substituted bisphosphonates **28a–f** (Table 3). 2-Methylsulfonyl derivative **26j** was hydrolyzed by sodium hydroxide in a mixture of water and THF [13] to give 2-hydroxy derivative **29a**; treatment of **26j** with sodium methylate in methanol [14] gave 2-methoxy derivative **29b**. Treatment of **29a** and **29b** with bromotrimethylsilane led to the decomposition of pyrimidine derivatives. 2-Methylsulfanyl derivative **21** was reduced with Raney-Nickel [11] to afford 4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine, which was subsequently deprotected by bromotrimethylsilane to give free phosphonic acid **30**.

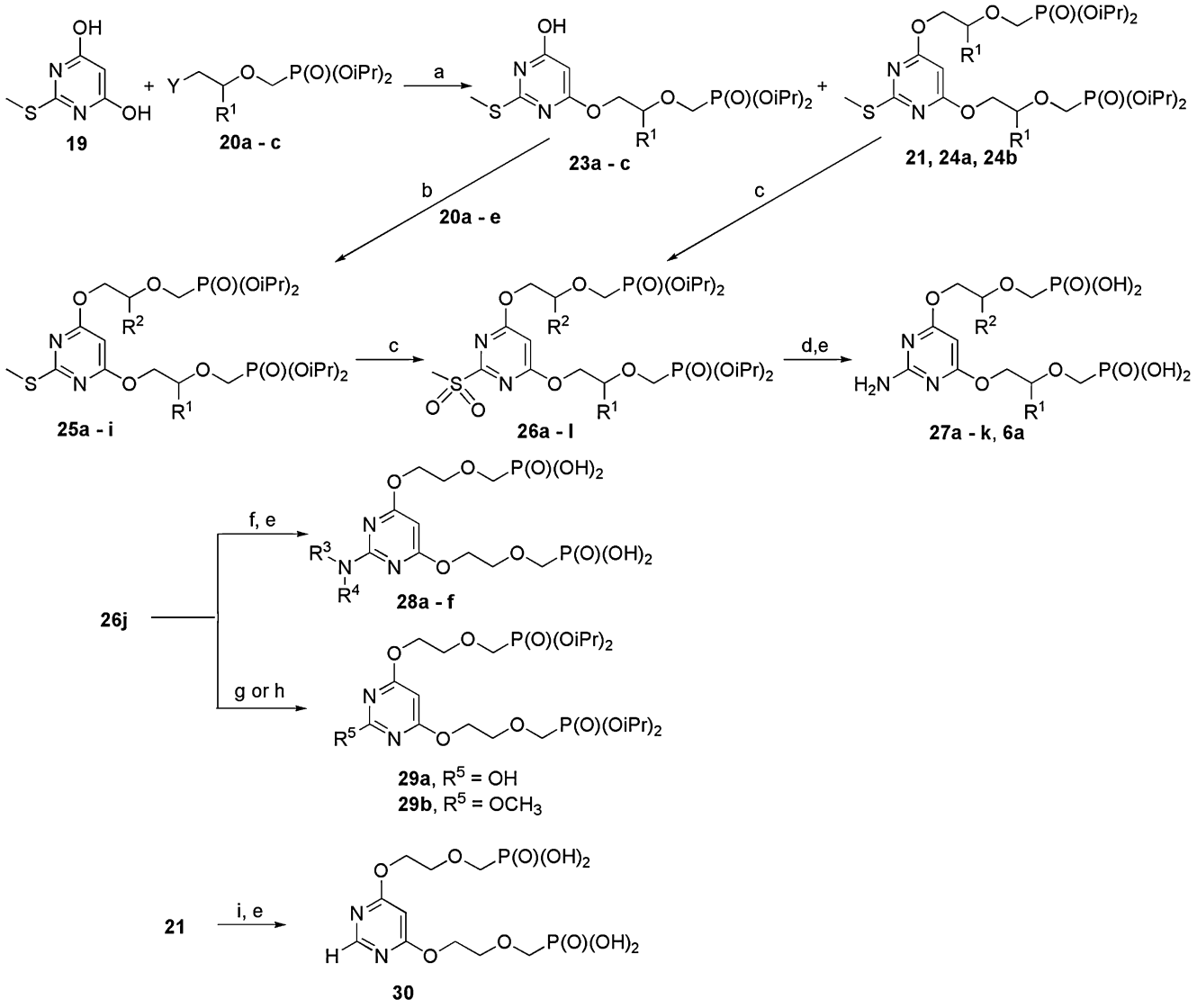
2.3. Bisphosphonates derived from 2-amino-4,6-disulfanylpyrimidne

Pyrimidine [15] **10** was converted to the disulfanyl analog **31** by reaction with thiourea (Scheme 4) [16]. Alkylation of **31** with phosphonates **20a–c** (Table 1) gave unequivocally *S*-alkylated products **32a–c** (Table 4). Sulfur derivatives are better nucleophiles than their oxygen and nitrogen analogs, so the alkylation of sulfur at positions 4 and 6 took place smoothly even at room temperature. Alkylation of pyrimidine **31** with 1 equiv of alkylating agent **20a** or **20b** gave monoalkylated products **34a** and **34b** together with dialkylated product. Further alkylation of **34a** and **34b** afforded pyrimidine with two different substituents **35a–c**. Diisopropyl esters of bisphosphonates **32a–c** and **35a–c** were cleaved by standard procedure with bromotrimethylsilane.

2.4. Alkoxyalkyl esters of bisphosphonates

For further biological activity screening lipid esters of compound **6a** and its 5-bromo and 5-methyl congener (Scheme 5) were prepared by the method described by J.R. Beadle and K.Y. Hostetler [17].

Pyrimidine **10** in neat ethyleneglycol in the presence of *t*-BuOK gave hydroxyethoxy derivative **37** in 71% yield (Scheme 5). Pyrimidine **37** in THF was treated with NaH, heated to 50 °C and then hexadecyloxyethyl toluenesulfonyloxymethylphosphonate (**38**) was added. Monoalkylated derivative **39a** was isolated together with bis derivative **40a**. Alkylation in DMF or in mixture of triethylamine and THF (1:1) gave lower yields; reaction in triethylamine [17] as solvent did not proceed at all. Bromination of **37** with elemental bromine in DMF/CCl_4 [9a] gave smoothly the 5-bromo derivative **41**. 5-Substituted derivatives **41** and **42** [Petr Jansa, unpublished results] were similarly converted to esters **39b** and **39c** and dialkylated products **40b** and **40c** by above described alkylation. Monoalkylated compounds **39a–c** were fully characterized and submitted for biological activity screening, however dialkylated products **40a–c** were nearly insoluble in any solvent; therefore their NMR spectra could not be measured. Thus compounds **40a–c** were characterized only by mass spectroscopy



Scheme 3. Synthesis of bisphosphonates by alkylation of 4,6-(dihydroxy)-2-(methylsulfanyl)pyrimidine.

and elemental analysis and were not tested for biological activity. Our attempts to convert compound **6a** to *cyclo*Sal, *cyclo*Amb [18] or POM [3] esters failed due to instability of ether bonds at positions 4 and 6 under reaction conditions.

Table 2
Substitution pattern of compounds **25**–**27**

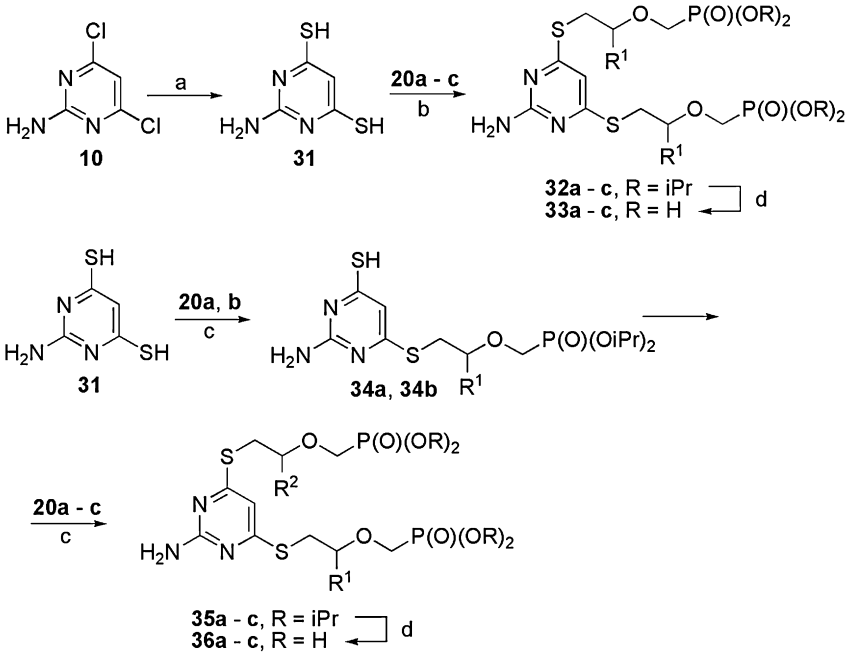
Compd. **25**, **26**	R^1	R^2	Product	R^1	R^2
25a, **26a**	H	(*S*)-CH_3	**27a**	H	(*S*)-CH_3
25b, **26b**	H	(*R*)-CH_3	**27b**	H	(*R*)-CH_3
25c, **26c**	H	(*S*)-CH_2OH	**27c**	H	(*S*)-CH_2OH
25d, **26d**	H	(*R*)-CH_2OH	**27d**	H	(*R*)-CH_2OH
25e, **26e**	(*S*)-CH_3	(*R*)-CH_3	**27e**	(*S*)-CH_3	(*R*)-CH_3
25f, **26f**	(*S*)-CH_3	(*S*)-CH_2OH	**27f**	(*S*)-CH_3	(*S*)-CH_2OH
25g, **26g**	(*S*)-CH_3	(*R*)-CH_2OH	**27g**	(*S*)-CH_3	(*R*)-CH_2OH
25h, **26h**	(*R*)-CH_3	(*S*)-CH_2OH	**27h**	(*R*)-CH_3	(*S*)-CH_2OH
25i, **26i**	(*R*)-CH_3	(*R*)-CH_2OH	**27i**	(*R*)-CH_3	(*R*)-CH_2OH
26j	H	H	**6a**	H	H
26k	(*S*)-CH_3	(*S*)-CH_3	**27j**	(*S*)-CH_3	(*S*)-CH_3
26l	(*R*)-CH_3	(*R*)-CH_3	**27k**	(*R*)-CH_3	(*R*)-CH_3

2.5. Capillary electrophoresis

Enantiomeric purity of compounds **27a** and **27b** was analyzed by capillary electrophoresis. Baseline separation of enantiomers **27a** and **27b**, with resolution 1.67, was achieved in chiral background electrolyte (BGE) composed of 50 mM borax, adjusted by NaOH to pH 10.0, with chiral selector β-cyclodextrin (20 mg/mL) (Fig. 3A). Compound **27a** was found enantiomerically pure as

Table 3
Substituents at position 2 of compound **28**

Compd. **28**	R^3	R^4
a	H	Cyclopropyl
b	H	Cyclopentyl
c	H	Methyl
d	H	Benzyl
e	H	4-Methoxybenzyl
f	–	Morpholino



a) 1. thiourea, 2. NaOH; b) 2 eq. NaH, DMF; c) 1 eq. NaH, DMF; d) $BrSiMe_3$, CH_3CN.

Scheme 4. Alkylation of 2-amino-4,6-(disulfanyl)pyrimidine.

demonstrated by single peak of CE analysis of this compound in chiral BGE (Fig. 3B) whereas a very small admixture of enantiomer **27a** was found in the CE analysis of compound **27b** (Fig. 3C).

It was confirmed that optically active phosphonates are stable and do not tend to racemize. No racemization did occur during the whole multistep synthesis from chiral precursors.

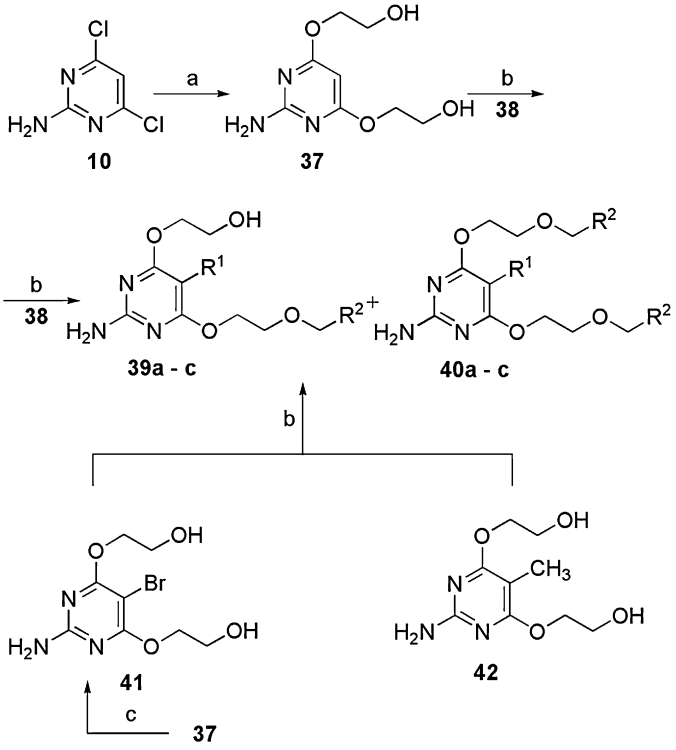
3. Conclusion

In conclusion, in the SAR studies of "open-ring" ANPs a series of bisphosphonates derived from 2-amino-4,6-(dihydroxy)pyrimidine were prepared. Bisphosphonates bearing two identical or different achiral or chiral phosphonoalkoxy chains were prepared either by nucleophilic aromatic substitution of 2-amino-4,6-dichloropyrimidine (**10**) or by alkylation of 4,6-(dihydroxy)-2-(methylsulfanyl)pyrimidine (**19**). The second method proved to be the universal method for regioselective preparation of *O*-alkylated pyrimidines at positions 4 and 6; furthermore 2-methylsulfanyl function is a versatile leaving group for introduction of various substituents at position 2 of the pyrimidine moiety. Disulfanylpyrimidine **31** was alkylated in the same manner to give exclusively *S*-alkylated product. Alkoxyalkyl esters of selected bisphosphonates were prepared to improve their bioavailability; however introduction of two lipid esters to bisphosphonates dramatically decreased their solubility. Enantiomeric purity of compounds **27a** and **27b** was successfully determined by capillary electrophoresis.

The whole series of bisphosphonates (**6a**, **13b**, **14b**, **18b**, **27a**–**k**, **28a**–**f**, **30**, **33a**–**c**, **36a**–**c** and **39a**–**c**) was investigated for their

Table 4
Substitution pattern of compounds **32**–**36**

Compd. **32**–**34**	R^1	Compd. **35**, **36**	R^1	R^2
a	H	**a**	H	(*S*)-CH_3
b	(*S*)-CH_3	**b**	H	(*R*)-CH_3
c	(*R*)-CH_3	**c**	(*S*)-CH_3	(*R*)-CH_3



38, $TsOCH_2P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$
39a, **40a**, R^1 = H, R^2 = $P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$
39b, **40b**, R^1 = Br, R^2 = $P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$
39c, **40c**, R^1 = CH_3, R^2 = $P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$

a) $HOCH_2CH_2OH$, *t*-BuOK; b) NaH, THF; c) Br_2/CCl_4/DMF.

Scheme 5. Synthesis of alkoxyalkyl esters of bisphosphonates.

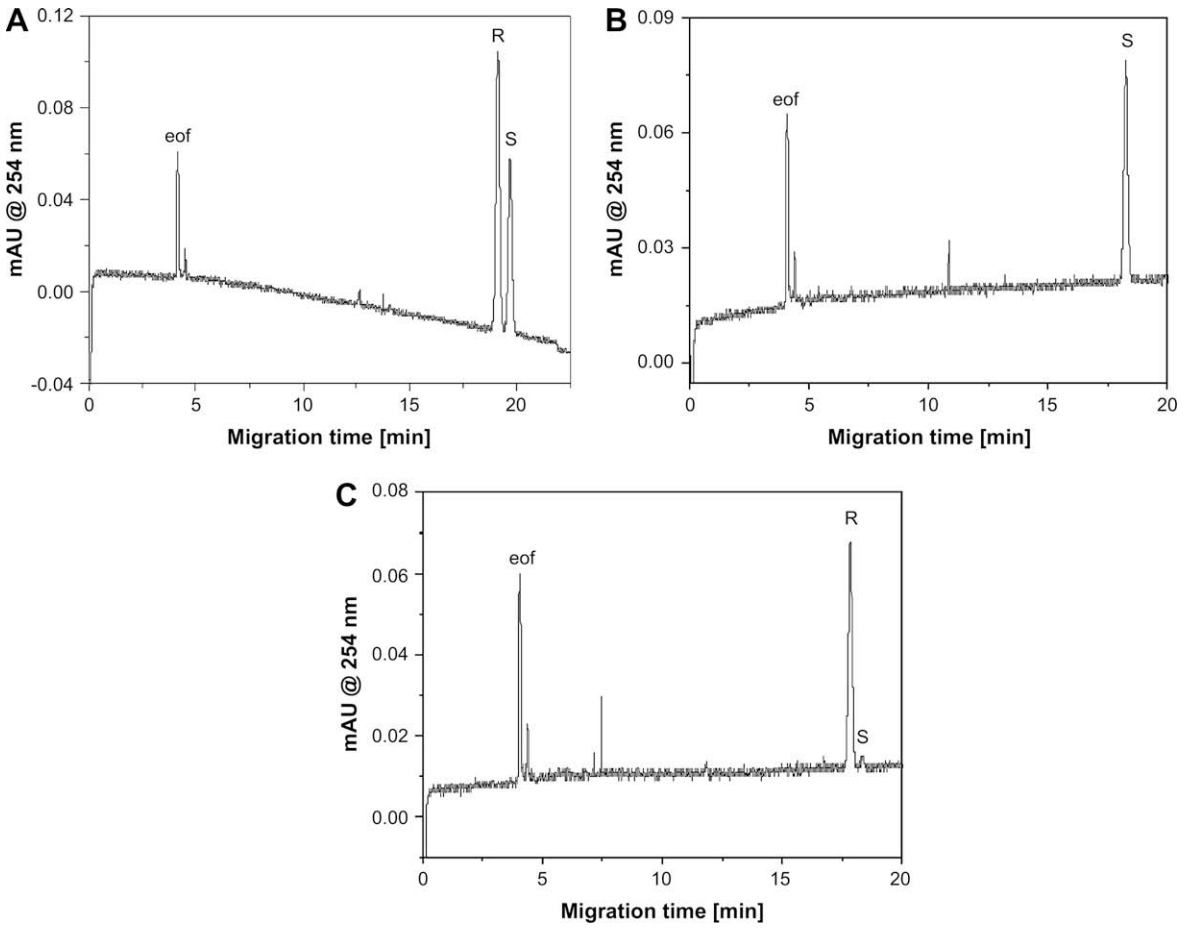


Fig. 3. A: CE separation of enantiomers **27a** (*S*), 0.1 mM and **27b** (*R*), 0.2 mM in chiral BGE (background electrolyte) composed of 50 mM borax, adjusted by NaOH to pH 10.0, with chiral selector β-cyclodextrin (20 mg/mL). B: CE analysis of enantiomer **27a** in the above chiral BGE. C: CE analysis of enantiomer **27b** in the above chiral BGE. eof = electroosmotic flow marker.

inhibitory activity against several DNA and retroviruses. None of the prepared bisphosphonates showed any appreciable antiviral activity. Finally, antiretroviral activity of resynthesized parent compound **6a** was not confirmed. The previously reported activity [8a] might probably had been caused by undetectable admixture of several orders more active monoalkylated 2-amino-4-hydroxy-6-[2-(phosphonomethoxy)ethoxy]pyrimidine. Compounds are devoid of any measurable toxicity to cell cultures.

4. Experimental section

4.1. Materials and general procedures

Solvents were dried by standard procedures. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone under argon. NMR spectra were recorded with Bruker Avance 500 (500 MHz for ^{1}H and 125.8 MHz for ^{13}C) and Bruker Avance 400 (^{1}H at 400, ^{13}C at 100.6 MHz) spectrometers in $CDCl_3$, DMSO-d_6, or D_2O. Chemical shifts (in ppm, δ scale) were referenced to TMS (for ^{1}H NMR spectra in $CDCl_3$) and/or to the solvent signal ($CDCl_3$ $\delta = 7.26$ ppm for ^{1}H NMR and $\delta = 77.0$ ppm for ^{13}C NMR; DMSO-d_6 for ^{1}H NMR $\delta = 2.5$ ppm and for ^{13}C $\delta = 39.7$). Chemical shifts in D_2O were referenced to 1,4-dioxane for ^{1}H NMR $\delta = 3.75$ and for ^{13}C NMR $\delta = 67.19$. Melting points were determined on a Büchi Melting Point B-545 apparatus and are uncorrected. TLC was performed on plates of Kieselgel 60 F254 (Merck). Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix) or on an LCQ classic spectrometer using electrospray ionization (ESI). Preparative HPLC purification was performed on a column packed with 10 μm C18 reversed phase (Luna), 250 × 21 mm; in ca. 300 mg portions of mixtures using linear gradient 0.1 M triethylammonium hydrogen carbonate (TEAB) in water and in 50% MeOH (linear gradient of TEAB in 50% MeOH, 0–100%).

All new compounds were fully characterized by mass spectrometry, elemental analysis or high resolution mass spectrometry (for intermediates) and NMR spectroscopy (including complete assignment of all NMR signals using a combination of H,H-COSY, H,C-HSQC, and H,C-HMBC methods). Diastereoisomers gave identical NMR spectra. To prove that we have two different diastereoisomers we prepared mixed samples of two diastereoisomers; two sets of signals were found for OCH_2-1′ protons in ^{1}H NMR spectra.

4.1.1. General procedure 1 (GP1) – deprotection of diisopropyl esters of bisphosphonates

Bisphosphonate (1 mmol) in acetonitrile (20 mL) was treated with bromotrimethylsilane (1.5 mL) at r.t. overnight. Volatiles were removed under reduced pressure, the residue was codistilled with water (3 × 50 mL) and 0.1 M TEAB (2 × 50 mL). Crude products were purified by preparative HPLC using linear gradient 0.1 M triethylammonium hydrogen carbonate in water and in 50% MeOH (linear gradient of TEAB in 50% MeOH, 0–100%) and triethylammonium salts of phosphonates were converted to free phosphonic acids by application onto a column of Dowex 50 × 8 in H^+ form and elution with water.

*4.1.2. General procedure 2 (GP2) – dialkylation of 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine (**19**) in DMSO*

Pyrimidine **19** (0.16 g, 1 mmol) in DMSO (5 mL) was treated with NaH (0.084 g, 60% in paraffin oil, 2.1 mmol) and heated at 80 °C for 30 min; appropriate phosphonates **20a–e** (2.1 mmol) were added and the resulting mixture was heated at 120 °C for 8–16 h. The mixture was cooled to r.t., DMSO was evaporated in vacuo at 60 °C. The residue was codistilled with DMF and EtOH, taken to $CHCl_3$ (100 mL) and washed with water (3 × 50 mL). Organic fraction was dried over $MgSO_4$ and evaporated in vacuo. The residue was separated by flash chromatography ($CHCl_3$/MeOH).

*4.1.3. General procedure 3 (GP3) – monoalkylation of 4,6-dihydroxy-2-(methylsulfanyl)-pyrimidine (**19**) in DMSO*

Pyrimidine **19** (0.16 g, 1 mmol) and NaH (0.04 g, 60% in paraffin oil, 1 mmol) in DMSO (5 mL) were heated at 60 °C for 30 min, phosphonates **20a–e** (1 mmol) in DMSO (1 mL) were added dropwise and the reaction mixture was heated at 120 °C for 8 h, cooled to r.t. and evaporated in vacuo at 60 °C. The residue was codistilled with DMF and EtOH, diluted with $CHCl_3$, washed with 3 portions of water and dried over $MgSO_4$. Products were separated by flash chromatography ($CHCl_3$/MeOH).

*4.1.4. General procedure 4 (GP4) – 2nd alkylation of monoalkylated product **23***

Pyrimidine **23** (1 mmol) and NaH (0.044 g, 60% in paraffin oil, 1.1 mmol) in DMF (5 mL) were heated at 40 °C for 30 min, appropriate phosphonates **20a–e** (1.1 mmol) were added and the mixture was stirred at 100 °C for 8–12 h, evaporated in vacuo, and codistilled with EtOH.

4.1.5. General procedure 5 (GP5) – oxidation of 2-methylsufanyl group to 2-methylsulfonyl group by m-*CPBA*

2-Methylsulfanyl derivative (1 mmol) in CH_2Cl_2 (6 mL) was treated with *m*-chloroperoxybenzoic acid (3 mmol) at r.t. for 3–12 h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $Na_2S_2O_5$, saturated $NaHCO_3$ and water. Organic fraction was dried over $MgSO_4$ and purified by flash chromatography ($CHCl_3$/MeOH).

4.1.6. General procedure 6 (GP6) – ammonolysis of 2-methylsulfonyl group to amino group

To a cooled (−78 °C) and stirred solution of compound **26** (1 mmol) in dry THF (30 mL), in a pressure tube, 20–30 mL of liquid ammonia was added. The pressure tube was sealed and allowed to warm to r.t. and the reaction mixture was stirred for 5–12 h. The reaction mixture was concentrated in vacuo and the crude product in $CHCl_3$ was applied onto a pad of silica gel and washed with 10% MeOH in $CHCl_3$ (150 mL).

*4.1.7. General procedure 7 (GP7) – alkylation of hydroxyethoxy derivatives **37**, **41** and **42** with phosphonate **38***

2-Hydroxyethoxy derivative (0.25 mmol), NaH (30 mg, 60% in paraffin oil, 0.75 mmol) and 4-dimethylaminopyridine (6 mg) in THF were heated at 50 °C for 30 min and phosphonate **38** (292 mg, 0.525 mmol) was added in one portion. The resulting mixture was heated at 70 °C for 16 h and evaporated in vacuo. The residue in $CHCl_3$ (50 mL) was washed with brine (2 × 50 mL) and water (1 × 50 mL) and evaporated under reduced pressure. Flash chromatography (EtOAc:EtOH:acetone:H_2O, 6:1:1:0.5 and EtOAc:EtOH:acetone:H_2O, 4:1:1:1) gave compounds **39** and **40**.

*4.2. 2-Amino-4,6-(2*R*,2′*R*)-bis(1,2-dihydroxypropoxy)pyrimidine (**12**)*

(*S*)-1,2-Isopropylideneglycerol (**11**) (7.53 mL, 61 mmol) in THF (15 mL) was added dropwise to a stirred suspension of NaH (2.5 g, 60% in paraffin oil, 61 mmol) in THF (80 mL) at r.t. After stirring for 1 h, pyrimidine **10** (5 g, 30.5 mmol) was added in one portion and the reaction mixture was refluxed for 6 h. After cooling to r.t., solvent was removed under reduced pressure and the residue was dissolved in hot $CHCl_3$ and filtered through Celite. Chromatography on silica gel ($CHCl_3$/MeOH 0–2%) afforded intermediate 2-amino-4,6-(*S*,*S*)-bis(2,2-dimethyl-4-methoxy-1,3-dioxolane)pyrimidine (10.1 g, 93%) as a yellow oil. ^{1}H NMR (DMSO-d_6): $\delta = 6.60$ (br s, 2H, NH_2), 5.36 (s, 1H, H-5), 4.33 (m, 2H, H-2′), 4.22 (dd, $J(1'a,2') = 4.6$, $J_{gem} = 11.1$, 2H, H-1′a), 4.16 (dd, $J(1'b,2') = 6.3$, $J_{gem} = 11.1$, 2H, H-1′b), 4.04 (dd, $J(3'a,2') = 6.4$, $J_{gem} = 8.4$, 2H, H-3′a), 3.68 (dd, $J(3'b,2') = 6.2$, $J_{gem} = 8.4$, 2H, H-3′b), 1.33 and 1.28 (2 × s, 2 × 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.19$ (2C, C-4 and C-6), 162.73 (C-2), 108.96 (2C, $CHMe_2$), 78.56 (C-5), 73.59 (2C, C-2′), 66.37 and 65.93 (2 × 2C, C-1′, C-3′), 26.81 and 25.52 (2 × 2C, CH_3) ppm. MS (FAB): m/z (%) = 356.1 (85) $[MH]^+$. Anal. $C_{16}H_{25}N_3O_6$ (C, H, N).

Solution of the intermediate (9 g, 25.3 mmol) in methanol/water mixture (1:4, 100 mL) was acidified with hydrochloric acid to pH 2 and stirred for 4 h at r.t. Reaction mixture was applied onto a column of Dowex 50 × 8, washed with water until neutral reaction of eluate and eluted with 2.5% ammonia. UV absorbing eluate was collected and evaporated. Crystallization from ethanol/ether mixture afforded **12** as a white solid (4.74 g, 66%); m.p. 110 °C. ^{1}H NMR (DMSO-d_6): $\delta = 6.50$ (br s, 2H, NH_2), 5.32 (s, 1H, H-5), 4.90 and 4.63 (2 × br s, 2 × 2H, OH), 4.17 (dd, $J(1'a,2') = 4.3$, $J_{gem} = 10.9$, 2H, H-1′a), 4.06 (dd, $J(1'b,2') = 6.6$, $J_{gem} = 10.9$, 2H, H-1′b), 3.72 (m, 2H, H-2′), 3.38 (d, $J(3',2') = 4.0$, 4H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.62$ (2C, C-4, C-6), 162.82 (C-2), 78.58 (C-5), 69.89 (2C, C-2′), 67.73 (2C, C-1′), 62.97 (C-3′) ppm. MS (FAB): m/z (%) = 276.1 (100) $[MH]^+$. Anal. $C_{10}H_{17}N_3O_6.½H_2O$ (C, H, N).

*4.3. 2-Amino-4,6-(2*R*,2′*R*)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]pyrimidine (**13a**)*

Compound **12** (3 g, 11.7 mmol) in pyridine (400 mL) was treated with DMTrCl (11.95 g, 35 mmol) and the mixture was stirred for 3 h. The reaction was quenched by addition of EtOH and the solvent was removed in vacuo. The residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$, and the separated organic layer was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue in DMF (150 mL) was treated with NaH (1 g, 60% suspension in mineral oil, 25 mmol) at 0 °C and stirred for 30 min; diisopropoxyphosphorylmethyl bromide (6.25 g, 25 mmol) was added and the mixture was stirred at r.t. overnight; the solvent was removed in vacuo and the residue was dissolved in 80% acetic acid (100 mL). After stirring at r.t. for 1 h, acetic acid was evaporated and the residue was codistilled with water. Flash chromatography in $CHCl_3$/MeOH (0–3%) afforded colorless oil (2.6 g, 39%). ^{1}H NMR (DMSO-d_6): $\delta = 6.57$ (br s, 2H, NH_2), 5.29 (s, 1H, H-5), 4.78 (t, $J(OH,3') = 5.4$, 2H, OH), 4.58 (m, 4H, CHipr.), 4.32 (dd, $J(1'a,2') = 3.6$, $J_{gem} = 11.5$, 2H, H-1′a), 4.19 (dd, $J(1'b,2') = 6.1$, $J_{gem} = 11.5$, 2H, H-1′b), 3.90 (dd, $J(P,CH) = 8.7$, $J_{gem} = 13.8$, 2H) and 3.86 (dd, $J(P,CH) = 8.9$, $J_{gem} = 13.8$, 2H, PCH_2), 3.69 (m, 2H, H-2′), 3.51 (t, $J(3',2') = J(3',OH) = 5.4$, 4H, H-3′), 1.235 (d, 6H), 1.23 (d, 6H), 1.22 (d, 6H) and 1.21 (d, $J(CH_3,CH) = 6.2$, 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.34$ (2C, C-4, C-6), 162.77 (C-2), 80.53 (d, 2C, $J(P,C) = 11.7$, C-2′), 78.46 (C-5), 70.37 (d, 2C) and 70.32 (d, 2C, $J(P,C) = 6.3$, CHipr.), 65.25 (2C, C-1′), 63.99 (d, 2C, $J(P,C) = 164.6$, PC), 60.08 (2C, C-3′), 23.99 (d, 4C, $J(P,C) = 3.9$) and 23.82 (d, 4C, $J(P,C) = 4.4$, CH_3) ppm. MS (FAB): m/z (%) = 632.6 (56) $[MH]^+$. Anal. $C_{24}H_{47}N_3O_{12}P_2$ (C, H, N, P).

*4.4. 2-Amino-4,6-(2*R*,2′*R*)-bis[2-(phosphonomethoxy)-3-hydroxypropoxy]pyrimidine (**13b**)*

Compound **13a** (2 g, 3.17 mmol) was deprotected by GP1 to give **13b** (1.05 g, 71%) as colorless foam. ^{1}H NMR (D_2O): $\delta = 4.56$ (dd,

J(1′a,2′) = 3.8, J_{gem} = 11.2, 2H, H-1′a), 4.43 (dd, J(1′b,2′) = 5.6, J_{gem} = 11.2, 2H, H-1′b), 3.94 (m, 2H, H-2′), 3.89 (dd, 2H) and 3.84 (dd, J(P,CH) = 9.3, J_{gem} = 13.3, 2H, PCH_2), 3.82 (dd, J(3′a,2′) = 4.3, J_{gem} = 12.2, 2H, H-3′a), 3.75 (dd, J(3′b,2′) = 5.7, J_{gem} = 12.2, 2H, H-3′b) ppm. ^{13}C NMR (D_2O): δ = 169.14 (2C, C-4, C-6), 155.56 (C-2), 79.62 (C-5), 79.57 (d, J(P,C) = 11.2, 2C, C-2′), 68.16 (2C, C-1′), 65.74 (d, J(P,C) = 158.7, 2C, PC), 60.11 (2C, C-3′) ppm. MS (FAB): m/z (%) = 464 (49) $[MH]^+$. $[\alpha]_D^{25} = -1.0$ (c 0.502, H_2O). Anal. $C_{12}H_{23}N_3O_{12}P_2.H_2O$ (C, H, N, P).

4.5. 2-Amino-4,6-(2S,2′S)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]pyrimidine (**14a**)

Prepared from **10** and **16** by the same procedure as compound **13a**. 2-Amino-4,6-(2*R*,2′*R*)-bis(2,2-dimethyl-4-methoxy-1,3-dioxolane)pyrimidine, yellow oil, yield 9.6 g (89%). ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with (2*S*,2′*S*) enantiomer. MS (FAB): m/z (%) = 356.0 (54) $[MH]^+$. Anal. $C_{16}H_{25}N_3O_6$ (C, H, N).

2-Amino-4,6-(2*S*,2′*S*)-bis(1,2-dihydroxypropoxy)pyrimidine, white solid, yield 5 g (70%); m.p. 99 °C. ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with (2*R*,2′*R*) enantiomer. MS (FAB): m/z (%) = 276.0 (80) $[MH]^+$. Anal. $C_{10}H_{17}N_3O_6.½H_2O$ (C, H, N).

2-Amino-4,6-(2*S*,2′*S*)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]pyrimidine (**14a**), colorless oil, yield 2.2 g (33%). ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with **13a**. MS (FAB): m/z (%) = 632.6 (100) $[MH]^+$. Anal. $C_{24}H_{47}N_3O_{12}P_2$ (C, H, N, P).

4.6. 2-Amino-4,6-(2S,2′S)-bis[2-(phosphonomethoxy)-3-hydroxypropoxy]pyrimidine (**14b**)

Prepared from **14a** by the same procedure as compound **13b**. Colorless foam, yield 0.95 g (64%). ^{1}H NMR (D_2O) and ^{13}C NMR (D_2O) identical with **13b**. MS (FAB): m/z (%) = 464 (15) $[MH]^+$. $[\alpha]_D^{25} = +1.9$ (c 0.267, H_2O). Anal. $C_{12}H_{23}N_3O_{12}P_2.H_2O$ (C, H, N, P).

4.7. 2-Amino-4-chloro-6-(2S)-(2,2-dimethyl-4-methoxy-1,3-dioxolane)pyrimidine (**15**)

Compound **11** (12.34 mL, 100 mmol) was added dropwise to the suspension of NaH (4 g, 60% suspension in mineral oil, 100 mmol) in THF (130 mL); the mixture was stirred for 1 h and pyrimidine **10** (16.4 g, 100 mmol) was added in one portion. Reaction mixture was heated at reflux for 6 h, cooled to r.t. and evaporated in vacuo. The residue in chloroform was washed with brine; the organic extract was dried over magnesium sulfate and evaporated. Chromatography in chloroform/methanol (0–3%) afforded 23.1 g (89%) of compound **15** as a white solid, m.p. 130 °C. ^{1}H NMR (DMSO-d_6): δ = 7.10 (br s, 2H, NH_2), 6.11 (s, 1H, H-5), 4.35 (m, 1H, H-2′), 4.28 (dd, J(1′a,2′) = 4.4, J_{gem} = 11.0, 1H, H-1′a), 4.21 (dd, J(1′b,2′) = 6.3, J_{gem} = 11.0, 1H, H-1′b), 4.04 (dd, J(3′a,2′) = 6.5, J_{gem} = 8.6, 1H, H-3′a), 3.70 (dd, J(3′b,2′) = 6.0, J_{gem} = 8.6, 1H, H-3′b), 1.32 and 1.27 (2 × s, 2 × 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.48 (C-6), 162.95 (C-2), 160.21 (C-4), 109.05 ($CHMe_2$), 94.48 (C-5), 73.35 (C-2′), 66.85 and 65.82 (C-1′, C-3′), 26.79 and 25.51 (CH_3) ppm. MS (ESI): m/z (%) = 282.43 (100) $[MNa]^+$, 260.0 (37) $[MH]^+$. Anal. $C_{10}H_{14}ClN_3O_3$ (C, H, Cl, N).

4.8. 2-Amino-4,6-(2R,2′S)-bis(1,2-dihydroxypropoxy)pyrimidine (**17**)

Compound **16** (1.43 mL, 11.6 mmol) was added dropwise to the suspension of NaH (0.46 g, 60% suspension in mineral oil, 11.6 mmol) in THF (10 mL); the mixture was stirred for 1 h and compound **15** (3 g, 11.6 mmol) in THF (5 mL) was added. Reaction mixture was heated at reflux for 6 h, filtered through Celite while hot, Celite was washed with chloroform and combined organic extracts were evaporated in vacuo. Flash chromatography in chloroform/methanol (0–2%) gave protected intermediate [2-amino-4,6-(2*R*,2′*S*)-bis(2,2-dimethyl-4-methoxy-1,3-dioxolane)pyrimidine, 2.95 g, 72%] as a yellow oil. ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with 2-amino-4,6-(2*S*,2′*S*)-bis(2,2-dimethyl-4-methoxy-1,3-dioxolane)pyrimidine. MS (FAB): m/z (%) = 356.0 (100) $[MH]^+$. Anal. $C_{16}H_{25}N_3O_6$ (C, H, N).

The intermediate was deprotected by the same procedure as was described for compound **12**. White solid, yield 69%; m.p. 116 °C. ^{1}H NMR (DMSO-d_6): δ = 6.51 (br s, 2H, NH_2), 5.32 (s, 1H, H-5), 4.90 and 4.65 (2 × br s, 2 × 2H, OH), 4.17 (dd, J(1′a,2′) = 4.3, J_{gem} = 10.9, 2H, H-1′a), 4.06 (dd, J(1′b,2′) = 6.6, J_{gem} = 10.9, 2H, H-1′b), 3.72 (m, 2H, H-2″), 3.38 (d, J(3′,2′) = 4.0, 4H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.62 (2C, C-4, C-6), 162.82 (C-2), 78.58 (C-5), 69.89 (2C, C-2′), 67.73 (2C, C-1′), 62.97 (C-3′) ppm. MS (FAB): m/z (%) = 276.1 (100) $[MH]^+$. Anal. $C_{10}H_{17}N_3O_6.½H_2O$ (C, H, N).

4.9. 2-Amino-4,6-(2S,2′R)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]pyrimidine (**18a**)

Prepared from compound **17** by the same procedure as was described for **13a**, colorless oil, yield 2.7 g (41%). ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with **13a**. MS (FAB): m/z (%) = 632.1 (100) $[MH]^+$. Anal. $C_{24}H_{47}N_3O_{12}P_2$ (C, H, N, P).

4.10. 2-Amino-4,6-(2S,2′R)-bis[2-(phosphonomethoxy)-3-hydroxypropoxy]pyrimidine (**18b**)

Prepared by the same procedure as compound **13b**, colorless foam, yield 63%. ^{1}H NMR (D_2O) and ^{13}C NMR (D_2O) identical with **13b**. MS (FAB): m/z (%) = 464 (35) $[MH]^+$. $[\alpha]_D^{25} = +0.02$ (c 0.383, H_2O). Anal. $C_{12}H_{23}N_3O_{12}P_2.H_2O$ (C, H, N, P).

4.11. 4,6-Bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]-2-(methylsulfanyl)pyrimidine (**21**) and 4-[2-(diisopropoxyphosphorylmethoxy)ethoxy]-1-[2-(diisopropoxyphosphorylmethoxy)ethyl]-2-(methylsulfanyl)pyrimidine-6(1H)-one (**22**)

GP2, pyrimidine **19** (3 g, 19 mmol), phosphonate **20a** (10.6 g, 40 mmol), yield 6.15 g (54%) of **21**, colorless syrup. ^{1}H NMR (DMSO-d_6): δ = 5.88 (s, 1H, H-5), 4.58 (dh, J(CH,P) = 7.7, J(CH,CH_3) = 6.3, 4H, CHipr.), 4.43 (m, 4H, H-1′), 3.82 (m, 4H, H-2′), 3.79 (d, J(C,H,P) = 8.3, 4H, PCH_2), 2.48 (s, 3H, SCH_3), 1.23 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.61 (C-2), 170.26 (2C, C-4,6), 85.55 (C-5), 70.57 (d, J(2′,P) = 11.9, C-2′), 70.36 (d, J(CH,P) = 6.2, CHipr.), 65.67 (C-1′), 64.95 (d, J(C,P) = 164.6, PCH_2), 24.00 (d, J(CH_3,P) = 3.7) and 23.85 (d, J(CH_3,P) = 4.6, CH_3ipr.), 13.66 (SCH_3) ppm. MS (ESI): m/z (%) = 625.1 (100) $[MNa]^+$, 603 (15) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{10}P_2S$ $[MH]^+$ 603.2192, found 603.2185. Anal. $C_{23}H_{45}N_2O_{10}P_2S$ (C, H, N, P, S).

Further elution of column gave **22**, yield 1.35 g (12%), colorless syrup. ^{1}H NMR (DMSO-d_6): δ = 5.40 (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 4.31 (m, 2H, H-1″), 4.10 (t, J(1′,2′) = 5.4, 2H, H-1′), 3.82 (m, 2H, H-2″), 3.79 (d, 2H) and 3.76 (d, J(P,CH) = 8.3, 2H, PCH_2), 3.75 (t, J(2′,1′) = 5.4, 2H, H-2′), 2.54 (s, 3H, SCH_3), 1.24 (d, 6H), 1.23 (d, 6H), 1.22 (d, 6H) and 1.20 (d, J(CH_3,CH) = 6.2, 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.34 (C-2), 162.75 (C-4), 159.48 (C-6), 85.65 (C-5), 70.76 (d, J(C,P) = 11.9, C-2′), 70.44 (d, J(C,P) = 11.89, C-2″), 70.04 (m, CHipr.), 69.53 (d, J(C,P) = 6.61, CHipr.), 65.31 (C-1′), 64.80 (d, J(C,P) = 164.41, PCH_2′), 64.75 (d, J(C,P) = 163.72, PCH_2″), 43.69 (C-1″), 23.61 (m, CH_3ipr.), 14.43 (SCH_3) ppm. MS (ESI): m/z (%) = 625.1 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{10}P_2S$ $[MH]^+$ 603.2192, found 603.2193. Anal. $C_{23}H_{45}N_2O_{10}P_2S$ (C, H, N, P, S).

*4.12. 6-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-4-hydroxy-2-(methylsulfanyl)pyrimidine (**23a**)*

GP3, pyrimidine **19** (3 g, 19 mmol), phosphonate **20a** (4.8 g, 18.1 mmol), yield 2.67 g (37%) of **23a**, colorless syrup. ^{1}H NMR (DMSO-d_6): $\delta = 12.32$ (br s, 1H, OH), 5.39 (br s, 1H, H-5), 4.59 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.8, 2H, CHipr.), 4.33 (m, 2H, H-1′), 3.80 (m, 2H, H-2′), 3.79 (d, J(CH_2,P) = 8.3, 2H, CH_2P), 2.47 (s, 3H, SCH_3), 1.24 (d, J(CH_3,CH) = 6.0, 6H) and (1.22 d, J(CH_3,CH) = 6.0, 6H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 169.19$ (C-4), 86.06 (C-5), 70.66 (d, J(2,P) = 11.9, C-2′), 70.43 (d, J(C,O,P) = 6.3, CHipr.), 65.83 (C-1′), 64.97 (d, J(C,P) = 164.5, CH_2P), 24.03 (d, J(CH_3,P) = 3.6) and 23.89 (d, J(CH_3,P) = 4.4, CH_3ipr.), 13.12 (SCH_3) ppm. MS (ESI): m/z (%) = 403 (100) [MNa]$^+$. HR MS (FAB) calcd. for $C_{14}H_{26}N_2O_6PS$ [MH]$^+$ 381.1249, found 381.1265. Compound **21** (4.18 g, 36%) was isolated as a side product.

*4.13. 6-(2S)-[2-(Diisopropoxyphosphorylmethoxy)propoxy]-4-hydroxy-2-(methylsulfanyl) pyrimidine (**23b**) and 4,6-(2S,2′S)-bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfanyl)pyrimidine (**24a**)*

GP3, from pyrimidine **19** (2 g, 12.6 mmol) and phosphonate **20b** (5.68 g, 13.9 mmol). Yield 1.25 g (25%) of **23b**, colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 12.3$ (br s, 1H, OH), 5.40 (br s, 1H, H-5), 4.58 (m, 2H, CHipr.), 4.23 (dd, $J_{gem} = 11.3$, J(1′a,2′) = 3.8, 1H, H-1′a), 4.16 (dd, $J_{gem} = 11.3$, J(1′b,2′) = 6.1, 1H, H-1′b), 3.87 (m, 2H, H-2′), 3.80 (m, 2H, OCH_2P), 2.47 (s, 3H, SCH_3), 1.22 (m, 12H, CH_3ipr.), 1.15 (d, J(CH_3,2′) = 6.4, 3H, H-3′) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 170.52$ (C-2), 169.15 (C-4), 162.50 (C-6), 86.08 (C-5), 75.24 (d, J(2′,P) = 12.8, C-2′), 70.32 (m, 2C, CHipr.), 69.20 (C-1′), 63.01 (d, J(OCH_2,P) = 165.2, OCH_2P), 23.92 (m, 4C, CH_3ipr.), 16.28 (C-3′), 13.10 (SCH_3) ppm. MS (FAB): m/z (%) = 395 (100) [MH]$^+$. HR MS (FAB) calcd. for $C_{15}H_{28}N_2O_6PS$ [MH]$^+$ 395.1405, found 395.1418.

Dialkylated derivative **24a** was isolated as a second product. Yield 1.75 g (22%), colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 5.69$ (s, 1H, H-5), 4.74 (m, 4H, CHipr.), 4.307 (d, J(1′,2′) = 5.17, 4H, H-1′), 3.89 (m, 2H, H-2′), 3.82 (d, J(CH_2,P) = 9.11, 4H, OCH_2P), 2.49 (s, 3H, SCH_3), 1.31 (m, 24H, CH_3ipr.), 1.23 (d, J(3′,2′) = 6.39, 6H, H-3′) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 170.92$ (C-2), 170.17 (C-4,6), 86.12 (C-5), 75.85 (d, J(2′,P) = 11.98, C-2′), 71.05 (d, J(C,P) = 6.68) and 70.96 (d, J(C,P) = 6.66, CHipr.), 69.35 (C-1′), 64.04 (d, J(C,P) = 168.56, PCH_2), 24.06 (d, J(C,P) = 3.69) and 23.92 (d, J(C,P) = 4.64, CH_3ipr.), 16.55 (C-3′), 14.00 (SCH_3) ppm. MS (ESI): m/z (%) = 653 (100) [MNa]$^+$, 631 (100) [MH]$^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{10}P_2S$ [MH]$^+$ 631.2583, found 631.2579.

*4.14. 6-(2R)-[2-(Diisopropoxyphosphorylmethoxy)propoxy]-4-hydroxy-2-(methylsulfanyl) pyrimidine (**23c**) and 4,6-(2R,2′R)-bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfanyl)pyrimidine (**24b**)*

GP3, from pyrimidine **19** (4 g, 25.2 mmol) and phosphonate **20c** (10.3 g, 25.2 mmol). Yield 2.5 g (25%) of compound **23c**, colorless oil. ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) identical with compound **23b**. MS (FAB): m/z (%) = 395 (100) [MH]$^+$. HR MS (FAB) calcd. for $C_{15}H_{28}N_2O_6PS$ [MH]$^+$ 395.1405, found 395.1413. Compound **24b** isolated as a colorless oil, yield 1.2 g (7.5%). ^{1}H NMR ($CDCl_3$) identical with compound **24a**. MS (FAB): m/z (%) = 631 (100) [MH]$^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{10}P_2S$ [MH]$^+$ 631.2583, found 631.2599.

*4.15. 4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(2S)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)-pyrimidine (**26a**)*

GP4, **23a** (800 mg, 2.1 mmol), **20b** (0.94 g, 2.3 mmol). The crude product was taken to $CHCl_3$, filtered through a pad of silica gel and washed with 10% MeOH in $CHCl_3$ (150 mL). The filtrate was evaporated in vacuo. The residue was without further purification oxidized by *m*-CPBA by GP5 to give **26a** (0.52 g, 38%), colorless oil. ^{1}H NMR (DMSO-d_6): $\delta = 6.52$ (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 4.52 (m, 2H, H-1′), 4.43 (dd, $J_{gem} = 11.4$, J(1″a,2″) = 3.5, 1H, H-1″a), 4.34 (dd, $J_{gem} = 11.4$, J(1″b,2″) = 6.0, 1H, H-1″b), 3.92 (m, 2H, H-2″), 3.86 (m, 2H, H-2′), 3.81 (m, 4H, 2 × PCH_2), 3.38 (s, 3H, SO_2CH_3), 1.19–1.24 (m, 24H, CH_3ipr.), 1.19 (d, J(CH_3, 2″) = 6.5, 3H, H-3″) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.51$ and 171.45 (C-4, C-6), 164.26 (C-2), 92.66 (C-5), 75.05 (d, J(2″,P) = 12.5, C-2″), 70.12–70.40 (m, 6C, CHipr., C-2′ and C-1″), 66.95 (C-1′), 64.95 (d, J(C,P) = 164.5, C-3′), 63.03 (d, J(C,P) = 165.3, C-3″), 38.94 (CH_3SO_2), 23.82–24.01 (m, 8C, CH_3ipr.), 16.21 (C-3″) ppm. MS (FAB): m/z (%) = 671 (23) [MNa]$^+$, 649 (32) [MH]$^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{12}P_2S$ [MH]$^+$ 649.2325, found 649.2328.

*4.16. 4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(2R)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26b**)*

Prepared by the same procedure as described for compound **26a** from **23a** (800 mg, 2.1 mmol) and phosphonate **20c**. Colorless oil, 460 mg (34%). ^{1}H NMR spectra identical with compound **26a**. MS (ESI): m/z (%) = 671.1 (100) [MNa]$^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{12}P_2S$ [MH]$^+$ 649.2325, found 649.2318.

*4.17. 4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(2S)-[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-2-(methylsulfonyl)pyrimidine (**26c**)*

Prepared by the same procedure (GP4 and GP5) as compound **26a** from **23a** (800 mg, 2.1 mmol) and phosphonate **20d** (1.07 g, 2.52 mmol). Colorless oil, 870 mg (62%). ^{1}H NMR ($CDCl_3$): $\delta = 6.21$ (s, 1H, H-5), 4.59 (m, 2H, H-1′), 4.51 (m, 2H, H-1″), 4.05 (dd, $J_{gem} = 14.2$, J(H,C,P) = 7.3, 1H, PCH_2″a), 3.95 (m, 2H, H-2′), 3.88 (m, 2H, H-2″), 3.83 (dd, $J_{gem} = 14.2$, J(H,C,P) = 8.4, 1H, PCH_2″b), 3.81 (d, J(H,C,P) = 8.2, 2H, PCH_2′), 3.80 (m, 1H, H-3″a), 3.70 (dd, $J_{gem} = 12.3$, J(3″b,2″) = 5.9, 1H, H-3″b), 3.30 (s, 3H, CH_3SO_2), 1.34 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 171.35$ (C-6), 171.21 (C-4), 164.18 (C-2), 93.69 (C-5), 81.49 (d, J(2″,P) = 7.4, C-2″), 71.78 (d, J(CH,P) = 6.6), 71.40 (d, J(CH,P) = 6.9) and 71.15 (d, J(CH,P) = 6.5, CHipr.), 70.73 (d, J(2′,P) = 10.5, C-2′), 66.86 (2C, C-1′,1″), 66.06 (d, J(C,P) = 167.7, PCH_2′), 65.35 (d, J(C,P) = 168.8, PCH_2″), 61.62 (C-3″), 38.88 (CH_3SO_2), 23.97 (m, CH_3ipr.) ppm. MS (ESI): m/z (%) = 687 (100) [MNa]$^+$, 665 (44) [MH]$^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{13}P_2S$ [MH]$^+$ 645.2274, found 645.2268.

*4.18. 4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(2R)-[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-2-(methylsulfonyl)pyrimidine (**26d**)*

Prepared by the same procedure (GP4 and GP5) as compound **26a** from **23a** (800 mg, 2.1 mmol) and phosphonate **20e** (1.07 g, 2.52 mmol). Colorless oil, 700 mg (50%). ^{1}H NMR ($CDCl_3$) spectra identical with compound **26c**. MS (ESI): m/z (%) = 687 (25) [MNa]$^+$, 665 (12) [MH]$^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{13}P_2S$ [MH]$^+$ 645.2274, found 645.2288.

*4.19. 4,6-(2R,2′S)-Bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26e**)*

Prepared by the same procedure (GP4 and GP5) as compound **26a** from **23b** (500 mg, 1.27 mmol) and phosphonate **20c** (0.57 g, 1.4 mmol). Colorless oil, 740 mg (88%). ^{1}H NMR (DMSO-d_6): $\delta = 6.35$ (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 4.43 (dd, J(1′a,2′) = 3.4, $J_{gem} = 11.4$, 2H, H-1′a), 4.34 (dd, J(1′b,2′) = 5.9, $J_{gem} = 11.4$, 2H, H-1′b), 3.92 (m, 2H,

H-2′), 3.82 (m, 4H, OCH_2P), 2.38 (s, 3H, SO_2CH_3), 1.18–1.24 (m, 30H, CH_3ipr., H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.50 (C-4,6), 164.25 (C-2), 92.66 (C-5), 75.05 (d, J(2′,P) = 12.7, C-2′), 70.30 (m, CHipr), 70.13 (C-1′), 63.04 (d, J(C,P) = 165.5, PCH_2), 38.95 (CH_3SO_2), 23.92 (m, CH_3ipr.), 16.22 (C-3′) ppm. MS (ESI): m/z (%) = 685 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{12}P_2S$ $[MH]^+$ 663.2476, found 663.2469.

4.20. 4-(2S)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(2S)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26f**)

GP4, GP5, **23b** (1.25 g, 3.17 mmol), **20d** (1.75 g, 4.12 mmol). Colorless oil, 394 mg (18%). ^1H NMR (DMSO-d_6): δ = 6.21 (s, 1H, H-5), 4.75 (m, 4H, CHipr.), 4.52 (dd, J_{gem} = 11.4, J(1″a,2″) = 5.9, 1H, H-1″a), 4.49 (dd, J_{gem} = 11.4, J(1″b,2″) = 4.8, 1H, H-1″b), 4.44 (dd, J_{gem} = 11.3, J(1′a,2′) = 3.8, 1H, H-1′a), 4.40 (dd, J_{gem} = 11.3, J(1′b,2′) = 6.2, 1H, H-1′b), 4.06 (dd, J_{gem} = 14.2, J(C,H,P) = 7.3, 1H, PCH_2″a), 3.95 (m, 2H, H-2′), 3.87 (m, 2H, H-2″), 3.83 (m, 4H, PCH_2″b, PCH_2′, H-3″), 3.70 (m, 2H, H-3b″), 3.30 (s, 3H, SO_2CH_3), 1.34 (m, 24H, CH_3ipr.), 1.28 (d, J(3′,2′) = 6.4, 3H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.39 (C-4), 171.20 (C-6), 164.17 (C-2), 93.65 (C-5), 81.53 (d, J(2″,P) = 7.5, C-2″), 75.54 (d, J(2′,P) = 11.9, C-2′), 71.81 (d, J(C,O,P) = 6.7), 71.42 (d, J(C,O,P) = 6.9) and 71.08 (d, 2C, J(C,O,P) = 6.7, CHipr.), 70.54 (C-1′), 66.83 (C-1″), 65.35 (d, J(C,P) = 168.8, PCH_2″), 64.00 (d, J(C,P) = 169.2, PCH_2′), 61.62 (C-3″), 38.89 (CH_3SO_2), 24.00 (m, CH_3ipr.), 16.34 (C-3′) ppm. MS (ESI): m/z (%) = 679 (10) $[MH]^+$, 701 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2429.

4.21. 4-(2R)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(2S)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26g**)

GP4, GP5, **23b** (1.25 g, 3.17 mmol), **20e** (1.75 g, 4.12 mmol). Colorless oil, 360 mg (16%). ^1H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with compound **26f**. MS (ESI): m/z (%) = 679 (6) $[MH]^+$, 701 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2438.

4.22. 4-(2S)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(2R)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26h**)

GP4, GP5, **23c** (1.25 g, 3.17 mmol), **20d** (1.75 g, 4.12 mmol). Colorless oil, 960 mg (42%). ^1H NMR (DMSO-d_6): δ = 6.21 (s, 1H, H-5), 4.75 (m, 4H, CHipr.), 4.51 (m, 2H, H-1″), 4.54 (dd, J_{gem} = 11.4, J(1′a,2′) = 3.9, 1H, H-1′a), 4.41 (dd, J_{gem} = 11.3, J(1′b,2′) = 6.1, 1H, H-1′b), 4.05 (dd, J_{gem} = 14.1, J(C,H,P) = 7.4, 1H, PCH_2″a), 3.95 (m, 2H, H-2′), 3.78–3.89 (m, 5H, H-2″, PCH_2″b, PCH_2′, H-3″), 3.70 (dd, J_{gem} = 12.4, J(3″b,2″) = 5.9, 1H, H-3″b), 3.30 (s, 3H, SO_2CH_3), 1.33 (m, 24H, CH_3ipr.), 1.28 (d, J(3′,2′) = 6.4, 3H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.35 (C-4), 171.17 (C-6), 164.14 (C-2), 93.60 (C-5), 81.43 (d, J(2″,P) = 7.7, C-2″), 75.51 (d, J(2′,P) = 11.8, C-2′), 71.75 (d, J(C,O,P) = 6.7), 71.37 (d, J(C,O,P) = 6.9), 71.06 (d, J(C,O,P) = 6.7) and 71.04 (d, J(C,O,P) = 6.5, CHipr.), 70.52 (C-1′), 66.84 (C-1″), 65.28 (d, J(C,P) = 168.8, PCH_2″), 63.95 (d, J(C,P) = 169.1, PCH_2′), 61.53 (C-3″), 38.89 (CH_3SO_2), 23.96 (m, CH_3ipr.), 16.30 (C-3′) ppm. MS (ESI): m/z (%) = 679 (35) $[MH]^+$, 701 (15) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2441.

4.23. 4-(2R)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(2R)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26i**)

GP3, GP4, **23c** (1.25 g, 3.17 mmol), **20e** (1.75 g, 4.12 mmol). Colorless oil, 1.05 g (46%). NMR spectra identical with **26h**. MS (ESI): m/z (%) = 679 (18) $[MH]^+$, 701 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2432.

4.24. 4,6-Bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]-2-(methylsulfonyl)pyrimidine (**26j**)

GP4, **21** (5.8 g, 9.62 mmol). Colorless oil, yield 4.2 g (69%). ^1H NMR (DMSO-d_6): δ = 6.20 (s, 1H, H-5), 4.58 (dh, J(CH,P) = 7.6, J(CH,CH_3) = 6.4, 4H, CHipr.), 4.58 (m, 4H, H-1′), 3.94 (m, 4H, H-2′), 3.80 (d, J(C,H,P) = 8.17, 4H, PCH_2), 3.30 (s, 3H, SO_2CH_3), 1.33 (d, 12H) and 1.32 (d, 12H, J(CH_3,CH) = 6.16, CH_3ipr.) ppm. MS (FAB): m/z (%) = 635 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{12}P_2S$ $[MH]^+$ 635.2168, found 635.2152.

4.25. 4,6-(2S,2′S)-Bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26k**)

GP5, **24a** (840 mg, 1.33 mmol). Yield 800 mg (72%), colorless oil. ^1H NMR (DMSO-d_6) identical with spectra of **26e**. MS (ESI): m/z (%) = 685 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{12}P_2S$ $[MH]^+$ 663.2476, found 663.2475.

4.26. 4,6-(2R,2′R)-Bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**26l**)

GP5, **24b** (1.2 g, 1.9 mmol). Yield 1.1 g (87%), colorless oil. ^1H NMR (DMSO-d_6) identical with spectra of **26e**. MS (ESI): m/z (%) = 685 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{12}P_2S$ $[MH]^+$ 663.2476, found 663.2484.

4.27. Preparation of compounds **27** – general method

2-Methylsulfonyl derivatives **26** were converted to 2-amino derivatives by GP6 and diisopropyl esters of 2-amino pyrimidines were without further purification deprotected to free phosphonic acids by GP1.

4.27.1. 2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(2S)-[2-(phosphonomethoxy)propoxy] pyrimidine (**27a**)

26a (500 mg, 0.77 mmol), freeze dried, white hydroscopic foam, yield 210 mg (61%). ^1H NMR (DMSO-d_6): δ = 5.92 (s, 1H, H-5), 4.52 (m, 5H, H-1′), 4.45 (dd, J_{gem} = 11.2, J(1″a,2′) = 2.9, 1H, H-1″a), 4.30 (dd, J_{gem} = 11.3, J(1″b,2″) = 6.3, 1H, H-1″b), 4.05 (dt, J(2″,3″) = J(2″,1″b) = 6.4, J(2″,1″a) = 3.0, 2H, H-2″), 3.98 (m, 2H, H-2′), 3.74–3.84 (m, 4H, PCH_2), 1.27 (d, J(3″,2″) = 6.5, 3H, H-3″) ppm. ^{13}C NMR (DMSO-d_6): δ = 169.98 (C-4), 169.91 (C-6), 156.14 (C-2), 79.67 (C-5), 76.06 (d, J(2″,P) = 11.0, C-2″), 72.42 (C-1), 70.72 (d, J(2′,P) = 10.3, C-2′), 67.19 (d, J(P,CH_2) = 157.2, PCH_2), 65.05 (d, J(P,CH_2) = 159.1, PCH_2), 15.72 (C-3″) ppm. MS (ESI): m/z (%) = 418 (100) $[MH]^+$. $[\alpha]_D^{25}$ = +12.1 (c 0.248, H_2O). Anal. $C_{11}H_{21}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.27.2. 2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(2R)-[2-(phosphonomethoxy)propoxy] pyrimidine (**27b**)

26b (400 mg, 0.62 mmol), freeze dried, white hydroscopic foam, yield 190 mg (70%). NMR spectrum identical with compound **27a**. MS (ESI): m/z (%) = 418 (100) $[MH]^+$. $[\alpha]_D^{25}$ = −10.3 (c 0.347, H_2O). Anal. $C_{11}H_{21}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.27.3. 2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(2S)-[2-(phosphonomethoxy)-3-hydroxypropoxy]pyrimidine (**27c**)

26c (870 mg, 1.3 mmol), freeze dried, white hydroscopic foam, yield 320 mg (54%). ^1H NMR (DMSO-d_6): δ = 5.36 (s, 1H, H-5), 4.29 (m, 2H, H-1′), 4.25 (dd, J_{gem} = 11.3, J(1″a,2″) = 4.3, 1H, H-1″a), 4.20 (dd, J_{gem} = 11.3, J(1″b,2″) = 5.7, 1H, H-1″b), 3.75 (m, 2H, H-2′), 3.71 (dd, J_{gem} = 13.6, J(H–C–P) = 8.7, 1H, OCH_2P″a), 3.67 (dd, J_{gem} = 13.6,

J(H–C–P) = 8.9, 1H, $OCH_2P''b$), 3.67 (m, 2H, H-2″), 3.58 (d, *J*(H–C–P) = 8.7, 2H, OCH_2P'), 3.51 (m, 2H, H-3″) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.46 (C-6), 171.36 (C-4), 162.82 (C-2), 80.41 (d, *J*(2″,P) = 9.9, C-2″), 78.64 (C-5), 70.76 (d, *J*(2′,P) = 11.4, C-2′), 66.97 (d, *J*(C,P) = 160.4, OCH_2P), 65.93 (d, *J*(C,P) = 160.4, OCH_2P''), 65.39 (C-1″), 64.93 (C-1′), 60.33 (C-3″) ppm. MS (ESI): *m*/*z* (%) = 434 (100) $[MH]^+$. $[\alpha]_D^{25} = +5.8$ (*c* 0.625, H_2O). Anal. $C_{11}H_{21}N_3O_{11}P_2.H_2O$ (C, H, N, P).

4.27.4. 2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(2R)-[2-(phosphonomethoxy)-3-hydroxypropoxy]pyrimidine (**27d**)

26d (700 mg, 1.05 mmol), freeze dried, white hydroscopic foam, yield 215 mg (45%). NMR spectrum identical with compound **27c**. MS (ESI): *m*/*z* (%) = 434 (100) $[MH]^+$. $[\alpha]_D^{25} = -6.7$ (*c* 0.341, H_2O). Anal. $C_{11}H_{21}N_3O_{11}P_2.H_2O$ (C, H, N, P).

4.27.5. 2-Amino-4,6-(2R,2′S)-bis[2-(phosphonomethoxy)-propoxy]pyrimidine (**27e**)

26e (840 mg, 1.27 mmol), freeze dried, white hydroscopic foam, yield 280 mg (49%). ^1H NMR (DMSO-d_6): δ = 3.58 (s, 1H, H-5), 4.18 (dd, J_{gem} = 11.1, *J*(1′a,2″) = 5.8, 2H, H-1′a), 4.12 (dd, J_{gem} = 11.1, *J*(1′b,2′) = 4.4, 2H, H-1″b), 3.82 (m, 2H, H-2′), 3.60 (d, *J*(CH_2,P) = 9.3, 4H, PCH_2), 1.14 (d, *J*(H-3′,H-2′) = 6.4, 6H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.40 (C-4, C-6), 162.77 (C-2), 78.54 (C-5), 74.96 (d, *J*(C-2′,P) = 11.4, C-2′), 68.58 (C-1′), 65.07 (d, *J*(CH_2,P) = 161.6, PCH_2), 16.80 (C-3″) ppm. MS (ESI): *m*/*z* (%) = 432.1 (100) $[MH]^+$, 454.1 (37) $[MNa]^+$. $[\alpha]_D^{25} = +0.9$ (*c* 0.521, H_2O). Anal. $C_{12}H_{23}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.27.6. 2-Amino-4-(2S)-[2-(phosphonomethoxy)-3-hydroxypropoxy]-6-(2S)-[2-(phosphonomethoxy)propoxy]pyrimidine (**27f**)

26f (394 mg, 0.64 mmol), freeze dried, white hydroscopic foam, yield 200 mg (67%). ^1H NMR (DMSO-d_6): δ = 5.36 (s, 1H, H-5), 4.26 (dd, J_{gem} = 11.3, *J*(1″a,2″) = 4.3, 1H, H-1″a), 4.20 (dd, J_{gem} = 11.3, *J*(1″b,2″) = 5.8, 1H, H-1″b), 4.16 (dd, J_{gem} = 11.1, *J*(1′a,2′) = 5.8, 1H, H-1′a), 4.12 (dd, J_{gem} = 11.1, *J*(1′b,2′) = 4.4, 1H, H-1′b), 3.81 (m, 1H, H-2′), 3.71 (dd, J_{gem} = 13.6, *J*(C–H–P) = 8.7, 1H, OCH_2,P″a), 3.67 (dd, J_{gem} = 13.6, *J*(C–H–P) = 8.9, 1H, OCH_2,P″b), 3.67 (m, 1H, H-2″), 3.60 (d, 2H, *J*(C–H–P) = 9.4, OCH_2,P′), 3.51 (m, 2H, H-3″), 1.13 (d, *J*(3′,2′) = 6.4, 3H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.48, 171.42 (C-4, C-6), 162.83 (C-2), 80.40 (d, *J*(2″,P) = 9.7, C-2″), 78.59 (C-5), 75.08 (d, *J*(2′,P) = 11.7, C-2′), 68.63 (C-1′), 66.00 (d, *J*(P,C) = 161.0, PCH_2''), 65.45 (C-1″), 65.01 (d, *J*(P,C) = 161.8, PCH_2'), 60.35 (C-3″), 16.83 (C-3′) ppm. MS (ESI): *m*/*z* (%) = 448.1 (100) $[MH]^+$. $[\alpha]_D^{25} = +12.2$ (*c* 0.181, H_2O). Analysis $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (C, H, N, P).

4.27.7. 2-Amino-4-(2R)-[2-(phosphonomethoxy)-3-hydroxypropoxy]-6-(2S)-[2-(phosphonomethoxy)propoxy]pyrimidine (**27g**)

From **26g** (360 mg, 0.58 mmol), freeze dried, white hydroscopic foam, yield 200 mg (73%). ^1H NMR (DMSO-d_6): δ = 5.36 (s, 1H, H-5), 4.25 (dd, J_{gem} = 11.3, *J*(1″a,2″) = 4.3, 1H, H-1″a), 4.20 (dd, J_{gem} = 11.3, *J*(1″b,2″) = 5.8, 1H, H-1″b), 4.17 (dd, J_{gem} = 11.1, *J*(1′a,2′) = 5.8, 1H, H-1′a), 4.11 (dd, J_{gem} = 11.1, *J*(1′b,2′) = 4.4, 1H, H-1′b), 3.81 (m, 1H, H-2′), 3.71 (dd, J_{gem} = 13.6, *J*(C–H–P) = 8.7, 1H, OCH_2,P″a), 3.67 (dd, J_{gem} = 13.6, *J*(C–H–P) = 8.9, 1H, OCH_2,P″b), 3.67 (m, 1H, H-2″), 3.60 (d, *J*(C–H–P) = 9.4, 2H, OCH_2,P′), 3.51 (m, 2H, H-3″), 1.13 (d, *J*(3′,2′) = 6.4, 3H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.48 and 171.42 (C-4, C-6), 162.83 (C-2), 80.40 (d, *J*(2″,P) = 9.7, C-2″), 78.59 (C-5), 75.08 (d, *J*(2′,P) = 11.7, C-2′), 68.63 (C-1′), 66.00 (d, *J*(P,C) = 161.0, PCH_2''), 65.45 (C-1″), 65.01 (d, *J*(P,C) = 161.8, PCH_2'), 60.35 (C-3″), 16.83 (C-3′) ppm. MS (ESI): *m*/*z* (%) = 448.1 (100) $[MH]^+$, 470.1 (35) $[MNa]^+$. $[\alpha]_D^{25} = +8.6$ (*c* 0.561, H_2O). Anal. $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (C, H, N, P).

4.27.8. 2-Amino-4-(2S)-[2-(phosphonomethoxy)-3-hydroxypropoxy]-6-(2R)-[2-(phosphonomethoxy)propoxy]pyrimidine (**27h**)

From **26h** (960 mg, 1.55 mmol), freeze dried, white hydroscopic foam, yield 605 mg (83%). NMR spectra identical with compound **27g**. MS (ESI): *m*/*z* (%) = 448.2 (100) $[MH]^+$. $[\alpha]_D^{25} = -4.4$ (*c* 0.182, H_2O). Anal. $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (C, H, N, P).

4.27.9. 2-Amino-4-(2R)-[2-(phosphonomethoxy)-3-hydroxypropoxy]-6-(2R)-[2-(phosphonomethoxy)propoxy]pyrimidine (**27i**)

From **26i** (1 g, 1.62 mmol), freeze dried, white hydroscopic foam, yield 616 mg (81%). NMR spectra identical with compound **39f**. MS (ESI): *m*/*z* (%) = 448.0 (100) $[MH]^+$. $[\alpha]_D^{25} = -5.8$ (*c* 0.312, H_2O). Anal. $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (C, H, N, P).

4.27.10. 2-Amino-4,6-(2S,2′S)-bis[2-(phosphonomethoxy)propoxy]pyrimidine (**27j**)

26k (350 mg, 0.58 mmol), yield 135 mg (52%), colorless foam. ^1H NMR (DMSO-d_6): δ = 3.57 (s, 1H, H-5), 4.17 (dd, J_{gem} = 11.1, *J*(1′a,2′) = 5.8, 2H, H-1′a), 4.11 (dd, J_{gem} = 11.1, *J*(1′b,2′) = 4.4, 2H, H-1′b), 3.81 (m, 2H, H-2′), 3.60 (d, *J*(CH_2,P) = 9.3, 4H, PCH_2), 1.14 (d, *J*(H-3′,H-2″) = 6.3, 6H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.41 (C-4, C-6), 162.80 (C-2), 78.54 (C-5), 75.02 (d, *J*(C-2′,P) = 11.6, C-2″), 68.59 (C-1′), 65.02 (d, *J*(CH_2,P) = 161.7, PCH_2), 16.82 (C-3′) ppm. MS (ESI): *m*/*z* (%) = 432.1 (100) $[MH]^+$; 454.1 (26) $[MNa]^+$. $[\alpha]_D^{25} = +20.9$ (*c* 0.291, H_2O). Anal. $C_{12}H_{23}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.27.11. 2-Amino-4,6-(2R,2′R)-bis[2-(phosphonomethoxy)-propoxy]pyrimidine (**27k**)

From **26l** (1.2 g, 1.8 mmol), white hydroscopic foam, yield 450 mg (55%). NMR spectra identical with compound **27j**. MS (ESI): *m*/*z* (%) = 432.0 (100) $[MH]^+$; 454.1 (14) $[MNa]^+$. $[\alpha]_D^{25} = -20.5$ (*c* 0.254, H_2O). Anal. $C_{12}H_{23}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.28. 2-Substituted bisphosphonates

4.28.1. 2-Cyclopropylamino-4,6-bis[2-(phosphonomethoxy)-ethoxy]pyrimidine (**28a**)

Compound **26j** (1 g, 1.57 mmol) in dry THF (45 mL) was treated with cyclopropylamine (5.5 mL) and the mixture was refluxed in a sealed flask for 2 h. Volatiles were removed in vacuo and the residue was purified by flash chromatography (EtOAc/EtOH 0–10%) to give 330 mg (34%) of 2-cyclopropylamino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine as a thick oil. ^1H NMR (DMSO-d_6): δ = 7.25 (br d, *J*(NH,CH) = 3.6, 1H, NH), 5.32 (s, 1H, H-5), 4.58 (m, 4H, P–OCH), 4.34 (m, 4H, C-1′), 3.79 (m, 4H, C-2′), 3.78 (d, *J*(OCH_2,P) = 8.3, 4H, PCH_2), 2.67 (m, 1H, CH cycloprop.), 1.23 (d, 12H) and 1.22 (d, *J*(CH_3,CH) = 6.3, 12H, CH_3), 0.62 (m, 2H) and 0.45 (m, 2H, CH_2 cycloprop.) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.99 2C (C-4, C-6), 162.51 (C-2), 78.66 (C-5), 70.80 (C-2′), 70.34 (d, 4C, *J*(CH,P) = 6.4, P–OCH), 64.96 (d, *J*(C-3′,P) = 164.5, P–OCH_2), 64.48 (C-1′), 23.87 (CH cycloprop.), 23.92 (CH_3), 6.34 (CH_2 cycloprop.) ppm. MS (FAB): *m*/*z* (%) = 612 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{25}H_{48}N_3O_{10}P_2$ $[MH]^+$ 612.2814, found 612.2813.

Diisopropyl esters were deprotected by GP1 to give **28a** (140 mg, 62%) as a white foam. ^1H NMR (D_2O): δ = 4.53 (m, 4H, H-1″), 3.98 (m, 4H, H-2′), 3.76 (d, *J*(PCH_2) = 9.2, 4H, PCH_2), 2.72 (m, 1H, CH cycloprop.), 0.93 and 0.71 (2 × m, 2 × 2H, CH_2 cycloprop.) ppm. ^{13}C NMR (D_2O): δ = 171.05 (C-4, C-6), 159.38 (C-2), 78.79 (C-5), 70.85 (d, *J*(C-2′,P) = 10.4, C-2′), 68.23 (C-1′), 67.60 (d, *J*(CH_2,P) = 156.4, PCH_2), 23.41 (CH cycloprop.), 7.19 (CH_2 cycloprop.) ppm. MS (ESI): *m*/*z* (%) = 444.1 (100) $[MH]^+$, 466.0 (26) $[MNa]^+$. Anal. $C_{13}H_{23}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.28.2. 2-Cyclopentylamino-4,6-bis[2-(phosphonomethoxy)-ethoxy]pyrimidine (**28b**)

Compound **26j** (1 g, 1.57 mmol) in dry THF (30 mL) was treated with cyclopentylamine (1.6 mL) and the mixture was refluxed in a sealed flask for 3 h. THF was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH

0–2%) to give 2-cyclopentylamino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine as an oil, yield 540 mg (54%). ^{1}H NMR ($CDCl_3$): $\delta = 5.37$ (s, 1H, H-5), 4.88 (d, J(NH-1′) = 7.0, 1H, NH), 4.76 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.7, 4H, CHipr.), 4.39 (m, 4H, H-1′), 4.18 (m, 1H, H-1″), 3.89 (m, 4H, H-2′), 3.82 (d, J(P,CH_2) = 8.2, 4H, PCH_2), 2.01 (m, 2H, H-2″a), 1.71 and 1.61 (2 × m, 2 × 2H, H-3″), 1.45 (m, 2H, H-2″b), 1.33 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 171.20$ (C-4, C-6), 161.10 (C-2), 79.48 (C-5), 71.33 (d, J(2′-P) = 11.0, C-2″), 71.08 (d, J(CH–P) = 6.6, CHipr.), 65.97 (d, J(3′-P) = 167.1, PCH_2), 64.76 (C-1′), 52.91 (C-1″), 33.29 (C-2″), 24.07 (d, J(CH_3-P) = 3.7) and 23.93 (d, J(CH_3-P) = 4.6, CH_3ipr.), 23.72 (C-3″) ppm. MS (FAB): m/z (%) = 640.5 (60) $[MH]^+$. HR MS (FAB) calcd. for $C_{27}H_{52}N_3O_{10}P_2$ $[MH]^+$ 640.3128, found 640.3140.

Diisopropyl esters were cleaved by GP1 to afford **28b** (220 mg, 53%) as a white hydroscopic foam. ^{1}H NMR (DMSO-d_6): $\delta = 5.34$ (s, 1H, H-5), 4.31 (m, 4H, H-1′), 4.09 (m, 1H, H-1″), 3.76 (m, 4H, H-2′), 3.58 (d, J(CH_2P) = 8.7, 4H, PCH_2), 1.88 (m, 2H, H-2″a), 1.65 (m, 2H, H-3″a), 1.49 (m, 4H, H-2″b and H-3″b) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.04$ (C-4, C-6), 161.23 (C-2), 78.17 (C-5), 70.73 (d, J(2,P) = 11.2, C-2′), 66.95 (d, J(3,P) = 160.3, PCH_2), 64.83 (C-1′), 52.60 (C-1″), 32.44 (C-2″), 23.71 (C-3″) ppm. MS (FAB): m/z (%) = 472.1 (100) $[MH]^+$. Anal. $C_{15}H_{27}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.28.3. 2-Methylamino-4,6-bis[2-(phosphonomethoxy)-ethoxy]pyrimidine (**28c**)

2-Methylsulfonyl derivative **26j** (1 g, 1.57 mmol) in EtOH (33.75 mL) was treated with methylamine (8 M solution in EtOH, 11.25 mL) and the reaction mixture was heated at 50 °C in a sealed tube for 6 h. Volatiles were removed under reduced pressure and the residue was purified by flash chromatography (EtOAc/EtOH 0–5%) to give colorless oil of 2-methylamino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine, yield 440 mg (48%). ^{1}H NMR (DMSO-d_6): $\delta = 6.97$ (br q, J(NH,CH_3) = 4.7, 1H, NH), 5.28 (s, 1H, H-5), 4.59 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.8, 4H, CHipr.), 4.33 (m, 4H, H-1′), 3.78 (m, 8H, H-2′, H-3′), 2.75 (d, J(CH_3,NH) = 4.7, 3H, CH_3NH) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 170.82$ (C-2), 161.74 (C-4, C-6), 77.83 (C-5), 70.36 (m, CHipr.), 64.97 (d, J(CH_2,P) = 164.2, CH_2P), 64.49 (C-1′), 27.86 (CH_3NH), 23.93 m (CH_3ipr.) ppm. MS (FAB): m/z (%) = 586.2 (75) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{46}N_3O_{10}P_2$ $[MH]^+$ 586.2658, found 586.2659.

Diisopropyl esters were deprotected by GP1 to give **28c** (210 mg, 67%) as a white hydroscopic foam. ^{1}H NMR (D_2O): $\delta = 4.54$ (m, 4H, H-1′), 3.98 (m, 4H, H-2′), 3.77 (d, J(CH_2,P) = 9.2, 4H, PCH_2), 3.00 (s, 3H, CH_3) ppm. ^{13}C NMR (D_2O): $\delta = 173.02$ (C-4, C-6), 162.95 (C-2), 70.72 d (C-2′), 69.21 (C-1″), 67.20 (d, PCH_2), 28.21 (CH_3) ppm. MS (FAB): m/z (%) = 418 (100) $[MH]^+$. Anal. $C_{11}H_{21}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.28.4. 2-Benzylamino-4,6-bis[2-(phosphonomethoxy)-ethoxy]pyrimidine (**28d**)

Pyrimidine **26j** (1 g, 1.57 mmol) in dry THF was treated with benzylamine (6 mL) and the reaction mixture was heated at 50 °C in a sealed tube for 4 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (EtOAc/EtOH 0–5%) to afford 2-benzylamino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (390 mg, 37%) as a colorless oil. ^{1}H NMR (DMSO-d_6): $\delta = 7.63$ (br t, J(NH,H-1″) = 6.4, 1H, NH), 7.15–7.34 (m, 5H, arom.), 5.29 (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 4.41 (d, J(H-1″,NH) = 6.3, 2H, H-1″), 4.29 (m, 4H, H-1′), 3.75 (m, 8H, H-2′, PCH_2), 1.23 (d, J(CH_3,CH) = 6.2, 12H) and 1.21 (d, J(CH_3,CH) = 6.2, 12H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 170.94$ (C-4, C-6), 161.46 (C-2), 140.71, 128.28, 127.42, 126.67 (arom.), 78.59 (C-5), 70.74 (C-2′), 70.35 (d, J(CH,P) = 6.3, 4C, CHipr.), 64.95 (d, J(CH_2,P) = 164.3, 2C, PCH_2), 64.58 (2C, C-1′), 44.39 (C-1″), 24.00 (d, J(CH_3,P) = 3.6, 4C) and 23.84 (d, J(CH_3,P) = 4.3, 4C, CH_3ipr.) ppm. MS (FAB): m/z (%) = 662 (25) $[MH]^+$. HR MS (FAB) calcd. for $C_{29}H_{50}N_3O_{10}P_2$ $[MH]^+$ 662.2971, found 662.2981.

The intermediate was treated with bromotrimethylsilane (GP1) to give **28d**, white hydroscopic foam, yield 120 mg (42%). ^{1}H NMR (DMSO-d_6): $\delta = 7.66$ (br t, J(NH, H-1″) = 6.0, 1H, NH), 7.16–7.34 (m, 5H, arom.), 5.37 (s, 1H, H-5), 4.42 (d, J(H-1″, NH) = 5.6, 2H, H-1″), 4.27 (m, 4H, H-1′), 3.71 (m, 4H, H-2′), 3.53 (m, 4H, PCH_2) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.15$ (C-4, C-6), 161.52 (C-2), 140.75, 128.35, 127.46, 126.70 (arom.), 78.68 (C-5), 70.60 (d, J(C-2′,P) = 10.9, C-2′), 67.18 (d, J(CH_2,P) = 160.6, PCH_2), 64.97 (C-1′), 44.41 (C-1″) ppm. MS (FAB): m/z (%) = 494 (100) $[MH]^+$, 516 (35) $[MNa]^+$. Anal. $C_{17}H_{25}N_3O_{10}P_2.H_2O$ (C, H, N, P).

4.28.5. 2-(4-Methoxybenzyl)amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**28e**)

2-Methylsulfonyl derivative **26j** (1 g, 1.57 mmol) in dry THF (30 mL) was treated with 4-methoxybenzylamine (0.61 mL, 4.1 mmol) and the reaction mixture was refluxed in a sealed tube for 2 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography to give 2-(4-methoxybenzyl)amino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (550 mg, 50%) as colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 7.25$ (m, 2H, Ph-2), 6.86 (m, 2H, Ph-3), 5.40 (s, 1H, H-5), 5.20 (t, J(NH,CH_2) = 5.9, 1H, NH), 4.75 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.7, 4H, CHipr.), 4.49 (d, J(CH_2,NH) = 5.9, 2H, Ph$\underline{CH_2}$NH), 4.33 (m, 4H, H-1), 3.86 (m, 4H, H-2), 3.81 (d, J(CH_2,P) = 8.3, 4H, PCH_2), 3.80 (s, 3H, OCH_3), 1.33 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 171.29$ (C-4, C-6), 161.20 (C-2), 158.73 (Ph-4), 131.41 (Ph-1), 128.74 (Ph-2), 113.85 (Ph-3), 80.01 (C-5), 71.29 (d, J(C-2′,P) = 11.1, C-2′), 71.08 (d, J(CH,P) = 6.6, CHipr.), 65.96 (d, J(CH_2,P) = 167.1, PCH_2), 64.86 (C-1′), 55.24 (OCH_3), 44.90 (Ph$\underline{CH_2}$NH), 24.06 (d, J(CH_3,CH) = 3.7) and 23.93 (d, J(CH_3,CH) = 4.6, CH_3ipr.) ppm. MS (FAB): m/z (%) = 692.2 (15) $[MH]^+$. HR MS (FAB) calcd. for $C_{30}H_{52}N_3O_{11}P_2$ $[MH]^+$ 692.3077, found 692.3074.

Diisopropyl esters were deprotected by GP1. The final product was applied onto a column of Dowex 50 × 8 in Na^+ form and eluted with water to give **28e** (160 mg, 23%) as a tetrasodium salt; white hydroscopic foam. ^{1}H NMR (DMSO-d_6): $\delta = 7.60$ (br t, J(NH,CH_2) = 6.4, NH), 7.23 (m, 2H, H-2″), 6.85 (m, 2H, H-3″), 5.36 (d, 1H, H-5), 4.33 (d, J(CH_2,NH) = 6.0, 2H, CH_2N), 4.25 (m, 4H, H-1′), 3.70 (s, 3H, OCH_3), 3.70 (m, 4H, H-2′), 3.50 (d, 4H, J(H,P) = 8.6, PCH_2). ^{13}C NMR (DMSO-d_6): $\delta = 171.14$ (C-4,6), 161.46 (C-2), 158.22 (C-4″), 132.68 (C-1″), 128.83 (C-2″), 113.76 (C-3″), 78.60 (C-5), 70.54 (d, J(2′,P) = 10.6, C-2′), 67.57 (d, J(C,P) = 159.5, PCH_2), 64.97 (C-1′), 55.21 (OCH_3), 43.80 (CH_2N). MS (ESI): m/z (%) = 524.1 (100) $[MH]^+$, 546.1 (69) $[MNa]^+$. Anal. $C_{18}H_{23}N_3Na_4O_{11}P_2$ (C, H, N, P).

4.28.6. 2-Morpholino-4,6-bis[2-(phosphonomethoxy)-ethoxy]pyrimidine (**28f**)

Compound **26j** (1 g, 1.57 mmol) in dry THF (30 mL) was treated with morpholine (1.4 mL) and the mixture was refluxed in a sealed flask for 2 h and the solvent was removed under reduced pressure. Flash chromatography ($CHCl_3$/MeOH 0–1%) gave thick oil of 2-morpholino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (720 mg, 71%). ^{1}H NMR ($CDCl_3$): $\delta = 5.39$ (s, 1H, C-5), 4.76 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.7, 4H, CHipr.), 4.41 (m, 4H, H-1′), 3.90 (m, 4H, H-2′), 3.82 (d, J(CH_2,P) = 8.2, 4H, PCH_2), 3.72 (s, 8H, H-2″, H-3″), 1.34 (d, J(CH_3,CH) = 6.3, 12H) and 1.33 (d, J(CH_3,CH) = 6.3, 12H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 171.13$ (C-4, C-6), 160.62 (C-2), 79.50 (C-5), 71.27 (d, J(2′,P) = 10.8, C-2′), 71.07 (d, J(CH,P) = 6.61, CHipr.), 66.74 (C-3″), 66.02 (d, J(CH,P) = 167.3, PCH_2), 64.80 (C-1′), 44.24 (C-2″), 24.07 (d, J(CH_3,P) = 3.5) and 23.94 (d, J(CH_3,P) = 4.5, CH_3ipr.) ppm. MS (FAB): m/z (%) = 642.5 (22) $[MH]^+$. HR MS (FAB) calcd. for $C_{26}H_{50}N_3O_{11}P_2$ $[MH]^+$ 642.2921, found 642.2911.

Deprotection of diisopropyl esters by GP1 gave **28f** (360 mg, 65%) as a white hydroscopic foam. ^{1}H NMR (DMSO-d_6): $\delta = 5.45$ (s, 1H, H-5), 4.33 (m, 4H, H-1′), 3.77 (m, 4H, H-2′), 3.64 (m, 8H,

CH_2–morpholine), 3.57 (d, J(P,CH) = 8.7, 4H, PCH_2) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.13 (2C, C-4, C-6), 160.51 (C-2), 78.84 (C-5), 70.62 (d, J(C-2,P) = 11.2, C-2′), 66.93 (d, J(C-3,P) = 160.4, PCH_2), 66.13 (C-3″), 65.10 (C-1′), 44.13 (C-2″) ppm. MS (ESI): m/z (%) = 474 (86) $[MH]^+$. Anal. $C_{14}H_{25}N_3O_{11}P_2.H_2O$ (C, H, N, P).

4.28.7. 4,6-Bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]-2-hydroxypyrimidine (**29a**)

Solution of NaOH (0.072 g) in water (10 mL) was added in one portion to the 2-methylsulfonyl derivative **26j** (1 g, 1.57 mmol) in THF (5 mL) and the resulting mixture was heated at 60 °C for 1 h. Reaction mixture was cooled to r.t., neutralized with acetic acid and volatiles were removed in vacuo. The residue was partitioned between $CHCl_3$ and water. Organic fraction was washed with water (3 × 50 mL) and dried over $MgSO_4$. Flash chromatography ($CHCl_3$/MeOH 0–5%) gave colorless oil, 450 mg (50%). ^1H NMR ($CDCl_3$): δ = 5.29 (s, 1H, H-5), 4.76 (dh, J(CH,P) = 7.7, J(CH,CH_3) = 6.2, CHipr.), 4.38 (m, 4H, H-1″), 3.92 (m, 4H, H-2′), 3.81 (d, J(CH_2,P) = 8.2, PCH_2), 1.34 and 1.33 (2 × d, J(CH_3,CH) = 6.2, 2 × 12H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 162.57 (C-4,6),158.72 (C-2), 75.35 (C-5), 71.18 (d, J(C,P) = 6.6, CHipr.), 70.59 (d, J(C,P) = 10.6, C-2′), 67.29 (C-1″), 66.08 (d, J(C,P) = 167.6, PCH_2), 24.05 (d, J(C,P) = 4.0, CH_3ipr.), 23.95 (d, J(C,P) = 4.6, CH_3ipr.) ppm. MS (FAB): m/z (%) = 573 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{22}H_{43}N_2O_{11}P_2$ $[MH]^+$ 573.2342, found 573.2347.

4.28.8. 2-Methoxy-4,6-bis[2-(diisopropoxyphosphorylmethoxy)-ethoxy]pyrimidine (**29b**)

26j (2 g, 3.15 mmol) in MeOH (40 mL) was treated with MeONa (1 M in MeOH, 3.5 mL) and the resulting mixture was heated at 80 °C for 4 h. Reaction mixture was cooled to r.t. and solvent was removed in vacuo. Flash chromatography afforded **29b** (600 mg, 32%) as an oil. ^1H NMR ($CDCl_3$): δ = 5.71 (s, 1H, H-5), 4.76 (dh, J(CH,CH_3) = 6.2, J(H,C,P) = 7.7, 4H, CHipr), 4.48 (m, 4H, H-1′), 3.94 (s, 3H, OCH_3), 3.92 (m, 4H, H-2″), 3.82 (d, J(H,C,P) = 8.2, 4H, OCH_2P), 1.33 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.99 (C-4, C-6), 164.52 (C-2), 84.20 (C-5), 71.08 (m, CHipr., H-2′), 65.97 (d, J(C,P) = 167.4, OCH_2P), 65.51 (C-1′), 54.65 (OCH_3), 24.05 (d, J(C,C,O,P) = 3.7) and 23.91 (d, J(C,C,O,P) = 4.6, CH_3ipr.) ppm. MS (FAB): m/z (%) = 587 (62) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{11}P_2$ $[MH]^+$ 587.2498, found 587.2516.

4.28.9. 4,6-Bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**30**)

Compound **21** (2 g, 3.22 mmol) in MeOH (200 mL) was treated with suspension of Raney-Nickel (ca. 15 g) in MeOH (30 mL). The reaction mixture was refluxed for 6 h, filtered while hot through Celite and the precipitate was washed with MeOH (500 mL). The filtrate was evaporated in vacuo, and the residue was purified by flash chromatography ($CHCl_3$/MeOH 0–5%) to give 4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine as a thick oil (1.5 g, 81%). ^1H NMR (DMSO-d_6): δ = 8.38 (d, J(H-2,H-5) = 0.9, 1H, H-2), 6.05 (d, J(H-5,H-2) = 0.9, 1H, H-5), 4.76 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.7, 4H, CHipr.), 4.50 (m, 4H, H-1′), 3.93 (m, 4H, H-2′), 3.83 (d, J(H,C,P) = 8.2, 4H, $POCH_2$), 1.33 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.51 (C-4, C-6), 157.21 (C-2), 91.15 (C-5), 71.12 (d, J(2′,P) = 10.8, C-2′), 71.09 (d, J(CH,P) = 6.7, CHipr.), 65.99 (d, J(C,P) = 167.3, $POCH_2$), 65.52 (C-1′), 24.05 (d, J(CH_3,P) = 3.7) and 23.93 (d, J(CH_3,P) = 4.6, CH_3ipr.) ppm. MS (ESI): m/z (%) = 579.2 (100) $[MNa]^+$. Anal. $C_{22}H_{42}N_2O_{10}P_2$ (C, H, N, P).

The intermediate was deprotected by bromotrimethylsilane (GP1) to give **30** (350 mg, 48%), freeze dried, white hydroscopic foam. ^1H NMR (DMSO-d_6): δ = 8.44 (d, J(H-2,H-5) = 0.9, 1H, H-2), 6.28 (d, J(H-5,H-2) = 0.9, 1H, H-5), 4.39 (m, 4H, H-1′), 3.80 (m, 4H, H-2′), 3.57 (d, J(H,C,P) = 8.7, 4H, OCH_2P) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.70 (C-4, C-6), 157.79 (C-2), 90.36 (C-5), 70.46 (d, J(2′,P) = 11.0, C-2′), 67.10 (d, J(C,P) = 160.2, PCH_2), 66.01 (C-1′) ppm. MS (ESI): m/z (%) = 389 (76) $[MH]^+$. Anal. $C_{10}H_{18}N_2O_{10}P_2.H_2O$ (C, H, N, P).

4.29. 4,6-Disulfanylpyrimidine derivatives

4.29.1. 2-Amino-4,6-disulfanylpyrimidine (**31**)

Thiourea (9.5 g, 90 mmol) was added to the solution of dichloropyrimidine **10** (5 g, 30 mmol) in EtOH (250 mL) and the reaction mixture was refluxed for 2 h. Solvent was removed in vacuo and the residue in aq. NaOH (0.5 M, 250 mL) was heated at 80 °C for 16 h. The reaction mixture was cooled to r.t., acidified with acetic acid to pH 4 and evaporated to half of its volume. The precipitate was filtered off, washed with water and dried to give yellow solid (4.78 g, 98%), m.p. 259 °C dec. ^1H NMR (DMSO-d_6): δ = 11.2–11.8 (br s, 2H, SH), 7.17 (br s, 2H, NH_2), 6.24 (s, 1H, H-5) ppm. ^{13}C NMR (DMSO-d_6): δ = 173.91 (2C, C-4, C-6), 149.10 (C-2), 116.36 (C-5) ppm. MS (EI): m/z (%) = 159.2 (100) $[M]^+$. Anal. $C_4H_5N_3S_2$ (C, H, N, S).

4.29.2. 2-Amino-4,6-bis{[2-(diisopropoxyphosphorylmethoxy)-ethyl]sulfanyl}pyrimidine (**32a**) and 2-amino-4,6-bis{[2-(phosphonomethoxy)ethyl]sulfanyl}pyrimidine (**33a**)

Phosphonate **20a** (6.9 g, 26.4 mmol) was added to a stirred mixture of disulfanylpyrimidine **31** (2 g, 12.56 mmol) and NaH (1.25 g, 60% in paraffin oil, 31 mmol) in DMF (50 mL) and the resulting mixture was stirred at r.t. for 24 h and evaporated in vacuo. The residue was adsorbed onto silica gel from methanol and separated by flash chromatography ($CHCl_3$/MeOH 0–5%) to give **32a** (5.6 g, 74%), pale yellow oil. ^1H NMR (DMSO-d_6): δ = 6.74 (br s, 2H, NH_2), 6.41 (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 3.79 (d, J(P,CH) = 8.3, 4H, PCH_2), 3.69 (t, J(1′,2′) = 6.4, 4H, H-1′), 3.26 (t, J(2″,1′) = 6.4, 4H, H-2″), 1.24 (d, 12H) and 1.23 (d, J(CH_3,CH) = 6.2, 12H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.60 (2C, C-4, C-6), 161.79 (C-2), 103.07 (C-5), 71.18 (d, J(P,C) = 12.2, 2C, C-2′), 70.35 (d, J(P,C) = 6.3, 4C, CHipr.), 64.81 (d, J(P,C) = 164.6, 2C, PCH_2), 27.60 (2C, C-1′), 24.03 (d, J(P,C) = 3.9, 4C) and 23.92 (d, J(P,C) = 4.4, 4C, CH_3) ppm. MS (ESI): m/z (%) = 604.2 (100) $[MH]^+$. Anal. $C_{22}H_{43}N_3O_8P_2S_2$ (C, H, N, P, S).

Subsequent deprotection of **32a** (2.6 g, 4.3 mmol) by GP1 gave free phosphonic acid **33a** (1.2 g, 65%) as a white foam. ^1H NMR (D_2O): δ = 6.75 (s, 1H, H-5), 3.97 (t, J(2′,1″) = 6.4, 4H, H-2′), 3.80 (d, J(P,CH) = 8.7, 4H, PCH_2), 3.45 (t, J(1′,2″) = 6.4, 4H, H-1′) ppm. ^{13}C NMR (D_2O): δ = 169.49 (2C, C-4, C-6), 161.20 (C-2), 104.15 (C-5), 70.59 (d, J(P,C) = 10.7, 2C, C-2′), 66.82 (d, J(P,C) = 156.2, 2C, PCH_2), 28.38 (2C, C-1′) ppm. MS (ESI): m/z (%) = 436 (35) $[MH]^+$. Anal. $C_{10}H_{19}N_3O_8P_2S_2$ (C, H, N, P, S).

4.29.3. 2-Amino-4,6-(2S,2′S)-bis{[2-(diisopropoxyphosphorylmethoxy)propyl]sulfanyl}pyrimidine (**32b**) and 2-amino-4,6-(2S,2′S)-bis{[2-(phosphonomethoxy)propyl]sulfanyl}pyrimidine (**33b**)

Prepared by the same procedure as compounds **32a** and **33a**. From pyrimidine **31** (200 mg, 1.25 mmol) and phosphonate **20b** (1.06 g, 2.6 mmol). Thick oil, 534 mg (68%). ^1H NMR (DMSO-d_6): δ = 6.69 (s, 2H, NH_2), 6.14 (s, 1H, H-5), 4.59 (m, 4H, CHipr.), 3.78 (d, J(P,CH) = 9.2, 4H, PCH_2), 3.74 (m, 2H, H-2′), 3.26 (dd, J(1″a,2′) = 5.5, J_{gem} = 13.6, 2H, H-1″a), 3.20 (dd, J(1′b,2′) = 5.8, J_{gem} = 13.6, 2H, H-1′b), 1.24 (d, 12H), 1.23 (d, 6H) and 1.16 (d, J(CH_3,CH) = 6.2, 6H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.63 (2C, C-4,6), 161.64 (C-2), 103.30 (C-5), 76.19 (d, J(P,C) = 12.7, 2C, C-2′), 70.30 (d, J(P,C) = 6.3, 4C, CHipr.), 62.84 (d, J(P,C) = 165.0, 2C, PCH_2), 33.21 (2C, C-1′), 24.02 (d, J(P,C) = 3.6, 4C) and 23.88 (d, J(P,C) = 4.6, 4C, CH_3ipr.), 18.73 (2C, C-3′) ppm. MS (ESI): m/z (%) = 654.2 (100) $[MNa]^+$.

Phosphonic acid **33b**, yield (280 mg, 73%), white foam. ^1H NMR (D_2O): δ = 6.81 (s, 1H, H-5), 3.94 (m, 2H, H-2′), 3.77 (dd, 2H) and 3.65 (dd, J(P,CH) = 9.3, J_{gem} = 13.2, 2H, PCH_2), 3.38 (dd, 2H) and 3.35 (dd, J(1′,2′) = 5.5, J_{gem} = 13.6, 2H, H-1″), 1.29 (d, J(3′,2″) = 6.2, 6H,

H-3′) ppm. MS (ESI): *m*/*z* (%) = 464.0 (100) $[MH]^+$. $[\alpha]_D^{25} = +48.3$ (*c* 0.357, H_2O). Anal. $C_{12}H_{23}N_3O_8P_2S_2.H_2O$ (C, H, N, P, S).

*4.29.4. 2-Amino-4,6-(2R,2′R)-bis{[2-(diisopropoxyphosphoryl-methoxy)propyl]sulfanyl}pyrimidine (**32c**) and 2-amino-4,6-(2R,2′R)-bis{[2-(phosphonomethoxy)propyl]sulfanyl}pyrimidine (**33c**)*

Prepared by the same procedure as compounds **32a** and **33a**. From pyrimidine **31** (500 mg, 3.1 mmol) and phosphonate **20c** (2.65 g, 6.5 mmol). Thick oil, 1.48 g (75%). NMR spectra identical with compound **32b**. MS (ESI): *m*/*z* (%) = 654.0 (100) $[MNa]^+$.

Phosphonic acid **33c**, yield 702 mg (71%), white foam. NMR spectra identical with compound **33b**. MS (ESI): *m*/*z* (%) = 464.0 (100) $[MH]^+$. $[\alpha]_D^{25} = -40.2$ (*c* 0.589, H_2O). Anal. $C_{12}H_{23}N_3O_8P_2S_2.H_2O$ (C, H, N, P, S).

*4.29.5. 2-Amino-6-{[2-(diisopropoxyphosphorylmethoxy)ethyl]-sulfanyl}-4-sulfanylpyrimidine (**34a**)*

To the solution of pyrimidine **31** (3 g, 18.84 mmol) and NaH (0.76 g, 60% in paraffin oil, 19 mmol) in DMF (70 mL) was added dropwise phosphonate **20a** (5 g, 19 mmol). The resulting mixture was stirred at r.t. for 3 days and evaporated in vacuo. The residue in $CHCl_3$ (200 mL) was washed with water (3 × 100 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue was separated by flash chromatography ($CHCl_3$/MeOH 0–5%) to give **32a** (3.18 g, 28%) and **34a** (2.92 g, 40%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6): δ = 11.90 (br s, 1H, SH), 7.00 (br s, 2H, NH_2), 6.33 (s, 1H, H-5), 4.59 (m, 2H, CHipr.), 3.78 (d, *J*(P,CH) = 8.3, 2H, PCH_2), 3.71 and 3.22 (2 × t, *J*(1′,2′) = 6.3, 2 × 2H, H-1′, H-2″), 1.24 (d, 6H) and 1.23 (d, *J*(CH_3,CH) = 6.2, 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 177.97 (C-4), 167.60 (C-2), 154.04 (C-6), 109.86 (C-5), 70.24 (d, *J*(P,C) = 12.2, C-2″), 70.36 (d, 2C, *J*(P,C) = 6.3, CHipr.), 64.83 (d, *J*(P,C) = 164.1, PCH_2), 28.33 (C-1′), 23.98 (d, 2C, *J*(P,C) = 3.9) and 23.91 (d, 2C, *J*(P,C) = 4.4, CH_3) ppm. MS (FAB): *m*/*z* (%) = 382 (100) $[MH]^+$. Anal. $C_{13}H_{24}N_3O_4PS_2$ (C, H, N, P, S).

*4.29.6. 2-Amino-6-(2S)-{[2-(diisopropoxyphosphorylmethoxy)-propyl]sulfanyl}-4-sulfanylpyrimidine (**34b**)*

To the solution of pyrimidine **31** (1 g, 6.3 mmol) and NaH (0.252 g, 60% in paraffin oil, 6.3 mmol) in DMF (25 mL) was added dropwise phosphonate **20b** (2.57 g, 6.3 mmol) at 0 °C. The resulting mixture was stirred at 60 °C for 8 h and evaporated in vacuo. The residue was purified by flash chromatography to give **32b** (750 mg, 19%) and **34b** (780 mg, 31%) as a thick oil. ^{1}H NMR (DMSO-d_6): δ = 11.88 (br s, 1H, SH), 7.00 (br s, 2H, NH_2), 6.33 (s, 1H, H-5), 4.59 (m, 2H, CHipr.), 3.79 (dd, 1H) and 3.75 (dd, J_{gem} = 13.8, *J*(P,CH) = 9.2, 1H, PCH_2), 3.74 (m, 1H, H-2′), 3.22 (dd, *J*(1″a,2′) = 5.4, J_{gem} = 13.6, 1H, H-1′a), 3.17 (dd, *J*(1′b,2′) = 5.8, J_{gem} = 13.6, 1H, H-1′b), 1.24 (d, 6H), 1.235 (d, 6H) and 1.18 (d, 3H, *J*(CH_3,CH) = 6.2, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 178.56 (C-4), 167.86 (C-2), 153.95 (C-6), 109.94 (C-5), 75.97 (d, *J*(P,C) = 12.8, C-2′), 70.37 and 70.34 (d, *J*(P,C) = 6.3, CHipr.), 62.78 (d, *J*(P,C) = 165.6, PCH_2), 34.35 (d, *J*(P,C) = 3.9, C-1′), 24.04 (d, 2C, *J*(P,C) = 3.0) and 23.90 (2C, *J*(P,C) = 4.6, CH_3), 18.17 (C-3′) ppm. MS (ESI): *m*/*z* (%) = 418 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{14}H_{27}N_3O_4PS_2$ $[MH]^+$ 396.1180, found 396.1176.

*4.29.7. 2-Amino-4-{[2-(diisopropoxyphosphorylmethoxy)-ethyl]sulfanyl}-6-(2S)-{[2-(diisopropoxyphosphorylmethoxy)-propyl]sulfanyl}pyrimidine (**35a**) and 2-amino-4-{[2-(phosphono-methoxy)ethyl]sulfanyl}-6-(2S)-{[2-(phosphonomethoxy)propyl]-sulfanyl}pyrimidine (**36a**)*

Monoderivative **34a** (300 mg, 0.79 mmol), NaH (0.035 g, 60% in paraffin oil, 0.87 mmol) and phosphonate **20b** (0.35 g, 0.86 mmol) in DMF (10 mL) were stirred at 60 °C for 4 h and evaporated in vacuo. The residue was treated with hot chloroform and filtered, and the filtrate was evaporated in vacuo. Flash chromatography afforded **35a** (400 mg, 82%) as an oil. ^{1}H NMR (DMSO-d_6): δ = 6.70 (br s, 2H, NH_2), 6.41 (s, 1H, H-5), 4.59 (m, 4H, CHipr.), 3.775 (d, 2H) and 3.77 (d, *J*(P,CH) = 8.4, 2H, PCH_2), 3.76 (m, 1H, H-2″), 3.71 (t, *J*(2′,1′) = 5.4, 2H, H-2′), 3.28 (dd, *J*(1″a,2″) = 6.1, J_{gem} = 13.6, 1H, H-1″a), 3.26 (t, *J*(1′,2′) = 5.4, 2H, H-1′), 3.20 (dd, *J*(1″b,2″) = 5.8, J_{gem} = 13.6, 1H, H-1″b), 1.245 (d, 12H) and 1.23 (d, *J*(CH_3,CH) = 6.2, 12H, CH_3ipr.), 1.18 (d, *J*(CH_3,CH) = 6.2, 3H, H-3″) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.71 and 167.47 (C-4,6), 161.70 (C-2), 103.83 (C-5), 75.70 (d, *J*(P,C) = 11.7, C-2″), 71.08 (d, 4C) and 70.64 (d, 4C, *J*(P,C) = 6.3, CHipr.), 70.03 (d, *J*(P,C) = 10.7, C-2′), 64.91 (d) and 62,05 (d, *J*(P,C) = 164.5, PCH_2), 32.10 (C-1″), 27.60 (C-1′), 24.66 (d, 4C) and 24.18 (d, 4C, *J*(P,C) = 3.9), 23.59 (d, 4C) and 23.32 (d, 4C, *J*(P,C) = 4.4, CH_3), 17.20 (C-3″) ppm. MS (FAB): *m*/*z* (%) = 618.2 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{46}N_3O_8P_2S_2$ $[MH]^+$ 618.2203, found 618.2205.

Diisopropylester **35a** was deprotected by GP1 to give **36a** (100 mg, 53%) as a white foam. ^{1}H NMR (D_2O): δ = 6.79 (s, 1H, H-5), 3.94 (m, 1H, H-2″), 3.87 (t, *J*(2′,1′) = 6.1, 2H, H-2′), 3.74 (dd, *J*(P,CH) = 9.2, J_{gem} = 13.3, 1H) and 3.70 (d, *J*(P,CH) = 8.9, 2H) and 3.66 (dd, *J*(P,CH) = 9.4, J_{gem} = 13.3, 1H, PCH_2), 3.38 (t, *J*(1′,2′) = 6.1, 2H, H-1′), 3.36 (dd, *J*(1″a,2″) = 5.4, J_{gem} = 14.4, 1H, H-1″a), 3.32 (dd, *J*(1″b,2″) = 6.1, J_{gem} = 14.4, 1H, H-1″b), 1.29 (d, *J*(3″,2″) = 6.3, 3H, H-3″). MS (ESI): *m*/*z* (%) = 450 (100) $[MH]^+$. $[\alpha]_D^{25} = +32.5$ (*c* 0.142, H_2O). Anal. $C_{11}H_{21}N_3O_8P_2S_2.H_2O$ (C, H, N, P, S).

*4.29.8. 2-Amino-4-{[2-(diisopropoxyphosphorylmethoxy)-ethyl]sulfanyl}-6-(2R)-{[2-(diisopropoxyphosphorylmethoxy)-propyl]sulfanyl}pyrimidine (**35b**) and 2-amino-4-{[2-(phosphonomethoxy)ethyl]sulfanyl}-6-(2R)-{[2-(phosphonomethoxy)propyl]sulfanyl}-pyrimidine (**36b**)*

Prepared by the same procedure as compounds **35a** and **36a** from pyrimidine **34a** and phosphonate **20c**.

35b: thick oil, yield 390 mg (80%). NMR spectra identical with compound **35a**. MS (FAB): *m*/*z* (%) = 618.0 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{46}N_3O_8P_2S_2$ $[MH]^+$ 618.2203, found 618.2206.

36b: white foam, yield 95 mg (50%). NMR spectra identical with compound **36a**. MS (ESI): *m*/*z* (%) = 450 (100) $[MH]^+$; 472 (50) $[MNa]^+$. $[\alpha]_D^{25} = -19.3$ (*c* 0.216, H_2O). Anal. $C_{11}H_{21}N_3O_8P_2S_2.H_2O$ (C, H, N, P, S).

*4.29.9. 2-Amino-4,6-(2R,2′S)-bis{[2-(diisopropoxyphosphoryl-methoxy)propyl]sulfanyl}pyrimidine (**35c**) and 2-amino-4-(2R,2′S)-bis{[2-(phosphonomethoxy)propyl]sulfanyl}pyrimidine (**36c**)*

Prepared by the same procedure as compounds **35a** and **36a** from pyrimidine **34b** (400 mg, 1.01 mmol) and phosphonate **20c** (0.45 g, 1.1 mmol).

35c: thick oil, yield 440 mg (71%). NMR spectra identical with compound **32b**. MS (ESI): *m*/*z* (%) = 654.0 (100) $[MNa]^+$.

36c: white foam, yield 180 mg (61%). ^{1}H NMR (D_2O): δ = 6.86 (s, 1H, H-5), 3.95 (m, 2H, H-2′), 3.79 (dd, J_{gem} = 13.2, *J*(CH_2,P) = 9.3, 2H) and 3.68 (dd, J_{gem} = 13.2, *J*(CH_2,P) = 9.5, 2H, PCH_2), 3.43 (dd, J_{gem} = 14.3, *J*(1,2) = 4.4, 2H) and 3.33 (dd, J_{gem} = 14.3, *J*(1,2) = 6.2, 2H, H-1′), 1.30 (d, *J*(CH_3,2′) = 6.7, 6H, H-3′) ppm. MS (ESI): *m*/*z* (%) = 464.0 (100) $[MH]^+$. $[\alpha]_D^{25} = +0.3$ (*c* 0.358, H_2O). Anal. $C_{12}H_{23}N_3O_8P_2S_2.H_2O$ (C, H, N, P, S).

4.30. Alkoxyalkyl esters of bisphosphonates

*4.30.1. 2-Amino-4,6-bis(2-hydroxyethoxy)pyrimidine (**37**)*

A solution of *t*-BuOK (13.5 g, 120 mmol) in ethyleneglycol (50 mL) was heated at 80 °C for 30 min and dichloropyrimidine **10** (5 g, 30 mmol) was added. The resulting mixture was stirred at 100 °C for 1 h, cooled to r.t. and neutralized by addition of Dowex 50 × 8; the resulting mixture was diluted with water (100 mL), applied onto a column of Dowex 50 × 8 and washed with water (1 L). Column was then eluted with 2.5% aq. ammonia, UV absorbing fraction was collected and evaporated under reduced pressure. The crude product was recrystallized from water to give a white solid

(4.65 g, 71%), m.p. 158 °C. ^{1}H NMR (DMSO-d_6): $\delta = 6.48$ (br s, 2H, NH_2), 5.32 (s, 1H, H-5), 4.82 (t, J(OH,2′) = 5.4, 2H, OH), 4.17 (t, J(1′,2′) = 5.1, 4H, H-1′), 3.63 (q, J(2′,1′) = J(2′,OH) = 5.2, 4H, H-2′) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.57$ (C-4, C-6), 162.87 (C-2), 76.69 (C-5), 67.53 (C-1′), 59.61 (C-2′) ppm. MS (ESI): m/z (%) = 216 (100) $[MH]^+$. Anal. $C_8H_{13}N_3O_4$ (C, H, N).

*4.30.2. Hexadecyloxyethyl toluenesulfonyloxymethylphosphonate, sodium salt (**38**)*

Prepared by previously described procedure [17a]; yield 47%. ^{1}H NMR ($CDCl_3$): $\delta = 7.77$ (br d, J(CH,CH) = 5.81, 2H, CHarom.), 7.30 (br d, J(CH,CH) = 6.0, 2H, CHarom.), 4.07 (br, 2H, PCH_2), 3.97 (br, 2H, OCH_2), 3.45 (br, 2H, OCH_2), 3.34 (br, 2H, OCH_2), 2.38 (s, 3H, CH_3arom.), 1.48 (br, 2H, $OCH_2\underline{CH_2}CH_2$), 1.25 (br s, 26H, 13 × CH_2), 0.87 (t, J(CH_3,CH_2) = 6.83, $\underline{CH_3}CH_2$) ppm. MS (ESI): m/z (%) = 579 (100) $[MNa]^+$. Anal. $C_{26}H_{46}NaO_7PS$ (C, H, N, P, S).

*4.30.3. 2-(Hexadecyloxy)ethyl 2-amino-4-(2-hydroxyethoxy)-6-[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**39a**)*

GP7, white solid (47 mg, 31%), m.p. 129 °C. ^{1}H NMR (DMSO-d_6): $\delta = 6.51$ (br s, 2H, NH_2), 5.31 (s, 1H, H-5), 4.24 (m, 2H, H-1′), 4.15 (t, J(1″,2″) = 5.2, 2H, H-1″), 3.75 (m, 2H, H-4′), 3.68 (m, 2H, H-2′), 3.62 (m, 2H, H-2″), 3.39 (m, 6H, H-3′, 5′, 6′), 1.45 (m, 2H, H-7′), 1.22 (m, 26H, alif.), 0.84 (t, J(CH_3,CH_2) = 6.9, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 171.52$ and 171.37 (C-4, C-6), 162.83 (C-2), 78.62 (C-5), 70.79 (d, J(5′,P) = 5.6, C-5′), 70.46 (C-6″), 69.99 (d, J(2′,P) = 8.9, C-2′), 67.47 (C-1″), 64.98 (C-1′), 62.88 (d, J(4′,P) = 5.1, C-4′), 59.55 (C-2″), 31.51 ($CH_3CH_2\underline{CH_2}$), 29.52, 29.25, 29.15, 28.92 and 25.90 (alif.), 22.31 ($CH_3\underline{CH_2}$), 14.18 (CH_3) ppm. MS (ESI): m/z (%) = 598.4 (80) $[M - H]^-$. Anal. $C_{27}H_{51}N_3NaO_8P$ (C, H, N, P).

*4.30.4. Bis[2-(hexadecyloxy)ethyl] 2-amino-4,6-bis-[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**40a**)*

GP7, white solid (59 mg, 24%), m.p. 184 °C. MS (ESI): m/z (%) = 962.5 (88) $[M - Na + H]^+$. Anal. $C_{46}H_{89}N_3Na_2O_{12}P_2$ (C, H, N, P).

*4.30.5. 2-(Hexadecyloxy)ethyl 2-amino-5-bromo-4-(2-hydroxyethoxy)-6-[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**39b**)*

GP7, white solid (44 mg, 26%), m.p. 83 °C. ^{1}H NMR (DMSO-d_6): $\delta = 6.72$ (br s, 2H, NH_2), 4.86 (br s, 1H, OH), 4.34 (m, 2H, H-1′), 4.25 (m, 2H, H-1″), 3.61–3.80 (m, 6H, H-2′, 4′, 2″), 3.38 (m, 6H, 2 × OCH_2, PCH_2), 1.44 (m, 2H, H-7′), 1.22 (m, 26H, alif.), 0.84 (t, J(CH_3,CH_2) = 6.8, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 166.40$ and 166.28 (C-4, C-6), 160.87 (C-2), 73.57 (C-5), 70.78 (d, J(5′,P) = 5.7, C-5′), 70.48 (C-6′), 69.88 (C-2′), 68.41 (C-1″), 66.20 (C-1′), 63.02 (C-4′), 59.42 (C-2″), 31.52 ($CH_3CH_2\underline{CH_2}$), 29.54, 29.22, 29.18, 28.94, 25.91 (alif.), 22.33 ($CH_3\underline{CH_2}$), 14.19 (CH_3) ppm. MS (ESI): m/z (%) = 654 (100) $[M - Na]^-$. Anal. $C_{27}H_{50}BrN_3NaO_8P$ (C, H, N, P).

*4.30.6. Bis[2-(hexadecyloxy)ethyl] 2-amino-5-bromo-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**40b**)*

GP7, white solid (61 mg, 23%), m.p. 160 °C dec. MS (ESI): m/z (%) = 1038 (26) $[M - Na]^-$, 1016 (63) $[M-(2 \times Na) + H]^-$. Anal. $C_{46}H_{88}BrN_3Na_2O_{12}P_2$ (C, H, N, P).

*4.30.7. 2-(Hexadecyloxy)ethyl 2-amino-4-(2-hydroxyethoxy)-5-methyl-6-[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**39c**)*

GP7, white solid (122 mg, 40%), m.p. 95 °C. ^{1}H NMR (DMSO-d_6): $\delta = 6.20$ (br s, 2H, NH_2), 4.85 (br, 1H, OH), 4.28 (m, 2H, H-1′), 4.19 (m, 2H, H-1″), 3.62–3.81 (m, 6H, H-2′, 4′, 2″), 3.39 (m, 6H, H-3′, 5′, 6′), 1.77 (s, 3H, 5-CH_3), 1.43 (m, 2H, H-7′), 1.22 (m, 26H, alif.), 0.84 (t, J(CH_3,CH_2) = 7.0, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 168.48$ and 168.32 (C-4, C-6), 160.37 (C-2), 86.86 (C-5), 70.73 (d, J(5′,P) = 5.5, C-5′), 70.52 (C-6′), 70.41 (C-2′), 67.49 (C-1″), 65.15 (C-1′), 63.11 (br, C-4′), 59.77 (C-2″), 31.57 ($CH_3CH_2\underline{CH_2}$), 29.55, 29.31, 29.21, 28.98, 25.94 (alif.), 22.37 ($CH_3\underline{CH_2}$), 14.24 (CH_3), 7.09 (5-CH_3) ppm. MS (ESI): m/z (%) = 590.3 (100) $[M - Na]^-$. Anal. $C_{28}H_{53}N_3NaO_8P$ (C, H, N, P).

*4.30.8. Bis[2-(hexadecyloxy)ethyl] 2-amino-5-methyl-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**40c**)*

GP7, white solid (106 mg, 21%), m.p. 142 °C. MS (ESI): m/z (%) = 952.5 (48) $[M - (2 \times Na) + H]^-$. Anal. $C_{47}H_{91}N_3Na_2O_{12}P_2$ (C, H, N, P).

*4.30.9. 2-Amino-5-bromo-4,6-bis(2-hydroxyethoxy)pyrimidine (**41**)*

Pyrimidine **37** (1 g, 4.65 mmol) in DMF (15 mL) was treated with bromine (0.7 M solution in CCl_4, 10 mL) and the mixture was stirred at r.t. overnight. The mixture was evaporated in vacuo and codistilled with EtOH (3 × 100 mL). The crude product was recrystallized from EtOH to give pale yellow needles (530 mg, 39%), m.p. 178 °C. ^{1}H NMR (DMSO-d_6): $\delta = 6.71$ (br s, 2H, NH_2), 4.26 (t, J(1′,2′) = 5.3, 4H, H-1′); 3.67 (t, J(2′,1′) = 5.3, 4H, H-2′) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 166.42$ (C-4, C-6), 160.88 (C-2), 73.60 (C-5), 68.46 (C-1′), 59.46 (C-2′) ppm. MS (ESI): m/z (%) = 294 (18) $[MH^+]$, 276 (100) $[M - H_2O + H]^+$. Anal. $C_8H_{12}BrN_3O_4$ (C, H, Br, N).

4.31. Capillary electrophoresis

Analyses were performed in a commercial P/ACE MDQ capillary electrophoresis (CE) apparatus (Beckman Coulter, Fullerton, CA, USA), equipped with an internally non-coated fused silica capillary with outer polyimide coating, total length 390 mm, effective length (from injection end to the detector) 288 mm, I.D./O.D. 50/375 μm (Polymicro Technologies, Phoenix, AR, USA). The analytes were monitored by UV–vis absorption spectrophotometric photodiode array detector (190–600 nm) at two wavelengths, 206 and 254 nm, respectively. The temperature of capillary liquid coolant was set at 20 °C.

The samples were injected hydrodynamically, by pressure 13.8 mbar for 10 s. The analytes were dissolved in deionized water, the concentration of enantiomers in their individual CZE analyses was 0.1 mM, whereas in enantiomeric mixtures the concentration of *R*-isomer was 0.2 mM and of *S*-isomer 0.1 mM, in order to distinguish their migration order. Separation voltage was 15 kV.

The analyses were performed both in non-chiral and chiral background electrolytes (BGEs) of the following composition:

Non-chiral BGEs: 25–50 mM borax, adjusted by NaOH to pH 10.0–10.5.

Chiral BGEs: 25–50 mM borax, adjusted by NaOH to pH 10.0–10.5 + chiral selector β-cyclodextrin (5–20 mg/mL).

4.32. Biological activity assays

In vitro cytostatic activity tests (cell growth inhibition) were performed with cultures of murine leukemia L1210 cells (ATCC CCL 219), human promyelocytic leukemia HL60 cells (ATCC CCL 240), human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2) and the human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119) [19].

The methodology of the antiviral activity assays followed previously described procedures [20,21].

Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

Acknowledgments

This study was performed as a part of research project OZ40550506 of the Institute of Organic Chemistry and Biochemistry, v.v.i. This study was supported by Centre of new antivirals and

antineoplastics 1M0508 by the Ministry of Education, Youth and Sports of the Czech Republic, by Gilead Sciences and IOCB Research Centre and by the NIH grant 1UC1AI062540-01. The authors' thanks are due to Prof. E. De Clercq and his group in the Rega Institute, Katholic University Leuven (Belgium) for the evaluation of antiviral activity; and to Dr I. Votruba of the Institute of Organic Chemistry and Biochemistry for the evaluation of cytostatic activity.

Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2008.09.031.

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