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Acyclic Nucleoside Bisphosphonates: Synthesis and Properties of Chiral 2-Amino-4,6-bis[(phosphonomethoxy)alkoxy]pyrimidines

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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.

Keywords

Acyclic Nucleoside Phosphonates; Pyrimidine; Bisphosphonates; Alkylation

1. Introduction

Acyclic nucleoside phosphonates [1] (ANPs) 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**, Figure 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**, **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

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by phosphonate-bearing building block afforded a mixture of *O*⁶- and *N*¹-regioisomers. 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–3d**) and 2-amino-4,6-dihydroxypyrimidine significantly inhibited replication of retroviruses and herpes viruses in cell culture. Compounds **3** can be considered as analogues 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 analogue 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** (Figure 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-(diisopropoxyphosphoryl)methoxy 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**) 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 equivalents 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 one equivalent of **11** and one equivalent 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 the 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 $\times$ 8 in H⁺ form.

Synthesis of bisphosphonates bearing two 2-(phosphonomethoxy)propoxy (PMPO) chains or two different phosphonoalkoxy chains by this method failed. Reaction of **10** with phosphonoalkoxyalkanols 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 the 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 dihydroxypyrimidine **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 **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 one equivalent of appropriate phosphonate **20** (Table 1) in DMSO afforded a mixture of mono- and dialkylated product **23** and **24** (Scheme 3, Table 1), respectively. Monoalkylated product **23** was subsequently alkylated by **20** in DMF to afford bisderivative 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. [13a]. 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-disulfanylpurimidine

Pyrimidine [15] **10** was converted to the disulfanyl analogue **31** by reaction with thiourea (Scheme 4) [16]. Alkylation of **31** with phosphonate **20a–c** (Table 1) gave unequivocally *S*-alkylated product **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 one equivalent of alkylating agent **20a** or **20b** gave monoalkylated product **34a** and **34b** together with dialkylated product. Further alkylation of **34a–b** 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 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 and elemental analysis and were not tested for biological activity. Our attempts to convert compound **6a** to *cycloSal*, *cycloAmb* [18] or POM [19] esters failed due to instability of ether bonds at positions 4 and 6 under reaction conditions.

2.5. Capillary electrophoresis

Enantiomeric purity of compounds **27a** and **27b** were 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), (Figure 3A). The compound **27a** was found enantiomerically pure as demonstrated by single peak of CE analysis of this compound in chiral BGE (Figure 3B) whereas a very small admixture of enantiomer **27a** was found in the CE analysis of compound **27b** (Figure 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 was 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 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 ^1H and 125.8 MHz for ^{13}C) and Bruker Avance 400 (^1H at 400, ^{13}C at 100.6 MHz) spectrometers in CDCl_3 , $\text{DMSO}-d_6$, or D_2O . Chemical shifts (in ppm, δ scale) were referenced to TMS (for ^1H NMR spectra in CDCl_3) and/or to the solvent signal (CDCl_3 δ = 7.26 ppm for ^1H NMR and δ = 77.0 ppm for ^{13}C NMR; $\text{DMSO}-d_6$ for ^1H NMR δ = 2.5 ppm and for ^{13}C δ = 39.7). Chemical shifts in D_2O were referenced to 1,4-dioxane for ^1H NMR δ = 3.75 and for ^{13}C NMR δ = 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 a LCQ classic spectrometer using electrospray ionization (ESI). Preparative HPLC purification was performed on a column packed with 10 μm C18 reversed phase (Luna), 250 $\times$ 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 ^1H , ^1H -COSY, ^1H , ^{13}C -HSQC, and ^1H , ^{13}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 ^1H NMR spectra.

4.1.1. General procedure 1 (GP1) – Deprotection of diisopropylesters 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 $\times$ 50 mL) and 0.1 M TEAB (2 $\times$ 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 $\times$ 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 $^\circ\text{C}$ for 30 min; appropriate phosphonate **20a–e** (2.1 mmol) was added and the resulting mixture was heated at 120 $^\circ\text{C}$ for 8–16 hr. The mixture was cooled to r.t., DMSO was evaporated in vacuo at 60 $^\circ\text{C}$. 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 MgSO₄ and taken down in vacuo. The residue was separated by flash chromatography (CHCl₃/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) was heated at 60 °C for 30 min., phosphonate **20a–e** (1 mmol) in DMSO (1 mL) was added dropwise and the reaction mixture was heated at 120 °C for 8 hr, cooled to r.t. and evaporated in vacuo at 60 °C. The residue was codistilled with DMF and EtOH, diluted with CHCl₃, washed with 3 portions of water and dried over MgSO₄. Products were separated by flash chromatography (CHCl₃/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) was heated at 40 °C for 30 min., appropriate phosphonate **20a–e** (1.1 mmol) was added and the mixture was stirred at 100 °C for 8–12 hr, evaporated in vacuo, and codistilled with EtOH.

4.1.5. General procedure 5 (GP5) – Oxidation of 2-methylsulfanyl group to 2-methylsulfonyl group by m-CPBA—2-Methylsulfanyl derivative (1 mmol) in CH₂Cl₂ (6 mL) was treated with m-chloroperoxybenzoic acid (3 mmol) at r.t. for 3–12 hr. The reaction mixture was diluted with CH₂Cl₂, washed with saturated aqueous Na₂S₂O₅, saturated NaHCO₃ and water. Organic fraction was dried over MgSO₄ and purified by flash chromatography (CHCl₃/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 hr. The reaction mixture was concentrated in vacuo and the crude product in CHCl₃ was applied onto a pad of silica gel and washed with 10% MeOH in CHCl₃ (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 was 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 hr and taken down in vacuo. The residue in CHCl₃ (50 mL) was washed with brine (2 × 50 mL) and water (1 × 50 mL) and evaporated under reduced pressure. Flash chromatography (EtOAc:EtOH:acetone:H₂O, 6:1:1:0.5 and EtOAc:EtOH:acetone:H₂O, 4:1:1:1) gave compounds **39** and **40**.

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

(S)-1,2-Isopropylidene-glycerol (**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 CHCl₃ and filtered through Celite. Chromatography on silica gel (CHCl₃/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. ¹H NMR (DMSO-*d*₆): δ = 6.60 (br s, 2H, NH₂), 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, CH₃) ppm. ¹³C NMR (DMSO-*d*₆): δ = 171.19 (2C, C-4 and C-6), 162.73 (C-2), 108.96 (2C, CHMe₂), 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 ($2 \times 2\text{C}$, CH_3) ppm. MS (FAB): m/z (%) = 356.1 (85) $[\text{MH}]^+$. Anal. $\text{C}_{16}\text{H}_{25}\text{N}_3\text{O}_6$ (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. ^1H NMR ($\text{DMSO}-d_6$): δ = 6.50 (br s, 2H, NH_2), 5.32 (s, 1H, H-5), 4.90 and 4.63 ($2 \times$ br s, $2 \times 2\text{H}$, OH), 4.17 (dd, $J(1'a,2') = 4.3$, $J_{\text{gem}} = 10.9$, 2H, H-1'a), 4.06 (dd, $J(1'b,2') = 6.6$, $J_{\text{gem}} = 10.9$, 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 ($\text{DMSO}-d_6$): 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) $[\text{MH}]^+$. Anal. $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_6 \cdot 1/2 \text{H}_2\text{O}$ (C, H, N).

4.3. 2-Amino-4,6-(2R,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 $\text{CHCl}_3/\text{MeOH}$ (0–3%) afforded colorless oil (2.6 g, 39%). ^1H NMR ($\text{DMSO}-d_6$): δ = 6.57 (br s, 2H, NH_2), 5.29 (s, 1H, H-5), 4.78 (t, $J(\text{OH},3') = 5.4$, 2H, OH), 4.58 (m, 4H, CH₂), 4.32 (dd, $J(1'a,2') = 3.6$, $J_{\text{gem}} = 11.5$, 2H, H-1'a), 4.19 (dd, $J(1'b,2') = 6.1$, $J_{\text{gem}} = 11.5$, 2H, H-1'b), 3.90 (dd, $J(\text{P},\text{CH}) = 8.7$, $J_{\text{gem}} = 13.8$, 2H) and 3.86 (dd, $J(\text{P},\text{CH}) = 8.9$, $J_{\text{gem}} = 13.8$, 2H, PCH_2), 3.69 (m, 2H, H-2'), 3.51 (t, $J(3',2') = J(3',\text{OH}) = 5.4$, 4H, H-3'), 1.235 (d, 6H), 1.23 (d, 6H), 1.22 (d, 6H) and 1.21 (d, $J(\text{CH}_3,\text{CH}) = 6.2$, 6H, CH_3) ppm. ^{13}C NMR ($\text{DMSO}-d_6$): δ = 171.34 (2C, C-4, C-6), 162.77 (C-2), 80.53 (d, 2C, $J(\text{P},\text{C}) = 11.7$, C-2'), 78.46 (C-5), 70.37 (d, 2C) and 70.32 (d, 2C, $J(\text{P},\text{C}) = 6.3$, CH₂), 65.25 (2C, C-1'), 63.99 (d, 2C, $J(\text{P},\text{C}) = 164.6$, PC), 60.08 (2C, C-3'), 23.99 (d, 4C, $J(\text{P},\text{C}) = 3.9$) and 23.82 (d, 4C, $J(\text{P},\text{C}) = 4.4$, CH_3) ppm. MS (FAB): m/z (%) = 632.6 (56) $[\text{MH}]^+$. Anal. $\text{C}_{24}\text{H}_{47}\text{N}_3\text{O}_{12}\text{P}_2$ (C, H, N, P).

4.4. 2-Amino-4,6-(2R,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. ^1H NMR (D_2O): δ = 4.56 (dd, $J(1'a,2') = 3.8$, $J_{\text{gem}} = 11.2$, 2H, H-1'a), 4.43 (dd, $J(1'b,2') = 5.6$, $J_{\text{gem}} = 11.2$, 2H, H-1'b), 3.94 (m, 2H, H-2'), 3.89 (dd, 2H) and 3.84 (dd, $J(\text{P},\text{CH}) = 9.3$, $J_{\text{gem}} = 13.3$, 2H, PCH_2), 3.82 (dd, $J(3'a,2') = 4.3$, $J_{\text{gem}} = 12.2$, 2H, H-3'a), 3.75 (dd, $J(3'b,2') = 5.7$, $J_{\text{gem}} = 12.2$, 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, $J(\text{P},\text{C}) = 11.2$, 2C, C-2'), 68.16 (2C, C-1'), 65.74 (d, $J(\text{P},\text{C}) = 158.7$, 2C, PC), 60.11 (2C, C-3') ppm. MS (FAB): m/z (%) = 464 (49) $[\text{MH}]^+$. $[\alpha]_D^{25} = -1.0$ (c 0.502, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{12}\text{P}_2 \cdot \text{H}_2\text{O}$ (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-(2R,2'R)-bis(2,2-dimethyl-4-methoxy-1,3-dioxolane)pyrimidine, yellow oil, yield 9.6 g (89%). ^1H 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) $[\text{MH}]^+$. Anal. $\text{C}_{16}\text{H}_{25}\text{N}_3\text{O}_6$ (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. ^1H 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) $[\text{MH}]^+$. 'Anal. $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_6 \cdot 1/2 \text{H}_2\text{O}$ (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%). ^1H NMR (DMSO- d_6) and ^{13}C NMR (DMSO- d_6) identical with **13a**. MS (FAB): m/z (%) = 632.6 (100) $[\text{MH}]^+$. Anal. $\text{C}_{24}\text{H}_{47}\text{N}_3\text{O}_{12}\text{P}_2$ (C, H, N, P).

4.6. 2-Amino-4,6-(2*S*,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%). ^1H NMR (D_2O) and ^{13}C NMR (D_2O) identical with **13b**. MS (FAB): m/z (%) = 464 (15) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}} = +1.9$ (c 0.267, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{12}\text{P}_2 \cdot \text{H}_2\text{O}$ (C, H, N, P).

4.7. 2-Amino-4-chloro-6-(2*S*)-(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 was taken to chloroform and 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. ^1H 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, $J(1'a,2') = 4.4$, $J_{\text{gem}} = 11.0$, 1H, H-1'a), 4.21 (dd, $J(1'b,2') = 6.3$, $J_{\text{gem}} = 11.0$, 1H, H-1'b), 4.04 (dd, $J(3'a,2') = 6.5$, $J_{\text{gem}} = 8.6$, 1H, H-3'a), 3.70 (dd, $J(3'b,2') = 6.0$, $J_{\text{gem}} = 8.6$, 1H, H-3'b), 1.32 and 1.27 ($2 \times$ s, $2 \times 3\text{H}$, 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) $[\text{MNa}]^+$, 260.0 (37) $[\text{MH}]^+$. Anal. $\text{C}_{10}\text{H}_{14}\text{ClN}_3\text{O}_3$ (C, H, Cl, N).

4.8. 2-Amino-4,6-(2*R*,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. ^1H 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) $[\text{MH}]^+$. Anal. $\text{C}_{16}\text{H}_{25}\text{N}_3\text{O}_6$ (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. ^1H NMR (DMSO- d_6): δ = 6.51 (br s, 2H, NH_2), 5.32 (s, 1H, H-5), 4.90 and 4.65 ($2 \times$ br s, $2 \times 2\text{H}$, OH), 4.17 (dd, $J(1'a,2') = 4.3$, $J_{\text{gem}} = 10.9$, 2H, H-1'a), 4.06 (dd, $J(1'b,2') = 6.6$, $J_{\text{gem}} = 10.9$, 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) $[\text{MH}]^+$. Anal. $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_6 \cdot 1/2 \text{H}_2\text{O}$ (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%). ^1H NMR (DMSO- d_6) and ^{13}C NMR (DMSO- d_6) identical with **13a**. MS (FAB): m/z (%) = 632.1 (100) $[\text{MH}]^+$. Anal. $\text{C}_{24}\text{H}_{47}\text{N}_3\text{O}_{12}\text{P}_2$ (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%. ^1H NMR (D_2O) and ^{13}C NMR (D_2O) identical with **13b**. MS (FAB): m/z (%) = 464 (35) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}} = +0.02$ (c 0.383, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{12}\text{P}_2 \cdot \text{H}_2\text{O}$ (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. ^1H NMR (DMSO- d_6): δ = 5.88 (s, 1H, H-5), 4.58 (dh, $J(\text{CH},\text{P}) = 7.7$, $J(\text{CH},\text{CH}_3) = 6.3$, 4H, CHipr.), 4.43 (m, 4H, H-1'), 3.82 (m, 4H, H-2'), 3.79 (d, $J(\text{C},\text{H},\text{P}) = 8.3$, 4H, PCH_2), 2.48 (s, 3H, SCH_3), 1.23 (m, 24H, CH_3 ipr.) 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, $J(2',\text{P}) = 11.9$, C-2'), 70.36 (d, $J(\text{CH},\text{P}) = 6.2$, CHipr.), 65.67 (C-1'), 64.95 (d, $J(\text{C},\text{P}) = 164.6$, PCH_2), 24.00 (d, $J(\text{CH}_3,\text{P}) = 3.7$) and 23.85 (d, $J(\text{CH}_3,\text{P}) = 4.6$, CH_3 ipr.), 13.66 (SCH_3) ppm. MS (ESI): m/z (%) = 625.1 (100) $[\text{MNa}]^+$, 603 (15) $[\text{MH}]^+$. HR MS (FAB) calcd. for $\text{C}_{23}\text{H}_{45}\text{N}_2\text{O}_{10}\text{P}_2\text{S}$ $[\text{MH}]^+$ 603.2192, found 603.2185. Anal. $\text{C}_{23}\text{H}_{45}\text{N}_2\text{O}_{10}\text{P}_2\text{S}$ (C, H, N, P, S).

Further elution of column gave **22**, yield 1.35 g (12%), colorless syrup. ^1H 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, $J(\text{P},\text{CH}) = 8.3$, 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, $J(\text{CH}_3,\text{CH}) = 6.2$, 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, $J(\text{C},\text{P}) = 11.9$, C-2'), 70.44 (d, $J(\text{C},\text{P}) = 11.89$, C-2''), 70.04 (m, CHipr.), 69.53 (d, $J(\text{C},\text{P}) = 6.61$, CHipr.), 65.31 (C-1'), 64.80 (d, $J(\text{C},\text{P}) = 164.41$, PCH_2 '), 64.75 (d, $J(\text{C},\text{P}) = 163.72$, PCH_2''), 43.69 (C-1''), 23.61 (m, CH_3 ipr.), 14.43 (SCH_3) ppm. MS (ESI): m/z (%) = 625.1 (100) $[\text{MNa}]^+$. HR MS (FAB) calcd. for $\text{C}_{23}\text{H}_{45}\text{N}_2\text{O}_{10}\text{P}_2\text{S}$ $[\text{MH}]^+$ 603.2192, found 603.2193. Anal. $\text{C}_{23}\text{H}_{45}\text{N}_2\text{O}_{10}\text{P}_2\text{S}$ (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. ^1H NMR (DMSO- d_6): δ = 12.32 (br s, 1H, OH), 5.39 (br s, 1H, H-5), 4.59 (dh, $J(\text{CH},\text{CH}_3) = 6.2$, $J(\text{CH},\text{P}) = 7.8$, 2H, CHipr.), 4.33 (m, 2H, H-1'), 3.80 (m, 2H, H-2'), 3.79 (d, $J(\text{CH}_2,\text{P}) = 8.3$, 2H, CH_2P), 2.47 (s, 3H, SCH_3), 1.24 (d, $J(\text{CH}_3,\text{CH}) = 6.0$, 6H) and (1.22 d, $J(\text{CH}_3,\text{CH}) = 6.0$, 6H, CH_3 ipr.) ppm. ^{13}C NMR (DMSO- d_6): δ = 169.19 (C-4), 86.06 (C-5), 70.66 (d, $J(2,\text{P}) = 11.9$, C-2'), 70.43 (d, $J(\text{C},\text{O},\text{P}) = 6.3$, CHipr.), 65.83 (C-1'), 64.97 (d, $J(\text{C},\text{P}) = 164.5$, CH_2P), 24.03 (d, $J(\text{CH}_3,\text{P}) = 3.6$) and 23.89 (d, $J(\text{CH}_3,\text{P}) = 4.4$, CH_3 ipr.), 13.12 (SCH_3) ppm. MS (ESI): m/z (%) = 403 (100) $[\text{MNa}]^+$. HR MS (FAB) calcd. for $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_6\text{PS}$ $[\text{MH}]^+$ 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. ^1H NMR (CDCl_3): δ = 12.3 (br s, 1H, OH), 5.40 (br s, 1H, H-5), 4.58 (m, 2H, CH₂ipr.), 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, OCH₂P), 2.47 (s, 3H, SCH₃), 1.22 (m, 12H, CH₃ipr.), 1.15 (d, $J(\text{CH}_3,2')$ = 6.4, 3H, H-3') ppm. ^{13}C NMR (CDCl_3): δ = 170.52 (C-2), 169.15 (C-4), 162.50 (C-6), 86.08 (C-5), 75.24 (d, $J(2',\text{P})$ = 12.8, C-2'), 70.32 (m, 2C, CH₂ipr.), 69.20 (C-1'), 63.01 (d, $J(\text{OCH}_2,\text{P})$ = 165.2, OCH₂P), 23.92 (m, 4C, CH₃ipr.), 16.28 (C-3'), 13.10 (SCH₃) ppm. MS (FAB): m/z (%) = 395 (100) [MH]⁺. HR MS (FAB) calcd. for C₁₅H₂₈N₂O₆PS [MH]⁺ 395.1405, found 395.1418.

Dialkylated derivative **24a** was isolated as a second product. Yield 1.75 g (22%), colorless oil. ^1H NMR (CDCl_3): δ = 5.69 (s, 1H, H-5), 4.74 (m, 4H, CH₂ipr.), 4.307 (d, $J(1',2')$ = 5.17, 4H, H-1'), 3.89 (m, 2H, H-2'), 3.82 (d, $J(\text{CH}_2,\text{P})$ = 9.11, 4H, OCH₂P), 2.49 (s, 3H, SCH₃), 1.31 (m, 24H, CH₃ipr.), 1.23 (d, $J(3',2')$ = 6.39, 6H, H-3') ppm. ^{13}C NMR (CDCl_3): δ = 170.92 (C-2), 170.17 (C-4,6), 86.12 (C-5), 75.85 (d, $J(2',\text{P})$ = 11.98, C-2'), 71.05 (d, $J(\text{C},\text{P})$ = 6.68) and 70.96 (d, $J(\text{C},\text{P})$ = 6.66, CH₂ipr.), 69.35 (C-1'), 64.04 (d, $J(\text{C},\text{P})$ = 168.56, PCH₂), 24.06 (d, $J(\text{C},\text{P})$ = 3.69) and 23.92 (d, $J(\text{C},\text{P})$ = 4.64, CH₃ipr.), 16.55 (C-3'), 14.00 (SCH₃) ppm. MS (ESI): m/z (%) = 653 (100) [MNa]⁺, 631 (100) [MH]⁺. HR MS (FAB) calcd. for C₂₅H₄₉N₂O₁₀P₂S [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. ^1H 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 C₁₅H₂₈N₂O₆PS [MH]⁺ 395.1405, found 395.1413. Compound **24b** isolated as a colorless oil, yield 1.2 g (7.5%). ^1H NMR (CDCl_3) identical with compound **24a**. MS (FAB): m/z (%) = 631 (100) [MH]⁺. HR MS (FAB) calcd. for C₂₅H₄₉N₂O₁₀P₂S [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 CHCl₃, filtered through a pad of silica gel and washed with 10% MeOH in CHCl₃ (150 mL). The filtrate was taken down in vacuo. The residue was without further purification oxidized by *m*-CPBA by GP5 to give **26a** (0.52 g, 38%), colorless oil. ^1H NMR (DMSO-*d*₆): δ = 6.52 (s, 1H, H-5), 4.58 (m, 4H, CH₂ipr.), 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 × PCH₂), 3.38 (s, 3H, SO₂CH₃), 1.19 – 1.24 (m, 24H, CH₃ipr.), 1.19 (d, $J(\text{CH}_3,2'')$ = 6.5, 3H, H-3'') ppm. ^{13}C NMR (DMSO-*d*₆): δ = 171.51 and 171.45 (C-4, C-6), 164.26 (C-2), 92.66 (C-5), 75.05 (d, $J(2'',\text{P})$ = 12.5, C-2''), 70.12 – 70.40 (m, 6C, CH₂ipr., C-2' and C-1''), 66.95 (C-1'), 64.95 (d, $J(\text{C},\text{P})$ = 164.5, C-3'), 63.03 (d, $J(\text{C},\text{P})$ = 165.3, C-3''), 38.94 (CH₃SO₂), 23.82 – 24.01 (m, 8C, CH₃ipr.), 16.21 (C-3'') ppm. MS (FAB): m/z (%) = 671 (23) [MNa]⁺, 649 (32) [MH]⁺. HR MS (FAB) calcd. for C₂₄H₄₇N₂O₁₂P₂S [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%). ¹H NMR spectra identical with compound **26a**. MS (ESI): *m/z* (%) = 671.1 (100) [MNa]⁺. HR MS (FAB) calcd. for C₂₄H₄₇N₂O₁₂P₂S [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%). ¹H NMR (CDCl₃): δ = 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, PCH₂'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, PCH₂'b), 3.81 (d, *J*(H,C,P) = 8.2, 2H, PCH₂'), 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, CH₃SO₂), 1.34 (m, 24H, CH₃ipr.) ppm. ¹³C NMR (CDCl₃): δ = 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, PCH₂'), 65.35 (d, *J*(C,P) = 168.8, PCH₂''), 61.62 (C-3''), 38.88 (CH₃SO₂), 23.97 (m, CH₃ipr.) ppm. MS (ESI): *m/z* (%) = 687 (100) [MNa]⁺, 665 (44) [MH]⁺. HR MS (FAB) calcd. for C₂₄H₄₇N₂O₁₃P₂S [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%). ¹H NMR (CDCl₃) spectra identical with compound **26c**. MS (ESI): *m/z* (%) = 687 (25) [MNa]⁺, 665 (12) [MH]⁺. HR MS (FAB) calcd. for C₂₄H₄₇N₂O₁₃P₂S [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%). ¹H NMR (DMSO-*d*₆): δ = 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, OCH₂P), 2.38 (s, 3H, SO₂CH₃), 1.18–1.24 (m, 30H, CH₃ipr., H-3') ppm. ¹³C 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, PCH₂), 38.95 (CH₃SO₂), 23.92 (m, CH₃ipr.), 16.22 (C-3') ppm. MS (ESI): *m/z* (%) = 685 (100) [MNa]⁺. HR MS (FAB) calcd. for C₂₅H₄₉N₂O₁₂P₂S [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, PCH₂'a), 3.95 (m, 2H, H-2'), 3.87 (m, 2H, H-2''), 3.83 (m, 4H, PCH₂'b, PCH₂', H-3''), 3.70 (m, 2H, H-3b''), 3.30 (s, 3H, SO₂CH₃), 1.34 (m, 24H, CH₃ipr.), 1.28 (d, *J*(3'2') = 6.4, 3H, H-3') ppm. ¹³C 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, $J(\text{C},\text{O},\text{P}) = 6.7$), 71.42 (d, $J(\text{C},\text{O},\text{P}) = 6.9$) and 71.08 (d, 2C, $J(\text{C},\text{O},\text{P}) = 6.7$, CHipr.), 70.54 (C-1'), 66.83 (C-1''), 65.35 (d, $J(\text{C},\text{P}) = 168.8$, PCH₂''), 64.00 (d, $J(\text{C},\text{P}) = 169.2$, PCH₂'), 61.62 (C-3''), 38.89 (CH₃SO₂), 24.00 (m, CH₃ipr.), 16.34 (C-3') ppm. MS (ESI): m/z (%) = 679 (10) [MH]⁺, 701 (100) [MNa]⁺. HR MS (FAB) calcd. for C₂₅H₄₉N₂O₁₃P₂S [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 ¹³C NMR (DMSO-*d*₆) identical with compound **26f**. MS (ESI): m/z (%) = 679 (6) [MH]⁺, 701 (100) [MNa]⁺. HR MS (FAB) calcd. for C₂₅H₄₉N₂O₁₃P₂S [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, $J_{\text{gem}} = 11.4$, $J(1'a,2') = 3.9$, 1H, H-1'a), 4.41 (dd, $J_{\text{gem}} = 11.3$, $J(1'b,2'') = 6.1$, 1H, H-1'b), 4.05 (dd, $J_{\text{gem}} = 14.1$, $J(\text{C},\text{H},\text{P}) = 7.4$, 1H, PCH₂'a), 3.95 (m, 2H, H-2'), 3.78–3.89 (m, 5H, H-2'', PCH₂'b, PCH₂'', H-3''), 3.70 (dd, $J_{\text{gem}} = 12.4$, $J(3''b,2'') = 5.9$, 1H, H-3''b), 3.30 (s, 3H, SO₂CH₃), 1.33 (m, 24H, CH₃ipr.), 1.28 (d, $J(3',2') = 6.4$, 3H, H-3') ppm. ¹³C NMR (DMSO-*d*₆): δ = 171.35 (C-4), 171.17 (C-6), 164.14 (C-2), 93.60 (C-5), 81.43 (d, $J(2'',\text{P}) = 7.7$, C-2''), 75.51 (d, $J(2',\text{P}) = 11.8$, C-2'), 71.75 (d, $J(\text{C},\text{O},\text{P}) = 6.7$), 71.37 (d, $J(\text{C},\text{O},\text{P}) = 6.9$), 71.06 (d, $J(\text{C},\text{O},\text{P}) = 6.7$) and 71.04 (d, $J(\text{C},\text{O},\text{P}) = 6.5$, CHipr.), 70.52 (C-1'), 66.84 (C-1''), 65.28 (d, $J(\text{C},\text{P}) = 168.8$, PCH₂''), 63.95 (d, $J(\text{C},\text{P}) = 169.1$, PCH₂'), 61.53 (C-3''), 38.89 (CH₃SO₂), 23.96 (m, CH₃ipr.), 16.30 (C-3') ppm. MS (ESI): m/z (%) = 679 (35) [MH]⁺, 701 (15) [MNa]⁺. HR MS (FAB) calcd. for C₂₅H₄₉N₂O₁₃P₂S [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 C₂₅H₄₉N₂O₁₃P₂S [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, $J(\text{CH},\text{P}) = 7.6$, $J(\text{CH},\text{CH}_3) = 6.4$, 4H, CHipr.), 4.58 (m, 4H, H-1'), 3.94 (m, 4H, H-2'), 3.80 (d, $J(\text{C},\text{H},\text{P}) = 8.17$, 4H, PCH₂), 3.30 (s, 3H, SO₂CH₃), 1.33 (d, 12H) and 1.32 (d, 12H, $J(\text{CH}_3,\text{CH}) = 6.16$, CH₃ipr.) ppm. MS (FAB): m/z (%) = 635 (100) [MH]⁺. HR MS (FAB) calcd. for C₂₃H₄₅N₂O₁₂P₂S [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 C₂₅H₄₉N₂O₁₂P₂S [MH]⁺ 663.2476, found 663.2475.

4.26. 4,6-(2R,2'R)-Bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (26I)

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) $[\text{MNa}]^+$. HR MS (FAB) calcd. for $\text{C}_{25}\text{H}_{49}\text{N}_2\text{O}_{12}\text{P}_2\text{S}$ $[\text{MH}]^+$ 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 hygroscopic 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, $J(1''\text{a},2')$ = 2.9, 1H, H-1'a), 4.30 (dd, J_{gem} = 11.3, $J(1''\text{b},2'')$ = 6.3, 1H, H-1''b), 4.05 (dt, $J(2'',3'')$ = $J(2'',1''\text{b})$ = 6.4, $J(2'',1''\text{a})$ = 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, $J(2'',\text{P})$ = 11.0, C-2''), 72.42 (C-1), 70.72 (d, $J(2',\text{P})$ = 10.3, C-2'), 67.19 (d, $J(\text{P},\text{CH}_2)$ = 157.2, PCH_2), 65.05 (d, $J(\text{P},\text{CH}_2)$ = 159.1, PCH_2), 15.72 (C-3'') ppm. MS (ESI): m/z (%) = 418 (100) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}}$ = +12.1 (c 0.248, H_2O). Anal. $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_{10}\text{P}_2\cdot\text{H}_2\text{O}$ (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 hygroscopic foam, yield 190 mg (70%). NMR spectrum identical with compound **27a**. MS (ESI): m/z (%) = 418 (100) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}}$ = -10.3 (c 0.347, H_2O). Anal. $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_{10}\text{P}_2\cdot\text{H}_2\text{O}$ (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 hygroscopic 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, $J(1''\text{a},2'')$ = 4.3, 1H, H-1'a), 4.20 (dd, J_{gem} = 11.3, $J(1''\text{b},2'')$ = 5.7, 1H, H-1''b), 3.75 (m, 2H, H-2'), 3.71 (dd, J_{gem} = 13.6, $J(\text{H-C-P})$ = 8.7, 1H, $\text{OCH}_2\text{P}''\text{a}$), 3.67 (dd, J_{gem} = 13.6, $J(\text{H-C-P})$ = 8.9, 1H, $\text{OCH}_2\text{P}''\text{b}$), 3.67 (m, 2H, H-2''), 3.58 (d, $J(\text{H-C-P})$ = 8.7, 2H, $\text{OCH}_2\text{P}'$), 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, $J(2'',\text{P})$ = 9.9, C-2''), 78.64 (C-5), 70.76 (d, $J(2',\text{P})$ = 11.4, C-2'), 66.97 (d, $J(\text{C},\text{P})$ = 160.4, OCH_2P), 65.93 (d, $J(\text{C},\text{P})$ = 160.4, $\text{OCH}_2\text{P}''$), 65.39 (C-1''), 64.93 (C-1'), 60.33 (C-3'') ppm. MS (ESI): m/z (%) = 434 (100) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}}$ = +5.8 (c 0.625, H_2O). Anal. $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_{11}\text{P}_2\cdot\text{H}_2\text{O}$ (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 hygroscopic foam, yield 215 mg (45%). NMR spectrum identical with compound **27c**. MS (ESI): m/z (%) = 434 (100) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}}$ = -6.7 (c 0.341, H_2O). Anal. $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_{11}\text{P}_2\cdot\text{H}_2\text{O}$ (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 hygroscopic foam, yield 280 mg (49%). ^1H NMR (DMSO- d_6): δ = 3.58 (s, 1H, H-5), 4.18 (dd, J_{gem} = 11.1, $J(1'\text{a},2')$ = 5.8, 2H, H-1'a), 4.12 (dd, J_{gem} = 11.1, $J(1'\text{b},2')$ = 4.4, 2H, H-1'b), 3.82 (m, 2H, H-2'), 3.60 (d, $J(\text{CH}_2,\text{P})$ = 9.3, 4H, PCH_2), 1.14 (d, $J(\text{H-3}',\text{H-2}')$ = 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, $J(\text{C-2}',\text{P})$ = 11.4, C-2'), 68.58

(C-1'), 65.07 (d, $J(\text{CH}_2, \text{P}) = 161.6$, PCH_2), 16.80 (C-3') ppm. MS (ESI): m/z (%) = 432.1 (100) $[\text{MH}]^+$, 454.1 (37) $[\text{MNa}]^+$. $[\alpha]^{25}_{\text{D}} = +0.9$ (c 0.521, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{10}\text{P}_2 \cdot \text{H}_2\text{O}$ (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 hygroscopic foam, yield 200 mg (67%). ^1H NMR ($\text{DMSO}-d_6$): $\delta = 5.36$ (s, 1H, H-5), 4.26 (dd, $J_{\text{gem}} = 11.3$, $J(1''\text{a}, 2'') = 4.3$, 1H, H-1''a), 4.20 (dd, $J_{\text{gem}} = 11.3$, $J(1''\text{b}, 2'') = 5.8$, 1H, H-1''b), 4.16 (dd, $J_{\text{gem}} = 11.1$, $J(1'\text{a}, 2') = 5.8$, 1H, H-1'a), 4.12 (dd, $J_{\text{gem}} = 11.1$, $J(1'\text{b}, 2') = 4.4$, 1H, H-1'b), 3.81 (m, 1H, H-2'), 3.71 (dd, $J_{\text{gem}} = 13.6$, $J(\text{C-H-P}) = 8.7$, 1H, $\text{OCH}_2\text{P}''\text{a}$), 3.67 (dd, $J_{\text{gem}} = 13.6$, $J(\text{C-H-P}) = 8.9$, 1H, $\text{OCH}_2\text{P}''\text{b}$), 3.67 (m, 1H, H-2''), 3.60 (d, 2H, $J(\text{C-H-P}) = 9.4$, $\text{OCH}_2\text{P}'$), 3.51 (m, 2H, H-3''), 1.13 (d, $J(3', 2') = 6.4$, 3H, H-3') ppm. ^{13}C NMR ($\text{DMSO}-d_6$): $\delta = 171.48$, 171.42 (C-4, C-6), 162.83 (C-2), 80.40 (d, $J(2'', \text{P}) = 9.7$, C-2''), 78.59 (C-5), 75.08 (d, $J(2', \text{P}) = 11.7$, C-2'), 68.63 (C-1'), 66.00 (d, $J(\text{P}, \text{C}) = 161.0$, PCH_2''), 65.45 (C-1''), 65.01 (d, $J(\text{P}, \text{C}) = 161.8$, PCH_2'), 60.35 (C-3''), 16.83 (C-3') ppm. MS (ESI): m/z (%) = 448.1 (100) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}} = +12.2$ (c 0.181, H_2O). Analysis $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{11}\text{P}_2 \cdot \text{H}_2\text{O}$ (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 hygroscopic foam, yield 200 mg (73%). ^1H NMR ($\text{DMSO}-d_6$): $\delta = 5.36$ (s, 1H, H-5), 4.25 (dd, $J_{\text{gem}} = 11.3$, $J(1''\text{a}, 2'') = 4.3$, 1H, H-1''a), 4.20 (dd, $J_{\text{gem}} = 11.3$, $J(1''\text{b}, 2'') = 5.8$, 1H, H-1''b), 4.17 (dd, $J_{\text{gem}} = 11.1$, $J(1'\text{a}, 2') = 5.8$, 1H, H-1'a), 4.11 (dd, $J_{\text{gem}} = 11.1$, $J(1'\text{b}, 2') = 4.4$, 1H, H-1'b), 3.81 (m, 1H, H-2'), 3.71 (dd, $J_{\text{gem}} = 13.6$, $J(\text{C-H-P}) = 8.7$, 1H, $\text{OCH}_2\text{P}''\text{a}$), 3.67 (dd, $J_{\text{gem}} = 13.6$, $J(\text{C-H-P}) = 8.9$, 1H, $\text{OCH}_2\text{P}''\text{b}$), 3.67 (m, 1H, H-2''), 3.60 (d, $J(\text{C-H-P}) = 9.4$, 2H, $\text{OCH}_2\text{P}'$), 3.51 (m, 2H, H-3''), 1.13 (d, $J(3', 2') = 6.4$, 3H, H-3') ppm. ^{13}C NMR ($\text{DMSO}-d_6$): $\delta = 171.48$ and 171.42 (C-4, C-6), 162.83 (C-2), 80.40 (d, $J(2'', \text{P}) = 9.7$, C-2''), 78.59 (C-5), 75.08 (d, $J(2', \text{P}) = 11.7$, C-2'), 68.63 (C-1'), 66.00 (d, $J(\text{P}, \text{C}) = 161.0$, PCH_2''), 65.45 (C-1''), 65.01 (d, $J(\text{P}, \text{C}) = 161.8$, PCH_2'), 60.35 (C-3''), 16.83 (C-3') ppm. MS (ESI): m/z (%) = 448.1 (100) $[\text{MH}]^+$, 470.1 (35) $[\text{MNa}]^+$. $[\alpha]^{25}_{\text{D}} = +8.6$ (c 0.561, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{11}\text{P}_2 \cdot \text{H}_2\text{O}$ (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 hygroscopic foam, yield 605 mg (83%). NMR spectra identical with compound **27g**. MS (ESI): m/z (%) = 448.2 (100) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}} = -4.4$ (c 0.182, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{11}\text{P}_2 \cdot \text{H}_2\text{O}$ (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 hygroscopic foam, yield 616 mg (81%). NMR spectra identical with compound **39f**. MS (ESI): m/z (%) = 448.0 (100) $[\text{MH}]^+$. $[\alpha]^{25}_{\text{D}} = -5.8$ (c 0.312, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{11}\text{P}_2 \cdot \text{H}_2\text{O}$ (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 ($\text{DMSO}-d_6$): $\delta = 3.57$ (s, 1H, H-5), 4.17 (dd, $J_{\text{gem}} = 11.1$, $J(1'\text{a}, 2') = 5.8$, 2H, H-1'a), 4.11 (dd, $J_{\text{gem}} = 11.1$, $J(1'\text{b}, 2') = 4.4$, 2H, H-1'b), 3.81 (m, 2H, H-2'), 3.60 (d, $J(\text{CH}_2, \text{P}) = 9.3$, 4H, PCH_2), 1.14 (d, $J(\text{H-3'}, \text{H-2'}) = 6.3$, 6H, H-3') ppm. ^{13}C NMR ($\text{DMSO}-d_6$): $\delta = 171.41$ (C-4, C-6), 162.80 (C-2), 78.54 (C-5), 75.02 (d, $J(\text{C-2'}, \text{P}) = 11.6$, C-2'), 68.59 (C-1'), 65.02 (d, $J(\text{CH}_2, \text{P}) = 161.7$, PCH_2), 16.82 (C-3') ppm. MS (ESI): m/z (%) = 432.1 (100) $[\text{MH}]^+$; 454.1 (26) $[\text{MNa}]^+$. $[\alpha]^{25}_{\text{D}} = +20.9$ (c 0.291, H_2O). Anal. $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_{10}\text{P}_2 \cdot \text{H}_2\text{O}$ (C, H, N, P).

4.27.11. 2-Amino-4,6-(2R,2'R)-bis[2-(phosphonomethoxy)propoxy]pyrimidine (27k)—From **26i** (1.2 g, 1.8 mmol), white hygroscopic foam, yield 450 mg (55%). NMR spectra identical with compound **27j**. MS (ESI): m/z (%) = 432.0 (100) $[MH]^+$; 454.1 (14) $[MNa]^+$. $[\alpha]^{25}_D = -20.5$ (c 0.254, H₂O). Anal. C₁₂H₂₃N₃O₁₀P₂·H₂O (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 hr. 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 (OCH₂,P) = 8.3, 4H, PCH₂), 2.67 (m, 1H, CH cycloprop.), 1.23 (d, 12H) and 1.22 (d, J (CH₃,CH) = 6.3, 12H, CH₃), 0.62 (m, 2H) and 0.45 (m, 2H, CH₂ cycloprop.) ppm. ¹³C 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-OCH₂), 64.48 (C-1'), 23.87 (CH cycloprop.), 23.92 (CH₃), 6.34 (CH₂ cycloprop.) ppm. MS (FAB): m/z (%) = 612 (100) $[MH]^+$. HR MS (FAB) calcd. for C₂₅H₄₈N₃O₁₀P₂ $[MH]^+$ 612.2814, found 612.2813. Diisopropyl esters were deprotected by GP1 to give **28a** (140 mg, 62%) as a white foam. ¹H NMR (D₂O): δ = 4.53 (m, 4H, H-1'), 3.98 (m, 4H, H-2'), 3.76 (d, J (P,CH₂) = 9.2, 4H, PCH₂), 2.72 (m, 1H, CH cycloprop.), 0.93 and 0.71 (2 × m, 2 × 2H, CH₂ cycloprop.) ppm. ¹³C NMR (D₂O): 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 (CH₂,P) = 156.4, PCH₂), 23.41 (CH cycloprop.), 7.19 (CH₂ cycloprop.) ppm. MS (ESI): m/z (%) = 444.1 (100) $[MH]^+$, 466.0 (26) $[MNa]^+$. Anal. C₁₃H₂₃N₃O₁₀P₂·H₂O (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 hr. THF was removed under reduced pressure and the residue was purified by flash chromatography (CHCl₃/MeOH 0–2%) to give 2-cyclopentylamino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine as an oil, yield 540 mg (54%). ¹H NMR (CDCl₃): δ = 5.37 (s, 1H, H-5), 4.88 (d, J (NH-1') = 7.0, 1H, NH), 4.76 (dh, J (CH,CH₃) = 6.2, J (CH,P) = 7.7, 4H, CH ipr.), 4.39 (m, 4H, H-1'), 4.18 (m, 1H, H-1''), 3.89 (m, 4H, H-2'), 3.82 (d, J (P,CH₂) = 8.2, 4H, PCH₂), 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, CH₃ipr.) ppm. ¹³C NMR (CDCl₃): δ = 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, CH ipr.), 65.97 (d, J (3'-P) = 167.1, PCH₂), 64.76 (C-1'), 52.91 (C-1''), 33.29 (C-2''), 24.07 (d, J (CH₃-P) = 3.7) and 23.93 (d, J (CH₃-P) = 4.6, CH₃ipr.), 23.72 (C-3'') ppm. MS (FAB): m/z (%) = 640.5 (60) $[MH]^+$. HR MS (FAB) calcd. for C₂₇H₅₂N₃O₁₀P₂ $[MH]^+$ 640.3128, found 640.3140.

Diisopropyl esters were cleaved by GP1 to afford **28b** (220 mg, 53%) as a white hygroscopic foam. ¹H NMR (DMSO-*d*₆): δ = 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 (CH₂P) = 8.7, 4H, PCH₂), 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. ¹³C NMR (DMSO-*d*₆): δ = 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, PCH₂), 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. C₁₅H₂₇N₃O₁₀P₂·H₂O (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%). ¹H NMR (DMSO-*d*₆): δ = 6.97 (br q, *J*(NH,CH₃) = 4.7, 1H, NH), 5.28 (s, 1H, H-5), 4.59 (dh, *J*(CH,CH₃) = 6.2, *J*(CH,P) = 7.8, 4H, CH₃ipr.), 4.33 (m, 4H, H-1'), 3.78 (m, 8H, H-2', H-3'), 2.75 (d, *J*(CH₃,NH) = 4.7, 3H, CH₃NH) ppm. ¹³C NMR (DMSO-*d*₆): δ = 170.82 (C-2), 161.74 (C-4, C-6), 77.83 (C-5), 70.36 (m, CH₃ipr.), 64.97 (d, *J*(CH₂,P) = 164.2, CH₂P), 64.49 (C-1'), 27.86 (CH₃NH), 23.93 m (CH₃ipr.) ppm. MS (FAB): *m/z* (%) = 586.2 (75) [MH]⁺. HR MS (FAB) calcd. for C₂₃H₄₆N₃O₁₀P₂ [MH]⁺ 586.2658, found 586.2659.

Diisopropyl esters were deprotected by GP1 to give **28c** (210 mg, 67%) as a white hygroscopic foam. ¹H NMR (D₂O): δ = 4.54 (m, 4H, H-1'), 3.98 (m, 4H, H-2'), 3.77 (d, *J*(CH₂,P) = 9.2, 4H, PCH₂), 3.00 (s, 3H, CH₃) ppm. ¹³C NMR (D₂O): δ = 173.02 (C-4, C-6), 162.95 (C-2), 70.72 d (C-2'), 69.21 (C-1'), 67.20 (d, PCH₂), 28.21 (CH₃) ppm. MS (FAB): *m/z* (%) = 418 (100) [MH]⁺. Anal. C₁₁H₂₁N₃O₁₀P₂·H₂O (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 hr. 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. ¹H NMR (DMSO-*d*₆): δ = 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, CH ipr.), 4.41 (d, *J*(H-1'',NH) = 6.3, 2H, H-1''), 4.29 (m, 4H, H-1'), 3.75 (m, 8H, H-2', PCH₂), 1.23 (d, *J*(CH₃,CH) = 6.2, 12H) and 1.21 (d, *J*(CH₃,CH) = 6.2, 12H, CH₃ ipr.) ppm. ¹³C NMR (DMSO-*d*₆): δ = 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, CH ipr.), 64.95 (d, *J*(CH₂,P) = 164.3, 2C, PCH₂), 64.58 (2C, C-1'), 44.39 (C-1''), 24.00 (d, *J*(CH₃,P) = 3.6, 4C) and 23.84 (d, *J*(CH₃,P) = 4.3, 4C, CH₃ipr.) ppm. MS (FAB): *m/z* (%) = 662 (25) [MH]⁺. HR MS (FAB) calcd. for C₂₉H₅₀N₃O₁₀P₂ [MH]⁺ 662.2971, found 662.2981.

The intermediate was treated with bromotrimethylsilane (GP1) to give **28d**, white hygroscopic foam, yield 120 mg (42%). ¹H NMR (DMSO-*d*₆): δ = 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, PCH₂) ppm. ¹³C NMR (DMSO-*d*₆): δ = 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*(CH₂,P) = 160.6, PCH₂), 64.97 (C-1'), 44.41 (C-1'') ppm. MS (FAB): *m/z* (%) = 494 (100) [MH]⁺, 516 (35) [MNa]⁺. Anal. C₁₇H₂₅N₃O₁₀P₂·H₂O (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-(diisopropoxy-phosphorylmethoxy)ethoxy]pyrimidine (550 mg, 50%) as colorless oil. ¹H NMR (CDCl₃): δ = 7.25 (m, 2H, Ph-2), 6.86 (m, 2H, Ph-3), 5.40 (s, 1H, H-5), 5.20 (t, *J*(NH,CH₂) = 5.9, 1H, NH), 4.75 (dh, *J*(CH,CH₃) = 6.2, *J*(CH,P) = 7.7, 4H, CH₃ipr.), 4.49 (d, *J*(CH₂,NH) = 5.9, 2H, PhCH₂NH), 4.33 (m, 4H, H-1), 3.86 (m, 4H, H-2), 3.81 (d, *J*(CH₂,P) = 8.3, 4H, PCH₂), 3.80 (s, 3H, OCH₃), 1.33 (m, 24H, CH₃ipr.) ppm. ¹³C NMR (CDCl₃): δ = 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, CH₃ipr.), 65.96 (d, *J*

(CH₂,P) = 167.1, PCH₂), 64.86 (C-1'), 55.24 (OCH₃), 44.90 (PhCH₂NH), 24.06 (d, *J* (CH₃,CH) = 3.7) and 23.93 (d, *J*(CH₃,CH) = 4.6, CH₃ipr.) ppm. MS (FAB): *m/z* (%) = 692.2 (15) [MH]⁺. HR MS (FAB) calcd. for C₃₀H₅₂N₃O₁₁P₂ [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 hygroscopic foam. ¹H NMR (DMSO-*d*₆): δ = 7.60 (br t, *J*(NH,CH₂) = 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*(CH₂,NH) = 6.0, 2H, CH₂N), 4.25 (m, 4H, H-1'), 3.70 (s, 3H, OCH₃), 3.70 (m, 4H, H-2'), 3.50 (d, 4H, *J*(H,P) = 8.6, PCH₂). ¹³C NMR (DMSO-*d*₆): δ = 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, PCH₂), 64.97 (C-1'), 55.21 (OCH₃), 43.80 (CH₂N). MS (ESI): *m/z* (%) = 524.1 (100) [MH]⁺, 546.1 (69) [MNa]⁺. Anal. C₁₈H₂₃N₃Na₄O₁₁P₂ (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 (CHCl₃/MeOH 0–1%) gave thick oil of 2-morpholino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (720 mg, 71%). ¹H NMR (CDCl₃): δ = 5.39 (s, 1H, C-5), 4.76 (dh, *J*(CH,CH₃) = 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*(CH₂,P) = 8.2, 4H, PCH₂), 3.72 (s, 8H, H-2'', H-3''), 1.34 (d, *J*(CH₃,CH) = 6.3, 12H) and 1.33 (d, *J*(CH₃,CH) = 6.3, 12H, CH₃ipr.) ppm. ¹³C NMR (CDCl₃): δ = 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, CH ipr.), 66.74 (C-3''), 66.02 (d, *J*(CH,P) = 167.3, PCH₂), 64.80 (C-1'), 44.24 (C-2''), 24.07 (d, *J*(CH₃,P) = 3.5) and 23.94 (d, *J*(CH₃,P) = 4.5, CH₃ipr.) ppm. MS (FAB): *m/z* (%) = 642.5 (22) [MH]⁺. HR MS (FAB) calcd. for C₂₆H₅₀N₃O₁₁P₂ [MH]⁺ 642.2921, found 642.2911.

Deprotection of diisopropyl esters by GP1 gave **28f** (360 mg, 65%) as a white hygroscopic foam. ¹H NMR (DMSO-*d*₆): δ = 5.45 (s, 1H, H-5), 4.33 (m, 4H, H-1'), 3.77 (m, 4H, H-2'), 3.64 (m, 8H, CH₂ – morpholine), 3.57 (d, *J*(P,CH) = 8.7, 4H, PCH₂) ppm. ¹³C 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, PCH₂), 66.13 (C-3''), 65.10 (C-1'), 44.13 (C-2'') ppm. MS (ESI): *m/z* (%) = 474 (86) [MH]⁺. Anal. C₁₄H₂₅N₃O₁₁P₂·H₂O (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 hr. Reaction mixture was cooled to r.t., neutralized with acetic acid and volatiles were removed in vacuo. The residue was partitioned between CHCl₃ and water. Organic fraction was washed with water (3 × 50 mL) and dried with MgSO₄. Flash chromatography (CHCl₃/MeOH 0–5%) gave colorless oil, 450 mg (50%). ¹H NMR (CDCl₃): δ = 5.29 (s, 1H, H-5), 4.76 (dh, *J*(CH,P) = 7.7, *J*(CH,CH₃) = 6.2, CHipr.), 4.38 (m, 4H, H-1'), 3.92 (m, 4H, H-2'), 3.81 (d, *J*(CH₂,P) = 8.2, PCH₂), 1.34 and 1.33 (2 × d, *J*(CH₃,CH) = 6.2, 2 × 12H, CH₃ipr.) ppm. ¹³C NMR (CDCl₃): 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, PCH₂), 24.05 (d, *J*(C,P) = 4.0, CH₃ipr.), 23.95 (d, *J*(C,P) = 4.6, CH₃ipr.) ppm. MS (FAB): *m/z* (%) = 573 (100) [MH]⁺. HR MS (FAB) calcd. for C₂₂H₄₃N₂O₁₁P₂ [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 (1M in MeOH, 3.5 mL) and the resulting mixture was heated at 80 °C for 4 hr. 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, $J(\text{CH}, \text{CH}_3)$ = 6.2, $J(\text{H}, \text{C}, \text{P})$ = 7.7, 4H, CH₃pr), 4.48 (m, 4H, H-1'), 3.94 (s, 3H, OCH₃), 3.92 (m, 4H, H-2'), 3.82 (d, $J(\text{H}, \text{C}, \text{P})$ = 8.2, 4H, OCH₂P), 1.33 (m, 24H, CH₃ipr.) ppm. ^{13}C NMR (CDCl_3): δ = 171.99 (C-4, C-6), 164.52 (C-2), 84.20 (C-5), 71.08 (m, CH₃pr., H-2'), 65.97 (d, $J(\text{C}, \text{P})$ = 167.4, OCH₂P), 65.51 (C-1'), 54.65 (OCH₃), 24.05 (d, $J(\text{C}, \text{C}, \text{O}, \text{P})$ = 3.7) and 23.91 (d, $J(\text{C}, \text{C}, \text{O}, \text{P})$ = 4.6, CH₃ipr.) ppm. MS (FAB): m/z (%) = 587 (62) $[\text{MH}]^+$. HR MS (FAB) calcd. for $\text{C}_{23}\text{H}_{45}\text{N}_2\text{O}_{11}\text{P}_2$ $[\text{MH}]^+$ 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 hr, 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 ($\text{CHCl}_3/\text{MeOH}$ 0–5%) to give 4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine as a thick oil (1.5 g, 81%). ^1H NMR ($\text{DMSO}-d_6$): δ = 8.38 (d, $J(\text{H}-2, \text{H}-5)$ = 0.9, 1H, H-2), 6.05 (d, $J(\text{H}-5, \text{H}-2)$ = 0.9, 1H, H-5), 4.76 (dh, $J(\text{CH}, \text{CH}_3)$ = 6.2, $J(\text{CH}, \text{P})$ = 7.7, 4H, CH₃pr.), 4.50 (m, 4H, H-1'), 3.93 (m, 4H, H-2'), 3.83 (d, $J(\text{H}, \text{C}, \text{P})$ = 8.2, 4H, POCH₂), 1.33 (m, 24H, CH₃ipr.) ppm. ^{13}C NMR ($\text{DMSO}-d_6$): δ = 170.51 (C-4, C-6), 157.21 (C-2), 91.15 (C-5), 71.12 (d, $J(2', \text{P})$ = 10.8, C-2'), 71.09 (d, $J(\text{CH}, \text{P})$ = 6.7, CH₃pr.), 65.99 (d, $J(\text{C}, \text{P})$ = 167.3, POCH₂), 65.52 (C-1'), 24.05 (d, $J(\text{CH}_3, \text{P})$ = 3.7) and 23.93 (d, $J(\text{CH}_3, \text{P})$ = 4.6, CH₃ipr.) ppm. MS (ESI): m/z (%) = 579.2 (100) $[\text{MNa}]^+$. Anal. $\text{C}_{22}\text{H}_{42}\text{N}_2\text{O}_{10}\text{P}_2$ (C, H, N, P).

The intermediate was deprotected by bromotrimethylsilane (GP1) to give **30** (350 mg, 48%), freeze dried, white hygroscopic foam. ^1H NMR ($\text{DMSO}-d_6$): δ = 8.44 (d, $J(\text{H}-2, \text{H}-5)$ = 0.9, 1H, H-2), 6.28 (d, $J(\text{H}-5, \text{H}-2)$ = 0.9, 1H, H-5), 4.39 (m, 4H, H-1'), 3.80 (m, 4H, H-2'), 3.57 (d, $J(\text{H}, \text{C}, \text{P})$ = 8.7, 4H, OCH₂P) ppm. ^{13}C NMR ($\text{DMSO}-d_6$): δ = 170.70 (C-4, C-6), 157.79 (C-2), 90.36 (C-5), 70.46 (d, $J(2', \text{P})$ = 11.0, C-2'), 67.10 (d, $J(\text{C}, \text{P})$ = 160.2, PCH₂), 66.01 (C-1') ppm. MS (ESI): m/z (%) = 389 (76) $[\text{MH}]^+$. Anal. $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_{10}\text{P}_2 \cdot \text{H}_2\text{O}$ (C, H, N, P).

4.29. 4,6-Disulfanylpurimidine derivatives

4.29.1. 2-Amino-4,6-disulfanylpurimidine (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 hr. Solvent was removed in vacuo and the residue in aq. NaOH (0.5 M, 250 mL) was heated at 80 °C for 16 hr. 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 ($\text{DMSO}-d_6$): δ = 11.2–11.8 (br s, 2H, SH), 7.17 (br s, 2H, NH₂), 6.24 (s, 1H, H-5) ppm. ^{13}C NMR ($\text{DMSO}-d_6$): δ = 173.91 (2C, C-4, C-6), 149.10 (C-2), 116.36 (C-5) ppm. MS (EI): m/z (%) = 159.2 (100) $[\text{M}]^+$. Anal. $\text{C}_4\text{H}_5\text{N}_3\text{S}_2$ (C, H, N, S).

4.29.2. 2-Amino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethyl]sulfanylpyrimidine (32a) and 2-amino-4,6-bis[2-(phosphonomethoxy)ethyl]sulfanylpyrimidine (33a)—Phosphonate **20a** (6.9 g, 26.4 mmol) was added to a stirred mixture of disulfanyl purimidine **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 hr and evaporated in vacuo. The residue was adsorbed onto silica gel from methanol and separated by flash chromatography ($\text{CHCl}_3/\text{MeOH}$ 0–5%) to give **32a** (5.6 g, 74%), pale yellow oil. ^1H NMR ($\text{DMSO}-d_6$): δ = 6.74 (br s, 2H, NH₂), 6.41 (s, 1H, H-5), 4.58 (m, 4H, CH₃pr.), 3.79 (d, $J(\text{P}, \text{CH})$ = 8.3, 4H, PCH₂), 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, $J(\text{CH}_3, \text{CH})$ = 6.2, 12H, CH₃) ppm. ^{13}C NMR ($\text{DMSO}-d_6$): δ = 167.60 (2C, C-4, C-6), 161.79 (C-2), 103.07 (C-5), 71.18 (d, $J(\text{P}, \text{C})$ = 12.2, 2C, C-2'), 70.35 (d, $J(\text{P}, \text{C})$ = 6.3, 4C,

CH₃pr.), 64.81 (d, $J(\text{P},\text{C}) = 164.6$, 2C, PCH₂), 27.60 (2C, C-1'), 24.03 (d, $J(\text{P},\text{C}) = 3.9$, 4C) and 23.92 (d, $J(\text{P},\text{C}) = 4.4$, 4C, CH₃) ppm. MS (ESI): m/z (%) = 604.2 (100) [MH]⁺. Anal. C₂₂H₄₃N₃O₈P₂S₂ (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 (D₂O): $\delta = 6.75$ (s, 1H, H-5), 3.97 (t, $J(2',1') = 6.4$, 4H, H-2'), 3.80 (d, $J(\text{P},\text{CH}) = 8.7$, 4H, PCH₂), 3.45 (t, $J(1',2') = 6.4$, 4H, H-1') ppm. ¹³C NMR (D₂O): $\delta = 169.49$ (2C, C-4, C-6), 161.20 (C-2), 104.15 (C-5), 70.59 (d, $J(\text{P},\text{C}) = 10.7$, 2C, C-2'), 66.82 (d, $J(\text{P},\text{C}) = 156.2$, 2C, PCH₂), 28.38 (2C, C-1') ppm. MS (ESI): m/z (%) = 436 (35) [MH]⁺. Anal. C₁₀H₁₉N₃O₈P₂S₂ (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): $\delta = 6.69$ (s, 2H, NH₂), 6.14 (s, 1H, H-5), 4.59 (m, 4H, CH₃pr.), 3.78 (d, $J(\text{P},\text{CH}) = 9.2$, 4H, PCH₂), 3.74 (m, 2H, H-2'), 3.26 (dd, $J(1'a,2') = 5.5$, $J_{\text{gem}} = 13.6$, 2H, H-1'a), 3.20 (dd, $J(1'b,2') = 5.8$, $J_{\text{gem}} = 13.6$, 2H, H-1'b), 1.24 (d, 12H), 1.23 (d, 6H) and 1.16 (d, $J(\text{CH}_3,\text{CH}) = 6.2$, 6H, CH₃pr.) ppm. ¹³C NMR (DMSO-*d*₆): $\delta = 167.63$ (2C, C-4,6), 161.64 (C-2), 103.30 (C-5), 76.19 (d, $J(\text{P},\text{C}) = 12.7$, 2C, C-2'), 70.30 (d, $J(\text{P},\text{C}) = 6.3$, 4C, CH₃pr.), 62.84 (d, $J(\text{P},\text{C}) = 165.0$, 2C, PCH₂), 33.21 (2C, C-1'), 24.02 (d, $J(\text{P},\text{C}) = 3.6$, 4C) and 23.88 (d, $J(\text{P},\text{C}) = 4.6$, 4C, CH₃pr.), 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 (D₂O): $\delta = 6.81$ (s, 1H, H-5), 3.94 (m, 2H, H-2'), 3.77 (dd, 2H) and 3.65 (dd, $J(\text{P},\text{CH}) = 9.3$, $J_{\text{gem}} = 13.2$, 2H, PCH₂), 3.38 (dd, 2H) and 3.35 (dd, $J(1',2') = 5.5$, $J_{\text{gem}} = 13.6$, 2H, H-1'), 1.29 (d, $J(3',2') = 6.2$, 6H, H-3') ppm. MS (ESI): m/z (%) = 464.0 (100) [MH]⁺. [α]_D²⁵ = +48.3 (c 0.357, H₂O). Anal. C₁₂H₂₃N₃O₈P₂S₂·H₂O (C, H, N, P, S).

4.29.4. 2-Amino-4,6-(2R,2'R)-bis{[2-(diisopropoxyphosphorylmethoxy)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]⁺. [α]_D²⁵ = -40.2 (c 0.589, H₂O). Anal. C₁₂H₂₃N₃O₈P₂S₂·H₂O (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 taken down in vacuo. The residue in CHCl₃ (200 mL) was washed with water (3 × 100 mL), dried over MgSO₄ and taken down under reduced pressure. The residue was separated by flash chromatography (CHCl₃/MeOH 0–5%) to give **32a** (3.18 g, 28%) and **34a** (2.92 g, 40%) as a pale yellow oil. ¹H NMR (DMSO-*d*₆): $\delta = 11.90$ (br s, 1H, SH), 7.00 (br s, 2H, NH₂), 6.33 (s, 1H, H-5), 4.59 (m, 2H, CH₃pr.), 3.78 (d, $J(\text{P},\text{CH}) = 8.3$, 2H, PCH₂), 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, $J(\text{CH}_3,\text{CH}) = 6.2$, 6H, CH₃) ppm. ¹³C NMR (DMSO-*d*₆): $\delta = 177.97$ (C-4), 167.60 (C-2), 154.04 (C-6), 109.86 (C-5), 70.24 (d, $J(\text{P},\text{C}) = 12.2$, C-2'), 70.36 (d, 2C, $J(\text{P},\text{C}) = 6.3$, CH₃pr.), 64.83 (d, $J(\text{P},\text{C}) = 164.1$, PCH₂), 28.33 (C-1'),

23.98 (d, 2C, $J(\text{P},\text{C}) = 3.9$) and 23.91 (d, 2C, $J(\text{P},\text{C}) = 4.4$, CH_3) ppm. MS (FAB): m/z (%) = 382 (100) $[\text{MH}]^+$. Anal. $\text{C}_{13}\text{H}_{24}\text{N}_3\text{O}_4\text{PS}_2$ (C, H, N, P, S).

4.29.6. 2-Amino-6-(2S)-[2-(diisopropoxyphosphorylmethoxy)propyl]sulfanyl-4-sulfanylpuridine (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 hr and taken down in vacuo. The residue was purified by flash chromatography to give **32b** (750 mg, 19%) and **34b** (780 mg, 31%) as a thick oil. ^1H NMR ($\text{DMSO}-d_6$): $\delta = 11.88$ (br s, 1H, SH), 7.00 (br s, 2H, NH_2), 6.33 (s, 1H, H-5), 4.59 (m, 2H, CH_{Hpr}), 3.79 (dd, 1H) and 3.75 (dd, $J_{\text{gem}} = 13.8$, $J(\text{P},\text{CH}) = 9.2$, 1H, PCH_2), 3.74 (m, 1H, H-2'), 3.22 (dd, $J(1'a,2') = 5.4$, $J_{\text{gem}} = 13.6$, 1H, H-1'a), 3.17 (dd, $J(1'b,2') = 5.8$, $J_{\text{gem}} = 13.6$, 1H, H-1'b), 1.24 (d, 6H), 1.235 (d, 6H) and 1.18 (d, 3H, $J(\text{CH}_3,\text{CH}) = 6.2$, CH_3) ppm. ^{13}C NMR ($\text{DMSO}-d_6$): $\delta = 178.56$ (C-4), 167.86 (C-2), 153.95 (C-6), 109.94 (C-5), 75.97 (d, $J(\text{P},\text{C}) = 12.8$, C-2'), 70.37 and 70.34 (d, $J(\text{P},\text{C}) = 6.3$, CH_{Hpr}), 62.78 (d, $J(\text{P},\text{C}) = 165.6$, PCH_2), 34.35 (d, $J(\text{P},\text{C}) = 3.9$, C-1'), 24.04 (d, 2C, $J(\text{P},\text{C}) = 3.0$) and 23.90 (2C, $J(\text{P},\text{C}) = 4.6$, CH_3), 18.17 (C-3') ppm. MS (ESI): m/z (%) = 418 (100) $[\text{MNa}]^+$. HR MS (FAB) calcd. for $\text{C}_{14}\text{H}_{27}\text{N}_3\text{O}_4\text{PS}_2$ $[\text{MH}]^+$ 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-(phosphonomethoxy)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) was stirred at 60 °C for 4 hr and taken down 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. ^1H NMR ($\text{DMSO}-d_6$): $\delta = 6.70$ (br s, 2H, NH_2), 6.41 (s, 1H, H-5), 4.59 (m, 4H, CH_{Hpr}), 3.775 (d, 2H) and 3.77 (d, $J(\text{P},\text{CH}) = 8.4$, 2H, PCH_2), 3.76 (m, 1H, H-2''), 3.71 (t, $J(2',1') = 5.4$, 2H, H-2'), 3.28 (dd, $J(1''a,2'') = 6.1$, $J_{\text{gem}} = 13.6$, 1H, H-1''a), 3.26 (t, $J(1',2') = 5.4$, 2H, H-1'), 3.20 (dd, $J(1''b,2'') = 5.8$, $J_{\text{gem}} = 13.6$, 1H, H-1''b), 1.245 (d, 12H) and 1.23 (d, $J(\text{CH}_3,\text{CH}) = 6.2$, 12H, $\text{CH}_{3\text{Hpr}}$), 1.18 (d, $J(\text{CH}_3,\text{CH}) = 6.2$, 3H, H-3'') ppm. ^{13}C NMR ($\text{DMSO}-d_6$): $\delta = 167.71$ and 167.47 (C-4,6), 161.70 (C-2), 103.83 (C-5), 75.70 (d, $J(\text{P},\text{C}) = 11.7$, C-2'), 71.08 (d, 4C) and 70.64 (d, 4C, $J(\text{P},\text{C}) = 6.3$, CH_{Hpr}), 70.03 (d, $J(\text{P},\text{C}) = 10.7$, C-2'), 64.91 (d) and 62.05 (d, $J(\text{P},\text{C}) = 164.5$, PCH_2), 32.10 (C-1''), 27.60 (C-1'), 24.66 (d, 4C) and 24.18 (d, 4C, $J(\text{P},\text{C}) = 3.9$), 23.59 (d, 4C) and 23.32 (d, 4C, $J(\text{P},\text{C}) = 4.4$, CH_3), 17.20 (C-3'') ppm. MS (FAB): m/z (%) = 618.2 (100) $[\text{MH}]^+$. HR MS (FAB) calcd. for $\text{C}_{23}\text{H}_{46}\text{N}_3\text{O}_8\text{P}_2\text{S}_2$ $[\text{MH}]^+$ 618.2203, found 618.2205.

Diisopropylester **35a** was deprotected by GP1 to give **36a** (100 mg, 53%) as a white foam. ^1H NMR (D_2O): $\delta = 6.79$ (s, 1H, H-5), 3.94 (m, 1H, H-2''), 3.87 (t, $J(2',1') = 6.1$, 2H, H-2'), 3.74 (dd, $J(\text{P},\text{CH}) = 9.2$, $J_{\text{gem}} = 13.3$, 1H) and 3.70 (d, $J(\text{P},\text{CH}) = 8.9$, 2H) and 3.66 (dd, $J(\text{P},\text{CH}) = 9.4$, $J_{\text{gem}} = 13.3$, 1H, PCH_2), 3.38 (t, $J(1',2') = 6.1$, 2H, H-1'), 3.36 (dd, $J(1''a,2'') = 5.4$, $J_{\text{gem}} = 14.4$, 1H, H-1''a), 3.32 (dd, $J(1''b,2'') = 6.1$, $J_{\text{gem}} = 14.4$, 1H, H-1''b), 1.29 (d, $J(3'',2'') = 6.3$, 3H, H-3''). MS (ESI): m/z (%) = 450 (100) $[\text{MH}]^+$. $[\alpha]_{\text{D}}^{25} = +32.5$ (c 0.142, H_2O). Anal. $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_8\text{P}_2\text{S}_2 \cdot \text{H}_2\text{O}$ (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) $[MNa]^+$. $[\alpha]^{25}_D = -19.3$ (c 0.216, H_2O). Anal. $C_{11}H_{21}N_3O_8P_2S_2 \cdot H_2O$ (C, H, N, P, S).

4.29.9. 2-Amino-4,6-(2R,2'S)-bis{[2-(diisopropoxyphosphorylmethoxy)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%). 1H 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) $[MH]^+$. $[\alpha]^{25}_D = +0.3$ (c 0.358, H_2O). Anal. $C_{12}H_{23}N_3O_8P_2S_2 \cdot H_2O$ (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 hr, cooled to r.t. and neutralized by addition of Dowex 50 $\times$ 8; the resulting mixture was diluted with water (100 mL), applied onto a column of Dowex 50 $\times$ 8 and washed with water (1L). 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. 1H NMR ($DMSO-d_6$): δ = 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 ($DMSO-d_6$): δ = 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 [24]; yield 47%. 1H NMR ($CDCl_3$): δ = 7.77 (br d, $J(CH,CH)$ = 5.81, 2H, $CH_{arom.}$), 7.30 (br d, $J(CH,CH)$ = 6.0, 2H, $CH_{arom.}$), 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, $CH_3_{arom.}$), 1.48 (br, 2H, $OCH_2CH_2CH_2$), 1.25 (br s, 26H, $13 \times CH_2$), 0.87 (t, $J(CH_3,CH_2)$ = 6.83, CH_3CH_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. 1H NMR ($DMSO-d_6$): δ = 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 ($DMSO-d_6$): δ = 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

(CH₃CH₂CH₂), 29.52, 29.25, 29.15, 28.92 and 25.90 (alif.), 22.31 (CH₃CH₂), 14.18 (CH₃) ppm. MS (ESI): m/z (%) = 598.4 (80) [M-H]⁻. Anal. C₂₇H₅₁N₃NaO₈P (C, H, N, P).

4.30.4. Bis[2-(Hexadecyloxy)ethyl] 2-amino-4,6-bis[2-(phosphonmethoxy)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. C₄₆H₈₉N₃Na₂O₁₂P₂ (C, H, N, P).

4.30.5. 2-(Hexadecyloxy)ethyl 2-amino-5-bromo-4-(2-hydroxyethoxy)-6-[2-(phosphonmethoxy)ethoxy]pyrimidine, sodium salt (39b)—GP7, white solid (44 mg, 26%), m.p. 83 °C. ¹H NMR (DMSO-*d*₆): δ = 6.72 (br s, 2H, NH₂), 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 × OCH₂, PCH₂), 1.44 (m, 2H, H-7'), 1.22 (m, 26H, alif.), 0.84 (t, *J*(CH₃,CH₂) = 6.8, 3H, CH₃) ppm. ¹³C NMR (DMSO-*d*₆): δ = 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 (CH₃CH₂CH₂), 29.54, 29.22, 29.18, 28.94, 25.91 (alif.), 22.33 (CH₃CH₂), 14.19 (CH₃) ppm. MS (ESI): m/z (%) = 654 (100) [M-Na]⁻. Anal. C₂₇H₅₀BrN₃NaO₈P (C, H, N, P).

4.30.6. Bis[2-(Hexadecyloxy)ethyl] 2-amino-5-bromo-4,6-bis[2-(phosphonmethoxy)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 × Na)+H]⁻. Anal. C₄₆H₈₈BrN₃Na₂O₁₂P₂ (C, H, N, P).

4.30.7. 2-(Hexadecyloxy)ethyl 2-amino-4-(2-hydroxyethoxy)-5-methyl-6-[2-(phosphonmethoxy)ethoxy]pyrimidine, sodium salt (39c)—GP7, white solid (122 mg, 40%), m.p. 95 °C. ¹H NMR (DMSO-*d*₆): δ = 6.20 (br s, 2H, NH₂), 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-CH₃), 1.43 (m, 2H, H-7'), 1.22 (m, 26H, alif.), 0.84 (t, *J*(CH₃,CH₂) = 7.0, 3H, CH₃) ppm. ¹³C NMR (DMSO-*d*₆): δ = 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 (CH₃CH₂CH₂), 29.55, 29.31, 29.21, 28.98, 25.94 (alif.), 22.37 (CH₃CH₂), 14.24 (CH₃), 7.09 (5-CH₃) ppm. MS (ESI): m/z (%) = 590.3 (100) [M-Na]⁻. Anal. C₂₈H₅₃N₃NaO₈P (C, H, N, P).

4.30.8. Bis[2-(Hexadecyloxy)ethyl] 2-amino-5-methyl-4,6-bis[2-(phosphonmethoxy)ethoxy]pyrimidine, sodium salt (40c)—GP7, white solid (106 mg, 21%), m.p. 142 °C. MS (ESI): m/z (%) = 952.5 (48) [M-(2 × Na)+H]⁻. Anal. C₄₇H₉₁N₃Na₂O₁₂P₂ (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 CCl₄, 10 mL) and the mixture was stirred at r.t. overnight. The mixture was taken down 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. ¹H NMR (DMSO-*d*₆): δ = 6.71 (br s, 2H, NH₂), 4.26 (t, *J*(1',2') = 5.3, 4H, H-1'); 3.67 (t, *J*(2',1') = 5.3, 4H, H-2') ppm. ¹³C NMR (DMSO-*d*₆): δ = 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 - H₂O+H]⁺. Anal. C₈H₁₂BrN₃O₄ (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) [20].

The methodology of the antiviral activity assays followed previously described procedures [21], [22].

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- 1a**, $R^1 = R^2 = H$ (PMEA)
1b, $R^1 = NH_2$, $R^2 = H$ (PMEDAP)
1c, $R^1 = H$, $R^2 = (R)\text{-CH}_3$ ((*R*)-PMPA)
1d, $R^1 = NH_2$, $R^2 = (R)\text{-CH}_3$ ((*R*)-PMPDAP)
1e, $R^1 = H$, $R^2 = (S)\text{-CH}_2OH$ ((*S*)-HPMPA)

2

- 3a**, $R^3 = R^4 = H$
3b, $R^3 = CH_3$, $R^4 = H$
3c, $R^3 = Br \text{ or } Cl$, $R^4 = H$
3d, $R^3 = H$, $R^4 = (R)\text{-CH}_3$

4

Figure 1.
Acyclic nucleoside phosphonates.

Figure 2.
Acyclic nucleoside bisphosphonates.

Figure 3.

A: CE separation of enantiomers **27a** (S), 0.1mM 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.

Scheme 1.
Synthesis of bis-HPMPO derivatives.

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.

Scheme 3.

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

a) 1. thiourea, 2. NaOH; b) 2 eq. NaH, DMF; c) 1 eq. NaH, DMF; d) BrSiMe₃, CH₃CN.

Scheme 4.

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

38, $\text{TsOCH}_2\text{P}(\text{O})[\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_{15}\text{CH}_3](\text{O}^-\text{Na}^+)$

39a, **40a**, $\text{R}^1 = \text{H}$, $\text{R}^2 = \text{P}(\text{O})[\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_{15}\text{CH}_3](\text{O}^-\text{Na}^+)$

39b, **40b**, $\text{R}^1 = \text{Br}$, $\text{R}^2 = \text{P}(\text{O})[\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_{15}\text{CH}_3](\text{O}^-\text{Na}^+)$

39c, **40c**, $\text{R}^1 = \text{CH}_3$, $\text{R}^2 = \text{P}(\text{O})[\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_{15}\text{CH}_3](\text{O}^-\text{Na}^+)$

a) $\text{HOCH}_2\text{CH}_2\text{OH}$, $t\text{-BuOK}$; b) NaH , THF; c) $\text{Br}_2/\text{CCl}_4/\text{DMF}$.

Scheme 5.

Synthesis of alkoxyalkyl esters of bisphosphonates.

Table 1Substitution 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	(<i>S</i>)-CH ₃	OTs	23b	(<i>S</i>)-CH ₃
c	(<i>R</i>)-CH ₃	OTs	23c	(<i>R</i>)-CH ₃
d	(<i>S</i>)-CH ₂ OH	OTs	24a	(<i>S</i>)-CH ₃
e	(<i>R</i>)-CH ₂ OH	OTs	24b	(<i>R</i>)-CH ₃

Table 2

Substitution pattern of compounds 25, 26 and 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 ₂ OH	27c	H	(S)-CH ₂ OH
25d, 26d	H	(R)-CH ₂ OH	27d	H	(R)-CH ₂ OH
25e, 26e	(S)-CH ₃	(R)-CH ₃	27e	(S)-CH ₃	(R)-CH ₃
25f, 26f	(S)-CH ₃	(S)-CH ₂ OH	27f	(S)-CH ₃	(S)-CH ₂ OH
25g, 26g	(S)-CH ₃	(R)-CH ₂ OH	27g	(S)-CH ₃	(R)-CH ₂ OH
25h, 26h	(R)-CH ₃	(S)-CH ₂ OH	27h	(R)-CH ₃	(S)-CH ₂ OH
25i, 26i	(R)-CH ₃	(R)-CH ₂ OH	27i	(R)-CH ₃	(R)-CH ₂ OH
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

Table 3Substituents 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

Table 4Substitution pattern of compounds **32**, **33**, **34**, **35** and **36**.

Compd. 32, 33, 34	R ¹	Compd. 35, 36	R ¹	R ²
a	H	a	H	(<i>S</i>)-CH ₃
b	(<i>S</i>)-CH ₃	b	H	(<i>R</i>)-CH ₃
c	(<i>R</i>)-CH ₃	c	(<i>S</i>)-CH ₃	(<i>R</i>)-CH ₃