1. Introduction

1.1. Acyclic nucleoside phosphonates

1.1.1. Overview of acyclic nucleoside phosphonates

Various nucleoside antimetabolites, modified either at heterocyclic base or sugar moiety, are widely used for treatment of cancer and leukemia,[1] as well as viral diseases.[2] Nucleoside analogues are in the most cases phosphorylated to their 5'-phosphates (mono-, di- and triphosphates) by cellular or viral kinases (nucleoside monophosphate kinases, nucleoside diphosphate kinases) or phosphotransferases to their active metabolites that interfere with natural metabolic pathways of nucleic acids and their precursors. The first phosphorylation step to 5'-monophosphate is crucial for antimetabolite activation however direct use of 5'-nucleotides is limited by enzymatic degradation of the phosphate during its membrane transport as well as in the cell pool. Furthermore, nucleoside analogues can undergo catabolic degradation similar to the natural nucleosides which lowers concentration of the active metabolite in the cell.[3] The second generation of nucleoside antimetabolites, e.g. C-nucleosides,[4] carbocyclic nucleosides[5] and acyclic nucleosides, circumvent intracellular degradation by their increased enzymatic and chemical stability.

Acyclic nucleosides, where the sugar moiety is replaced with *N*-alkyl chain, are powerful antivirals active against herpes viruses. Among them, acyclovir[6] (Zovirax™) is used for treatment of HSV-1 and 2 infections, and ganciclovir[7] is used for CMV infections (Figure 1). Acyclic nucleosides are phosphorylated selectively in infected cells by viral thymidine kinase and inhibit viral DNA polymerase. On the contrary, (*S*)-DHPA[8] [(*S*)-9-(2,3-dihydroxypropyl)adenine], which is active against a

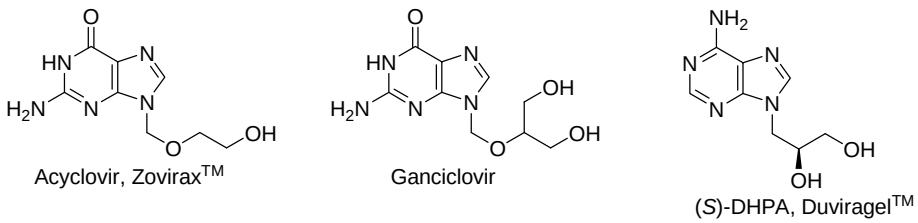


Figure 1. Acyclic nucleosides.

broad range of both DNA and RNA viruses, interferes with the (*S*)-adenosylhomocysteine (SAH) hydrolase and does not require phosphorylation.

Acyclic nucleoside phosphonates (ANPs) are acyclic nucleotide analogues where the sugar moiety is replaced with *N*-alkoxy chain and the 5'-phosphate is replaced with chemically stable phosphonate (change of -C-O-P- to -O-C-P-). The phosphonate group is isosteric and isopolar to the phosphate moiety, it is recognized by enzymes as a nucleotide analogue, however resists to enzymatic degradation. ANPs represent a key class of nucleotide analogues with a broad spectrum of antiviral, cytostatic and antiparasitic activity.[9] In the cell, ANPs are phosphorylated by nucleotide kinases to the corresponding diphosphates (ANPpp) that inhibit DNA polymerases and/or reverse transcriptase and their incorporation into the growing DNA chain leads to chain termination.[10] The antiviral activity of ANPs is probably result of the higher affinity of the diphosphorylated ANP metabolite for viral DNA polymerases than for the cellular DNA polymerases. The most important ANPs that were launched to the market are listed below:

9-[2-(Phosphonomethoxy)ethyl]adenine[11] (PMEA, adefovir, Figure 2) is active against DNA viruses (including herpesviruses and hepadnaviruses) and retroviruses.[12] PMEA was originally developed as an anti-HIV drug, but the clinical trials were discontinued due to the side effects at the therapeutic dose. However, its orally bioavailable prodrug, bis(pivaloyloxymethyl) ester (adefovir dipivoxil),[13] was approved for treatment of chronic hepatitis B (Hepsera®).[14]

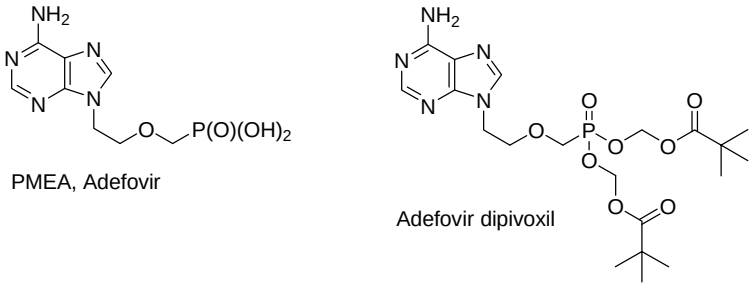


Figure 2. Adefovir.

Substitution at position 2 of the side chain by methyl group leads to another structural group of biologically active compounds. The adenine derivative, 9-(*R*)-[2-(phosphonomethoxy)propyl]adenine (PMPA, tenofovir, Figure 3),[15] is a promising anti-HIV agent. Its prodrug, the bis(isopropoxycarbonyloxymethyl) ester, was approved for treatment of AIDS (Viread®, Tenofovir disoproxil fumarate)[16] and

chronic hepatitis B infections.[17,18] Tenofovir was also developed in a double combination with Emtricitabine (Truvada®) as well as in a triple combination in a single pill with Emtricitabine and Efavirenz (Atripla™) for treatment of AIDS. The antiviral activity spectrum of tenofovir includes hepadna- and retroviruses. The efficacy of tenofovir in the prevention of retrovirus infection was described and is a subject of further studies.[19]

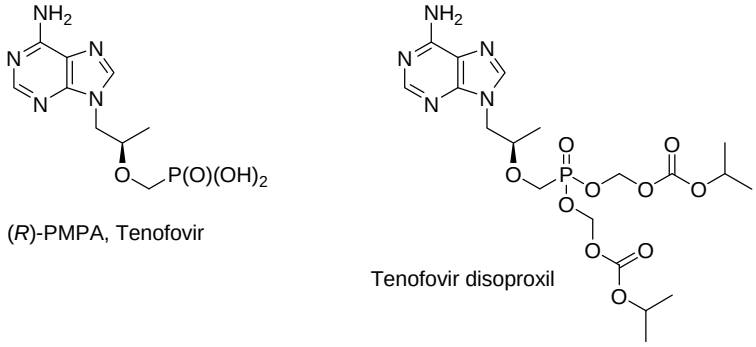


Figure 3. Tenofovir.

The third type of ANPs is represented by (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine [(*S*)-HPMPC, cidofovir, Figure 4].[20] Cidofovir is active against virtually all DNA viruses, including polyoma-, papilloma-, adeno-, herpes-, and poxviruses.[21] Cidofovir was licensed for the treatment of CMV retinitis in AIDS patients (Vistide™, intravenous administration at a dose of 5 mg/kg once every other week). However, it was successfully used in the treatment of HPV-associated diseases (hypopharyngeal papilloma,[22] laryngeal papilloma,[23] recurrent respiratory papillomatosis,[24] and plantar warts[25]), pox-associated diseases (molluscum contagiosum[26]) or orf-virus infections (ecthyma contagiosum[27]) in immunosuppressed patients.

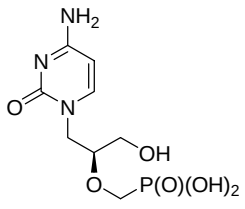


Figure 4. Cidofovir.

Except for the cytosine derivative (cidofovir), the choice of the heterocyclic base is limited mostly to adenine, guanine and diaminopurine derivatives[28]and to their 8-aza[29] and 3-deaza congeners.[30] The 2,6-diaminopurine and guanine ANP derivatives are often very potent antivirals[31] and/or exhibit also antitumor properties.[32] The pharmacophore of purine acyclic nucleoside phosphonates is characterized by the presence of amino groups at the pyrimidine part of the purine system. While N^6-substitution in adenine and 2,6-diaminopurine derivatives still preserves the biological activity in the PME and PMP series,[33] other alterations of amino groups generally result in complete loss of activity.[34,40e] It was shown that N^6-substituted derivatives of 2,6-diaminopurine are converted to their 2,6-diaminopurine and subsequently to guanine counterparts by N^6-methyl-AMP aminohydrolase in the cell.[35] The N^6-substituted PMEG prodrug (GS-9219) is nowadays entering the Phase I clinical trials against hematologic cancers.[36]

Acyclic nucleoside phosphonates derived from 2,4-diamino-6-hydroxypyrimidine, where the alkoxyalkylphosphonate side chain is attached to the oxygen atom at the position 6 of the pyrimidine moiety, are considered as the second generation of ANPs (Figure 5).[37]

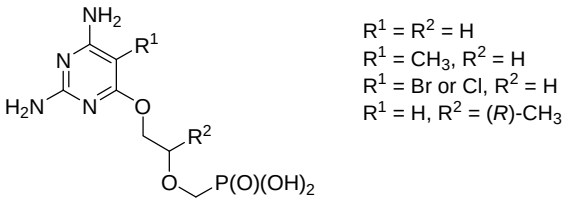


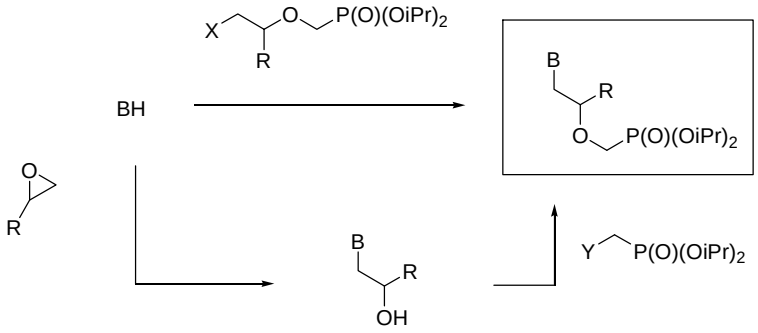
Figure 5. Open-ring ANPs.

These compounds can be considered as 2,6-diaminopurine analogues with an open imidazole ring. 2,4-Diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine shows inhibitory activity against both DNA and retroviruses comparable to adefovir and tenofovir.[38] Further SAR studies showed that 5-substituted derivatives of 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy]pyrimidine markedly inhibited retrovirus replication in cell culture. The 5-methyl derivative was inhibitory to human immunodeficiency virus and Moloney murine sarcoma virus-induced cytopathicity in cell culture but also cytostatic to CEM cell cultures. Also the 5-halogen-substituted derivatives showed a pronounced antiretroviral activity,

comparable to that of the reference drugs adefovir and tenofovir, but were devoid of any measurable toxicity *in vitro*.[39]

1.1.2. Synthesis of acyclic nucleoside phosphonates

Generally, there are two main methods of synthesis of ANPs[9b] (Scheme 1): a) direct alkylation of the heterocyclic base with phosphonate-bearing building block containing a suitable leaving group (Cl, OTs) in the presence of a strong base (NaH, Cs_2CO_3, DBU) in DMF as a solvent; b) alkylation of the heterocyclic base to the corresponding hydroxyalkyl derivative followed by etherification with diisopropoxyphosphorylmethyl bromide or *p*-toluenesulfonate. The diisopropyl esters are routinely converted to phosphonic acids by treatment with trimethylsilyl bromide followed by hydrolysis. Phosphonate containing building blocks[40] are prepared by Arbuzov reaction of triisopropyl phosphite with an appropriate alkyl halide.

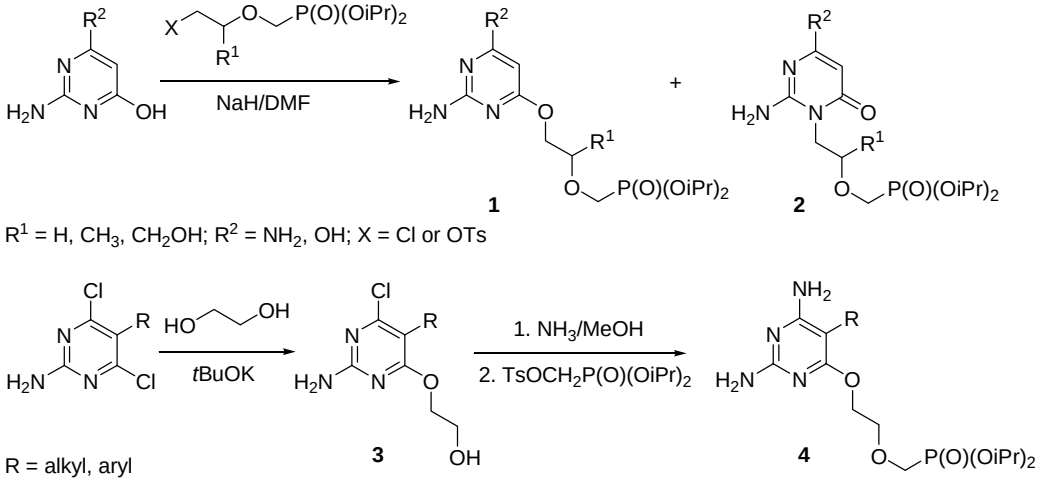


B = heterocyclic base; R = H, CH_3, CH_2OH; X = Cl, OTs; Y = Br, OTs.

Scheme 1. Methods of synthesis of ANPs.

Open-ring derivatives were prepared by alkylation of 2,4-diamino-6-hydroxypyrimidine with diisopropoxyphosphorylmethoxyalkyl chloride or *p*-toluenesulfonate to afford a mixture of O^6 and N^1 regioisomers **1** and **2**, respectively (Scheme 2). Alternative synthesis of open-ring ANPs starting from 2-amino-4,6-dichloropyrimidine that gives exclusively O^6-alkylated product was also developed.[41] The 4,6-dichloropyrimidine was converted to its 2-hydroxyethoxy derivative **3** by reaction with ethyleneglycol in the presence of base (*t*BuOK) and etherification of **3** with diisopropyl *p*-toluenesulfonyloxymethylphosphonate gave the diisopropyl

phosphonomethoxyethoxy congener **4**. The carba-analogues of open-ring ANPs were prepared from 2-amino-4,6-dichloropyrimidine by Sonogashira cross-coupling followed by reduction.[42] The replacement of the C–O moiety by the C–C bond resulted in the loss of antiviral activity.

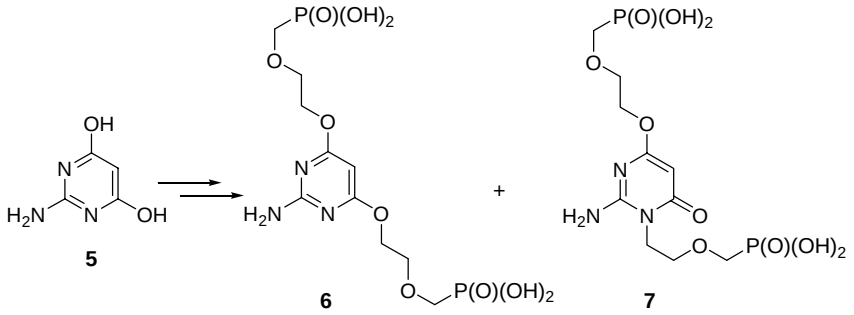


Scheme 2. Methods of synthesis of open-ring ANPs.

1.2. Bisphosphonates

1.2.1. Acyclic nucleoside bisphosphonates

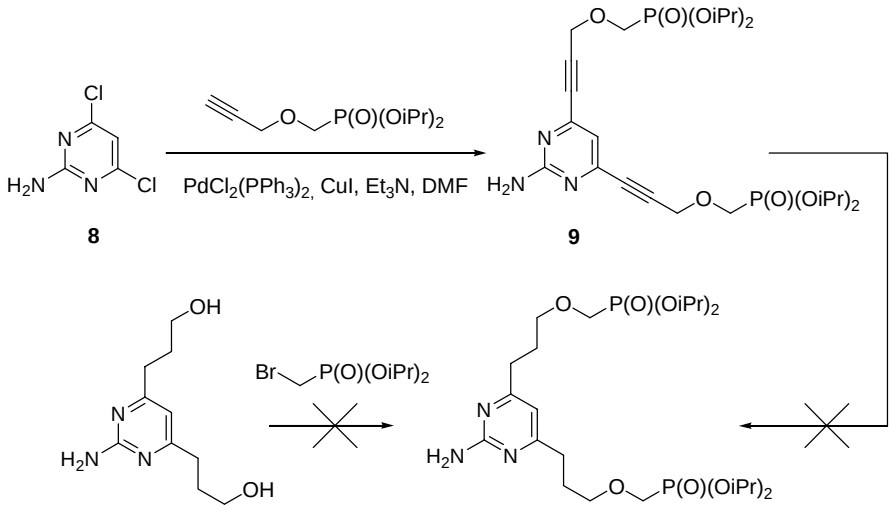
The isomeric bisphosphonates **6** and **7** (Scheme 3) bearing two phosphonoalkoxy chains may be considered as a second group of "open-ring" ANPs and potential antivirals.[37] Bisphosphonates derived from 2-amino-4,6-dihydroxypyrimidine, i. e. symmetrical 2-amino-4,6-bis[2-(phosphonomethoxy)eth-



Scheme 3. Acyclic nucleoside bisphosphonates.

oxy]pyrimidine (**6**) and 2-amino-4-[2-(phosphonomethoxy)ethoxy]-1-[2-(phosphonomethoxy)ethyl]pyrimidin-6(1*H*)-one (**7**) were prepared by direct alkylation of 2-amino-4,6-dihydroxypyrimidine (**5**) with 2-(diisopropoxyphosphorylmethoxy)ethyl chloride in the presence of NaH as a base in 5% and 8% yield, respectively, and the regioisomers were separated by chromatography. The symmetrical O^4,O^6 dialkylated bisphosphonate **6** was described to possess antiviral activity (EC_{50} = 0.1066 μmol/ml).

Among the products prepared in these studies, the dialkynyl compound **9** was prepared from 2-amino-4,6-dichloropyrimidine (**8**) by Sonogashira cross-coupling (Scheme 4).[42] Attempts to prepare the carba-analogue of **6** failed due to instability of the dialkynyl precursor **9** under reaction conditions. The attempts at dialkylation of the bis(3-hydroxypropyl)pyrimidine by diisopropyl bromomethylphosphonate also completely failed.



Scheme 4. Synthesis of carba-analogues of bisphosphonates.

In the long-term exploration of the SAR of modified ANPs performed in our group, a series of bifunctional acyclic nucleoside phosphonates depicted in Figure 6 was prepared by Silvie Vrbková.[43] Selected bisphosphonates were converted to their lipophilic esters to decrease polarity of the bisphosphonate moiety and facilitate their penetration through the cell membrane. Their cytostatic, antiviral and anti-osteoporotic activities were studied; the (2S,3S)-9-{3-(hydroxy)-1,4-[bis(phosphonomethoxy)]butan-2-yl}guanine showed promising cytostatic activity (liposomes were used for the transport into the cell). All the compounds were

generally prepared by alkylation of heterocyclic base with a suitably functionalized bisphosphonate-building block.

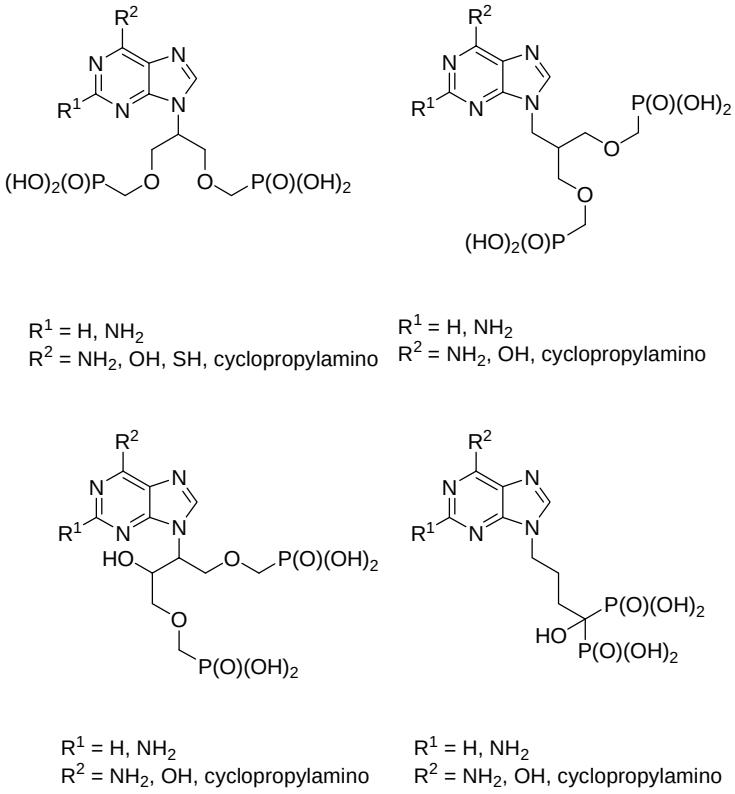
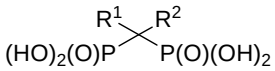


Figure 6. Acyclic nucleoside bisphosphonates.

1.2.2. Other bisphosphonates and bisphosphates

The bisphosphonates (BPs) showed in Figure 7 are metabolically stable structural analogues of pyrophosphate. BPs have a high affinity for bone mineral and were found to prevent calcification and/or pathological calcification. BPs are currently in clinical use for the following indications: a) as a bone markers in nuclear medicine for the diagnosis of bone metastases and other bone lesions; b) osteolytic bone diseases or bone resorption, including osteoporosis, tumor-related bone destruction and Paget's disease; c) inhibition of abnormal calcification.[44]



Bisphosphonate	R^1	R^2
Medronic acid	H	H
Oxidronic acid	H	OH
Clodronic acid	Cl	Cl
Etidronic acid	CH_3	OH
Tiludronic acid	4-chlorophenylsulfanyl	OH
Pamidronic acid	2-aminoethyl	OH
Alendronic acid	3-aminopropyl	OH
Ibandronic acid	*N*-methyl-*N*-pentyl-2-aminoethyl	OH
Neridronic acid	5-aminopentyl	OH
Incadronic acid	*N*-cycloheptylamino	H
Risendronic acid	(pyridine-3-yl)methyl	OH
Zoledronic acid	(imidazol-1-yl)methyl	OH

Figure 7. Bisphosphonates in clinical practice.

Purine and pyrimidine nucleotides act as extracellular signaling molecules through activation of P2 receptors. These receptors can be divided into two categories: P2X (ligand-gated ion channels) and P2Y (G protein-coupled) nucleotide receptors.[45] Various bisphosphates of naturally occurring nucleosides, e. g. adenosine 2',5'-diphosphate and adenosine 3',5'-diphosphate[46] were described as agonists or competitive antagonists of $P2Y_1$ receptor. Also, acyclic analogues of deoxyadenosine 3',5'-diphosphates were shown to be $P2Y_1$ receptor antagonists[47] (Figure 8).

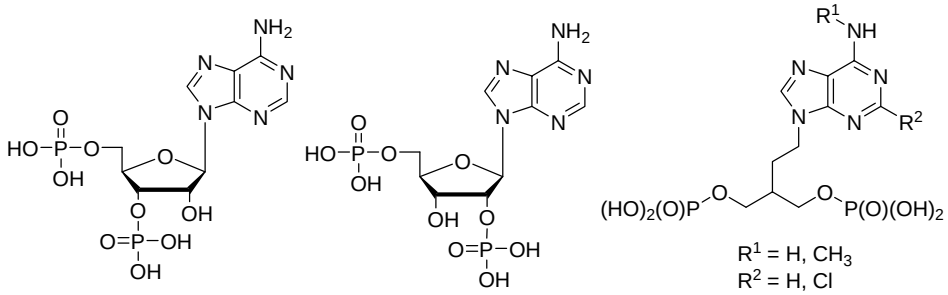
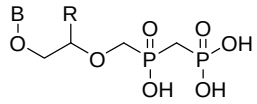


Figure 8. Bisphosphates as $P2Y_1$ receptor antagonists.

1.3. Phosphonomethylphosphinates

Phosphonomethylphosphinate contain P-C-P-C unit, this system can be considered as a nonhydrolyzable mimic of diphosphate moiety, where the bridging oxygen atoms are replaced with methylene groups. Analogues of acyclic nucleoside diphosphates containing phosphonomethylphosphinyl system derived from 1-hydroxypyrimidines and 9-hydroxypurines were previously described in the literature (Figure 9).[48] Adenine and guanine derivatives were reported to possess moderate antiviral activity against HSV-1, VZV and visna virus.



B = adenin-9-yl, guanin-9-yl, cytosin-1-yl, uracil-1-yl and thymin-1-yl
R = H, CH_2OH

Figure 9. Analogues of acyclic nucleoside diphosphates containing phosphonomethylphosphinyl moiety.

Modified triphosphates of carbocyclic nucleoside analogues containing phosphonomethylphosphinyl unit and their stability towards alkaline phosphatases were also described.[49]

Several farnesyl pyrophosphate (FPP) analogues containing stable phosphonomethylphosphinyl moiety that mimics the terminal diphosphate were reported in the literature. FPP mimics shown in Figure 10 inhibit squalene

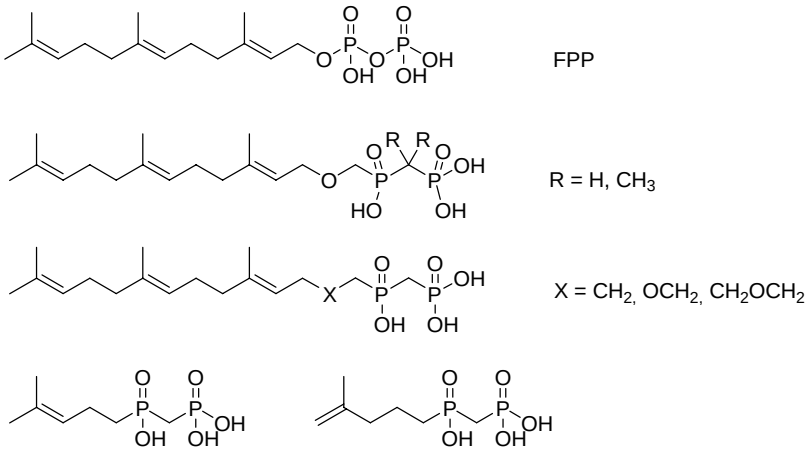


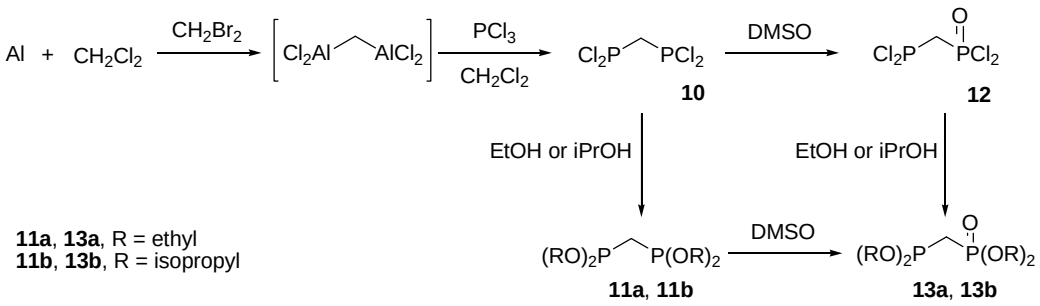
Figure 10. Farnesyl pyrophosphate and isopentenyl diphosphate mimics.

synthetase[50] and/or protein:farnesyl transferase.[51] Squalene synthetase catalyses the condensation of 2 molecules of farnesyl pyrophosphate and reductive rearrangement of the resulting presqualene pyrophosphate to produce squalene.[52] The inhibitors of this enzyme are attractive because of its strategic location in the cholesterol biosynthesis pathway.

Dimethylallyl diphosphate and isopentenyl diphosphate analogues (Figure 10) are competitive inhibitors of farnesyl diphosphate synthetase. The isopentenyl derivative is a substrate for farnesyl diphosphate synthetase and generates a nonreactive phosphonomethylphosphinyl product that can inhibit normal isoprenoid reactions that utilize FPP as a substrate, including squalene synthetase and geranylgeranyl diphosphate synthetase etc.[53]

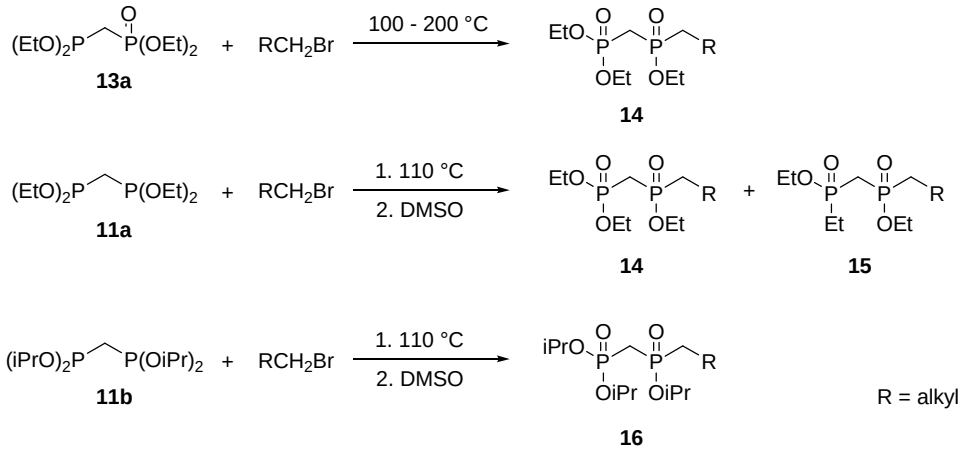
Phosphonomethylphosphinate derivatives were also described as transcarbamoylase[54] and/or glutamine synthetase[55] inhibitors.

Synthesis of phosphonomethylphosphinates was first described by Lehmkuhl and Schäfer[56] and Novikova et. al.[57] The synthetic route to diphosphonites **11** and phosphonomethylphosphonites **13** is outlined in Scheme 5; aluminum was refluxed in dichloromethane with catalytic amount of dibromomethane to give bis(dichloroaluminum)methane, which was not isolated, but added directly to a mixture of methylene chloride and phosphorus trichloride to give bis(dichlorophosphino)methane (**10**). The diphosphonites **11** were prepared by addition of **10** to dry isopropyl or ethyl alcohol and a base (triethyl amine or pyridine). Compounds **10** and **11a** and **11b** can be oxidized with 1 eq. of DMSO to give **12** and **13a** and **13b**, respectively.



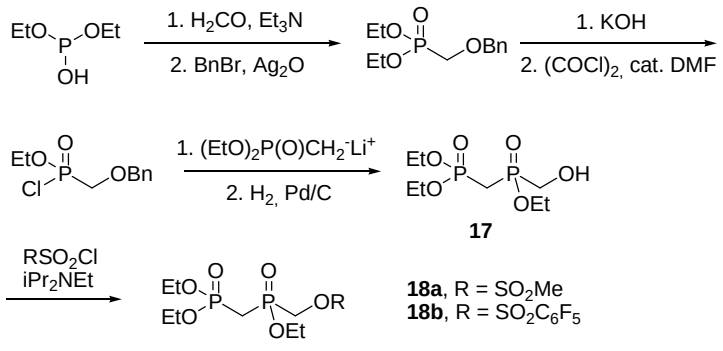
Scheme 5. Synthesis of diphosphonites **11a**, **11b** and phosphonomethylphosphonites **13a**, **13b**.

Phosphonite **13a** reacts with alkyl bromide to give a product of Arbuzov reaction **14** in approx. 40–50% yield (Scheme 6). The tetraethyl ester **11a** gives under Arbuzov reaction conditions mixture of phosphinates **14** and **15**. The ethylphosphinate **15** results from the reaction of ethyl bromide, the product of the first Arbuzov reaction, with the terminal phosphonite prior to oxidation with DMSO. The side product **15** is avoided by using the more sterically hindered isopropyl ester **11b**; Arbuzov reaction affords the desired triisopropyl ester **16** (yield 40%) only.[53,55]



Scheme 6. Arbuzov reaction of phosphonites.

Another synthetic approach to phosphonomethylphosphinates was developed by Flohr et. al.[54] (Scheme 7). Alcohol **17** was prepared from diethyl phosphite in 6 steps in overall yield of 30%. Although attempts to triflate **17** failed, mesylate **18a** was readily formed, but its reactivity in subsequent S_N2 reaction was too low. Pentafluorophenylsulfonate **18b** was prepared in 84% yield and its reactivity was sufficient to allow introduction of a variety of amine nucleophiles under mild reaction conditions.



Scheme 7. Synthesis of phosphonomethylphosphinate from diethyl phosphite.

1.4. dUTPase

Deoxyuridine nucleotidohydrolase (dUTPase) is an enzyme essential in both eukaryotes and prokaryotes;[58] the enzyme catalyzes hydrolysis of dUTP into dUMP and diphosphate using Mg^{2+} as a cofactor.[59] The enzyme supplies the dUMP substrate for dTTP synthesis and, by maintaining low dUTP/dTTP ratio in the cell, minimizes uracil misincorporation into DNA.[60] dUTPases can be divided into three groups. Mammalian and avian herpes viruses contain the monomeric form of the enzyme; protozoan parasites and the bacterium *Campylobacter jejuni* encode a dimeric form of dUTPase; and finally the most studied family of dUTPases found in eukaryotes, prokaryotes and viruses contains the trimeric form of the enzyme. The monomeric and trimeric forms have similar enzymatic properties. In contrast, the dimeric enzymes possess no similarity to members of the other classes in sequence, structure or enzymatic characteristics. In general, the sequence homology among trimeric dUTPases is relatively high (e. g. viral enzymes show 65% sequence identity with the human enzyme).[61]

Large effort is undertaken to develop leads for the treatment of tuberculosis infections in the human population. Approximately one third of the world's human population is infected with *M. tuberculosis* and nearly two million people die every year from the infection.[62] The emergence of drug resistant strains of *M. tuberculosis* has made the search for new drugs more urgent. *M. tuberculosis* dUTPase was recognized as a one of valid targets for drug design. The human dUTPase shares 34% sequence identity with the *M. tuberculosis* enzyme and so could be inhibited by the same drug designed to inhibit the *M. tuberculosis* enzyme.[63] Cross-reaction could be prevented by detailed study of the active site of the enzyme and SAR study of inhibitors.

Previously described inhibitors (Figure 11) of dUTPase include nonhydrolyzable analogues of nucleoside triphosphates, where the α,β bridging oxygen atom is replaced by a methylene[64] or an imido[65] group. 2′-Deoxyuridine derivatives containing either a triphenylmethyl or triphenylsilyl substituent at the 5′ position[66] and their acyclic analogues[67] inhibit selectively *Plasmodium falciparum* dUTPase.

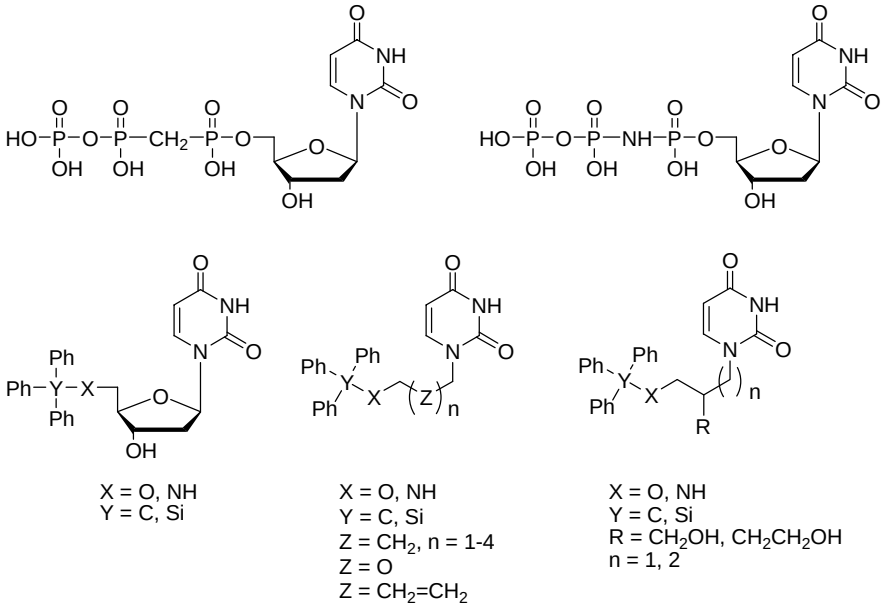


Figure 11. Inhibitors of dUTPase.

2. Aims of the work

Acyclic nucleoside phosphonates are an interesting and important class of biologically active compounds possessing antiviral and cytostatic activities. This thesis is a part of the long-term exploration of the structure-activity relationship of modified ANPs performed by our group.

In the first part of my work I have developed the regioselective synthesis of bisphosphonates in order to study their biological and physical properties. The aims of this project can be summarized by the following terms:

- To synthesize a series of bisphosphonates derived from 2-amino-4,6-dihydroxypyrimidine bearing two identical or diverse phosphonomethoxy-alkoxy chains.
- To develop regioselective synthesis of *O*-regioisomers from either 2-amino-4,6-dichloropyrimidine or 2-substituted 4,6-dihydroxypyrimidine.
- To prepare substituted bisphosphonates at positions 2, 4 and 6 of the pyrimidine ring.
- To synthesize lipophilic esters of bisphosphonates.
- To evaluate biological activities and physical properties of prepared bisphosphonates.

In the second part of my work I focused on the synthesis of acyclic nucleoside phosphonomethylphosphinates as analogues of acyclic nucleoside diphosphates, biologically interesting compounds that have not yet received much attention. The aims of the project were:

- The preparation of acyclic nucleoside phosphonomethylphosphinates of natural heterocyclic bases and evaluation of their biological properties.
- The preparation of acyclic uridine phosphonomethylphosphinates and their corresponding phosphates as potential inhibitors of dUTPase.

3. Results and Discussion

3.1. Acyclic nucleoside bisphosphonates

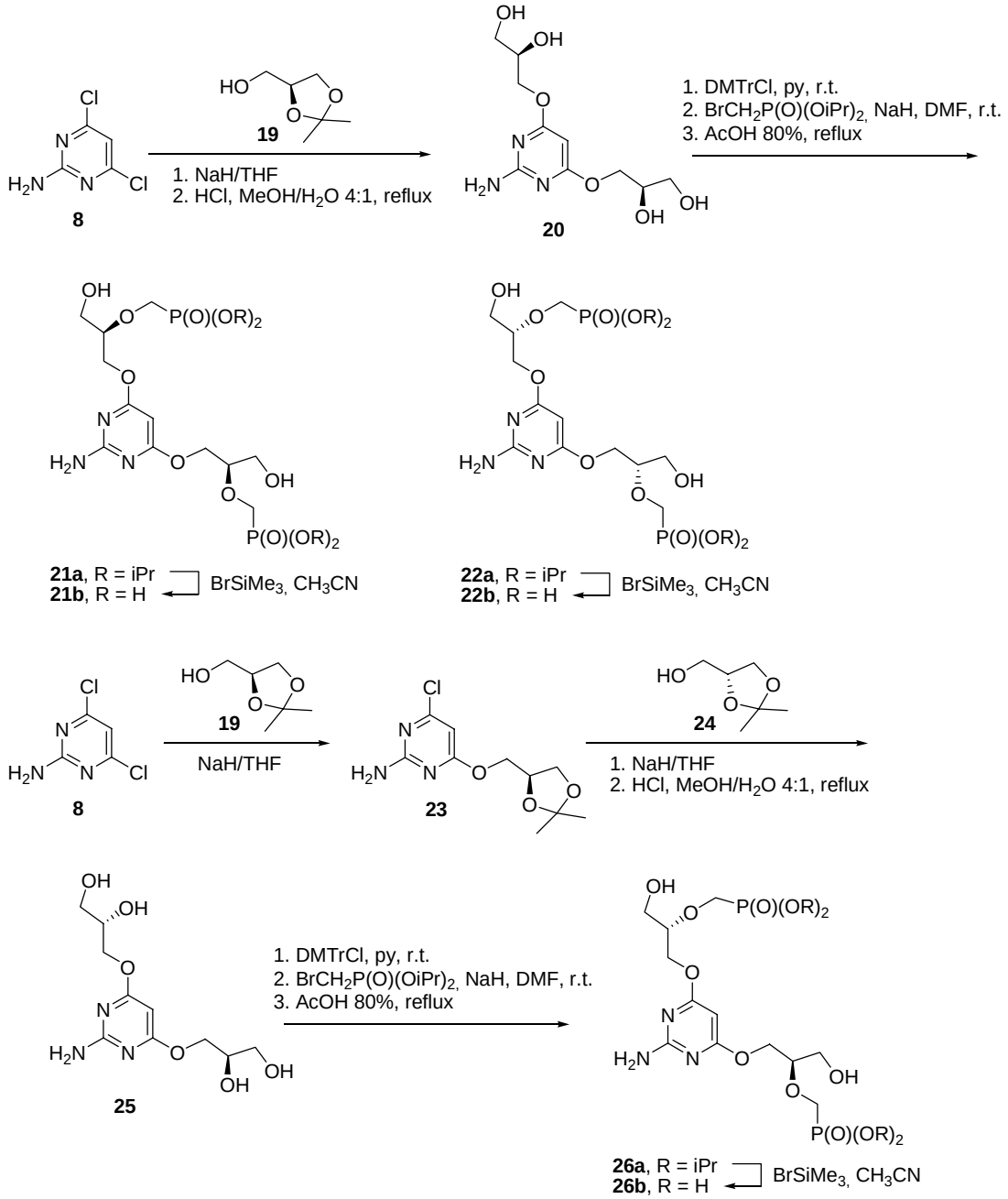
3.1.1. Synthesis of bisphosphonates from 2-amino-4,6-dichloro-pyrimidine

The bisphosphonate **6** was originally prepared by alkylation of 2-amino-4,6-dihydroxypyrimidine (**5**).[37] Since this reaction affords a mixture of *O*– and *N*–alkylated products **6** and **7**, I have decided to use a completely different synthetic strategy and use nucleophilic aromatic substitution of 2-amino-4,6-dichloropyrimidine with appropriate alcohol to form ether bonds at positions 4 and 6 of the pyrimidine ring. Formation of *N*-alkylated regioisomer by this reaction is not possible.

3.1.1.1. Synthesis of bis-HPMPO derivatives

Bisphosphonates bearing two 3-hydroxy-2-(phosphonomethoxy)propoxy (HPMPO) side chains at positions 4 and 6 of the pyrimidine moiety were prepared by stepwise synthesis starting from 2-amino-4,6-dichloropyrimidine (**8**) and enantiomericaly pure 1,2-isopropylideneglycerol **19** and **24**, respectively (Scheme 8). Reaction of **8** with 2 equivalents of (*S*)-1,2-isopropylideneglycerol (**19**) was performed in THF in the presence of NaH as a base; subsequent deprotection by diluted hydrochloric acid gave 2,3-dihydroxypropoxy derivative **20** in 64% yield. Primary hydroxyl groups were protected by treatment with 4,4´-dimethoxytrityl chloride and alkylation of secondary hydroxyl groups by diisopropoxyphosphorylmethyl bromide followed by deprotection with acetic acid afforded bis-HPMPO derivative **21a**. Diisopropyl esters were cleaved under standard conditions (bromotrimethylsilane in acetonitrile, followed by hydrolysis) to afford free phosphonic acid **21b**. The enantiomer **22b** was prepared by the same procedure as compound **21b** from pyrimidine **8** and (*R*)-1,2-isopropylideneglycerol (**24**). The diastereosisomer **26b** was prepared by reaction of pyrimidine **8** with one equivalent of **19** and one equivalent of NaH in THF; the monoalkylated product **23** was treated

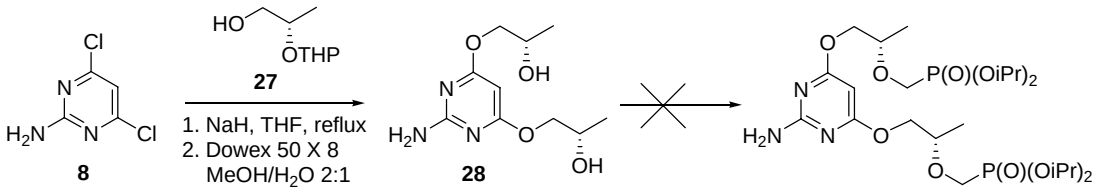
with **24** under the same conditions to afford dihydroxypropoxy derivative **25**. Further procedure was identical with that described for the compound **21b**. Compounds **21b**, **22b** and **26b** were purified by preparative HPLC; triethylammonium salts of phosphonic acids were converted to the free phosphonic acids on a column of Dowex 50 × 8 in H^+ form.



Scheme 8. Synthesis of bis-HPMPO derivatives from 2-amino-4,6-dichloropyrimidine (**8**).

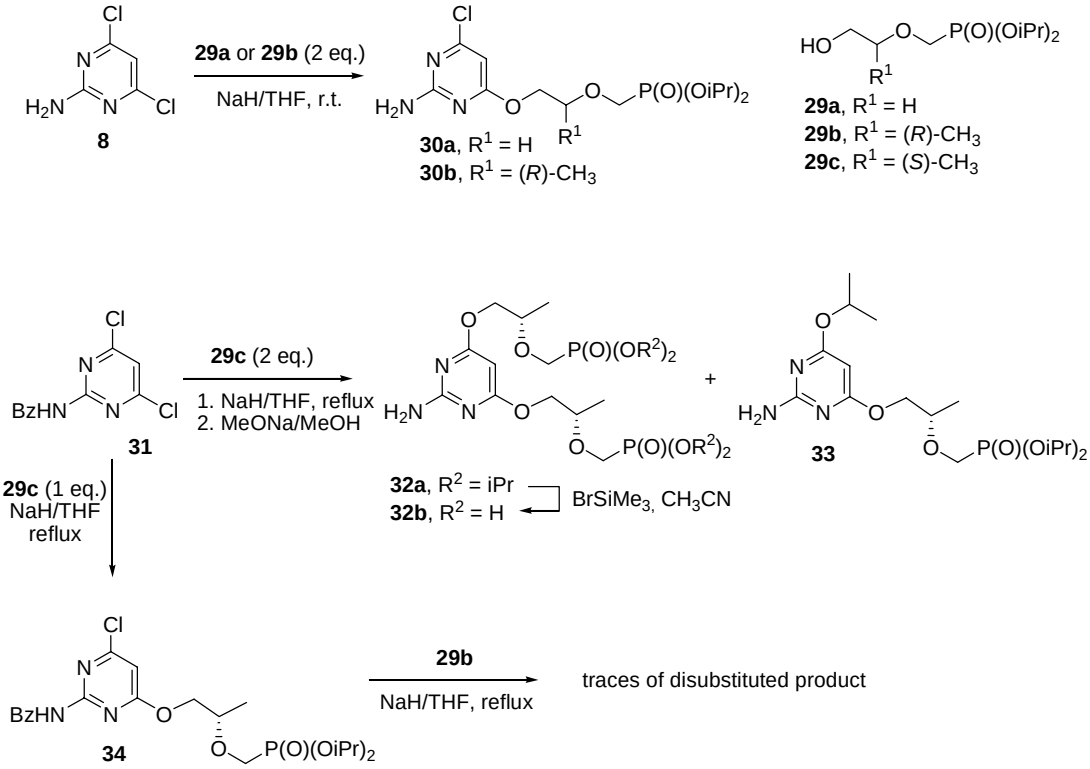
3.1.1.2. Synthesis of bis-PMPO derivatives

Synthesis of bisphosphonates bearing two 2-(phosphonomethoxy)propoxy (PMPO) chains by the above method failed. Reaction of **8** with 1,2-propanediol **27**[27,68] gave the desired bis-2-hydroxypropoxy derivative **28**, however this compound is further unreactive and my attempts for alkylation with diisopropoxyphosphorylmethyl bromide were unsuccessful (Scheme 9).



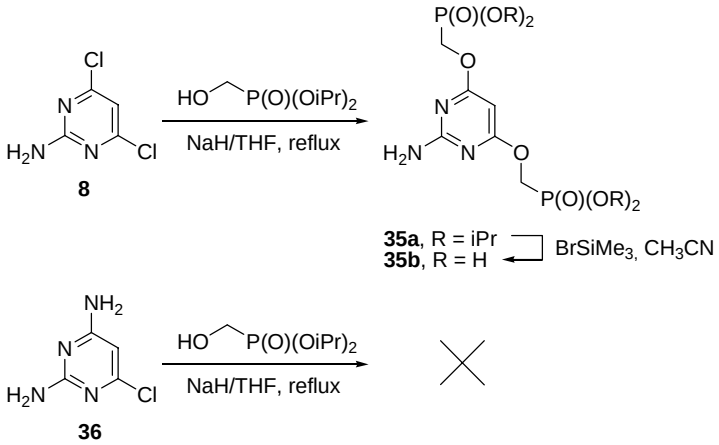
Scheme 9. Synthesis of bis-PMPO derivatives from 2-amino-4,6-dichloropyrimidine (**8**).

In contrast to the stepwise synthesis is direct reaction of pyrimidine **8** with chiral phosphonate bearing building block **29a**–**c**[28,40c] (Scheme 10). While reaction of **8** with isopropylideneglycerol **19** and **24**, respectively, afforded desired dialkylated product in nearly quantitave yield, reaction of **8** with 2 equivalents of phosphonate **29** gave product of monosubstitution **30** only. Reaction of **8** with synthon **29** was performed either in THF, DMF or dioxane using NaH, DBU, *t*BuONa, K_2CO_3 or Cs_2CO_3 as a base or in toluene in the presence of KOH, K_2CO_3 and 18-crown-6 as an activator;[69] the best results were achieved using THF as a solvent and NaH as a base. The problem was partially solved by protection of amino group in position 2 by benzoylation using benzoylcyanide. The reaction of protected pyrimidine **31**[70] with 2 equivalents of phosphonate **29c** in presence of 2.2 eq. NaH in THF furnished desired disubstituted product **32a** in 18% yield together with 4-isopropoxy derivative **33** in 26% yield. The benzoyl group was deprotected using sodium methoxide in methanol at r.t. Monosubstituted derivative **34** was subsequently treated with synthon **29b** under the same conditions however only traces of disubstituted product (up to 5%) were obtained. The pyrimidine **34** was unreactive toward nucleophilic aromatic substitution (starting material was recovered) just like the pyrimidine with unprotected amino group; heating of the reaction mixture or larger excess of base led to the decomposition of the starting material due to instability of the ether bond at the position 6 of the pyrimidine.



Scheme 10. Synthesis of bis-PMPO derivatives from 2-amino-4,6-dichloropyrimidine (**8**).

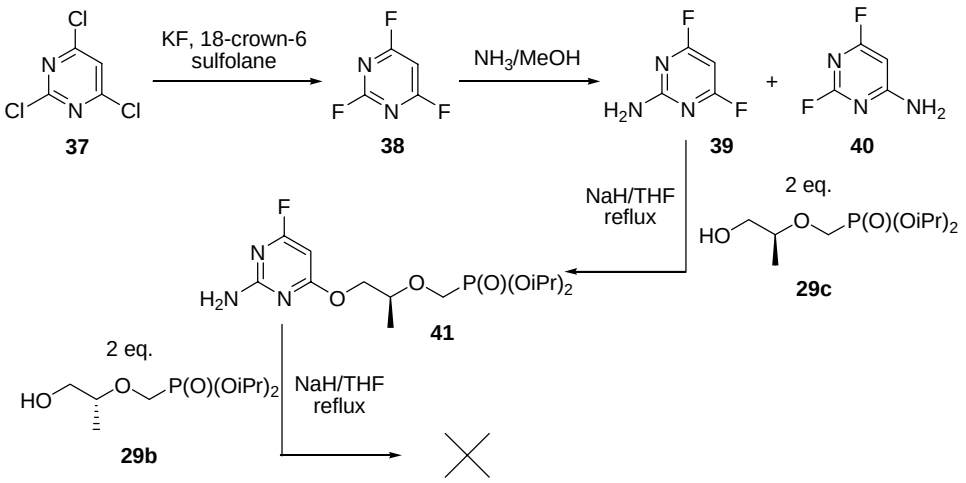
Pyrimidine **8** reacted with diisopropyl hydroxymethylphosphonate under standard conditions (NaH/THF) to afford bisderivative **35a** in 28% yield, on contrary 2,4-diamino-6-chloropyrimidine (**36**) was completely unreactive toward the above reaction (Scheme 11).



Scheme 11. Synthesis of bisphosphonate **35b**.

3.1.1.3. Comparison of reactivity of chloro- and fluoropyrimidines toward S_NAr

To enhance reactivity of pyrimidine ring toward aromatic nucleophilic substitution[71] I have converted **8** to its 2-amino-4,6-difluoro congener **39** (ref. 72) however the fluoropyrimidines showed the same low reactivity as the chloropyrimidines (Scheme 12). Commercially available 2,4,6-trichloropyrimidine (**37**) was treated with potassium fluoride in the presence of catalytic amount of 18-crown-6 in sulfolane to give 2,4,6-trifluoropyrimidine (**38**).[73] Reaction of **38** with methanolic ammonia gave mixture of 2- and 4-amino difluoropyrimidine **39** and **40**; regioisomers were readily separated by crystallization. Unfortunately, difluoropyrimidine **39** upon reaction with phosphonate **29c** gave further unreactive monosubstituted product **41**.



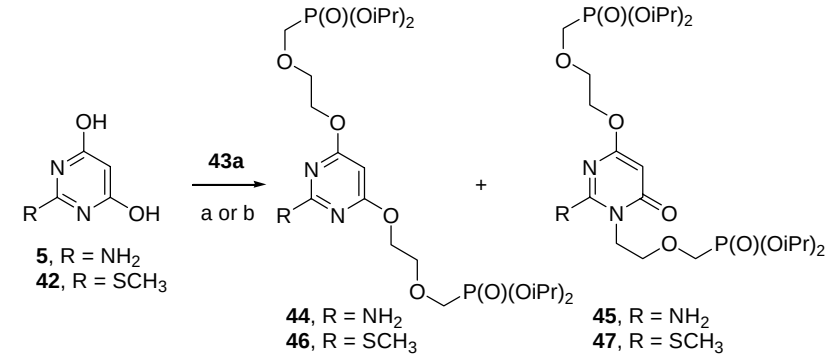
Scheme 12. Reactivity of 2-amino-4,6-difluoropyrimidine (**39**).

To conclude this part, S_NAr is widely applied for the preparation of functionalized pyrimidines from halopyrimidines,[74] nevertheless it was not efficient for reaction of 2-amino-4,6-dichloropyrimidine (**8**) with phosphonoalkoxyalkanols **29**. Reaction of pyrimidine **8** with aliphatic alcohol under standard conditions (NaH/THF) proceeds smoothly and affords disubstituted product in a good yield. On contrary, reaction with phosphonoalkoxyalkanol gives only monosubstituted product that is further unreactive toward S_NAr under mild conditions. The ether bond at position 6 is relatively labile and under harsh reaction conditions decomposes or is

subjected to transetherification. The exchange of chlorine atom to fluorine at positions 4 and 6 of the pyrimidine ring did not improve the reactivity toward S_NAr.

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

Since synthesis of bisphosphonates bearing two different chains from **8** failed, I focused again on alkylation of dihydroxypyrimidine **5** and also 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine (**42**) with phosphonate **43a.**[40] Alkylation of **5** in DMF gave mixture of compounds **44** and **45** in approximately 1:1 ration in a very low yield. Alkylation of **5** in DMSO slightly increased the yield and the yield of *O*-alkylated product **44** was two fold higher compared to *N*-alkylated product **45**. Alkylation of commercially available pyrimidine **42** in DMSO gave *O*–alkylated regioisomer **46** as a major product in 54% yield; regioisomer **47** was formed in 12% yield (Scheme 13). Alkylation of **42** in DMF did not proceed at all because of poor solubility of disodium salt of pyrimidine **42** in DMF; this might be also a reason of low yield in the reaction described in the literature.[37] Thus, the alkylation of pyrimidine **42** was the method of choice for the synthesis of bisphosphonates. This method afforded compound **6** in 18% overall yield compared to the previously



Cl O P(O)(OiPr)2

43a

Starting mat.	Product	R. conditions	Yield (%)
5	**44**	a	5
5	**45**	a	8
5	**44**	b	12
5	**45**	b	6
42	**46**	b	54
42	**47**	b	12

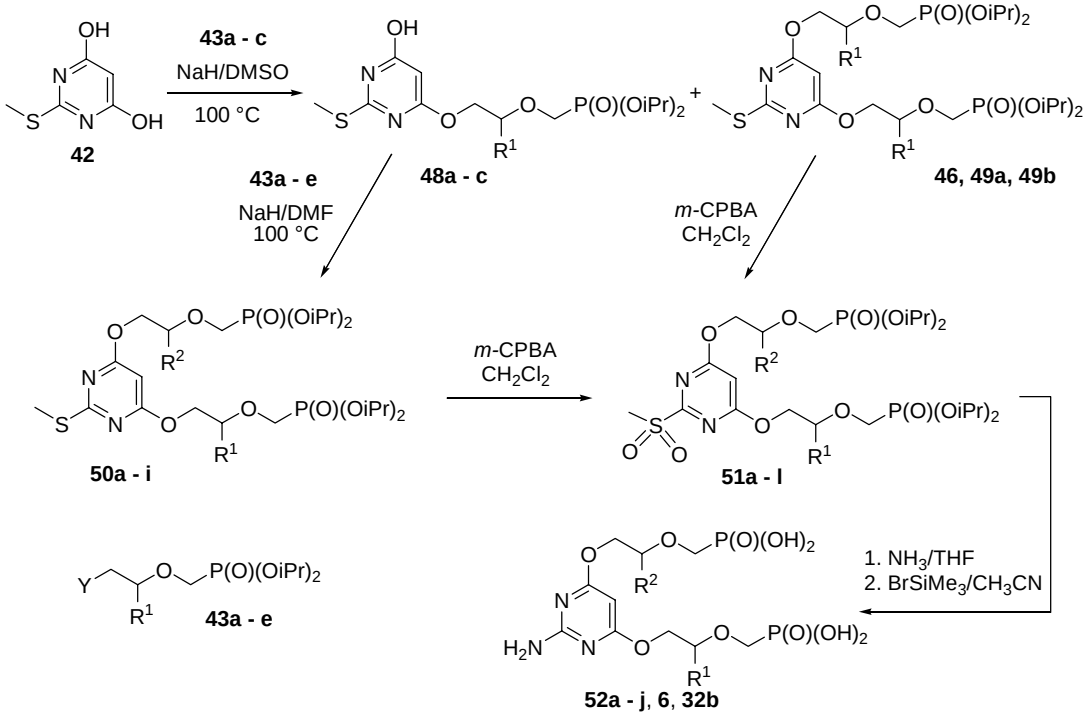
Reaction conditions: a) NaH/DMF; b) NaH/DMSO.

Scheme 13. Comparison of reactivity of compounds **5** and **42**.

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

3.1.2.1. Synthesis of bisphosphonates

Alkylation of pyrimidine **42** with one equivalent of appropriate phosphonate **43**[40] (Table 1) in DMSO afforded mixture of mono- and dialkylated products **48** and **49** (Scheme 14, Table 1), respectively. Monoalkylated product **48** was subsequently alkylated with **43** in DMF to afford bisderivative bearing two different substituents **50a**–**i** (Table 2). 2-Methylsulfanyl group of compounds **46**, **49a**–**b** and **50a**–**i** (Table 2) was oxidized by *m*-CPBA in dichloromethane[75] to give 2-methylsulfonyl derivatives **51a**–**l**, which were further converted to 2-amino congeners using liquid ammonia in THF at r.t.[75a] Final deprotection of diisopropyl esters by bromotrimethylsilane afforded free phosphonic acids **6** and **52a**–**j**.



Scheme 14. Synthesis of bisphosphonates by alkylation of pyrimidine **42**.

Compd. **43**	R^1, R^2	Y	Compd. **48**	R^1	Compd. **49**	R^1
a	H	Cl	**a**	H	-	-
b	(*S*)-CH_3	OTs	**b**	(*S*)-CH_3	**a**	(*S*)-CH_3
c	(*R*)-CH_3	OTs	**c**	(*R*)-CH_3	**b**	(*R*)-CH_3
d	(*S*)-CH_2OH	OTs	-	-	-	-
e	(*R*)-CH_2OH	OTs	-	-	-	-

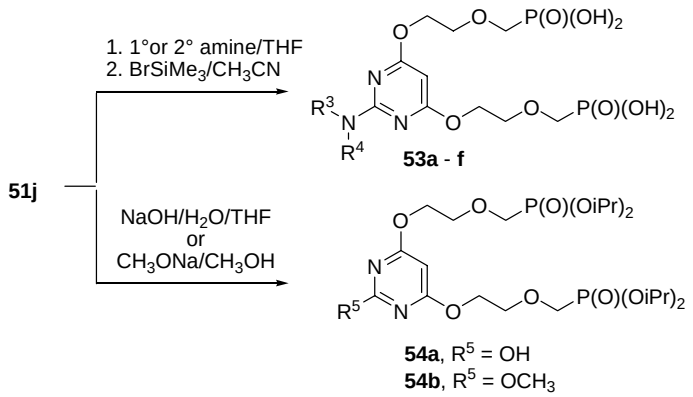
Table 1. Substitution patterns of compounds **43**, **48** and **49**.

Compd. **50**	R^1	R^2	Compd. **51**	R^1	R^2	Product	R^1	R^2
a	H	(*S*)-CH_3	**a**	H	(*S*)-CH_3	**52a**	H	(*S*)-CH_3
b	H	(*R*)-CH_3	**b**	H	(*R*)-CH_3	**52b**	H	(*R*)-CH_3
c	H	(*S*)-CH_2OH	**c**	H	(*S*)-CH_2OH	**52c**	H	(*S*)-CH_2OH
d	H	(*R*)-CH_2OH	**d**	H	(*R*)-CH_2OH	**52d**	H	(*R*)-CH_2OH
e	(*S*)-CH_3	(*R*)-CH_3	**e**	(*S*)-CH_3	(*R*)-CH_3	**52e**	(*S*)-CH_3	(*R*)-CH_3
f	(*S*)-CH_3	(*S*)-CH_2OH	**f**	(*S*)-CH_3	(*S*)-CH_2OH	**52f**	(*S*)-CH_3	(*S*)-CH_2OH
g	(*S*)-CH_3	(*R*)-CH_2OH	**g**	(*S*)-CH_3	(*R*)-CH_2OH	**52g**	(*S*)-CH_3	(*R*)-CH_2OH
h	(*R*)-CH_3	(*S*)-CH_2OH	**h**	(*R*)-CH_3	(*S*)-CH_2OH	**52h**	(*R*)-CH_3	(*S*)-CH_2OH
i	(*R*)-CH_3	(*R*)-CH_2OH	**i**	(*R*)-CH_3	(*R*)-CH_2OH	**52i**	(*R*)-CH_3	(*R*)-CH_2OH
-	-	-	**j**	H	H	**6**	H	H
-	-	-	**k**	(*S*)-CH_3	(*S*)-CH_3	**32b**	(*S*)-CH_3	(*S*)-CH_3
-	-	-	**l**	(*R*)-CH_3	(*R*)-CH_3	**52j**	(*R*)-CH_3	(*R*)-CH_3

Table 2. Substitution patterns of compounds **50**, **51** and **52**.

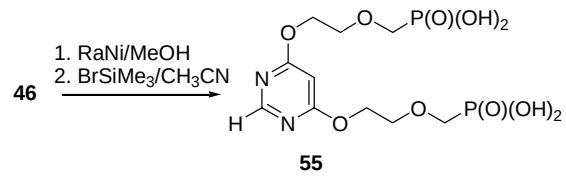
3.1.2.2. Synthesis of 2-substituted bisphosphonates

Compound **51j** upon treatment with primary or secondary amine[75b] and subsequent deprotection with bromotrimethylsilane afforded N^2-substituted bisphosphonates **53a**–**f** (Scheme 15, Table 3). 2-Methylsulfonyl derivative **51j** was hydrolyzed by sodium hydroxide in a mixture of water and THF[76] to give 2-hydroxy derivative **54a**; treatment of **51j** with sodium methylate in methanol[77] gave 2-methoxy derivative **54b**, however treatment of **54a** and **54b** with bromotrimethylsilane led to their decomposition. 2-Methylsulfanyl derivative **46** was reduced with Raney-Nickel[28] to afford 4,6-bis[2-(phosphonomethoxy)ethoxy]-pyrimidine, which was deprotected by bromotrimethylsilane to give free phosphonic acid **55**.



Compd. **53**	R^3	R^4
a	H	cyclopropyl
b	H	cyclopentyl
c	H	methyl
d	H	benzyl
e	H	4-methoxybenzyl
f	morpholine	-

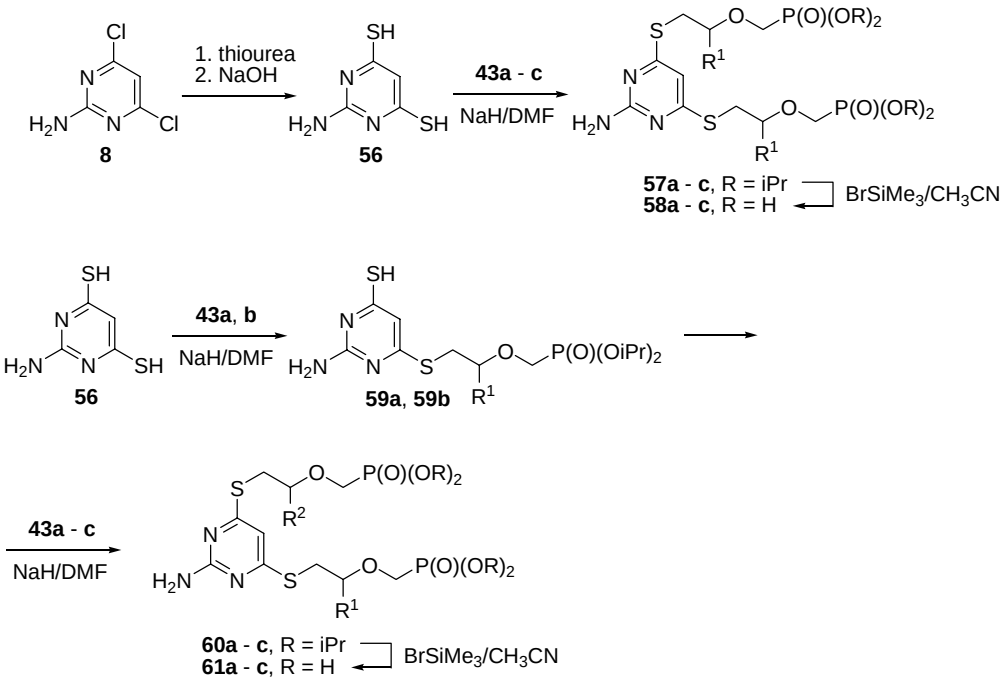
Table 3.



Scheme 15. Synthesis of 2-substituted bisphosphonates.

3.1.3. Bisphosphonates derived from 2-amino-4,6-disulfanyl-pyrimidine

Pyrimidine **8** was converted to the disulfanyl analogue **56** by reaction with thiourea (Scheme 16).[78,79] Alkylation of **56** with phosphonate **43a**–**c** (Table 1) gave unequivocally *S*–alkylated product **57a**–**c** (Scheme 16, 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 **56** with one equivalent of alkylating agent **43a** or **43b** gave monoalkylated products **59a** and **59b** together with dialkylated products. Further alkylation of **59a**–**b** afforded pyrimidines with two different substituents **60a**–**c**. Diisopropyl esters of bisphosphonates **57a**–**c** and **60a**–**c** were cleaved by standard procedure with bromotrimethylsilane to give bisphosphonates **58a**–**c** and **61a**–**c**.



Scheme 16. Synthesis of bisphosphonates from 2-amino-4,6-disulfanylpyrimidine.

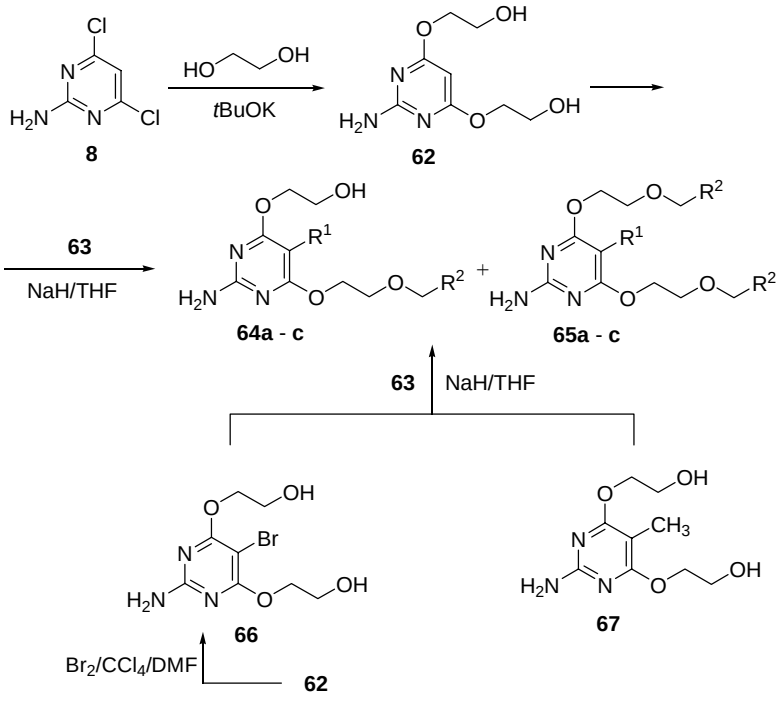
Compd. **57**, **58**, **59**	R^1	Compd. **60**, **61**	R^1	R^2
a	H	**a**	H	(*S*)-CH_3
b	(*S*)-CH_3	**b**	H	(*R*)-CH_3
c	(*R*)-CH_3	**c**	(*S*)-CH_3	(*R*)-CH_3

Table 4. Substitution pattern of compounds **57**–**61**.

3.1.4. Alkoxyalkyl esters of bisphosphonates

For further biological activity screening lipid esters of compound **6** and its 5-bromo and 5-methyl congener (Scheme 17) were prepared by method described by J. R. Beadle and K. Y. Hostetler.[80]

Pyrimidine **8** in neat ethylene glycol in the presence of *t*BuOK gave hydroxyethoxy derivative **62** in 71% yield (Scheme 17). Pyrimidine **62** in THF was treated with NaH, heated to 50 °C and then hexadecyloxyethyl toluenesulfonyloxymehylphosphonate (**63**) was added. Monoalkylated derivative **64a** was isolated together with bisderivative **65a**. Alkylation in DMF or in a mixture of triethylamine and THF (1:1) gave lower yields; reaction in triethylamine[80] as a solvent did not proceed at all. Bromination of **62** with elemental bromine in DMF/CCl_4[39a] gave smoothly the 5-bromo derivative **66**. 5-Substituted derivatives **66** and **67** (ref. 41) were similarly converted to esters **64b** and **64c** and dialkylated products **65b** and **65c** by the above described alkylation. Monoalkylated compounds **64a**–**c** were fully characterized and submitted for biological activity screening



63, $TsOCH_2P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$
64a, **65a**, R^1 = H, R^2 = $P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$
64b, **65b**, R^1 = Br, R^2 = $P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$
64c, **65c**, R^1 = CH_3, R^2 = $P(O)[O(CH_2)_2O(CH_2)_{15}CH_3](O^-Na^+)$

Scheme 17. Synthesis of alkoxyalkyl esters of bisphosphonates.

however dialkylated products **65a**–**c** were nearly insoluble in any solvent; therefore their NMR spectra could not be measured. Hence compounds **65a**–**c** were characterized only by mass spectroscopy and elemental analysis and were not tested for biological activity. Our attempts to convert compound **6** to *cyclo*Sal, *cyclo*Amb[81] or POM[82] esters failed due to instability of ether bonds at positions 4 and 6 under reaction conditions.

3.1.5. Properties of bisphosphonates

3.1.5.1. Capillary zone electrophoresis

Enantiomerical purity of compounds **52a** and **52b** were successfully analyzed by capillary zone electrophoresis (CZE experiments were performed by Dr. Veronika Šolínová and Dr. Václav Kašička). Baseline separation of enantiomers **52a** and **52b**, with resolution 1.67, was achieved 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), (Figure 12). The compound **52a** was found enantiomerically pure as demonstrated by single peak of CZE analysis of this compound in chiral BGE (Figure 13) whereas a very small admixture of enantiomer **52a** was found in the CZE analysis of compound **52b** (Figure 14).

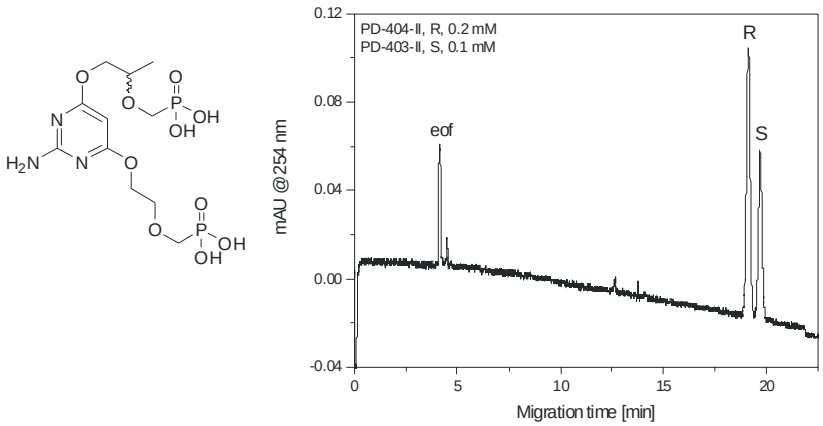


Figure 12. CZE separation of enantiomers **52a** (*S*, PD-403-II), 0.1mM and **52b** (*R*, PD-404-II), 0.2 mM in chiral BGE composed of 50 mM borax, adjusted by NaOH to pH 10.0, with chiral selector β-cyclodextrin (20 mg/ml); eof = electroosmotic flow marker.

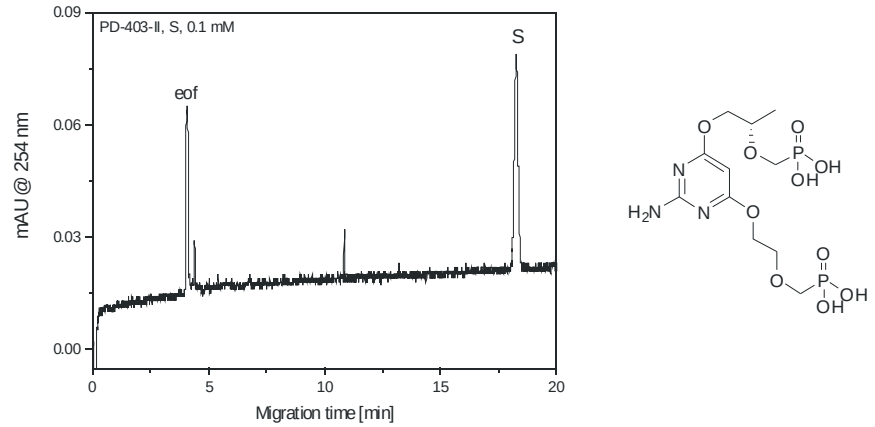


Figure 13. CZE analysis of enantiomer **52a** (PD-403-II) in the above chiral BGE.

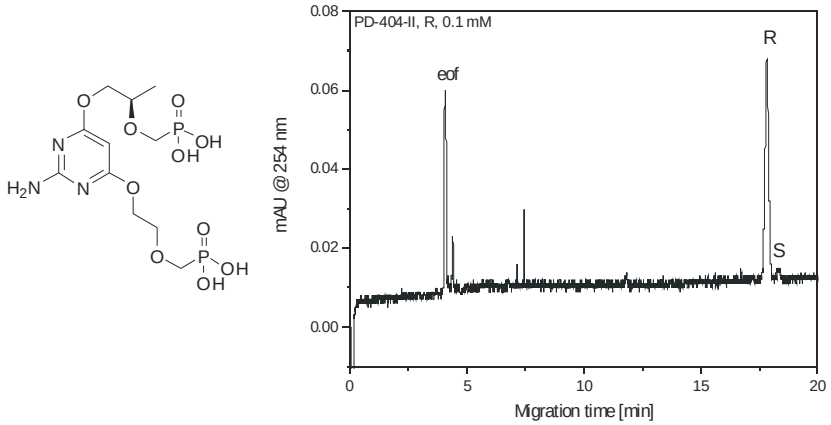


Figure 14. CE analysis of enantiomer **52b** (PD-404-II) in the above chiral BGE.

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.

3.1.5.2. Biological activity

The whole series of bisphosphonates (**6**, **21b**, **22b**, **26b**, **32b**, **35b**, **52a–j**, **53a–f**, **54a**, **54b**, **55**, **58a–c**, **61a–c** and **64a–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 **6** was not confirmed. The previously reported activity[37] might have been caused by undetectable admixture of several orders more active monoalkylated 2-amino-4-hydroxy-6-[2-(phosphonomethoxy)ethoxy]pyrimidine. The compounds are devoid of any measurable toxicity to cell cultures. The bisphosphonates neither inhibit *Mycobacterium tuberculosis* dUTPase nor HIV integrase.

3.1.6. 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 diverse achiral or chiral phosphonoalkoxy chains were prepared either by nucleophilic aromatic substitution of 2-amino-4,6-dichloropyrimidine (**8**) or by alkylation of 4,6-(dihydroxy)-2-(methylsulfanyl)pyrimidine (**42**). The second method proved to be the universal method for regioselective preparation of *O*–alkylated pyrimidines at positions 4 and 6. Furthermore, the methylsulfanyl function is versatile leaving group for introduction of various substituents at position 2 of the pyrimidine moiety. Disulfanylpyrimidine **56** 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 dramatically decreased solubility of bisphosphonates. Enantiomerical purity of compounds **52a** and **52b** was successfully determined by capillary zone electrophoresis and it was confirmed that optically active phosphonates do not tend to racemize.

Prepared bisphosphonates and their esters were tested for their cytostatic and antiviral activity however compounds did not show any appreciable biological activity or toxicity.

Since the phosphonic acids are known to form strong complexes with various metal ions it is obvious goal to study complexation properties of prepared bisphosphonates where these properties could be expected to be more enhanced. Metal ion binding properties of bisphosphonates will be studied in collaboration with Assoc. Prof. Petr Hermann (Charles University). Stability constants of complexes with various biogenic metal ions will be determined and compared with data

obtained for acyclic nucleoside phosphonates.[83] Potential chelating properties of bisphosphonates will be examined.

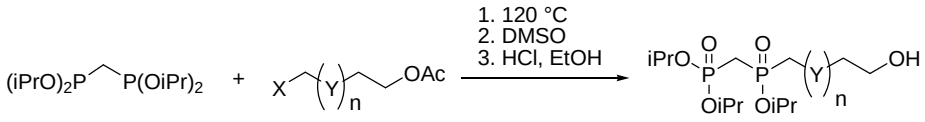
3.2. Acyclic nucleoside phosphonomethylphosphinates

In the second part of my work, I focused on the synthesis of acyclic nucleoside phosphonomethylphosphinates, e.g. 2-[(hydroxy)(phosphonomethyl)phosphoryl-methoxy]ethyl purines (A, G) and pyrimidines (C, U, T) (Scheme 19), chemically and enzymatically stable analogues of acyclic nucleoside diphosphates with the PME side chain and studied the influence of introduction of diphosphonate moiety instead of the phosphonate to their biological activities.

Analogues of dUDP and dUTP containing phosphonomethylphosphinate moiety (Scheme 20) were prepared as well. Their inhibitory activity to *Mycobacterium Tuberculosis* dUTPase was studied.

3.2.1. Acyclic nucleoside phosphonomethylphosphinates with natural heterocyclic bases

The acyclic nucleoside diphosphate analogues were prepared by Mitsunobu reaction of suitably protected heterocyclic bases with functionalized alcohols **69a**–**e** bearing the phosphonomethylphosphinyl unit. Alcohols **69a**–**e** were prepared by Arbuzov reaction of alkyl bromides **68a** (ref. 84) and **68b** and alkyl iodides **68c**–**e** with air and moisture sensitive phosphonite **11b** (Scheme 18).[55,57a] The corresponding alkyl chlorides were unreactive at 120 °C and heating to higher temperature led to the decomposition of starting materials. My attempts to perform the reaction under microwave heating were unsuccessful. The terminal phosphonite was oxidized with DMSO to phosphonate and the acetyl protecting group was removed by hydrolysis with hydrochloric acid. Alcohols **69a**–**e** were prepared in



11b **68a-e** **69a-e**

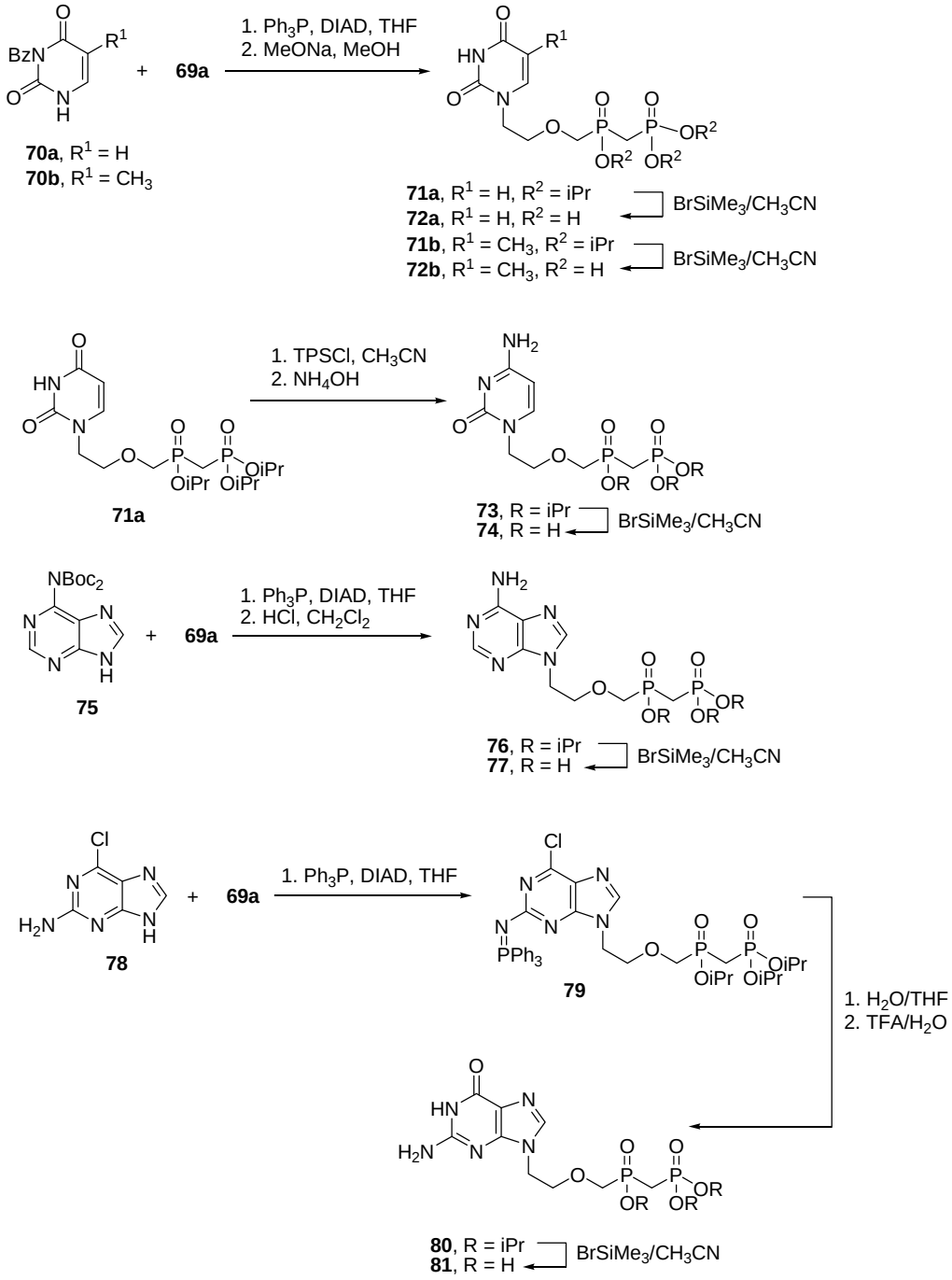
68a, X= Br, Y = O, n = 1
68b, X = Br, Y = CH_2, n = 1
68c, X = I, Y = CH_2, n = 2
68d, X = I, Y = CH_2, n = 3
68e, X = I, Y = CH_2O, n = 1

69a, Y = O, n = 1
69b, Y = CH_2, n = 1
69c, Y = CH_2, n = 2
69d, Y = CH_2, n = 3
69e, Y = CH_2O, n = 1

Scheme 18. Arbuzov reaction of diphosphonite **11b** with alkyl halides.

overall yield 30–40%. This method proved to be better in my hands than the previously described Arbuzov reaction of phosphonomethylphosphonite with alkyl halide[48] due to the poor yields of the synthesis of the starting phosphonomethylphosphonite,[57,53] that is accompanied by formation of side products and complicated separation. Isopropyl esters were used instead of ethyl esters to suppress side Arbuzov reaction with ethyl bromide.

Uracil and thymine derivatives **72a** and **72b** were prepared by alkylation of N^3-benzoyl uracil **70a** and thymine **70b** (ref. 85) with alcohol **69a** under Mitsunobu conditions[86] in 79% and 52% yield, respectively (Scheme 19). Isopropyl esters were deprotected by standard procedure employing trimethylsilyl bromide in acetonitrile. The uracil derivative **71a** was converted to the cytosine analogue **73** by treatment with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) followed by amination by ammonium hydroxide;[87] treatment with trimethylsilyl bromide afforded **74** in 46% yield. Adenine nucleoside diphosphonate **76** was prepared by Mitsunobu reaction of **69a** with N^6-amino bis-Boc adenine **75** (ref. 88) followed by hydrolysis of the bis-Boc protecting group with hydrochloric acid in dichloromethane.[89] The guanine counterpart **80** was prepared analogically starting from 2-amino-6-chloropurine (**78**) by alkylation with **69a** to give predominantly N^2-triphenylphosphoranylidene derivative **79**. Compound **79** was easily hydrolyzed by refluxing in THF/H_2O mixture[90] to afford 2-amino derivative which was subsequently converted to guanine **80** by treatment with trifluoroacetic acid. Deprotectection of **76** and **80** by trimethylsilyl bromide gave free acids **77** and **81**.

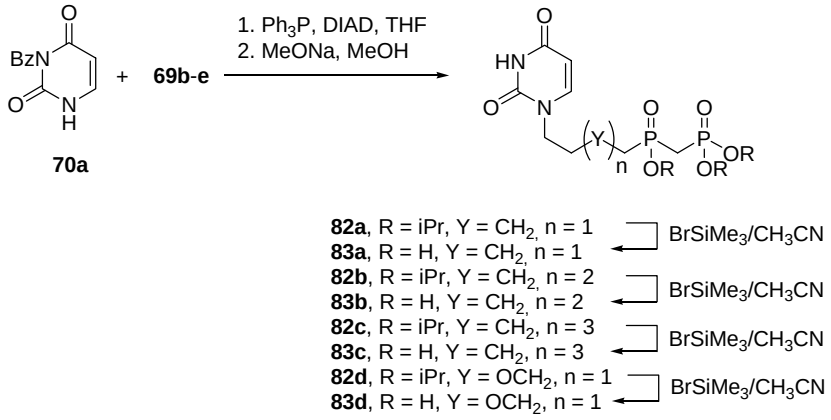


Scheme 19. Synthesis of acyclic nucleoside phosphonomethylphosphinates by Mitsunobu reaction.

3.2.2. Analogues of dUDP and dUTP

In addition to the 1-{2-[(hydroxy)(phosphonomethyl)phosphorylmethoxy]-ethyl}uracil (**72a**) with methoxyethyl side chain, the series of uracil derivatives bearing carbon side chain (4, 5 and 6 carbon atoms) as well as the ethoxyethyl side chain was prepared to study potential inhibitory activity of these compounds to

dUTPase (Scheme 20). Compounds **82** and **83** were prepared by the same procedure as was described for compounds **71** and **72**; N^3-benzoyl uracil (**70a**) was alkylated with alcohols **69b**–**e** by Mitsunobu reaction, benzoyl group was removed by action of sodium methoxide and subsequent treatment with trimethylsilyl bromide gave phosphonomethylphosphinates **83a**–**d**.



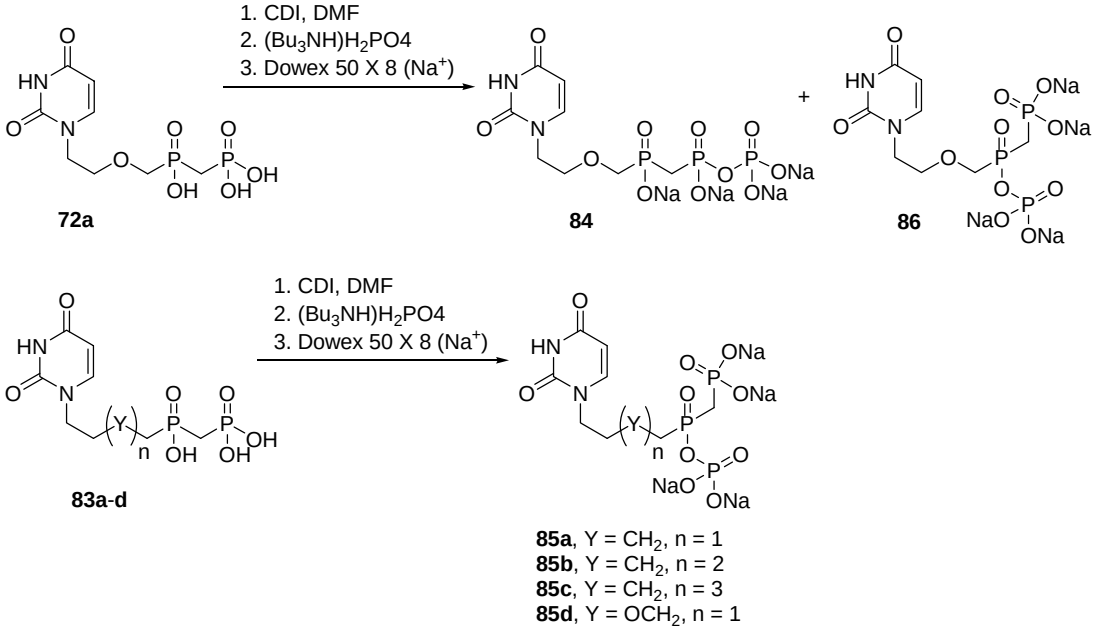
Scheme 20. Synthesis of analogues of dUDP.

Since the natural substrate of dUTPase is 2'-deoxyuridine triphosphate, I tried to convert the diphosphonates **72a** and **83a**–**d** to the corresponding triphosphate analogues (Scheme 21). My attempts to prepare triphosphate mimics by morpholidate method,[91] that is widely used for conversion of ANPs to phosphates, were unsuccessful because the morpholidate of **72a** was formed in a low yield. Synthesis of triphosphate analogues by methods employing diphenyl chlorophosphate[92] or benzyl hydrogen phosphoramidate[93] failed as well. Phosphates were finally prepared by activation of phosphonate with 1,1'-carbonyldiimidazole (CDI) followed by addition of the tri-*n*-butylammonium phosphate in DMF.[94] The phosphor-1-imidazolidate of **72a** was also prepared by reaction with imidazole in the presence of 2,2'-dithiodipyridine and triphenylphosphine,[95] however yields obtained by this method were lower compared to the reaction with CDI.

Reaction of **72a** with CDI followed by tri-*n*-butylammonium phosphate in DMF gave expected triphosphate analogue **84** in 5% yield (Scheme 21). Compound **84** was purified by anion exchange chromatography (eluted with TEAB) and the triethyl ammonium salt was finally converted to the corresponding sodium salt using DOWEX 50 × 8 (Na^+ form). The identity was determined by ^1H and ^{31}P NMR and its purity (determined by HPLC) exceeded 98%. Compounds **83a**–**d** were similarly

converted to their phosphate counterparts by above described method and isolated as their sodium salts however only branched phosphates **85a**–**d** were isolated as major products in approximately 15–30% yield. The branched structure of compounds **85a**–**d** was confirmed by ^{31}P NMR spectra. In proton decoupled spectrum P-β and P-γ appeared as doublets with coupling constant to P-α 2 Hz and 28 Hz respectively (see Table 5, Figure 15). The alpha position of P-α was confirmed by the highest value of its chemical shift (close to 50 ppm) and by ^{31}P NMR spectrum without proton decoupling where P-α exhibited splitting due to coupling with two neighboring methylene groups.

Compound **84** is relatively stable, it decomposes in neutral aqueous solution to the starting diphosphonate **72a** and phosphate, however only 5% of diphosphonate **72a** appeared in neutral aqueous solution during 24 h period at room temperature. On contrary, very low stability of compounds **85a**–**d** was observed; we managed to prepare compounds **85a**–**d** in 90–98% purity (compounds tend to decompose to the starting diphosphonate and phosphate during purification and evaporation).



Scheme 21. Phosphorylation of compounds **72a** and **83a**–**d**.

To explain whether the branched phosphates are formed immediately during the reaction or are formed by intramolecular migration of the terminal phosphate from its linear counterpart, we followed the course of the reaction of **72a** with CDI and tri-*n*-

butylammonium phosphate by ^{31}P NMR. Immediately after addition of CDI, signals of the starting material disappeared however an unidentifiable mixture of several products was observed. After 2 hr the spectrum was not further changing and tri-*n*-butylammonium phosphate was added to the reaction mixture (the imidazolide of **72a** was not isolated due to its instability). It was revealed that both (branched and linear) phosphates were formed in approximately 2:1 ratio after 6hr. We used ^{31}P-^{31}P-COSY to identify the signals of linear and branched triphosphate analogues. Finally, the reaction mixture was separated, the linear phosphate **84** was isolated in 7% yield together with branched phosphate **86** in 13% yield (Scheme 21).

The identical experiment for compounds **83a** and **83d** showed the same results, both branched and linear phosphates were formed. The ratio of branched and linear phosphate was from 2:1 to 10:1.

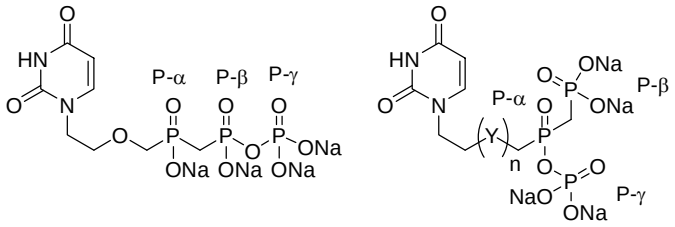


Figure 15. Labelling of phosphorus atoms for ^{31}P NMR.

Compound	P-α	P-β	P-γ
72a	36.32 d, *J*(P-α,P-β) = 9.0	18.18 d, *J*(P-β,P-α) = 9.0	-
84	30.98 d, *J*(P-α,P-β) = 6.2	6.92 dd, *J*(P-β,P-α) = 6.2, *J*(P-β,P-γ) = 25.2	-5.51 d, *J*(P-γ,P-β) = 25.2
86	34.5 dd, *J*(P-α,P-γ) = 28.6, *J*(P-α,P-β) = 7.2	11.4 d, *J*(P-β,P-α) = 7.2	-5.4 d, *J*(P-γ,P-α) = 28.7
83a	49.0 d, *J*(P-α,P-β) = 7.0	15.6 d, *J*(P-β,P-α) = 7.0	-
85a	46.2 dd, *J*(P-α,P-γ) = 28.3, *J*(P-α,P-β) = 6.0	10.9 d, *J*(P-β,P-α) = 6.0	-5.1 d, *J*(P-γ,P-α) = 28.3
83b	49.8 d, *J*(P-α,P-β) = 6.7	14.56 d, *J*(P-β,P-α) = 6.7	-
85b	50.1 dd, *J*(P-α,P-γ) = 28.7, *J*(P-α,P-β) = 2.1	9.61 d, *J*(P-β,P-α) = 2.1	-4.4 d, *J*(P-γ,P-α) = 28.7
83c	50.53 d, *J*(P-α,P-β) = 7.8	14.80 d, *J*(P-β,P-α) = 7.8	-
85c	50.4 dd, *J*(P-α,P-γ) = 28.4, *J*(P-α,P-β) = 2.0	9.7 d, *J*(P-β,P-α) = 2.0	-4.3 d, *J*(P-γ,P-α) = 28.4
83d	44.3 d, *J*(P-α,P-β) = 9.2	15.8 d, *J*(P-β,P-α) = 9.2	-
85d	49.3 dd, *J*(P-α,P-γ) = 27.3, *J*(P-α,P-β) = 1.8	9.5 d, *J*(P-β,P-α) = 1.8	-4.4 d, *J*(P-γ,P-α) = 27.3

Table 5. ^{31}P NMR chemical shifts (ppm) and interaction constants (Hz) of comp. **72a** and **83**–**86**.

From NMR titration studies (performed by Dr. Martin Dračínský) of **72a**, pKa values of phosphonomethylphosphinyl residue were determined (Figure 16 and 17). pKa of phosphinate (P-α, Figure 16) and pKa_1 of phosphonate (P-β, Figure 17) are around 1.8 and 2.7, respectively. The pKa values indicate that both groups are acidic enough to undergo reaction with CDI and phosphate and explain formation of mixture of branched and linear phosphates. This explanation is also supported by our observation of formation of mixture of products in the reaction of **72a** with CDI. The intramolecular migration of the phosphate group was not observed.

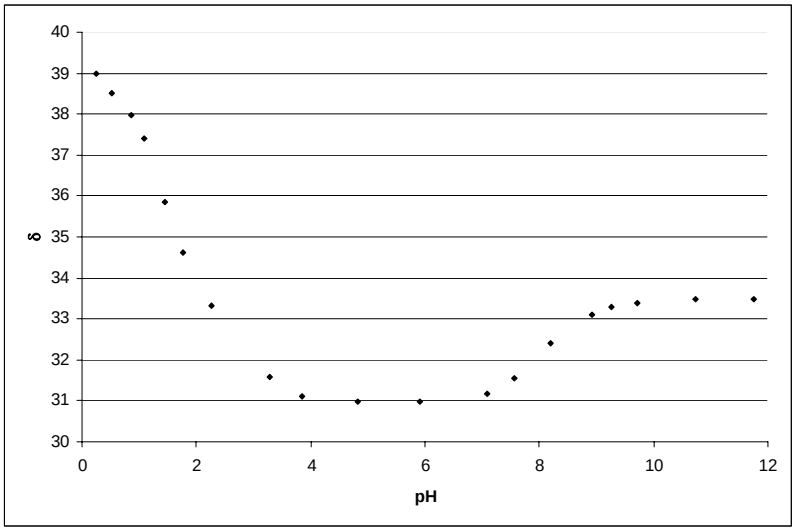


Figure 16. pH dependence of the chemical shifts δ (ppm) of P-α of compound **72a**.

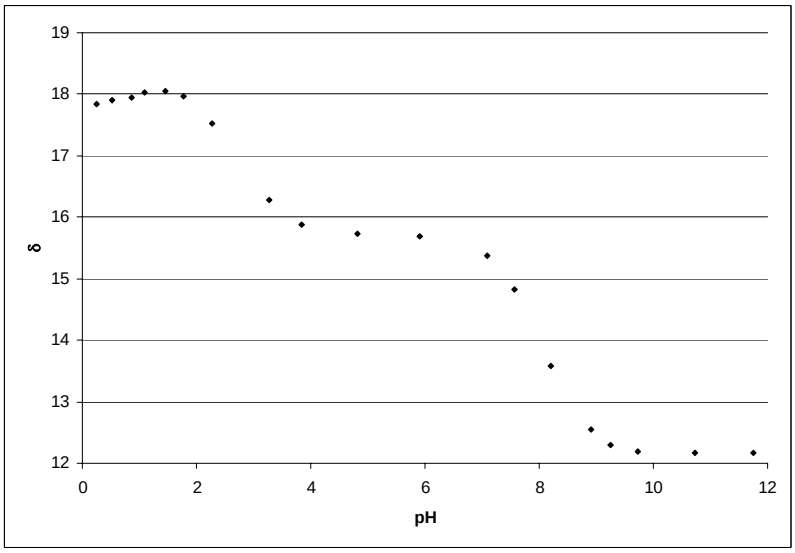


Figure 17. pH dependence of the chemical shifts δ (ppm) of P-β of compound **72a**.

The stability of linear and branched triphosphate analogues was also studied using *ab initio* quantum chemical calculations (performed by Dr. Jindřich Fanfrlík). We compared thermodynamic stability of linear and branched phosphates (Figure 18) with carbon side chain (**87a** and **87b**) and methoxyethyl side chain (**88a** and **88b**). The triphosphate analogues were for calculations taken as protonated (the molecule **87b** corresponds to compound **85a**, **88a** corresponds to **84** and **88b** to compound **86**). In the both cases, the branched phosphates were more stable (see Table 6).

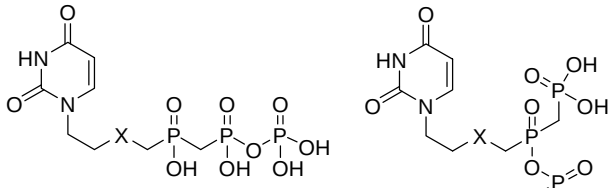


Figure 18. Molecules studied by *ab initio* quantum chemical calculations.

Molecule	ΔE	$\Delta\Delta G_{HYD}$	ΔE + $\Delta\Delta G_{HYD}$
87a	0.0	0.0	0.0
87b	-9.1	4.9	-4.2
88a	0.0	0.0	0.0
88b	-10.5	4.5	-6.0

Table 6. Relative gas phase energies (ΔE) and relative hydration free energies ($\Delta\Delta G_{HYD}$) in kcal/mol.

3.2.3. Conclusion

In conclusion, a series of acyclic nucleoside diphosphate analogues of purine and pyrimidine nucleotides containing stable phosphonomethylphosphinyl unit were prepared by improved previously described methods. Alcohols **69a**–**e** were prepared by Arbuzov reaction of diphosphonite **11b** with acetyl alkyl bromides and alkyl iodides in overall 30–40% yield. Suitably protected heterocyclic bases were coupled with functionalized alcohols **69a**–**e** by Mitsunobu reaction and finally deprotected by standard procedures.

Phosphonomethylphosphinates **72a** and **83a–d** were successfully converted to their phosphate counterparts by conversion of phosphonomethylphosphinates to the corresponding imidazolides with CDI and subsequent reaction with tri-*n*-butylammonium phosphate. Interestingly, branched phosphates **85a–d** and **86** were isolated as major products unlike the expected linear phosphates that were in minority. The detailed ^{31}P NMR studies of the course of the reaction showed that both branched and linear phosphates are formed immediately during the reaction. The intramolecular phosphate migration was not observed. pKa values of phosphonate and phosphinate moiety of the compound **72a** were determined by ^{31}P NMR titration studies and are around 2.7 and 1.8, respectively; that explains reactivity of both phosphonate and phosphinate residue with CDI. In addition, the thermodynamic stability calculations showed that the branched phosphates are more stable.

Compounds **72a**, **72b**, **74**, **77**, **81** and **83a–d** were screened for cytostatic and antiviral activity and none of the tested compounds exhibited any significant biological activity or cytotoxicity. dUDP and dUTP analogues **72a**, **83a–d**, **84**, **85a–d** and **86** were tested for their potency to inhibit *Mycobacterium tuberculosis* dUTPase however none of the analogues inhibited the enzyme.

These data indicate that phosphonomethylphosphinyl system is not optimal analogue of the natural diphosphate in nucleotides. This effect may be due to the differences between the pKa's of the phosphonomethylphosphinyl analogue and the normal diphosphate, small geometric differences between C–P and O–P bonds and differences in metal ion binding properties.[96,64]

4. Summary

A number of novel compounds was synthesized in order to enlarge the family of acyclic nucleoside phosphonates and to further explore the structure-activity relationship of this important and interesting class of nucleotide analogues.

In summary, array of acyclic nucleoside bisphosphonates bearing PMEO, PMPO and HPMPO side chains was prepared. Further, a series of acyclic nucleoside phosphonomethylphosphinates, nonhydrolyzable analogues of acyclic nucleoside diphosphates, was prepared.

Bisphosphonates bearing two HPMPO side chains were prepared from 2-amino-4,6-dichloropyrimidine by nucleophilic aromatic substitution with isopropylideneglycerol and subsequent alkylation with diisopropyl bromomethylphosphonate. However, this synthetic strategy was not suitable for preparation of other bisphosphonates due to a low reactivity of 2-amino-4,6-dichloropyrimidine and its 4,6-difluoro congener.

Alkylation of 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine in DMSO that gives predominantly *O*-alkylated regioisomers was finally used for synthesis of a large number of bisphosphonates. Bisphosphonates bearing two identical or diverse phosphonomethoxyalkoxy chains were prepared as well as 2-substituted bis-PME derivatives. The methylsulfanyl and/or the methylsulfonyl group proved to be suitable leaving group for introduction of various substituents at position 2 of the pyrimidine ring. Liphophilic esters of bisphosphonates were prepared to decrease their polarity. However, their introduction dramatically decreased solubility of bisphosphonates.

A series of bisphosphonates with phosphonomethoxyalkylsulfanyl side chain was prepared by alkylation of 2-amino-4,6-disulfanylpyrimidine with phosphonate bearing building block. Owing to better nucleophilicity of sulphur compared to oxygen and nitrogen, the alkylation gives exclusively *S*-alkylated product.

Antiviral and cytostatic activities of bisphosphonates were studied however compounds do not possess any significant biological activity or toxicity.

In the second part of my work, I prepared a series of 2-[(hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl purines (A, G) and pyrimidines (C, U, T) as chemically and enzymatically stable analogues of acyclic

nucleoside diphosphates. The diphosphonate derivatives were prepared by Mitsunobu reaction of suitably protected heterocyclic bases and alcohols containing phosphonomethylphosphinyl moiety. In addition, acyclic analogues of dUDP bearing phosphonomethylphosphinylalkyl and alkoxyalkyl side chains were prepared. Their phosphorylation to dUTP analogues gave mixture of α- and β-phosphates. The ^{31}P NMR study of the course of the phosphorylation reaction and measurement of pKa of the phosphonomethylphosphinate moiety showed that both phosphinate and phosphonate hydroxyl groups react with 1,1′-carbonyldiimidazole and phosphate to give a mixture of α- and β-phosphate in approx. 2:1 to 10:1 ratio.

Prepared phosphonomethylphosphinates were tested for cytostatic and antiviral activity and none of the tested compounds exhibited any significant biological activity or cytotoxicity. The dUDP and dUTP analogues do not inhibit the *Mycobacterium tuberculosis* dUTPase. In conclusion, the substitution of diphosphate moiety by nonhydrolyzable phosphonomethylphosphinate system led to the loss of antiviral and cytostatic activity. It probably results from differences between the pKa's of the phosphonomethylphosphinate analogue and the normal diphosphate, geometric differences between C–P and O–P bonds and differences in metal ion binding properties.

5. List of publications of the author related to the thesis

Petra Doláková, Martin Dračínský, Milena Masojídková, Veronika Šolínová, Václav Kašička and Antonín Holý, "Acyclic Nucleoside Bisphosphonates: Synthesis and Properties of Chiral 2-Amino-4,6-bis[(phosphonomethoxy)alkoxy]pyrimidines", *European Journal of Medicinal Chemistry*, 2008, in press.

Petra Doláková, Martin Dračínský, Jindřich Fanfrlík, and Antonín Holý, "Synthesis of Analogues of Acyclic Nucleoside Diphosphates Containing Phosphonomethylphosphinyl Moiety and Studies of Their Phosphorylation Reaction", *European Journal of Organic Chemistry*, 2008, submitted.

6. Experimental part

6.1. General procedures

Solvents were dried by standard procedures. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone under argon. NMR spectra were recorded with Bruker Avance 500 (500 MHz for ^{1}H and 125.8 MHz for ^{13}C) and Bruker Avance 400 (^{1}H at 400, ^{13}C at 100.6 MHz) spectrometers in $CDCl_3$, DMSO-d_6, or D_2O. Chemical shifts (in ppm, δ scale) were referenced to TMS (for ^{1}H NMR spectra in $CDCl_3$) and/or to the solvent signal ($CDCl_3$ δ = 7.26 ppm for ^{1}H NMR and δ = 77.0 ppm for ^{13}C NMR; DMSO-d_6 for ^{1}H NMR δ = 2.5 ppm and for ^{13}C δ = 39.7). Chemical shifts in D_2O were referenced to 1,4-dioxane for ^{1}H NMR δ = 3.75 and for ^{13}C NMR δ = 67.19. Chemical shifts for ^{31}P spectra were referenced to H_3PO_4 (δ = 0 ppm). For ^{31}P NMR data see Table 5. Melting points were determined on a Büchi Melting Point B-545 aparatus 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 × 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%) or using linear gradient of H_2O/MeOH (0–100%). Preparative HPLC purification of triphosphate analogues was performed on a column packed with POROS ® HQ 50 μm (50 ml) using gradient of TEAB in water (0–0.4 M).

The nomenclature of compounds used in this thesis corresponds to nomenclature used in the chemistry of nucleic acids.

All new compounds were fully characterized by mass spectrometry (Mass spectrometry department, IOCB), elemental analysis (Analytical laboratory, IOCB) or high resolution mass spectrometry (for intermediates) and NMR spectroscopy (including complete assignment of all NMR signals using a combination of H,H-

COSY, H,C-HSQC, and H,C-HMBC methods). NMR spectra of compounds **20**–**35** were measured and interpreted by Dr. Milena Masojídková and NMR spectra of compounds **38**–**86** were measured and interpreted by Dr. Martin Dračínský.

Diastereoisomers of bisphosphonates gave identical NMR spectra. To prove that we have two different diasostereoisomers we prepared mixed samples of two diastereosisomers; two sets of signals were found for OCH_2-1´ protons of bisphosphonates in ^1H NMR spectra.

4,6-Dihydroxy-2-(methylsulfanyl)pyrimidine (**42**) was prepared by previously described method[97] and purchased from Aldrich. Tetraisopropyl methylenediphosphonite (**11b**) was prepared according to the previously described procedure[55] and purchased from Sigma–Aldrich as well. 5-Cloropentyl acetate, 4-bromobutyl acetate and 6-chloro-1-hexanol were purchased from Aldrich. 2-(2-Chloroethoxy)ethanol was purchased from Janssen Chimica.

General procedure 1 (GP1) – Alkylation of dichloropyrimidines **8** and **31** and difluoropyrimidine **39**

Phosphonate **29** (6.6 mmol) was dissolved in dry THF (6 ml) and NaH (0.26 g, 60% in paraffin oil, 6.6 mmol) was added in one portion at 0 °C and the reaction mixture was stirred for 0.5 h or sonicated for 1 min. Pyrimidine **8** (0.5 g, 3 mmol) or **31** (0.8 g, 3 mmol) or **39** (0.4 g, 3 mmol) was added and the resulting mixture was stirred at r.t. for 4–24 h and the solvent was evaporated under reduced pressure.

General procedure 2 (GP2) – Deprotection of diisopropyl esters of bisphosphonates

Bisphosphonate (1 mmol) in acetonitrile (20 ml) was treated with bromotrimethylsilane (1.5 ml) at r.t. overnight. Volatiles were removed under reduced pressure, the residue was codistilled with water (3 × 50 ml) and 0.1 M TEAB (2 × 50 ml). Crude products were purified by preparative HPLC using linear gradient 0.1 M triethylammonium hydrogen carbonate in water and in 50% MeOH (linear gradient of TEAB in 50% MeOH, 0–100%) and triethylammonium salts of

phosphonates were converted to free phosphonic acids by application onto a column of Dowex 50 × 8 in H^+ form and elution with water.

General procedure 3 (GP3) – Dialkylation of 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine (**42**) in DMSO

Pyrimidine **42** (0.16 g, 1 mmol) in DMSO (5 ml) was treated with NaH (0.084 g, 60% in paraffin oil, 2.1 mmol) and heated at 80 °C for 30 min; appropriate phosphonate **43a**–**e** (2.1 mmol) was added and the resulting mixture was heated at 120 °C for 8–16 hr. The mixture was cooled to r.t., DMSO was evaporated in vacuo at 60 °C. The residue was codistilled with DMF and EtOH, taken to $CHCl_3$ (100 ml) and washed with water (3 × 50 ml). Organic fraction was dried over $MgSO_4$ and taken down in vacuo. The residue was separated by flash chromatography ($CHCl_3$/MeOH).

General procedure 4 (GP4) – Monoalkylation of 4,6-dihydroxy-2-(methylsulfanyl)pyrimidine (**42**) in DMSO

Pyrimidine **42** (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., appropriate phosphonate **43a**–**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 $CHCl_3$, washed with 3 portions of water and dried over $MgSO_4$. Products were separated by flash chromatography ($CHCl_3$/MeOH).

General procedure 5 (GP5) – 2^{nd} Alkylation of monoalkylated product **48**

Pyrimidine **48** (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 **43a**–**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.

General procedure 6 (GP6) – Oxidation of 2-methylsufanyl group of compounds **49** and **50** to 2-methylsulfonyl group by *m*-CPBA

2-Methylsulfanyl derivative (1 mmol) in CH_2Cl_2 (6 ml) was treated with *m*-chloroperbenzoic acid (3 mmol) at r.t. for 3–12 hr. The reaction mixture was diluted with CH_2Cl_2, washed with saturated aqueous $Na_2S_2O_3$, saturated $NaHCO_3$ and water. Organic fraction was dried over $MgSO_4$ and purified by flash chromatography ($CHCl_3$/MeOH).

General procedure 7 (GP7) – Ammonolysis of 2-methylsulfonyl group to amino group

To a cooled (-78 °C) and stirred solution of compound **51** (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 $CHCl_3$ was applied onto a pad of silica gel and washed with 10% MeOH in $CHCl_3$ (150 ml).

General procedure 8 (GP8) – Alkylation of hydroxyethoxy derivatives **62**, **66** and **67** with phosphonate **63**

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 **63** (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 $CHCl_3$ (50 ml) was washed with brine (2 × 50 ml) and water (1 × 50 ml) and evaporated under reduced pressure. Flash chromatography (EtOAc/EtOH/acetone/H_2O, 6:1:1:0.5 and EtOAc/EtOH/acetone/H_2O, 4:1:1:1) gave compounds **64** and **65**.

General procedure 9 (GP9) – Arbuzov reaction

Alkylhalide **68a**–**e** (1 mmol) was added dropwise to diisopropyl methylenediphosphonite (**11b**) (1 mmol) at r.t. and the resulting mixture was heated

at 120 °C for 6 h under argon atmosphere. The reaction mixture was cooled, DMSO (0.15 ml) was added and the resulting mixture was heated at 60 °C for 2 h. The mixture was partioned between water and $CHCl_3$. The organic fraction was washed with 3 portions of water and taken down in vacuo. The residue in EtOH (3 ml) was treated with HCl (2 M, 1.2 ml) and heated under reflux for 2 h. The mixture was cooled to r.t., neutralized with aqueous ammonia (25%) and evaporated. The residue in $CHCl_3$ was washed with water, dried over $MgSO_4$ and purified by chromatography on silica gel ($CHCl_3$/MeOH 0–5%).

General procedure 10 (GP10) – Mitsunobu reaction

To a suspension of triphenylphosphine (394 mg, 1.6 mmol) in dry THF (6 ml), diisopropyl azodicarboxylate (DIAD, 273 μl, 1.4 mmol) was added and the solution was stirred at 0 °C for 0.5 h. The prepared complex was added dropwise to a suspension of the purine or the pyrimidine base **70a**, **70b**, **75** or **78** (1.1 mmol) and appropriate alcohol **69a**–**e** (0.5 mmol) in dry THF (3 ml) at –40 °C under argon. The reaction mixture was warmed to r.t., stirred overnight and the solvent was removed.

General procedure 11 (GP11) – Deprotection of triisopropyl esters of phosphonomethylphosphinates

To the solution of triisopropyl ester of phosphonomethylphosphinates **71a**–**b**, **73**, **76**, **80** and **82a**–**d** (1 mmol) in dry CH_3CN (30 ml) Me_3SiBr (3 ml) was added and the mixture was stirred at r.t. overnight. The solvent was removed in vacuo and the residue was codistilled with water. The crude product was purified by preparative HPLC (linear gradient of methanol (0–100%) in water).

6.2. Bisphosphonates

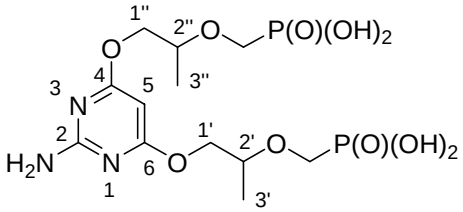


Figure 19. Numbering of the bisphosphonates for NMR analysis.

2-Amino-4,6-(2*R*,2′*R*)-bis(1,2-dihydroxypropoxy)pyrimidine (**20**)

(*S*)-1,2-Isopropylideneglycerol (**19**) (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 **8** (5 g, 30.5 mmol) was added in one portion and the reaction mixture was refluxed for 6 h. After cooling to r.t., solvent was removed under reduced pressure and the residue was dissolved in hot $CHCl_3$ and filtered through Celite. Chromatography on silica gel ($CHCl_3$/MeOH 0–2%) afforded intermediate 2-amino-4,6-(*S*,*S*)-bis[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]pyrimidine (10.1 g, 93%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ = 6.60 (br s, 2H, NH_2), 5.36 (s, 1H, H-5), 4.33 (m, 2H, H-2′), 4.22 (dd, *J*(1′a,2′) = 4.6, *J*gem = 11.1, 2H, H-1′a), 4.16 (dd, *J*(1′b,2′) = 6.3, *J*gem = 11.1, 2H, H-1′b), 4.04 (dd, *J*(3′a,2′) = 6.4, *J*gem = 8.4, 2H, H-3′a), 3.68 (dd, *J*(3′b,2′) = 6.2, *J*gem = 8.4, 2H, H-3′b), 1.33 and 1.28 (2 × s, 2 × 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.19 (2C, C-4 and C-6), 162.73 (C-2), 108.96 (2C, $CHMe_2$), 78.56 (C-5), 73.59 (2C, C-2′), 66.37 and 65.93 (2 × 2C, C-1′, C-3′), 26.81 and 25.52 (2 × 2C, CH_3) ppm. MS (FAB): m/z (%) = 356.1 (85) $[MH]^+$. For $C_{16}H_{25}N_3O_6$ (355.38) calcd. C 54.07, H 7.09, N 11.82, O 27.01; found C 53.98, H 7.08, N 11.79.

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 **20** as a white solid (4.74 g, 66%); m.p. 110 °C. ^{1}H NMR (DMSO-d_6): δ = 6.50 (br s, 2H, NH_2), 5.32 (s, 1H, H-5), 4.90 and 4.63 (2 × br s, 2 × 2H, OH), 4.17 (dd, *J*(1′a,2′) = 4.3,

*J*gem = 10.9, 2H, H-1´a), 4.06 (dd, *J*(1´b,2´) = 6.6, *J*gem = 10.9, 2H, H-1´b), 3.72 (m, 2H, H-2´), 3.38 (d, *J*(3´,2´) = 4.0, 4H, H-3´) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.62 (2C, C-4, C-6), 162.82 (C-2), 78.58 (C-5), 69.89 (2C, C-2´), 67.73 (2C, C-1´), 62.97 (C-3´) ppm. MS (FAB): m/z (%) = 276.1 (100) $[MH]^+$. For $C_{10}H_{17}N_3O_6$.1/2 H_2O (284.26) calcd. C 42.25, H 6.38, N 14.78, O 36.58; found C 42.35, H 6.22, N 14.60.

2-Amino-4,6-(2*R*,2´*R*)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-pyrimidine (**21a**)

Compound **20** (3 g, 11.7 mmol) in pyridine (400 ml) was treated with DMTrCl (11.95 g, 35 mmol) and the mixture was stirred for 3 h. The reaction was quenched by addition of EtOH and the solvent was removed in vacuo. The residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$, and the separated organic layer was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue in DMF (150 ml) was treated with NaH (1 g, 60% suspension in mineral oil, 25 mmol) at 0 °C and stirred for 30 min; diisopropoxyphosphorylmethyl bromide (6.25 g, 25 mmol) was added and the mixture was stirred at r.t. overnight; the solvent was removed in vacuo and the residue was dissolved in 80% acetic acid (100 ml). After stirring at r.t. for 1 h, acetic acid was evaporated and the residue was codistilled with water. Flash chromatography in $CHCl_3$/MeOH (0–3%) afforded colorless oil (2.6 g, 39%). ^{1}H NMR (DMSO-d_6): δ = 6.57 (br s, 2H, NH_2), 5.29 (s, 1H, H-5), 4.78 (t, *J*(OH,3´) = 5.4, 2H, OH), 4.58 (m, 4H, CHipr.), 4.32 (dd, *J*(1´a,2´) = 3.6, *J*gem = 11.5, 2H, H-1´a), 4.19 (dd, *J*(1´b,2´) = 6.1, *J*gem = 11.5, 2H, H-1´b), 3.90 (dd, *J*(P,CH) = 8.7, *J*gem = 13.8, 2H) and 3.86 (dd, *J*(P,CH) = 8.9, *J*gem = 13.8, 2H, PCH_2), 3.69 (m, 2H, H-2´), 3.51 (t, *J*(3´,2´) = *J*(3´,OH) = 5.4, 4H, H-3´), 1.235 (d, 6H), 1.23 (d, 6H), 1.22 (d, 6H) and 1.21 (d, *J*(CH_3,CH) = 6.2, 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.34 (2C, C-4, C-6), 162.77 (C-2), 80.53 (d, 2C, *J*(P,C) = 11.7, C-2´), 78.46 (C-5), 70.37 (d, 2C) and 70.32 (d, 2C, *J*(P,C) = 6.3, CHipr.), 65.25 (2C, C-1´), 63.99 (d, 2C, *J*(P,C) = 164.6, PC), 60.08 (2C, C-3´), 23.99 (d, 4C, *J*(P,C) = 3.9) and 23.82 (d, 4C, *J*(P,C) = 4.4, CH_3) ppm. MS (FAB): m/z (%) = 632.6 (56) $[MH]^+$. For $C_{24}H_{47}N_3O_{12}P_2$ (631.59) calcd. C 45.64, H 7.50, N 6.65, O 30.40, P 9.81; found C 45.49, H 7.62, N 6.59; P 9.75.

2-Amino-4,6-(2*R*,2´*R*)-bis[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine (**21b**)

Compound **21a** (2 g, 3.17 mmol) was deprotected by GP2 to give **21b** (1.05 g, 71%) as colorless foam. ^{1}H NMR (D_2O): δ = 4.56 (dd, *J*(1´a,2´) = 3.8, *J*gem = 11.2, 2H, H-1´a), 4.43 (dd, *J*(1´b,2´) = 5.6, *J*gem = 11.2, 2H, H-1´b), 3.94 (m, 2H, H-2´), 3.89 (dd, 2H) and 3.84 (dd, *J*(P,CH) = 9.3, *J*gem = 13.3, 2H, PCH_2), 3.82 (dd, *J*(3´a,2´) = 4.3, *J*gem = 12.2, 2H, H-3´a), 3.75 (dd, *J*(3´b,2´) = 5.7, *J*gem = 12.2, 2H, H-3´b) ppm. ^{13}C NMR (D_2O): δ = 169.14 (2C, C-4, C-6), 155.56 (C-2), 79.62 (C-5), 79.57 (d, *J*(P,C) = 11.2, 2C, C-2´), 68.16 (2C, C-1´), 65.74 (d, *J*(P,C) = 158.7, 2C, PC), 60.11 (2C, C-3´) ppm. MS (FAB): m/z (%) = 464 (49) $[MH]^+$. For $C_{12}H_{23}N_3O_{12}P_2.H_2O$ (481.27) calcd. C 29.95, H 5.24, N 8.73, O 43.22, P 12.87; found C 29.83, H 5.27, N 8.61, P 12.59. $[\alpha]^{25}_D$ = -1.0 (c 0.502, H_2O).

2-Amino-4,6-(2*S*,2´*S*)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-pyrimidine (**22a**)

Prepared from **8** and **24** by the same procedure as compound **21a**.

2-Amino-4,6-(2*R*,2´*R*)-bis[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]pyrimidine, yellow oil, yield 9.6 g (89%). ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with (2*S*,2´*S*) enantiomer. MS (FAB): m/z (%) = 356.0 (54) $[MH]^+$. For $C_{16}H_{25}N_3O_6$ (355.38) calcd. C 54.07, H 7.09, N 11.82, O 27.01; found C 54.02, H 6.99, N 11.53.

2-Amino-4,6-(2*S*,2´*S*)-bis(1,2-dihydroxypropoxy)pyrimidine, white solid, yield 5 g (70%); m.p. 99 °C. ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with (2*R*,2´*R*) enantiomer. MS (FAB): m/z (%) = 276.0 (80) $[MH]^+$. For $C_{10}H_{17}N_3O_6.1/2$ H_2O (284.26) calcd. C 42.25, H 6.38, N 14.78, O 36.58; found C 42.34, H 6.17, N 14.62.

2-Amino-4,6-(2*S*,2´*S*)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]pyrimidine (**22a**), colorless oil, yield 2.2 g (33%). ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with **21a**. MS (FAB): m/z (%) = 632.6 (100) $[MH]^+$. For $C_{24}H_{47}N_3O_{12}P_2$ (631.59) calcd. C 45.64, H 7.50, N 6.65, O 30.40, P 9.81; found C 45.45, H 7.49, N 6.55; P 9.92.

2-Amino-4,6-(2*S*,2´*S*)-bis[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine (**22b**)

Prepared from **22a** by GP2. Colorless foam, yield 0.95 g (64%). ^{1}H NMR (D_2O) and ^{13}C NMR (D_2O) identical with **21b**. MS (FAB): m/z (%) = 464 (15) $[MH]^+$. For $C_{12}H_{23}N_3O_{12}P_2.H_2O$ (481.27) calcd. C 29.95, H 5.24, N 8.73, O 43.22, P 12.87; found C 30.29, H 5.18, N 8.55, P 12.74. $[\alpha]^{25}_D$ = +1.9 (c 0.267, H_2O).

2-Amino-4-chloro-6-(2*S*)-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]pyrimidine (**23**)

Compound **19** (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 **8** (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 **23** as a white solid, m.p. 130 °C. ^{1}H NMR (DMSO-d_6): δ = 7.10 (br s, 2H, NH_2), 6.11 (s, 1H, H-5), 4.35 (m, 1H, H-2´), 4.28 (dd, *J*(1´a,2´) = 4.4, *J*gem = 11.0, 1H, H-1´a), 4.21 (dd, *J*(1´b,2´) = 6.3, *J*gem = 11.0, 1H, H-1´b), 4.04 (dd, *J*(3´a,2´) = 6.5, *J*gem = 8.6, 1H, H-3´a), 3.70 (dd, *J*(3´b,2´) = 6.0, *J*gem = 8.6, 1H, H-3´b), 1.32 and 1.27 (2 × s, 2 × 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.48 (C-6), 162.95 (C-2), 160.21 (C-4), 109.05 ($CHMe_2$), 94.48 (C-5), 73.35 (C-2´), 66.85 and 65.82 (C-1´, C-3´), 26.79 and 25.51 (CH_3) ppm. MS (ESI): m/z (%) = 282.43 (100) $[MNa]^+$, 260.0 (37) $[MH]^+$. For $C_{10}H_{14}ClN_3O_3$ (259.68) calcd. C 46.25, H 5.43, Cl 13.65, N 16.18, O 18.48; found C 46.19, H 5.31, Cl 13.41, N 16.02.

2-Amino-4,6-(2*R*,2´*S*)-bis(1,2-dihydroxypropoxy)pyrimidine (**25**)

Compound **24** (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 **23** (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-1,3-dioxolan-4-yl)methoxy]-pyrimidine, 2.95 g, 72%] as a yellow oil. ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with 2-amino-4,6-(2*S*,2´*S*)-bis[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]pyrimidine. MS (FAB): m/z (%) = 356.0 (100) $[MH]^+$. For $C_{16}H_{25}N_3O_6$ (355.38) calcd. C 54.07, H 7.09, N 11.82, O 27.01; found C 53.97, H 7.31, N 11.74.
The intermediate was deprotected by the same procedure as was described for compound **20** (HCl/MeOH/H_2O) to give **25**, white solid, yield 69%; m.p. 116 °C. ^{1}H NMR (DMSO-d_6): δ = 6.51 (br s, 2H, NH_2), 5.32 (s, 1H, H-5), 4.90 and 4.65 (2 × br s, 2 × 2H, OH), 4.17 (dd, *J*(1´a,2´) = 4.3, *J*gem = 10.9, 2H, H-1´a), 4.06 (dd, *J*(1´b,2´) = 6.6, *J*gem = 10.9, 2H, H-1´b), 3.72 (m, 2H, H-2´), 3.38 (d, *J*(3´,2´) = 4.0, 4H, H-3´) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.62 (2C, C-4, C-6), 162.82 (C-2), 78.58 (C-5), 69.89 (2C, C-2´), 67.73 (2C, C-1´), 62.97 (C-3´) ppm. MS (FAB): m/z (%) = 276.1 (100) $[MH]^+$. For $C_{10}H_{17}N_3O_6.1/2\ H_2O$ (284.26) calcd. C 42.25, H 6.38, N 14.78, O 36.58; found C 42.24, H 6.20, N 14.60.

2-Amino-4,6-(2*R*,2´*S*)-bis[2-(diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-pyrimidine (**26a**)

Prepared from compound **25** by the same procedure as was described for **21a**, colorless oil, yield 2.7 g (41%). ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) identical with **21a**. MS (FAB): m/z (%) = 632.1 (100) $[MH]^+$. For $C_{24}H_{47}N_3O_{12}P_2$ (631.59) calcd. C 45.64, H 7.50, N 6.65, O 30.40, P 9.81; found C 45.59, H 7.31, N 6.72; P 9.60.

2-Amino-4,6-(2*R*,2´*S*)-bis[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine (**26b**)

GP2, colorless foam, yield 63%. ^{1}H NMR (D_2O) and ^{13}C NMR (D_2O) identical with **21b**. MS (FAB): m/z (%) = 464 (35) $[MH]^+$. For $C_{12}H_{23}N_3O_{12}P_2.H_2O$ (481.27) calcd. C 29.95, H 5.24, N 8.73, O 43.22, P 12.87; found C 30.25, H 5.18, N 8.51, P 12.93. $[\alpha]^{25}_D$ = +0.02 (c 0.383, H_2O).

2-Amino-4,6-(2*S*,2′*S*)-bis-(2-hydroxypropoxy)pyrimidine (**28**)

Solution of compound **27** (1.92 g, 12 mmol) in THF (12 ml) was treated with NaH (0.48 g, 60% in paraffin oil, 12 mmol) at 0 °C, after 0.5 h pyrimidine **8** (1 g, 6 mmol) was added and the reaction mixture was heated at 60 °C for 6 h. The solvent was evaporated and the residue in methanol (30 ml) and water (10 ml) mixture was refluxed with Dowex 50 × 8 (H^+ form) resin for 2 h. The reaction mixture was applied onto a column of Dowex 50 × 8, washed with water and eluted with 2.5% ammonia. The UV-absorbing eluate was collected and evaporated. Flash chromatography ($CHCl_3$/MeOH) gave 1.3 g (87%) of **28** as an colorless oil. ^{1}H NMR (DMSO-d_6): δ = 6.50 (br s, 2H, NH_2), 5.31 (s, 1H, H-5), 4.81 (d, *J*(OH,2′) = 5.0, 2H, OH), 4.02 (dd, *J*(1′a,2′) = 6.6, *J*gem = 10.6, 2H, H-1′a), 3.96 (dd, *J*(1′b,2′) = 4.8, *J*gem = 10.6, 2H, H-1′b), 3.87 (m, 2H, H-2′), 1.08 (d, *J*(CH_3,2′) = 6.4, 6H, CH_3) ppm. MS (FAB): m/z (%) = 244 (100) $[MH]^+$. For $C_{10}H_{17}N_3O_4$ (243.26) calcd. C 49.37, H 7.04, N 17.27, O 26.31; found C 49.54, H 6.95, N 17.22.

2-Amino-4-chloro-6-[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (**30a**)

GP1, purified by flash chromatography ($CHCl_3$/MeOH), crystallized from EtOAc, white crystalline product, yield 21%, m.p. 96 °C. ^{1}H NMR (DMSO-d_6): δ = 7.08 (br s, 2H, NH_2), 6.06 (d, 1H, H-5), 4.59 (m, 2H, CHipr.), 4.37 (m, 2H, H-1′), 3.80 (m, 2H, H-2′), 3.78 (d, *J*(P,CH) = 8.3, 2H, PCH_2), 1.24 and 1.23 (2 × d, *J*(CH_3,CH) = 6.2, 2 × 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.55 (C-6), 162.97 (C-2), 160.11 (C-4), 94.46 (C-5), 71.71 (d, *J*(P,C) = 11.7, C-2′), 70.34 (d, *J*(P,C) = 6.3, CHipr), 65.14 (C-1′), 64.99 (d, *J*(P,C) = 164.6, PCH_2), 24.00 and 23.97 (2 × d, *J*(P,C) = 3.4, CH_3) ppm. MS (FAB): m/z (%) = 368 (100) $[MH]^+$. For $C_{13}H_{23}ClN_3O_5P$ (367.76) calcd. C 42.46, H 6.30, Cl 9.64, N 11.43, O 21.75, P 8.42; found C 42.58, H 6.44, Cl 9.31, N 11.21, P 8.66.

2-Amino-4-chloro-6-(2*R*)-[2-(diisopropoxyphosphorylmethoxy)propoxy]pyrimidine (**30b**)

GP1, flash chromatography ($CHCl_3$/MeOH) afforded colorless oil, yield 33%. ^{1}H NMR (DMSO-d_6): δ = 7.07 (br s, 2H, NH_2), 6.07 (s, 1H, H-5), 4.58 (m, 2H, CHipr.), 4.25 (dd, *J*(1´a,2´) = 3.8, *J*gem = 11.4, 1H, H-1´a), 4.20 (dd, *J*(1´b,2´) = 6.1, *J*gem = 11.4, 1H, H-1´b), 3.86 (m, 1H, H-2´), 3.81 and 3.77 (2 × dd, *J*(P,CH) = 9.0, *J*gem = 13.7, 2 × 1H, PCH_2), 1.25 (d, 3H), 1.24 (d, 3H), 1.225 (d, 3H) and 1.21 (d, *J*(CH_3,CH) = 6.2, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.58 (C-6), 162.96 (C-2), 160.11 (C-4), 94.43 (C-5), 75.11 (d, *J*(P,C) = 12.7, C-2´), 70.28 and 70.25 (2 × d, *J*(P,C) = 6.4, CHipr.), 68.70 (C-1´), 63.07 (d, *J*(P,C) = 165.0, PCH_2), 23.97 (d, *J*(P,C) = 3.9, 2C), 23.83 and 23.79 (2 × d, *J*(P,C) = 3.4, CH_3), 16.26 (C-3´) ppm. MS (FAB): m/z (%) = 382 (60) $[MH]^+$. For $C_{14}H_{25}ClN_3O_5P$ (381.79) calcd. C 44.04, H 6.60, Cl 9.29, N 11.01, O 20.95, P 8.11; found C 43.92, H 6.50, Cl 9.36, N 11.21, P 8.20.

2-Amino-4,6-(2*S*,2´*S*)-bis[2-(diisopropoxyphosphorylmethoxy)propoxy]pyrimidine (**32a**) and 2-amino-4-(*2S*)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-6-(2-propoxy)pyrimidine (**33**)

GP1, the residue was dissolved in $CHCl_3$, washed with brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue in MeOH (50 ml) was treated with MeONa (1M, 5 ml) at r.t. overnight. The reaction mixture was neutralized with acetic acid and the solvent was removed under reduced pressure. Flash chromatography ($CHCl_3$/MeOH) gave compound **33**, colorless syrup, yield 26%. ^{1}H NMR (DMSO-d_6): δ = 6.48 (br s, 2H, NH_2), 5.24 (s, 1H, H-5), 5.16 (sept, *J*(CH,CH_3) = 6.2, 1H, OCHipr.), 4.57 (m, 2H, POCH), 4.13 (dd, *J*(1´a,2´) = 4.2, *J*gem = 11.4, 1H, H-1´a), 4.12 (dd, *J*(1´b,2´) = 6.0, *J*gem = 11.4, 1H, H-1´b), 3.83 (m, 1H, H-2´), 3.81 and 3.77 (2 × dd, *J*(P,CH) = 9.0, *J*gem = 13.7, 2 × 1H, PCH_2), 1.23 (d, 3H), 1.225 (d, 3H), 1.22 (d, 9H), 1.21 (d, 3H) and 1.13 (d, *J*(CH_3,CH) = 6.2, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.30 and 171.02 (C-4, C-6), 162.88 (C-2), 78.96 (C-5), 75.40 (d, *J*(P,C) = 12.7, C-2´), 70.36 and 70.31 (2 × d, *J*(P,C) = 6.3, POCH), 68.21 (C-1´), 67.67 (OCH), 62.99 (d, *J*(P,C) = 165.0, PCH_2), 24.02 (d,

J(P,C) = 3.4, 2C), 23.85 (d, *J*(P,C) = 4.4, 2C, CH_3), 22.11 (2C, CH_3), 16.43 (C-3′) ppm. MS (FAB): m/z (%) = 406.2 (100) $[MH]^+$. For $C_{17}H_{32}N_3O_6P$ (405.43) calcd. C 50.36, H 7.96, N 10.36, O 23.68, P 7.64; found C 50.51, H 8.12, N 10.21, P 7.53. $[\alpha]^{25}_D$ = +3.2 (c 0.347, MeOH).

Further elution of column gave **32a** as colorless syrup, yield 18%. ^{1}H NMR (DMSO-*d*$_6$): δ = 6.56 (br s, 2H, NH_2), 5.29 (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 4.18 (dd, *J*(1′a,2′) = 3.8, *J*gem = 11.2, 2H, H-1′a), 4.12 (dd, *J*(1′b,2′) = 6.2, *J*gem = 11.2, 2H, H-1′b), 3.83 (m, 2H, H-2′), 3.81 and 3.77 (2 × dd, *J*(P,CH) = 9.2, Jgem = 13.8, 2 × 2H, PCH_2), 1.235, 1.23, 1.22, 1.21, 1.13 (d, *J*(CH_3,CH) = 6.2, 24H, CH_3) ppm. ^{13}C NMR (DMSO-*d*$_6$): δ = 171.32 (2C, C-4, C-6), 162.78 (C-2), 78.45 (C-5), 75.37 (d, *J*(P,C) = 12.7, 2C, C-2′), 70.35 (d, 2C) and 70.30 (d, *J*(P,C) = 6.3, 2C, CHipr.), 68.33 (2C, C-1′), 63.10 (d, *J*(P,C) = 165.5, 2C, PCH_2), 24.02 (d, *J*(P,C) = 3.9, 4C) and 23.86 (d, *J*(P,C) = 4.4, 4C, CH_3), 16.40 (2C, C-3′) ppm. MS (FAB): m/z (%) = 600 (56) $[MH]^+$. For $C_{24}H_{47}N_3O_{10}P_2$ (599.59) calcd. C 48.08, H 7.90, N 7.01, O 26.68, P 10.33; found C 48.01, H 7.63, N 6.92, P 10.15. $[\alpha]^{25}_D$ = +16.8 (c 0.169, MeOH).

2-Amino-4,6-(2*S*,2′*S*)-bis[2-(phosphonomethoxy)propoxy]pyrimidine (**32b**)

GP2, from **32a** (350 mg, 0.58 mmol), yield 135 mg (52%), colorless foam. ^{1}H NMR (DMSO-*d*$_6$): δ = 3.57 (s, 1H, H-5), 4.17 (dd, *J*gem = 11.1, *J*(1′a,2′) = 5.8, 2H, H-1′a), 4.11 (dd, *J*gem = 11.1, *J*(1′b,2′) = 4.4, 2H, H-1′b), 3.81 (m, 2H, H-2′), 3.60 (d, *J*(CH_2,P) = 9.3, 4H, PCH_2), 1.14 (d, *J*(3′,2′) = 6.3, 6H, H-3′) ppm. ^{13}C NMR (DMSO-*d*$_6$): δ = 171.41 (C-4, C-6), 162.80 (C-2), 78.54 (C-5), 75.02 (d, *J*(2′,P) = 11.6, C-2′), 68.59 (C-1′), 65.02 (d, *J*(CH_2,P) = 161.7, PCH_2), 16.82 (C-3′) ppm. MS (ESI): m/z (%) = 432.1 (100) $[MH]^+$; 454.1 (26) $[MNa]^+$. For $C_{12}H_{23}N_3O_{10}P_2.H_2O$ (449.09) calcd. C 32.08, H 5.61, N 9.35, O 39.17, P 13.79; found C 32.34, H 5.51, N 9.17, P 13.83. $[\alpha]^{25}_D$ = +20.9 (c 0.291, H_2O).

N-{4-Chloro-6-(2*S*)-[2-(diisopropoxyphosphorylmethoxy)propoxy]pyrimidin-2-yl}-benzamide (**34**)

Phosphonate **29c** (1.12 g, 4.4 mol) in dry THF (20 ml) was treated with NaH (0.156 g, 60% in paraffin oil, 3.9 mmol), after stirring for 0.5 h pyrimidine **31** (1 g, 3.7

mmol) was added and the resulting mixture was stirred at r.t. for 2 h. Reaction mixture was neutralized with acetic acid and evaporated in vacuo. The residue was dissolved in $CHCl_3$, washed with brine and dried over $MgSO_4$. Flash chromatography ($CHCl_3$/MeOH) afforded pale yellow oil, 1.04 g (57%). ^{1}H NMR (DMSO-*d*$_6$): δ = 11.18 (br s, 1H, NH), 7.92 (d, 2H), 7.60 (t, 1H) and 7.51 (t, 2H, arom.), 6.82 (s, 1H, H-5), 4.53 (m, 2H, CHipr.), 4.41 (dd, *J*(1´a,2´) = 3.8, *J*gem = 11.3, 1H, H-1´a), 4.30 (dd, *J*(1´b,2´) = 5.8, *J*gem = 11.3, 1H, H-1´b), 3.92 (m, 1H, H-2´), 3.73 (d, *J*(P,C) = 9.1, PCH_2), 1.24 and 1.23 (2 × d, *J*(CH_3,CH) = 6.2, 2 × 6H, CH_3ipr.), 1.18 (d, *J*(CH_3,2´) = 6.5, H-3´) ppm. MS (FAB): m/z (%) = 486 (80) $[MH]^+$. For $C_{21}H_{29}ClN_3O_6P$ (485.89) calcd. C 51.91, H 6.02, Cl 7.30, N 8.65, O 19.76, P 6.37; found C 51.83, H 6.31, Cl 7.29, N 8.52, P 6.21.

2-Amino-4,6-bis-(diisopropoxyphosphorylmethoxy)pyrimidine (**35a**)

Diisopropyl hydroxymethylphosphonate (2.55 g, 13 mmol) in THF (10 ml) was treated with NaH (0.52 g, 60% in paraffin oil, 13 mmol) and the reaction mixture was stirred at r.t. for 0.5 h; pyrimidine **8** (1 g, 6.1 mmol) was added and the resulting mixture was stirred overnight. Volatiles were removed under reduced pressure. Flash chromatography ($CHCl_3$/MeOH) and crystallization from EtOAc/light petroleum mixture gave **35a** (0.84 g, 28%) as a white solid, m.p. 138 °C. ^{1}H NMR (DMSO-*d*$_6$): δ = 6.81 (br s, 2H, NH_2), 5.46 (s, 1H, H-5), 4.64 (m, 4H, CHipr.), 4.59 (d, *J*(P,C) = 8.2, 4H, PCH_2), 1.25 and 1.21 (2 × d, *J*(CH_3,CH) = 6.2, 2 × 12H, CH_3) ppm. ^{13}C NMR (DMSO-*d*$_6$): δ = 170.84 (d, *J*(P,C) = 9.3, 2C, C-4, C-6), 162.33 (C-2), 78.70 (C-5), 70.88 (d, *J*(P,C) = 6.4, 4C, CHipr.), 58.54 (d, *J*(P,C) = 167.5, 2C, PC), 23.98 (d, *J*(P,C) = 3.4, 4C) and 23.82 (d, *J*(P,C) = 4.9, 4C, CH_3) ppm. MS (FAB): m/z (%) = 484 (100) $[MH]^+$, 506 (100) $[MNa]^+$. For $C_{18}H_{35}N_3O_8P_2$ (483.43) calcd. C 44.72, H 7.30, N 8.69, O 26.48, P 12.81; found C 44.61, H 7.28, N 8.62, P 12.89.

2-Amino-4,6-bis-(phosphonomethoxy)pyrimidine (**35b**)

GP2, **35a** (670 mg, 1.39 mmol), yield 280 mg (64%), white foam. ^{1}H NMR (D_2O): δ = 5.84 (s, 1H, H-5), 4.07 (d, *J*(P,CH) = 9.4, 4H, PCH_2). MS (FAB): m/z (%) = 316

(100) $[MH]^+$. For $C_6H_{11}N_3O_8P_2$ (315.11) calcd. C 22.87, H 3.52, N 13.33, O 40.62, P 19.66; found C 22.74, H 3.52, N 13.21, P 19.55.

2-Amino-4,6-difluoropyrimidine (**39**) and 4-amino-2,6-difluoropyrimidine (**40**)

2,4,6-Trifluoropyrimidine (**38**) (5.7 g, 43 mmol) in MeOH (50 ml) was treated with methanolic ammonia (1.5 M, 10 ml) at -40 °C, the reaction mixture in a sealed flask was allowed to warm to r.t. The crystalline product was filtered off and the crude product was recrystallized from EtOH to give **39** (4.15 g 74%), m.p. not melting bellow 300 °C, dec. ^{1}H NMR (DMSO-d_6): δ = 7.54 (br s, 2H, NH_2), 6.13 (t, 1H, J(H,F) = 1.1) ppm. ^{19}F NMR (DMSO-d_6): δ = -54.81 (br s) ppm. ^{13}C NMR (DMSO-d_6): δ = 172.25 (dd, J(C,F) = 249.3, J(C,F) = 22.8, C-4, C-6), 163.06 (t, J(C,F) = 24.2, C-2), 78.59 (t, J(C,F) = 39.1, C-5) ppm. MS (FAB): m/z (%) = 132 (8) $[MH]^+$. The mother liquor was purified by flash chromatography (EtOAc/light petroleum) to give **40** (0.52 g, 9%), m.p. 211–212 °C. ^{1}H NMR (DMSO-d_6): δ = 7.78 (br s, 2H, NH_2), 9.56 (d, 1H, J(H,F) = 2.8) ppm. ^{19}F NMR (DMSO-d_6): δ = -42.46 (br s), -63.47 (br s) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.14 (dd, J(C,F) = 243.2, J(C,F) = 19.0, C-6), 169.35 (dd, J(C,F) = 11.7, J(C,F) = 18.6, C-4), 161.84 (dd, J(C,F) = 210.2, J(C,F) = 24.2, C-2), 83.83 (dd, J(C,F) = 33.2, J(C,F) = 5.8, C-5) ppm. MS (FAB): m/z (%) = 132 (12) $[MH]^+$.

2-Amino-6-(2*S*)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-4-fluoropyrimidine (**41**)

GP1, purified by flash chromatography (EtOAc/light petroleum), yellow oil, yield 68%. ^{1}H NMR (DMSO-d_6): δ = 7.06 (br s, 2H, NH_2), 5.65 (s, 1H, H-5), 4.58 (m, 1H, H-3'), 4.26 (dd, J(1'a,2') = 3.8, Jgem = 11.3, 1H, H-1'a), 4.20 (dd, J(1'b,2') = 6.2, Jgem = 11.3, 1H, H-1'b), 3.85 (m, 2H, H-2'), 4.12 and 3.99 (dd, J(P,CH) = 10.2, Jgem = 13.2, 2H, PCH_2), 1.23 and 1.22 and 1.215 (d, J(H,P) = 6.2, 9H, CH_3ipr.), 1.15 (d, J(H,P) = 6.5, 3H, CH_3ipr.). MS (FAB): m/z (%) = 388.1 (100) $[MNa]^+$.

4,6-Bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]-2-(methylsulfanyl)pyrimidine (**46**) and 4-[2-(diisopropoxyphosphorylmethoxy)ethoxy]-1-[2-(diisopropoxy-phosphorylmethoxy)ethyl]-2-(methylsulfanyl)pyrimidine-6(1*H*)-one (**47**)

GP3, pyrimidine **42** (3 g, 19 mmol), phosphonate **43a** (10.6 g, 40 mmol), yield 6.15 g (54%) of **46**, colorless syrup. ^{1}H NMR (DMSO-d_6): δ = 5.88 (s, 1H, H-5), 4.58 (dh, J(CH,P) = 7.7, J(CH,CH_3) = 6.3, 4H, CHipr.), 4.43 (m, 4H, H-1′), 3.82 (m, 4H, H-2′), 3.79 (d, J(C,H,P) = 8.3, 4H, PCH_2), 2.48 (s, 3H, SCH_3), 1.23 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.61 (C-2), 170.26 (2C, C-4,6), 85.55 (C-5), 70.57 (d, J(2′,P) = 11.9, C-2′), 70.36 (d, J(CH,P) = 6.2, CHipr.), 65.67 (C-1′), 64.95 (d, J(C,P) = 164.6, PCH_2), 24.00 (d, J(CH_3,P) = 3.7) and 23.85 (d, J(CH_3,P) = 4.6, CH_3ipr.), 13.66 (SCH_3) ppm. MS (ESI): m/z (%) = 625.1 (100) $[MNa]^+$, 603 (15) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{10}P_2S$ $[MH]^+$ 603.2192, found 603.2185. $C_{23}H_{45}N_2O_{10}P_2S$ (602.61) calcd. C 45.84, H 7.36, N 4.65, O 26.55, P 10.28, S 5.32; found C 45.71, H 7.49, N 4.55, P 10.11, S 5.48.

Further elution of column gave **47**, yield 1.35 g (12%), colorless syrup. ^{1}H NMR (DMSO-d_6): δ = 5.40 (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 4.31 (m, 2H, H-1′′), 4.10 (t, J(1′,2′) = 5.4, 2H, H-1′), 3.82 (m, 2H, H-2′′), 3.79 (d, 2H) and 3.76 (d, J(P,CH) = 8.3, 2H, PCH_2), 3.75 (t, J(2′,1′) = 5.4, 2H, H-2′), 2.54 (s, 3H, SCH_3), 1.24 (d, 6H), 1.23 (d, 6H), 1.22 (d, 6H) and 1.20 (d, J(CH_3,CH) = 6.2, 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.34 (C-2), 162.75 (C-4), 159.48 (C-6), 85.65 (C-5), 70.76 (d, J(C,P) = 11.9, C-2′), 70.44 (d, J(C,P) = 11.89, C-2′′), 70.04 (m, CHipr.), 69.53 (d, J(C,P) = 6.61, CHipr.), 65.31 (C-1′), 64.80 (d, J(C,P) = 164.41, PCH_2′), 64.75 (d, J(C,P) = 163.72, PCH_2′′), 43.69 (C-1′′), 23.61 (m, CH_3ipr.), 14.43 (SCH_3) ppm. MS (ESI): m/z (%) = 625.1 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{10}P_2S$ $[MH]^+$ 603.2192, found 603.2193. For $C_{23}H_{45}N_2O_{10}P_2S$ (602.61) calcd. C 45.84, H 7.36, N 4.65, O 26.55, P 10.28, S 5.32; found C 45.92, H 7.54, N 4.51, P 10.02, S 5.59.

6-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-4-hydroxy-2-(methylsulfanyl)-pyrimidine (**48a**)

GP4, pyrimidine **42** (3 g, 19 mmol), phosphonate **43a** (4.8 g, 18.1 mmol), yield 2.67 g (37%) of **48a**, colorless syrup. ^{1}H NMR (DMSO-d_6): δ = 12.32 (br s, 1H, OH), 5.39 (br s, 1H, H-5), 4.59 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.8, 2H, CHipr.), 4.33 (m, 2H, H-1′), 3.80 (m, 2H, H-2′), 3.79 (d, J(CH_2,P) = 8.3, 2H, CH_2P), 2.47 (s, 3H, SCH_3), 1.24 (d, J(CH_3,CH) = 6.0, 6H) and (1.22 d, J(CH_3,CH) = 6.0, 6H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): δ = 169.19 (C-4), 86.06 (C-5), 70.66 (d, J(2,P) = 11.9, C-2′), 70.43 (d, J(C,O,P) = 6.3, CHipr.), 65.83 (C-1′), 64.97 (d, J(C,P) = 164.5, CH_2P), 24.03 (d, J(CH_3,P) = 3.6) and 23.89 (d, J(CH_3,P) = 4.4, CH_3ipr.), 13.12 (SCH_3) ppm. MS (ESI): m/z (%) = 403 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{14}H_{26}N_2O_6PS$ $[MH]^+$ 381.1249, found 381.1265.
Compound **46** (4.18 g, 36%) was isolated as a side product.

6-(*2S*)-[2-(Diisopropoxyphosphorylmethoxy)propoxy]-4-hydroxy-2-(methyl-sulfanyl)pyrimidine (**48b**) and 4,6-(2*S*,2′*S*)-bis[2-(diisopropoxyphosphorylmethoxy)-propoxy]-2-(methylsulfanyl)pyrimidine (**49a**)

GP4, from pyrimidine **42** (2 g, 12.6 mmol) and phosphonate **43b** (5.68 g, 13.9 mmol). Yield 1.25 g (25%) of **48b**, colorless oil. ^{1}H NMR ($CDCl_3$): δ = 12.3 (br s, 1H, OH), 5.40 (br s, 1H, H-5), 4.58 (m, 2H, CHipr.), 4.23 (dd, *J*gem = 11.3, J(1′a,2′) = 3.8, 1H, H-1′a), 4.16 (dd, *J*gem = 11.3, J(1′b,2′) = 6.1, 1H, H-1′b), 3.87 (m, 2H, H-2′), 3.80 (m, 2H, OCH_2P), 2.47 (s, 3H, SCH_3), 1.22 (m, 12H, CH_3ipr.), 1.15 (d, J(CH_3,2′) = 6.4, 3H, H-3′) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.52 (C-2), 169.15 (C-4), 162.50 (C-6), 86.08 (C-5), 75.24 (d, J(2′,P) = 12.8, C-2′), 70.32 (m, 2C, CHipr.), 69.20 (C-1′), 63.01 (d, J(OCH_2,P) = 165.2, OCH_2P), 23.92 (m, 4C, CH_3ipr.), 16.28 (C-3′), 13.10 (SCH_3) ppm. MS (FAB): m/z (%) = 395 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{15}H_{28}N_2O_6PS$ $[MH]^+$ 395.1405, found 395.1418.
Dialkylated derivative **49a** was isolated as the second product. Yield 1.75 g (22%), colorless oil. ^{1}H NMR ($CDCl_3$): δ = 5.69 (s, 1H, H-5), 4.74 (m, 4H, CHipr.), 4.307 (d, J(1′,2′) = 5.17, 4H, H-1′), 3.89 (m, 2H, H-2′), 3.82 (d, J(CH_2,P) = 9.11, 4H, OCH_2P), 2.49 (s, 3H, SCH_3), 1.31 (m, 24H, CH_3ipr.), 1.23 (d, J(3′,2′) = 6.39, 6H, H-

3′) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.92 (C-2), 170.17 (C-4,6), 86.12 (C-5), 75.85 (d, *J*(2′,P) = 11.98, C-2′), 71.05 (d, *J*(C,P) = 6.68) and 70.96 (d, *J*(C,P) = 6.66, CHipr.), 69.35 (C-1′), 64.04 (d, *J*(C,P) = 168.56, PCH_2), 24.06 (d, *J*(C,P) = 3.69) and 23.92 (d, *J*(C,P) = 4.64, CH_3ipr.), 16.55 (C-3′), 14.00 (SCH_3) ppm. MS (ESI): m/z (%) = 653 (100) $[MNa]^+$, 631 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{10}P_2S$ $[MH]^+$ 631.2583, found 631.2579.

6-(*2R*)-[2-(Diisopropoxyphosphorylmethoxy)propoxy]-4-hydroxy-2-(methylsulfanyl)pyrimidine (**48c**) and 4,6-(2*R*,2′*R*)-bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfanyl)pyrimidine (**49b**)

GP4, from pyrimidine **42** (4 g, 25.2 mmol) and phosphonate **43c** (10.3 g, 25.2 mmol). Yield 2.5 g (25%) of compound **48c**, colorless oil. ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) identical with compound **48b**. MS (FAB): m/z (%) = 395 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{15}H_{28}N_2O_6PS$ $[MH]^+$ 395.1405, found 395.1413. Compound **49b** isolated as a colorless oil, yield 1.2 g (7.5%). ^{1}H NMR ($CDCl_3$) identical with compound **49a**. MS (FAB): m/z (%) = 631 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{10}P_2S$ $[MH]^+$ 631.2583, found 631.2599.

4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(*2S*)-[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**51a**)

GP5, **48a** (800 mg, 2.1 mmol), **43b** (0.94 g, 2.3 mmol). The crude product was taken to $CHCl_3$, filtered through a pad of silica gel and washed with 10% MeOH in $CHCl_3$ (150 ml). The filtrate was taken down in vacuo. The residue was without further purification oxidized with *m*-CPBA by GP6 to give **51a** (0.52 g, 38%), colorless oil. ^{1}H NMR (DMSO-d_6): δ = 6.52 (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 4.52 (m, 2H, H-1′), 4.43 (dd, *J*gem = 11.4, *J*(1′′a,2′′) = 3.5, 1H, H-1′′a), 4.34 (dd, *J*gem = 11.4, *J*(1′′b,2′′) = 6.0, 1H, H-1′′b), 3.92 (m, 2H, H-2′′), 3.86 (m, 2H, H-2′), 3.81 (m, 4H, 2 × PCH_2), 3.38 (s, 3H, SO_2CH_3), 1.19–1.24 (m, 24H, CH_3ipr.), 1.19 (d, *J*(CH_3, 2′′) = 6.5, 3H, H-3′′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.51 and 171.45 (C-4, C-6), 164.26 (C-2), 92.66 (C-5), 75.05 (d, *J*(2′′,P) = 12.5, C-2′′), 70.12–70.40 (m, 6C, CHipr., C-2′ and C-1′′), 66.95 (C-1′), 64.95 (d, *J*(C,P) = 164.5, C-3′), 63.03 (d,

J(C,P) = 165.3, C-3´´), 38.94 (CH_3SO_2), 23.82–24.01 (m, 8C, CH_3ipr.), 16.21 (C-3´´) ppm. MS (FAB): m/z (%) = 671 (23) $[MNa]^+$, 649 (32) $[MH]^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{12}P_2S$ $[MH]^+$ 649.2325, found 649.2328.

4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(*2R*)-[2-(diisopropoxyphosphoryl-methoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**51b**)

Prepared by the same procedure as described for compound **51a** from **48a** (800 mg, 2.1 mmol) and phosphonate **43c**. Colorless oil, 460 mg (34%). ^{1}H NMR spectra identical with compound **51a**. MS (ESI): m/z (%) = 671.1 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{12}P_2S$ $[MH]^+$ 649.2325, found 649.2318.

4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(2*S*)-[2-(diisopropoxyphosphoryl-methoxy)-3-hydroxypropoxy]-2-(methylsulfonyl)pyrimidine (**51c**)

Prepared by the same procedure (GP5 and GP6) as compound **51a** from **48a** (800 mg, 2.1 mmol) and phosphonate **43d** (1.07 g, 2.52 mmol). Colorless oil, 870 mg (62%). ^{1}H NMR ($CDCl_3$): 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, PCH_2´´a), 3.95 (m, 2H, H-2´), 3.88 (m, 2H, H-2´´), 3.83 (dd, *J*gem = 14.2, *J*(H,C,P) = 8.4, 1H, PCH_2´´b), 3.81 (d, *J*(H,C,P) = 8.2, 2H, PCH_2´), 3.80 (m, 1H, H-3´´a), 3.70 (dd, *J*gem = 12.3, *J*(3´´b,2´´) = 5.9, 1H, H-3´´b), 3.30 (s, 3H, CH_3SO_2), 1.34 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): 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, PCH_2´), 65.35 (d, *J*(C,P) = 168.8, PCH_2´´), 61.62 (C-3´´), 38.88 (CH_3SO_2), 23.97 (m, CH_3ipr.) ppm. MS (ESI): m/z (%) = 687 (100) $[MNa]^+$, 665 (44) $[MH]^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{13}P_2S$ $[MH]^+$ 645.2274, found 645.2268.

4-[2-(Diisopropoxyphosphorylmethoxy)ethoxy]-6-(2*R*)-[2-(diisopropoxyphosphoryl-methoxy)-3-hydroxypropoxy]-2-(methylsulfonyl)pyrimidine (**51d**)

Prepared by the same procedure (GP5 and GP6) as compound **51a** from **48a** (800 mg, 2.1 mmol) and phosphonate **43e** (1.07 g, 2.52 mmol). Colorless oil, 700 mg (50%). ^{1}H NMR ($CDCl_3$) spectra identical with compound **51c**. MS (ESI): m/z (%) = 687 (25) $[MNa]^+$, 665 (12) $[MH]^+$. HR MS (FAB) calcd. for $C_{24}H_{47}N_2O_{13}P_2S$ $[MH]^+$ 645.2274, found 645.2288.

4,6-(2*R*,2′*S*)-Bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)-pyrimidine (**51e**)

GP5 and GP6, from **48b** (500 mg, 1.27 mmol) and phosphonate **43c** (0.57 g, 1.4 mmol). Colorless oil, 740 mg (88%). ^{1}H 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, Jgem = 11.4, 2H, H-1′a), 4.34 (dd, *J*(1′b,2′) = 5.9, Jgem = 11.4, 2H, H-1′b), 3.92 (m, 2H, H-2′), 3.82 (m, 4H, OCH_2P), 2.38 (s, 3H, SO_2CH_3), 1.18–1.24 (m, 30H, CH_3ipr., H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.50 (C-4,6), 164.25 (C-2), 92.66 (C-5), 75.05 (d, *J*(2′,P) = 12.7, C-2′), 70.30 (m, CHipr), 70.13 (C-1′), 63.04 (d, *J*(C,P) = 165.5, PCH_2), 38.95 (CH_3SO_2), 23.92 (m, CH_3ipr.), 16.22 (C-3′) ppm. MS (ESI): m/z (%) = 685 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{12}P_2S$ $[MH]^+$ 663.2476, found 663.2469.

4-(2*S*)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(*2S*)-[2-(diiso-propoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**51f**)

GP5 and GP6, **48b** (1.25 g, 3.17 mmol) and **43d** (1.75 g, 4.12 mmol). Colorless oil, 394 mg (18%). ^{1}H NMR (DMSO-d_6): δ = 6.21 (s, 1H, H-5), 4.75 (m, 4H, CHipr.), 4.52 (dd, *J*gem = 11.4, *J*(1′′a,2′′) = 5.9, 1H, H-1′′a), 4.49 (dd, *J*gem = 11.4, *J*(1′′b,2′′) = 4.8, 1H, H-1′′b), 4.44 (dd, *J*gem = 11.3, *J*(1′a,2′) = 3.8, 1H, H-1′a), 4.40 (dd, *J*gem = 11.3, *J*(1′b,2′) = 6.2, 1H, H-1′b), 4.06 (dd, *J*gem = 14.2, *J*(C,H,P) = 7.3, 1H, PCH_2′′a), 3.95 (m, 2H, H-2′), 3.87 (m, 2H, H-2′′), 3.83 (m, 4H, PCH_2′′b, PCH_2′, H-3′′), 3.70 (m, 2H, H-3b′′), 3.30 (s, 3H, SO_2CH_3), 1.34 (m, 24H, CH_3ipr.), 1.28 (d, *J*(3′,2′) = 6.4, 3H, H-3′) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.39 (C-4),

171.20 (C-6), 164.17 (C-2), 93.65 (C-5), 81.53 (d, *J*(2´´,P) = 7.5, C-2´´), 75.54 (d, *J*(2´,P) = 11.9, C-2´), 71.81 (d, *J*(C,O,P) = 6.7), 71.42 (d, *J*(C,O,P) = 6.9) and 71.08 (d, 2C, *J*(C,O,P) = 6.7, CHipr.), 70.54 (C-1´), 66.83 (C-1´´), 65.35 (d, *J*(C,P) = 168.8, PCH_2´´), 64.00 (d, *J*(C,P) = 169.2, PCH_2´), 61.62 (C-3´´), 38.89 (CH_3SO_2), 24.00 (m, CH_3ipr.), 16.34 (C-3´) ppm. MS (ESI): m/z (%) = 679 (10) $[MH]^+$, 701 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2429.

4-(2*R*)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(*2S*)-[2-(diiso-propoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**51g**)

GP5, GP6, **48b** (1.25 g, 3.17 mmol), **43e** (1.75 g, 4.12 mmol). Colorless oil, 360 mg (16%). ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) spectra identical with compound **51f**. MS (ESI): m/z (%) = 679 (6) $[MH]^+$, 701 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2438.

4-(2*S*)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(*2R*)-[2-(diiso-propoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**51h**)

GP5, GP6, **48c** (1.25 g, 3.17 mmol), **43d** (1.75 g, 4.12 mmol). Colorless oil, 960 mg (42%). ^{1}H NMR (DMSO-d_6): δ = 6.21 (s, 1H, H-5), 4.75 (m, 4H, CHipr.), 4.51 (m, 2H, H-1´´), 4.54 (dd, *J*gem = 11.4, *J*(1´a,2´) = 3.9, 1H, H-1´a), 4.41 (dd, *J*gem = 11.3, *J*(1´b,2´) = 6.1, 1H, H-1´b), 4.05 (dd, *J*gem = 14.1, *J*(C,H,P) = 7.4, 1H, PCH_2´´a), 3.95 (m, 2H, H-2´), 3.78–3.89 (m, 5H, H-2´´, PCH_2´´b, PCH_2´, H-3´´), 3.70 (dd, *J*gem = 12.4, *J*(3´´b,2´´) = 5.9, 1H, H-3´´b), 3.30 (s, 3H, SO_2CH_3), 1.33 (m, 24H, CH_3ipr.), 1.28 (d, *J*(3´,2´) = 6.4, 3H, H-3´) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.35 (C-4), 171.17 (C-6), 164.14 (C-2), 93.60 (C-5), 81.43 (d, *J*(2´´,P) = 7.7, C-2´´), 75.51 (d, *J*(2´,P) = 11.8, C-2´), 71.75 (d, *J*(C,O,P) = 6.7), 71.37 (d, *J*(C,O,P) = 6.9), 71.06 (d, *J*(C,O,P) = 6.7) and 71.04 (d, *J*(C,O,P) = 6.5, CHipr.), 70.52 (C-1´), 66.84 (C-1´´), 65.28 (d, *J*(C,P) = 168.8, PCH_2´´), 63.95 (d, *J*(C,P) = 169.1, PCH_2´), 61.53 (C-3´´), 38.89 (CH_3SO_2), 23.96 (m, CH_3ipr.), 16.30 (C-3´) ppm. MS (ESI): m/z (%) = 679 (35) $[MH]^+$, 701 (15) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2441.

4-(2*R*)-[2-(Diisopropoxyphosphorylmethoxy)-3-hydroxypropoxy]-6-(*2R*)-[2-(diiso-propoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)pyrimidine (**51i**)

GP5, GP6, **48c** (1.25 g, 3.17 mmol), **43e** (1.75 g, 4.12 mmol). Colorless oil, 1.05 g (46%). NMR spectra identical with **51h**. MS (ESI): m/z (%) = 679 (18) $[MH]^+$, 701 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{13}P_2S$ $[MH]^+$ 679.2430, found 679.2432.

4,6-Bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]-2-(methylsulfonyl)pyrimidine (**51j**)

GP6, **46** (5.8 g, 9.62 mmol). Colorless oil, yield 4.2 g (69%). ^{1}H NMR (DMSO-d_6): δ = 6.20 (s, 1H, H-5), 4.58 (dh, J(CH,P) = 7.6, J(CH,CH_3) = 6.4, 4H, CHipr.), 4.58 (m, 4H, H-1´), 3.94 (m, 4H, H-2´), 3.80 (d, J(C,H,P) = 8.17, 4H, PCH_2), 3.30 (s, 3H, SO_2CH_3), 1.33 (d,12H) and 1.32 (d, 12H, J(CH_3,CH) = 6.16, CH_3ipr.) ppm. MS (FAB): m/z (%) = 635 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{12}P_2S$ $[MH]^+$ 635.2168, found 635.2152.

4,6-(2*S*,2´*S*)-Bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)-pyrimidine (**51k**)

GP6, **49a** (840 mg, 1.33 mmol). Yield 800 mg (72%), colorless oil. ^{1}H NMR (DMSO) spectrum identical with spectrum of **51e**. MS (ESI): m/z (%) = 685 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{12}P_2S$ $[MH]^+$ 663.2476, found 663.2475.

4,6-(2*R*,2´*R*)-Bis[2-(diisopropoxyphosphorylmethoxy)propoxy]-2-(methylsulfonyl)-pyrimidine (**51l**)

GP6, **49b** (1.2 g, 1.9 mmol). Yield 1.1 g (87%), colorless oil. ^{1}H NMR (DMSO-d_6) spectrum identical with spectrum of **51e**. MS (ESI): m/z (%) = 685 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{25}H_{49}N_2O_{12}P_2S$ $[MH]^+$ 663.2476, found 663.2484.

Preparation of compounds **52** – general method: 2-Methylsulfonyl derivatives **51** were converted to 2-amino congeners by GP7 and diisopropyl esters of 2-amino pyrimidines were without further purification deprotected to free phosphonic acids by GP2.

2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(*2S*)-[2-(phosphonomethoxy)propoxy] pyrimidine (**52a**)

From **51a** (500 mg, 0.77 mmol), freeze dried, white hydroscopic foam, yield 210 mg (61%). ^{1}H NMR (DMSO-d_6): δ = 5.92 (s, 1H, H-5), 4.52 (m, 5H, H-1′), 4.45 (dd, *J*gem = 11.2, *J*(1′′a,2′) = 2.9, 1H, H-1′′a), 4.30 (dd, *J*gem = 11.3, *J*(1′′b,2′′) = 6.3, 1H, H-1′′b), 4.05 (dt, *J*(2′′,3′′) = *J*(2′′,1′′b) = 6.4, *J*(2′′,1′′a) = 3.0, 2H, H-2′′), 3.98 (m, 2H, H-2′), 3.74–3.84 (m, 4H, PCH_2), 1.27 (d, *J*(3′′,2′′) = 6.5, 3H, H-3′′) ppm. ^{13}C NMR (DMSO-d_6): δ = 169.98 (C-4), 169.91 (C-6), 156.14 (C-2), 79.67 (C-5), 76.06 (d, *J*(2′′,P) = 11.0, C-2′′), 72.42 (C-1), 70.72 (d, *J*(2′,P) = 10.3, C-2′), 67.19 (d, *J*(P,CH_2) = 157.2, PCH_2), 65.05 (d, *J*(P,CH_2) = 159.1, PCH_2), 15.72 (C-3′′) ppm. MS (ESI): m/z (%) = 418 (100) $[MH]^+$. For $C_{11}H_{21}N_3O_{10}P_2.H_2O$ (435.26) calcd. C 30.35, H 5.33, N 9.65, O 40.43, P 14.23; found C 30.42, H 5.01, N 9.56, P 14.21. $[\alpha]^{25}_D$ = +12.1 (c 0.248, H_2O).

2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(*2R*)-[2-(phosphonomethoxy)propoxy]pyrimidine (**52b**)

From **51b** (400 mg, 0.62 mmol), freeze dried, white hydroscopic foam, yield 190 mg (70%). NMR spectra identical with compound **52a**. MS (ESI): m/z (%) = 418 (100) $[MH]^+$. For $C_{11}H_{21}N_3O_{10}P_2.H_2O$ (435.26) calcd. C 30.35, H 5.33, N 9.65, O 40.43, P 14.23; found C 30.38, H 5.06, N 9.42, P 14.22. $[\alpha]^{25}_D$ = -10.3 (c 0.347, H_2O).

2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(2*S*)-[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine (**52c**)

From **51c** (870 mg, 1.3 mmol), freeze dried, white hydroscopic foam, yield 320 mg (54%). ^{1}H NMR (DMSO-d_6): δ = 5.36 (s, 1H, H-5), 4.29 (m, 2H, H-1′), 4.25 (dd,

*J*gem = 11.3, *J*(1´´a,2´´) = 4.3, 1H, H-1´´a), 4.20 (dd, *J*gem = 11.3, *J*(1´´b,2´´) = 5.7, 1H, H-1´´b), 3.75 (m, 2H, H-2´), 3.71 (dd, *J*gem = 13.6, *J*(H-C-P) = 8.7, 1H, OCH_2P´´a), 3.67 (dd, *J*gem = 13.6, *J*(H-C-P) = 8.9, 1H, OCH_2P´´b), 3.67 (m, 2H, H-2´´), 3.58 (d, *J*(H-C-P) = 8.7, 2H, OCH_2P´), 3.51 (m, 2H, H-3´´) ppm. ^{13}C NMR (DMSO-*d*$_6$): δ = 171.46 (C-6), 171.36 (C-4), 162.82 (C-2), 80.41 (d, *J*(2´´,P) = 9.9, C-2´´), 78.64 (C-5), 70.76 (d, *J*(2´,P) = 11.4, C-2´), 66.97 (d, *J*(C,P) = 160.4, OCH_2P), 65.93 (d, *J*(C,P) = 160.4, OCH_2P´´), 65.39 (C-1´´), 64.93 (C-1´), 60.33 (C-3´´) ppm. MS (ESI): m/z (%) = 434 (100) $[MH]^+$. For $C_{11}H_{21}N_3O_{11}P_2.H_2O$ (451.26) calcd. C 29.28, H 5.14, N 9.31, O 42.55, P 13.73; found C 29.47, H 4.98, N 9.14, P 13.77. $[\alpha]^{25}_D$ = +5.8 (c 0.625, H_2O).

2-Amino-4-[2-(phosphonomethoxy)ethoxy]-6-(2*R*)-[3-hydroxy-2-(phosphono-methoxy)propoxy]pyrimidine (**52d**)

From **51d** (700 mg, 1.05 mmol), freeze dried, white hydroscopic foam, yield 215 mg (45%). NMR spectra identical with compound **52c**. MS (ESI): m/z (%) = 434 (100) $[MH]^+$. For $C_{11}H_{21}N_3O_{11}P_2.H_2O$ (451.26) calcd. C 29.28, H 5.14, N 9.31, O 42.55, P 13.73; found C 29.37, H 4.97, N 9.44, P 13.92. $[\alpha]^{25}_D$ = -6.7 (c 0.341, H_2O).

2-Amino-4,6-(2*R*,2´*S*)-bis[2-(phosphonomethoxy)propoxy]pyrimidine (**52e**)

From **51e** (840 mg, 1.27 mmol), freeze dried, white hydroscopic foam, yield 280 mg (49%). ^{1}H NMR (DMSO-*d*$_6$): δ = 3.58 (s, 1H, H-5), 4.18 (dd, *J*gem = 11.1, *J*(1´a,2´) = 5.8, 2H, H-1´a), 4.12 (dd, *J*gem = 11.1, *J*(1´b,2´) = 4.4, 2H, H-1´b), 3.82 (m, 2H, H-2´), 3.60 (d, *J*(CH_2,P) = 9.3, 4H, PCH_2), 1.14 (d, *J*(H-3´,H-2´) = 6.4, 6H, H-3´) ppm. ^{13}C NMR (DMSO-*d*$_6$): δ = 171.40 (C-4, C-6), 162.77 (C-2), 78.54 (C-5), 74.96 (d, *J*(C-2´,P) = 11.4, C-2´), 68.58 (C-1´), 65.07 (d, *J*(CH_2,P) = 161.6, PCH_2), 16.80 (C-3´) ppm. MS (ESI): m/z (%) = 432.1 (100) $[MH]^+$, 454.1 (37) $[MNa]^+$. For $C_{12}H_{23}N_3O_{10}P_2.H_2O$ (449.29) calcd. C 32.08, H 5.61, N 9.35, O 39.17, P 13.79; found C 32.19, H 5.57, N 9.16, P 13.62. $[\alpha]^{25}_D$ = +0.9 (c 0.521, H_2O).

2-Amino-4-(2*S*)-[3-hydroxy-2-(phosphonomethoxy)propoxy]-6-(*2S*)-[2-(phosphono-methoxy)propoxy]pyrimidine (**52f**)

From **51f** (394 mg, 0.64 mmol), freeze dried, white hydroscopic foam, yield 200 mg (67%). ^{1}H NMR (DMSO-d_6): δ = 5.36 (s, 1H, H-5), 4.26 (dd, *J*gem = 11.3, *J*(1´´a,2´´) = 4.3, 1H, H-1´´a), 4.20 (dd, *J*gem = 11.3, *J*(1´´b,2´´) = 5.8, 1H, H-1´´b), 4.16 (dd, *J*gem = 11.1, *J*(1´a,2´) = 5.8, 1H, H-1´a), 4.12 (dd, *J*gem = 11.1, *J*(1´b,2´) = 4.4, 1H, H-1´b), 3.81 (m, 1H, H-2´), 3.71 (dd, *J*gem = 13.6, *J*(C-H-P) = 8.7, 1H, OCH_2,P´´a), 3.67 (dd, *J*gem = 13.6, *J*(C-H-P) = 8.9, 1H, OCH_2,P´´b), 3.67 (m, 1H, H-2´´), 3.60 (d, 2H, *J*(C-H-P) = 9.4, OCH_2,P´), 3.51 (m, 2H, H-3´´), 1.13 (d, *J*(3´,2´) = 6.4, 3H, H-3´) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.48, 171.42 (C-4, C-6), 162.83 (C-2), 80.40 (d, *J*(2´´,P) = 9.7, C-2´´), 78.59 (C-5), 75.08 (d, *J*(2´,P) = 11.7, C-2´), 68.63 (C-1´), 66.00 (d, *J*(P,C) = 161.0, PCH_2´´), 65.45 (C-1´´), 65.01 (d, *J*(P,C) = 161.8, PCH_2´), 60.35 (C-3´´), 16.83 (C-3´) ppm. MS (ESI): m/z (%) = 448.1 (100) $[MH]^+$. For $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (465.28) calcd. C 30.98, H 5.42, N 9.03, O 41.26, P 13.31; found C 30.82, H 5.39, N 8.83, P 13.15. $[\alpha]^{25}_D$ = +12.2 (c 0.181, H_2O).

2-Amino-4-(2*R*)-[3-hydroxy-2-(phosphonomethoxy)propoxy]-6-(*2S*)-[2-(phosphono-methoxy)propoxy]pyrimidine (**52g**)

From **51g** (360 mg, 0.58 mmol), freeze dried, white hydroscopic foam, yield 200 mg (73%). ^{1}H NMR (DMSO-d_6): δ = 5.36 (s, 1H, H-5), 4.25 (dd, *J*gem = 11.3, *J*(1´´a,2´´) = 4.3, 1H, H-1´´a), 4.20 (dd, *J*gem = 11.3, *J*(1´´b,2´´) = 5.8, 1H, H-1´´b), 4.17 (dd, *J*gem = 11.1, *J*(1´a,2´) = 5.8, 1H, H-1´a), 4.11 (dd, *J*gem = 11.1, *J*(1´b,2´) = 4.4, 1H, H-1´b), 3.81 (m, 1H, H-2´), 3.71 (dd, *J*gem = 13.6, *J*(C-H-P) = 8.7, 1H, OCH_2,P´´a), 3.67 (dd, *J*gem = 13.6, *J*(C-H-P) = 8.9, 1H, OCH_2,P´´b), 3.67 (m, 1H, H-2´´), 3.60 (d, *J*(C-H-P) = 9.4, 2H, OCH_2,P´), 3.51 (m, 2H, H-3´´), 1.13 (d, *J*(3´,2´) = 6.4, 3H, H-3´) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.48 and 171.42 (C-4, C-6), 162.83 (C-2), 80.40 (d, *J*(2´´,P) = 9.7, C-2´´), 78.59 (C-5), 75.08 (d, *J*(2´,P) = 11.7, C-2´), 68.63 (C-1´), 66.00 (d, *J*(P,C) = 161.0, PCH_2´´), 65.45 (C-1´´), 65.01 (d, *J*(P,C) = 161.8, PCH_2´), 60.35 (C-3´´), 16.83 (C-3´) ppm. MS (ESI): m/z (%) = 448.1 (100) $[MH]^+$, 470.1 (35) $[MNa]^+$. For $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (465.28) calcd. C 30.98, H

5.42, N 9.03, O 41.26, P 13.31; found C 30.68, H 5.42, N 8.96, P 13.25. $[\alpha]^{25}_{D}$ = +8.6 (c 0.561, H_2O).

2-Amino-4-(2*S*)-[3-hydroxy-2-(phosphonomethoxy)propoxy]-6-(*2R*)-[2-(phosphono-methoxy)propoxy]pyrimidine (**52h**)

From **51h** (960 mg, 1.55 mmol), freeze dried, white hydroscopic foam, yield 605 mg (83%). NMR spectra identical with compound **51g**. MS (ESI): m/z (%) = 448.2 (100) $[MH]^+$. For $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (465.28) calcd. C 30.98, H 5.42, N 9.03, O 41.26, P 13.31; found C 31.13, H 5.25, N 9.03, P 13.21. $[\alpha]^{25}_{D}$ = -4.4 (c 0.182, H_2O).

2-Amino-4-(2*R*)-[3-hydroxy-2-(phosphonomethoxy)propoxy]-6-(*2R*)-[2-(phosphonomethoxy)propoxy]pyrimidine (**52i**)

From **51i** (1 g, 1.62 mmol), freeze dried, white hydroscopic foam, yield 616 mg (81%). NMR spectra identical with compound **52f**. MS (ESI): m/z (%) = 448.0 (100) $[MH]^+$. For $C_{12}H_{23}N_3O_{11}P_2.H_2O$ (465.28) calcd. C 30.98, H 5.42, N 9.03, O 41.26, P 13.31; found C 30.79, H 5.47, N 8.93, P 13.14. $[\alpha]^{25}_{D}$ = -5.8 (c 0.312, H_2O).

2-Amino-4,6-(2*R*,2´*R*)-bis[2-(phosphonomethoxy)propoxy]pyrimidine (**52j**)

From **51l** (1.2 g, 1.8 mmol), white hydroscopic foam, yield 450 mg (55%). NMR spectra identical with compound **32b**. MS (ESI): m/z (%) = 432.0 (100) $[MH]^+$; 454.1 (14) $[MNa]^+$. For $C_{12}H_{23}N_3O_{10}P_2.H_2O$ (449.09) calcd. C 32.08, H 5.61, N 9.35, O 39.17, P 13.79; found C 32.26, H 5.45, N 9.40, P 14.06. $[\alpha]^{25}_{D}$ = -20.5 (c 0.254, H_2O).

2-Cyclopropylamino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**53a**)

Compound **51j** (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. ^{1}H NMR (DMSO-d_6): δ = 7.25 (br d, J(NH,CH) = 3.6, 1H, NH), 5.32 (s, 1H, H-5), 4.58 (m, 4H, P-OCH), 4.34 (m, 4H, C-1′), 3.79 (m, 4H, C-2′), 3.78 (d, J(OCH_2,P) = 8.3, 4H, PCH_2), 2.67 (m, 1H, CH cycloprop.), 1.23 (d, 12H) and 1.22 (d, J(CH_3,CH) = 6.3, 12H, CH_3), 0.62 (m, 2H) and 0.45 (m, 2H, CH_2 cycloprop.) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.99 2C (C-4, C-6), 162.51 (C-2), 78.66 (C-5), 70.80 (C-2′), 70.34 (d, 4C, J(CH,P) = 6.4, P-OCH), 64.96 (d, J(C-3′,P) = 164.5, P-OCH_2), 64.48 (C-1′), 23.87 (CH cycloprop.), 23.92 (CH_3), 6.34 (CH_2 cycloprop.) ppm. MS (FAB): m/z (%) = 612 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{25}H_{48}N_3O_{10}P_2$ $[MH]^+$ 612.2814, found 612.2813.

Diisopropyl esters were deprotected by GP2 to give **53a** (140 mg, 62%) as a white foam. ^{1}H NMR (D_2O): δ = 4.53 (m, 4H, H-1′), 3.98 (m, 4H, H-2′), 3.76 (d, J(P,CH_2) = 9.2, 4H, PCH_2), 2.72 (m, 1H, CH cycloprop.), 0.93 and 0.71 (2 × m, 2 × 2H, CH_2 cycloprop.) ppm. ^{13}C NMR (D_2O): δ = 171.05 (C-4, C-6), 159.38 (C-2), 78.79 (C-5), 70.85 (d, J(C-2′,P) = 10.4, C-2′), 68.23 (C-1′), 67.60 (d, J(CH_2,P) = 156.4, PCH_2), 23.41 (CH cycloprop.), 7.19 (CH_2 cycloprop.) ppm. MS (ESI): m/z (%) = 444.1 (100) $[MH]^+$, 466.0 (26) $[MNa]^+$. For $C_{13}H_{23}N_3O_{10}P_2.H_2O$ (461.30) calcd. C 33.85, H 5.46, N 9.11, O 38.15, P 13.43; found C 33.79, H 5.29, N 9.00, P 13.25.

2-Cyclopentylamino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**53b**)

Compound **51j** (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 ($CHCl_3$/MeOH 0–2%) to give 2-cyclopentylamino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine as an oil, yield 540 mg (54%). ^{1}H NMR ($CDCl_3$): δ = 5.37 (s, 1H, H-5), 4.88 (d, J(NH-1′) = 7.0, 1H, NH), 4.76 (dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.7, 4H, CHipr.), 4.39 (m, 4H, H-1′), 4.18 (m, 1H, H-1′′), 3.89 (m, 4H, H-2′), 3.82 (d, J(P,CH_2) = 8.2, 4H, PCH_2), 2.01 (m, 2H, H-2′′a), 1.71 and 1.61 (2 × m, 2 × 2H, H-3′′), 1.45 (m, 2H, H-2′′b), 1.33 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): 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, PCH_2), 64.76 (C-1′), 52.91 (C-1′′), 33.29 (C-2′′), 24.07 (d, J(CH_3-P) = 3.7) and

23.93 (d, *J*(CH_3-P) = 4.6, CH_3ipr.), 23.72 (C-3´´) ppm. MS (FAB): m/z (%) = 640.5 (60) $[MH]^+$. HR MS (FAB) calcd. for $C_{27}H_{52}N_3O_{10}P_2$ $[MH]^+$ 640.3128, found 640.3140.

Diisopropyl esters were cleaved by GP2 to afford **53b** (220 mg, 53%) as a white hydroscopic foam. ^{1}H 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*(CH_2P) = 8.7, 4H, PCH_2), 1.88 (m, 2H, H-2´´a), 1.65 (m, 2H, H-3´´a), 1.49 (m, 4H, H-2´´b and H-3´´b) ppm. ^{13}C NMR (DMSO-*d*$_6$): 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, PCH_2), 64.83 (C-1´), 52.60 (C-1´´), 32.44 (C-2´´), 23.71 (C-3´´) ppm. MS (FAB): m/z (%) = 472.1 (100) $[MH]^+$. For $C_{15}H_{27}N_3O_{10}P_2.H_2O$ (489.35) calcd. C 36.82, H 5.97, N 8.59, O 35.96, P 12.66; found C 37.00, H 5.87, N 8.49, P 12.36.

2-Methylamino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**53c**)

2-Methylsulfonyl derivative **51j** (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-(diisopropoxyphosphoryl-methoxy)ethoxy]pyrimidine, yield 440 mg (48%). ^{1}H NMR (DMSO-*d*$_6$): δ = 6.97 (br q, *J*(NH,CH_3) = 4.7, 1H, NH), 5.28 (s, 1H, H-5), 4.59 (dh, *J*(CH,CH_3) = 6.2, *J*(CH,P) = 7.8, 4H, CHipr.), 4.33 (m, 4H, H-1´), 3.78 (m, 8H, H-2´, H-3´), 2.75 (d, *J*(CH_3,NH) = 4.7, 3H, CH_3NH) ppm. ^{13}C NMR (DMSO-*d*$_6$): δ = 170.82 (C-2), 161.74 (C-4, C-6), 77.83 (C-5), 70.36 (m, CHipr.), 64.97 (d, *J*(CH_2,P) = 164.2, CH_2P), 64.49 (C-1´), 27.86 (CH_3NH), 23.93 m (CH_3ipr.) ppm. MS (FAB): m/z (%) = 586.2 (75) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{46}N_3O_{10}P_2$ $[MH]^+$ 586.2658, found 586.2659.

Diisopropyl esters were deprotected by GP2 to give **53c** (210 mg, 67%) as a white hydroscopic foam. ^{1}H NMR (D_2O): δ = 4.54 (m, 4H, H-1´), 3.98 (m, 4H, H-2´), 3.77 (d, *J*(CH_2,P) = 9.2, 4H, PCH_2), 3.00 (s, 3H, CH_3) ppm. ^{13}C NMR (D_2O): δ = 173.02 (C-4, C-6), 162.95 (C-2), 70.72 d (C-2´), 69.21 (C-1´), 67.20 (d, PCH_2), 28.21 (CH_3) ppm. MS (FAB): m/z (%) = 418 (100) $[MH]^+$. For $C_{11}H_{21}N_3O_{10}P_2.H_2O$ (435.26)

calcd. C 30.35, H 5.33, N 9.65, O 40.43, P 14.23, found C 30.25, H 5.26, N 9.47, P 14.09.

2-Benzylamino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**53d**)

Pyrimidine **51j** (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. ^{1}H 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´, PCH_2), 1.23 (d, J(CH_3,CH) = 6.2, 12H) and 1.21 (d, J(CH_3,CH) = 6.2, 12H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): 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, CHipr.), 64.95 (d, J(CH_2,P) = 164.3, 2C, PCH_2), 64.58 (2C, C-1´), 44.39 (C-1´´), 24.00 (d, J(CH_3,P) = 3.6, 4C) and 23.84 (d, J(CH_3,P) = 4.3, 4C, CH_3ipr.) ppm. MS (FAB): m/z (%) = 662 (25) $[MH]^+$. HR MS (FAB) calcd. for $C_{29}H_{50}N_3O_{10}P_2$ $[MH]^+$ 662.2971, found 662.2981.
The intermediate was treated with bromotrimethylsilane (GP2) to give **53d**, white hydroscopic foam, yield 120 mg (42%). ^{1}H 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, PCH_2) ppm. ^{13}C 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(CH_2,P) = 160.6, PCH_2), 64.97 (C-1´), 44.41 (C-1´´) ppm. MS (FAB): m/z (%) = 494 (100) $[MH]^+$, 516 (35) $[MNa]^+$. For $C_{17}H_{25}N_3O_{10}P_2.H_2O$ (511.36) calcd. C 39.93, H 5.32, N 8.22, O 34.42, P 12.11; found C 39.80, H 5.13, N 8.03, P 11.93.

2-(4-Methoxybenzyl)amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**53e**)

2-Methylsulfonyl derivative **51j** (1 g, 1.57 mmol) in dry THF (30 ml) was treated with 4-methoxybenzylamine (0.61 ml, 4.1 mmol) and the reaction mixture was

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

Diisopropyl esters were deprotected by GP2. The final product was applied onto a column of Dowex 50 × 8 in Na^+ form and eluted with water to give **53e** (160 mg, 23%) as a tetrasodium salt; white hydroscopic foam. ^{1}H NMR (DMSO-d_6): δ = 7.60 (br t, *J*(NH,CH_2) = 6.4, NH), 7.23 (m, 2H, H-2′′), 6.85 (m, 2H, H-3′′), 5.36 (d, 1H, H-5), 4.33 (d, *J*(CH_2,NH) = 6.0, 2H, CH_2N), 4.25 (m, 4H, H-1′), 3.70 (s, 3H, OCH_3), 3.70 (m, 4H, H-2′), 3.50 (d, 4H, *J*(H,P) = 8.6, PCH_2). ^{13}C NMR (DMSO-d_6): 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, PCH_2), 64.97 (C-1′), 55.21 (OCH_3), 43.80 (CH_2N). MS (ESI): m/z (%) = 524.1 (100) $[MH]^+$, 546.1 (69) $[MNa]^+$. For $C_{18}H_{23}N_3Na_4O_{11}P_2$ (611.29) calcd. C 35.37, H 3.79, N 6.87, Na 15.04, O 28.79, P 10.13; found C 35.09, H 3.98, N 6.71, P 10.12.

2-Morpholino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**53f**)

Compound **51j** (1 g, 1.57 mmol) in dry THF (30 ml) was treated with morpholine (1.4 ml) and the mixture was refluxed in a sealed flask for 2 h and the solvent was removed under reduced pressure. Flash chromatography ($CHCl_3$/MeOH 0–1%) gave thick oil of 2-morpholino-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]-pyrimidine (720 mg, 71%). ^{1}H NMR ($CDCl_3$): δ = 5.39 (s, 1H, C-5), 4.76 (dh,

J(CH,CH_3) = 6.2, *J*(CH,P) = 7.7, 4H, CHipr.), 4.41 (m, 4H, H-1´), 3.90 (m, 4H, H-2´), 3.82 (d, *J*(CH_2,P) = 8.2, 4H, PCH_2), 3.72 (s, 8H, H-2´´, H-3´´), 1.34 (d, *J*(CH_3,CH) = 6.3, 12H) and 1.33 (d, *J*(CH_3,CH) = 6.3, 12H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): 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, PCH_2), 64.80 (C-1´), 44.24 (C-2´´), 24.07 (d, *J*(CH_3,P) = 3.5) and 23.94 (d, *J*(CH_3,P) = 4.5, CH_3ipr.) ppm. MS (FAB): m/z (%) = 642.5 (22) $[MH]^+$. HR MS (FAB) calcd. for $C_{26}H_{50}N_3O_{11}P_2$ $[MH]^+$ 642.2921, found 642.2911.

Deprotection of diisopropyl esters by GP2 gave **53f** (360 mg, 65%) as a white hydroscopic foam. ^{1}H 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, CH_2 – morpholine), 3.57 (d, *J*(P,CH) = 8.7, 4H, PCH_2) ppm. ^{13}C NMR (DMSO-d_6): δ = 171.13 (2C, C-4, C-6), 160.51 (C-2), 78.84 (C-5), 70.62 (d, *J*(C-2,P) = 11.2, C-2´), 66.93 (d, *J*(C-3,P) = 160.4, PCH_2), 66.13 (C-3´´), 65.10 (C-1´), 44.13 (C-2´´) ppm. MS (ESI): m/z (%) = 474 (86) $[MH]^+$. For $C_{14}H_{25}N_3O_{11}P_2.H_2O$ (491.31) calcd. C 34.22, H 5.54, N 8.55, O 39.08, P 12.61; found C 34.32, H 5.55, N 8.47, P 12.47.

4,6-Bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]-2-hydroxypyrimidine (**54a**)

Solution of NaOH (0.072 g) in water (10 ml) was added in one portion to the 2-methylsulfonyl derivative **51j** (1 g, 1.57 mmol) in THF (5 ml) and the resulting mixture was heated at 60 °C for 1hr. Reaction mixture was cooled to r.t., neutralized with acetic acid and volatiles were removed in vacuo. The residue was partioned between $CHCl_3$ and water. Organic fraction was washed with water (3 × 50 ml) and dried with $MgSO_4$. Flash chromatography ($CHCl_3$/MeOH 0–5%) gave colorless oil, 450 mg (50%). ^{1}H NMR ($CDCl_3$): δ = 5.29 (s, 1H, H-5), 4.76 (dh, *J*(CH,P) = 7.7, *J*(CH,CH_3) = 6.2, CHipr.), 4.38 (m, 4H, H-1´), 3.92 (m, 4H, H-2´), 3.81 (d, *J*(CH_2,P) = 8.2, PCH_2), 1.34 and 1.33 (2 × d, *J*(CH_3,CH) = 6.2, 2 × 12H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 162.57 (C-4,6),158.72 (C-2), 75.35 (C-5), 71.18 (d, *J*(C,P) = 6.6, CHipr.), 70.59 (d, *J*(C,P) = 10.6, C-2´), 67.29 (C-1´), 66.08 (d, *J*(C,P) = 167.6, PCH_2), 24.05 (d, *J*(C,P) = 4.0, CH_3ipr.), 23.95 (d, *J*(C,P) = 4.6, CH_3ipr.) ppm. MS (FAB): m/z (%) = 573 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{22}H_{43}N_2O_{11}P_2$ $[MH]^+$ 573.2342, found 573.2347.

2-Methoxy-4,6-bis[2-(diisopropoxyphosphorylmethoxy)ethoxy]pyrimidine (**54b**)

51j (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 **54b** (600 mg, 32%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 5.71 (s, 1H, H-5), 4.76 (dh, *J*(CH,CH_3) = 6.2, *J*(H,C,P) = 7.7, 4H, CHipr), 4.48 (m, 4H, H-1′), 3.94 (s, 3H, OCH_3), 3.92 (m, 4H, H-2′), 3.82 (d, *J*(H,C,P) = 8.2, 4H, OCH_2P), 1.33 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.99 (C-4, C-6), 164.52 (C-2), 84.20 (C-5), 71.08 (m, CHipr., H-2′), 65.97 (d, *J*(C,P) = 167.4, OCH_2P), 65.51 (C-1′), 54.65 (OCH_3), 24.05 (d, *J*(C,C,O,P) = 3.7) and 23.91 (d, *J*(C,C,O,P) = 4.6, CH_3ipr.) ppm. MS (FAB): m/z (%) = 587 (62) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{45}N_2O_{11}P_2$ $[MH]^+$ 587.2498, found 587.2516.

4,6-Bis[2-(phosphonomethoxy)ethoxy]pyrimidine (**55**)

Compound **46** (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 ($CHCl_3$/MeOH 0–5%) to give 4,6-bis[2-(diisopropoxy-phosphorylmethoxy)ethoxy]pyrimidine as a thick oil (1.5 g, 81%). ^{1}H NMR (DMSO-d_6): δ = 8.38 (d, *J*(H-2,H-5) = 0.9, 1H, H-2), 6.05 (d, *J*(H-5,H-2) = 0.9, 1H, H-5), 4.76 (dh, *J*(CH,CH_3) = 6.2, *J*(CH,P) = 7.7, 4H, CHipr.), 4.50 (m, 4H, H-1′), 3.93 (m, 4H, H-2′), 3.83 (d, *J*(H,C,P) = 8.2, 4H, $POCH_2$), 1.33 (m, 24H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.51 (C-4, C-6), 157.21 (C-2), 91.15 (C-5), 71.12 (d, *J*(2′,P) = 10.8, C-2′), 71.09 (d, *J*(CH,P) = 6.7, CHipr.), 65.99 (d, *J*(C,P) = 167.3, $POCH_2$), 65.52 (C-1′), 24.05 (d, *J*(CH_3,P) = 3.7) and 23.93 (d, *J*(CH_3,P) = 4.6, CH_3ipr.) ppm. MS (ESI): m/z (%) = 579.2 (100) $[MNa]^+$. For $C_{22}H_{42}N_2O_{10}P_2$ (556.52) calcd. C 47.48, H 7.61, N 5.03, O 28.75, P 11.13; found C 47.32, H 7.61, N 4.80, P 11.16.

The intermediate was deprotected by bromotrimethylsilane (GP2) to give **55** (350 mg, 48%), freeze dried, white hydroscopic foam. ^{1}H NMR (DMSO-d_6): δ = 8.44 (d,

J(H-2,H-5) = 0.9, 1H, H-2), 6.28 (d, *J*(H-5,H-2) = 0.9, 1H, H-5), 4.39 (m, 4H, H-1′), 3.80 (m, 4H, H-2′), 3.57 (d, *J*(H,C,P) = 8.7, 4H, OCH_2P) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.70 (C-4, C-6), 157.79 (C-2), 90.36 (C-5), 70.46 (d, *J*(2′,P) = 11.0, C-2′), 67.10 (d, *J*(C,P) = 160.2, PCH_2), 66.01 (C-1′) ppm. MS (ESI): m/z (%) = 389 (76) $[MH]^+$. For $C_{10}H_{18}N_2O_{10}P_2.H_2O$ (406.22) calcd. C 29.57, H 4.96, N 6.90, O 43.32, P 15.25; found C 29.72, H 4.78, N 6.86, P 15.08.

2-Amino-4,6-disulfanylpyrimidine (**56**)

Thiourea (9.5 g, 90 mmol) was added to the solution of dichloropyrimidine **8** (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 (DMSO-d_6): δ = 11.2–11.8 (br s, 2H, SH), 7.17 (br s, 2H, NH_2), 6.24 (s, 1H, H-5) ppm. ^{13}C NMR (DMSO-d_6): δ = 173.91 (2C, C-4, C-6), 149.10 (C-2), 116.36 (C-5) ppm. MS (EI): m/z (%) = 159.2 (100) $[M]^+$. For $C_4H_5N_3S_2$ (159.23) calcd. C 30.17, H 3.16, N 26.39, S 40.27; found C 30.12, H 3.00, N 26.08, S 40.39.

2-Amino-4,6-bis{[2-(diisopropoxyphosphorylmethoxy)ethyl]sulfanyl}pyrimidine (**57a**) and 2-amino-4,6-bis{[2-(phosphonomethoxy)ethyl]sulfanyl}pyrimidine (**58a**)

Phosphonate **43a** (6.9 g, 26.4 mmol) was added to a stirred mixture of disulfanyl pyrimidine **56** (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 ($CHCl_3$/MeOH 0–5%) to give **57a** (5.6 g, 74%), pale yellow oil. ^1H NMR (DMSO-d_6): δ = 6.74 (br s, 2H, NH_2), 6.41 (s, 1H, H-5), 4.58 (m, 4H, CHipr.), 3.79 (d, *J*(P,CH) = 8.3, 4H, PCH_2), 3.69 (t, *J*(1′,2′) = 6.4, 4H, H-1′), 3.26 (t, *J*(2′,1′) = 6.4, 4H, H-2′), 1.24 (d, 12H) and 1.23 (d, *J*(CH_3,CH) = 6.2, 12H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.60 (2C, C-4, C-6), 161.79 (C-2), 103.07 (C-5), 71.18 (d, *J*(P,C) = 12.2, 2C, C-2′), 70.35 (d, *J*(P,C) = 6.3, 4C, CHipr.), 64.81 (d,

J(P,C) = 164.6, 2C, PCH_2), 27.60 (2C, C-1´), 24.03 (d, *J*(P,C) = 3.9, 4C) and 23.92 (d, *J*(P,C) = 4.4, 4C, CH_3) ppm. MS (ESI): m/z (%) = 604.2 (100) $[MH]^+$. For $C_{22}H_{43}N_3O_8P_2S_2$ (603.67) calcd. C 43.77, H 7.18, N 6.96, O 21.20, P 10.26, S 10.62; found C 43.64, H 7.22, N 7.19, P 9.99, S 10.80.

Subsequent deprotection of **57a** (2.6 g, 4.3 mmol) by GP2 gave free phosphonic acid **58a** (1.2 g, 65%) as a white foam. ^{1}H NMR (D_2O): δ = 6.75 (s, 1H, H-5), 3.97 (t, *J*(2´,1´) = 6.4, 4H, H-2´), 3.80 (d, *J*(P,CH) = 8.7, 4H, PCH_2), 3.45 (t, *J*(1´,2´) = 6.4, 4H, H-1´) ppm. ^{13}C NMR (D_2O): δ = 169.49 (2C, C-4, C-6), 161.20 (C-2), 104.15 (C-5), 70.59 (d, *J*(P,C) = 10.7, 2C, C-2´), 66.82 (d, *J*(P,C) = 156.2, 2C, PCH_2), 28.38 (2C, C-1´) ppm. MS (ESI): m/z (%) = 436 (35) $[MH]^+$. For $C_{10}H_{19}N_3O_8P_2S_2$ (435.35) calcd. C 27.59, H 4.40, N 9.65, O 29.40, P 14.23, S 14.73; found C 27.69, H 4.69, N 9.61, P 14.12, S 14.59.

2-Amino-4,6-(2*S*,2´*S*)-bis{[2-(diisopropoxyphosphorylmethoxy)propyl]sulfanyl}-pyrimidine (**57b**) and 2-amino-4,6-(2*S*,2´*S*)-bis{[2-(phosphonomethoxy)propyl]-sulfanyl}pyrimidine (**58b**)

Prepared by the same procedure as compounds **57a** and **58a**. From pyrimidine **56** (200 mg, 1.25 mmol) and phosphonate **43b** (1.06 g, 2.6 mmol). Thick oil, 534 mg (68%). ^{1}H NMR (DMSO-d_6): δ = 6.69 (s, 2H, NH_2), 6.14 (s, 1H, H-5), 4.59 (m, 4H, CHipr.), 3.78 (d, *J*(P,CH) = 9.2, 4H, PCH_2), 3.74 (m, 2H, H-2´), 3.26 (dd, *J*(1´a,2´) = 5.5, *J*gem = 13.6, 2H, H-1´a), 3.20 (dd, *J*(1´b,2´) = 5.8, *J*gem = 13.6, 2H, H-1´b), 1.24 (d, 12H), 1.23 (d, 6H) and 1.16 (d, *J*(CH_3,CH) = 6.2, 6H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.63 (2C, C-4,6), 161.64 (C-2), 103.30 (C-5), 76.19 (d, *J*(P,C) = 12.7, 2C, C-2´), 70.30 (d, *J*(P,C) = 6.3, 4C, CHipr.), 62.84 (d, *J*(P,C) = 165.0, 2C, PCH_2), 33.21 (2C, C-1´), 24.02 (d, *J*(P,C) = 3.6, 4C) and 23.88 (d, *J*(P,C) = 4.6, 4C, CH_3ipr.), 18.73 (2C, C-3´) ppm. MS (ESI): m/z (%) = 654.2 (100) $[MNa]^+$.

Phosphonic acid **58b**, yield (280 mg, 73%), white foam. ^{1}H NMR (D_2O): δ = 6.81 (s, 1H, H-5), 3.94 (m, 2H, H-2´), 3.77 (dd, 2H) and 3.65 (dd, *J*(P,CH) = 9.3, *J*gem = 13.2, 2H, PCH_2), 3.38 (dd, 2H) and 3.35 (dd, *J*(1´,2´) = 5.5, *J*gem = 13.6, 2H, H-1´), 1.29 (d, *J*(3´,2´) = 6.2, 6H, H-3´) ppm. MS (ESI): m/z (%) = 464.0 (100) $[MH]^+$. For $C_{12}H_{23}N_3O_8P_2S_2.H_2O$ (481.4) calcd. C 29.94, H 5.23, N 8.73, O 29.91, P 12.87, S

13.32; found C 30.15, H 5.11, N 8.54, P 12.65, S 13.50. $[\alpha]^{25}_D$ = +48.3 (c 0.357, H_2O).

2-Amino-4,6-(2*R*,2′*R*)-bis{[2-(diisopropoxyphosphorylmethoxy)propyl]sulfanyl}-pyrimidine (**57c**) and 2-amino-4,6-(2*R*,2′*R*)-bis{[2-(phosphonomethoxy)propyl]-sulfanyl}pyrimidine (**58c**)

Prepared by the same procedure as compounds **57a** and **58a**. From pyrimidine **56** (500 mg, 3.1 mmol) and phosphonate **43c** (2.65 g, 6.5 mmol). Thick oil, 1.48 g (75%). NMR spectra identical with compound **57b**. MS (ESI): m/z (%) = 654.0 (100) $[MNa]^+$.

Phosphonic acid **58c**, yield 702 mg (71%), white foam. NMR spectra identical with compound **58b**. MS (ESI): m/z (%) = 464.0 (100) $[MH]^+$. For $C_{12}H_{23}N_3O_8P_2S_2.H_2O$ (481.4) calcd. C 29.94, H 5.23, N 8.73, O 29.91, P 12.87, S 13.32; found C 30.10, H 5.25, N 8.65, P 12.67, S 13.28. $[\alpha]^{25}_D$ = -40.2 (c 0.589, H_2O).

2-Amino-6-{[2-(diisopropoxyphosphorylmethoxy)ethyl]sulfanyl}-4-sulfanyl-pyrimidine (**59a**)

To the solution of pyrimidine **56** (3 g, 18.84 mmol) and NaH (0.76 g, 60% in paraffin oil, 19 mmol) in DMF (70 ml) phosphonate **43a** was added dropwise (5 g, 19 mmol). The resulting mixture was stirred at r.t. for 3 days and taken down in vacuo. The residue in $CHCl_3$ (200 ml) was washed with water (3 × 100 ml), dried over $MgSO_4$ and taken down under reduced pressure. The residue was separated by flash chromatography ($CHCl_3$/MeOH 0–5%) to give **57a** (3.18 g, 28%) and **59a** (2.92 g, 40%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6): δ = 11.90 (br s, 1H, SH), 7.00 (br s, 2H, NH_2), 6.33 (s, 1H, H-5), 4.59 (m, 2H, CHipr.), 3.78 (d, *J*(P,CH) = 8.3, 2H, PCH_2), 3.71 and 3.22 (2 × t, *J*(1′,2′) = 6.3, 2 × 2H, H-1′, H-2′), 1.24 (d, 6H) and 1.23 (d, *J*(CH_3,CH) = 6.2, 6H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 177.97 (C-4), 167.60 (C-2), 154.04 (C-6), 109.86 (C-5), 70.24 (d, *J*(P,C) = 12.2, C-2′), 70.36 (d, 2C, *J*(P,C) = 6.3, CHipr.), 64.83 (d, *J*(P,C) = 164.1, PCH_2), 28.33 (C-1′), 23.98 (d, 2C, *J*(P,C) = 3.9) and 23.91 (d, 2C, *J*(P,C) = 4.4, CH_3) ppm. MS (FAB): m/z (%) =

382 (100) $[MH]^+$. For $C_{13}H_{24}N_3O_4PS_2$ (381.45) calcd. C 40.93, H 6.34, N 11.02, O 16.78, P 8.12, S 16.81; found C 40.81, H 6.52, N 10.86, P 8.01, S 17.02.

2-Amino-6-(*2S*)-{[2-(diisopropoxyphosphorylmethoxy)propyl]sulfanyl}-4-sulfanyl-pyrimidine (**59b**)

To the solution of pyrimidine **56** (1 g, 6.3 mmol) and NaH (0.252 g, 60% in paraffin oil, 6.3 mmol) in DMF (25 ml) phosphonate **43b** wad added dropwise (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 **57b** (750 mg, 19%) and **59b** (780 mg, 31%) as an thick oil. ^{1}H NMR (DMSO-d_6): δ = 11.88 (br s, 1H, SH), 7.00 (br s, 2H, NH_2), 6.33 (s, 1H, H-5), 4.59 (m, 2H, CHipr.), 3.79 (dd, 1H) and 3.75 (dd, *J*gem = 13.8, *J*(P,CH) = 9.2, 1H, PCH_2), 3.74 (m, 1H, H-2´), 3.22 (dd, *J*(1´a,2´) = 5.4, *J*gem = 13.6, 1H, H-1´a), 3.17 (dd, *J*(1´b,2´) = 5.8, *J*gem = 13.6, 1H, H-1´b), 1.24 (d, 6H), 1.235 (d, 6H) and 1.18 (d, 3H, *J*(CH_3,CH) = 6.2, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 178.56 (C-4), 167.86 (C-2), 153.95 (C-6), 109.94 (C-5), 75.97 (d, *J*(P,C) = 12.8, C-2´), 70.37 and 70.34 (d, *J*(P,C) = 6.3, CHipr.), 62.78 (d, *J*(P,C) = 165.6, PCH_2), 34.35 (d, *J*(P,C) = 3.9, C-1´), 24.04 (d, 2C, *J*(P,C) = 3.0) and 23.90 (2C, *J*(P,C) = 4.6, CH_3), 18.17 (C-3´) ppm. MS (ESI): m/z (%) = 418 (100) $[MNa]^+$. HR MS (FAB) calcd. for $C_{14}H_{27}N_3O_4PS_2$ $[MH]^+$ 396.1180, found 396.1176.

2-Amino-4-{[2-(diisopropoxyphosphorylmethoxy)ethyl]sulfanyl}-6-(*2S*)-{[2-(diiso-propoxyphosphorylmethoxy)propyl]sulfanyl}pyrimidine (**60a**) and 2-amino-4-{[2-(phosphonomethoxy)ethyl]sulfanyl}-6-(*2S*)-{[2-(phosphonomethoxy)propyl]-sulfanyl}pyrimidine (**61a**)

Monoderivative **59a** (300 mg, 0.79 mmol), NaH (0.035 g, 60% in paraffin oil, 0.87 mmol) and phosphonate **43b** (0.35 g, 0.86 mmol) in DMF (10 ml) was stirred at 60 °C for 4hr and taken down in vacuo. The residue was treated with hot chloroform and filtered, and the filtrate was evaporated in vacuo. Flash chromatography afforded **60a** (400 mg, 82%) as an oil. ^{1}H NMR (DMSO-d_6): δ = 6.70 (br s, 2H, NH_2), 6.41 (s, 1H, H-5), 4.59 (m, 4H, CHipr.), 3.775 (d, 2H) and 3.77 (d, *J*(P,CH) = 8.4, 2H, PCH_2), 3.76 (m, 1H, H-2´´), 3.71 (t, *J*(2´,1´) = 5.4, 2H, H-2´), 3.28 (dd, *J*(1´´a,2´´) =

6.1, *J*gem = 13.6, 1H, H-1´´a), 3.26 (t, *J*(1´,2´) = 5.4, 2H, H-1´), 3.20 (dd, *J*(1´´b,2´´) = 5.8, *J*gem = 13.6, 1H, H-1´´b), 1.245 (d, 12H) and 1.23 (d, *J*(CH_3,CH) = 6.2, 12H, CH_3ipr.), 1.18 (d, *J*(CH_3,CH) = 6.2, 3H, H-3´´) ppm. ^{13}C NMR (DMSO-d_6): δ = 167.71 and 167.47 (C-4,6), 161.70 (C-2), 103.83 (C-5), 75.70 (d, *J*(P,C) = 11.7, C-2´´), 71.08 (d, 4C) and 70.64 (d, 4C, *J*(P,C) = 6.3, CHipr.), 70.03 (d, *J*(P,C) = 10.7, C-2´), 64.91 (d) and 62,05 (d, *J*(P,C) = 164.5, PCH_2), 32.10 (C-1´´), 27.60 (C-1´), 24.66 (d, 4C) and 24.18 (d, 4C, *J*(P,C) = 3.9), 23.59 (d, 4C) and 23.32 (d, 4C, *J*(P,C) = 4.4, CH_3), 17.20 (C-3´´) ppm. MS (FAB): m/z (%) = 618.2 (100) $[MH]^+$. HR MS (FAB) calcd. for $C_{23}H_{46}N_3O_8P_2S_2$ $[MH]^+$ 618.2203, found 618.2205.

Diisopropylester **60a** was deprotected by GP2 to give **61a** (100 mg, 53%) as a white foam. ^{1}H NMR (D_2O): δ = 6.79 (s, 1H, H-5), 3.94 (m, 1H, H-2´´), 3.87 (t, *J*(2´,1´) = 6.1, 2H, H-2´), 3.74 (dd, *J*(P,CH) = 9.2, *J*gem = 13.3, 1H) and 3.70 (d, *J*(P,CH) = 8.9, 2H) and 3.66 (dd, *J*(P,CH) = 9.4, *J*gem = 13.3, 1H, PCH_2), 3.38 (t, *J*(1´,2´) = 6.1, 2H, H-1´), 3.36 (dd, *J*(1´´a,2´´) = 5.4, *J*gem = 14.4, 1H, H-1´´a), 3.32 (dd, *J*(1´´b,2´´) = 6.1, *J*gem = 14.4, 1H, H-1´´b), 1.29 (d, *J*(3´´,2´´) = 6.3, 3H, H-3´´). MS (ESI): m/z (%) = 450 (100) $[MH]^+$. For $C_{11}H_{21}N_3O_8P_2S_2.H_2O$ (467.39) calcd. C 28.27, H 4.96, N 8.99, O 30.81, P 13.25, S 13.72; found C 28.39, H 4.91, N 9.11, P 13.12, S 13.69. $[\alpha]^{25}_D$ = +32.5 (c 0.142, H_2O).

2-Amino-4-{[2-(diisopropoxyphosphorylmethoxy)ethyl]sulfanyl}-6-(*2R*)-{[2-(diisopropoxyphosphorylmethoxy)propyl]sulfanyl}pyrimidine (**60b**) and 2-amino-4-{[2-(phosphonomethoxy)ethyl]sulfanyl}-6-(*2R*)-{[2-(phosphonomethoxy)propyl]-sulfanyl}pyrimidine (**61b**)

Prepared by the same procedure as compounds **60a** and **61a** from pyrimidine **59a** and phosphonate **43c**.

60b: thick oil, yield 390 mg (80%). NMR spectra identical with compound **60a**. 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.

61b: white foam, yield 95 mg (50%). NMR spectra identical with compound **61a**. MS (ESI): m/z (%) = 450 (100) $[MH]^+$; 472 (50) $[MNa]^+$. For $C_{11}H_{21}N_3O_8P_2S_2.H_2O$ (467.39) calcd. C 28.27, H 4.96, N 8.99, O 30.81, P 13.25, S 13.72; found C 28.14, H 4.72, N 8.87, P 13.29, S 13.80. $[\alpha]^{25}_D$ = -19.3 (c 0.216, H_2O).

2-Amino-4,6-(2*R*,2´*S*)-bis{[2-(diisopropoxyphosphorylmethoxy)propyl]sulfanyl-pyrimidine (**60c**) and 2-amino-4,6-(2*R*,2´*S*)-bis{[2-(phosphonomethoxy)propyl]-sulfanyl}pyrimidine (**61c**)

Prepared by the same procedure as compounds **60a** and **61a** from pyrimidine **59b** (400 mg, 1.01 mmol) and phosphonate **43c** (0.45 g, 1.1 mmol).

60c: thick oil, yield 440 mg (71%). NMR spectra identical with compound **57b**. MS (ESI): m/z (%) = 654.0 (100) $[MNa]^+$.

61c: white foam, yield 180 mg (61%). ^{1}H NMR (D_2O): δ = 6.86 (s, 1H, H-5), 3.95 (m, 2H, H-2´), 3.79 (dd, *J*gem = 13.2, *J*(CH_2,P) = 9.3, 2H) and 3.68 (dd, *J*gem = 13.2, *J*(CH_2,P) = 9.5, 2H, PCH_2), 3.43 (dd, *J*gem = 14.3, *J*(1,2) = 4.4, 2H) and 3.33 (dd, *J*gem = 14.3, *J*(1,2) = 6.2, 2H, H-1´), 1.30 (d, *J*(CH_3,2´) = 6.7, 6H, H-3´) ppm. MS (ESI): m/z (%) = 464.0 (100) $[MH]^+$. For $C_{12}H_{23}N_3O_8P_2S_2.H_2O$ (481.4) calcd. C 29.94, H 5.23, N 8.73, O 29.91, P 12.87, S 13.32; found C 30.07, H 5.03, N 8.52, P 13.01, S 13.24. $[\alpha]^{25}_D$ = +0.3 (c 0.358, H_2O).

2-Amino-4,6-bis(2-hydroxyethoxy)pyrimidine (**62**)

A solution of *t*-BuOK (13.5 g, 120 mmol) in ethyleneglycol (50 ml) was heated at 80 °C for 30 min. and dichloropyrimidine **8** (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 × 8; the resulting mixture was diluted with water (100 ml), applied onto a column of Dowex 50 × 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. ^{1}H 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) $[MH]^+$. For $C_8H_{13}N_3O_4$ (215.21) calcd. C 44.65, H 6.09, N 19.53, O 29.74; found C 44.50, H 6.04, N 19.23.

Hexadecyloxyethyl toluenesulfonyloxymehylphosphonate, sodium salt (**63**)

Prepared by previously described procedure;[80] yield 47%. ^{1}H NMR ($CDCl_3$): δ = 7.77 (br d, *J*(CH,CH) = 5.81, 2H, CHarom.), 7.30 (br d, *J*(CH,CH) = 6.0, 2H, CHarom.), 4.07 (br, 2H, PCH_2), 3.97 (br, 2H, OCH_2), 3.45 (br, 2H, OCH_2), 3.34 (br, 2H, OCH_2), 2.38 (s, 3H, CH_3arom.), 1.48 (br, 2H, $OCH_2\underline{CH_2}CH_2$), 1.25 (br s, 26H, 13 × CH_2), 0.87 (t, *J*(CH_3,CH_2) = 6.83, $\underline{CH_3}CH_2$) ppm. MS (ESI): m/z (%) = 579 (100) $[MNa]^+$. For $C_{26}H_{46}NaO_7PS$ (556.67) calcd. C 56.10, H 8.33, Na 4.13, O 20.12, P 5.56, S 5.76; found C 55.92, H 8.58, P 5.31, S 5.93.

2-(Hexadecyloxy)ethyl 2-amino-4-(2-hydroxyethoxy)-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine, sodium salt (**64a**)

GP8, white solid (47 mg, 31%), m.p. 129 °C. ^{1}H 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 ($CH_3CH_2\underline{CH_2}$), 29.52, 29.25, 29.15, 28.92 and 25.90 (alif.), 22.31 ($CH_3\underline{CH_2}$), 14.18 (CH_3) ppm. MS (ESI): m/z (%) = 598.4 (80) $[M-H]^-$. For $C_{27}H_{51}N_3NaO_8P$ (599.67) calcd. C 54.08, H 8.57, N 7.01, Na 3.83, O 21.34, P 5.17; found C 53.89, H 9.15, N 7.25, P 5.01.

Bis[2-(Hexadecyloxy)ethyl] 2-amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimi-dine, sodium salt (**65a**)

GP8, white solid (59 mg, 24%), m.p. 184 °C. MS (ESI): m/z (%) = 962.5 (88) $[M-Na+H]^+$. For $C_{46}H_{89}N_3Na_2O_{12}P_2$ (984.14) calcd. C 56.14, H 9.12, N 4.27, Na 4.67, O 19.51, P 6.29; found C 56.00, H 9.39, N 3.99, P 6.03.

2-(Hexadecyloxy)ethyl 2-amino-5-bromo-4-(2-hydroxyethoxy)-6-[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**64b**)

GP8, white solid (44 mg, 26%), m.p. 83 °C. ^{1}H NMR (DMSO-d_6): δ = 6.72 (br s, 2H, NH_2), 4.86 (br s, 1H, OH), 4.34 (m, 2H, H-1′), 4.25 (m, 2H, H-1′′), 3.61– 3.80 (m, 6H, H-2′, 4′, 2′′), 3.38 (m, 6H, 2 × OCH_2, PCH_2), 1.44 (m, 2H, H-7′), 1.22 (m, 26H, alif.), 0.84 (t, J(CH_3,CH_2) = 6.8, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): 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 ($CH_3CH_2\underline{CH_2}$), 29.54, 29.22, 29.18, 28.94, 25.91 (alif.), 22.33 ($CH_3\underline{CH_2}$), 14.19 (CH_3) ppm. MS (ESI): m/z (%) = 654 (100) [M-Na]$^-$. For $C_{27}H_{50}BrN_3NaO_8P$ (678.57) calcd. C 47.79, H 7.43, Br 11.78, N 6.19, Na 3.39, O 18.86, P 4.56; found C 47.52, H 7.61, Br 11.53, N 6.00, P 4.76.

Bis[2-(Hexadecyloxy)ethyl] 2-amino-5-bromo-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**65b**)

GP8, white solid (61 mg, 23%), m.p. 160 °C dec. MS (ESI): m/z (%) = 1038 (26) [M-Na]$^-$, 1016 (63) [M-(2 × Na)+H]$^-$. For $C_{46}H_{88}BrN_3Na_2O_{12}P_2$ (1063.03) calcd. C 51.97, H 8.34, Br 7.52, N 3.95, Na 4.33, O 18.06, P 5.83; found C 51.68, H 8.69, Br 7.76, N 3.86, P 5.89.

2-(Hexadecyloxy)ethyl 2-amino-4-(2-hydroxyethoxy)-5-methyl-6-[2-(phosphonomethoxy)ethoxy] pyrimidine, sodium salt (**64c**)

GP8, white solid (122 mg, 40%), m.p. 95 °C. ^{1}H NMR (DMSO-d_6): δ = 6.20 (br s, 2H, NH_2), 4.85 (br, 1H, OH), 4.28 (m, 2H, H-1′), 4.19 (m, 2H, H-1′′), 3.62–3.81 (m, 6H, H-2′, 4′, 2′′), 3.39 (m, 6H, H-3′, 5′, 6′), 1.77 (s, 3H, 5-CH_3), 1.43 (m, 2H, H-7′), 1.22 (m, 26H, alif.), 0.84 (t, J(CH_3,CH_2) = 7.0, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): 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 ($CH_3CH_2\underline{CH_2}$), 29.55, 29.31, 29.21, 28.98, 25.94 (alif.), 22.37 ($CH_3\underline{CH_2}$), 14.24 (CH_3), 7.09 (5-CH_3) ppm. MS (ESI): m/z (%) = 590.3

(100) [M-Na]$^-$. For $C_{28}H_{53}N_3NaO_8P$ (613.70) calcd. C 54.80, H 8.70, N 6.85, Na 3.75, O 20.86, P 5.05; found C 54.59, H 8.96, N 6.80, P 4.98.

Bis[2-(Hexadecyloxy)ethyl] 2-amino-5-methyl-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine, sodium salt (**65c**)

GP8, white solid (106 mg, 21%), m.p. 142 °C. MS (ESI): m/z (%) = 952.5 (48) [M-(2 × Na)+H]$^-$. For $C_{47}H_{91}N_3Na_2O_{12}P_2$ (998.17) calcd. C 56.55, H 9.19, N 4.21, Na 4.61, O 19.23, P 6.21; found C 56.31, H 9.32, N 4.03, P 6.44.

2-Amino-5-bromo-4,6-bis(2-hydroxyethoxy)pyrimidine (**66**)

Pyrimidine **62** (1 g, 4.65 mmol) in DMF (15 ml) was treated with bromine (0.7 M solution in CCl_4, 10 ml) and the mixture was stirred at r.t. overnight. The mixture was 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. ^{1}H NMR (DMSO-d_6): δ = 6.71 (br s, 2H, NH_2), 4.26 (t, J(1′,2′) = 5.3, 4H, H-1′), 3.67 (t, J(2′,1′) = 5.3, 4H, H-2′) ppm. ^{13}C NMR (DMSO-d_6): 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 – H_2O+H]$^+$. For $C_8H_{12}BrN_3O_4$ (294.1) calcd. C 32.67, H 4.11, Br 27.17, N 14.29, O 21.76; found C 32.56, H 4.01, Br 27.54, N 14.32.

Capillary zone 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).

6.3. Phosphonomethylphosphinates

5-Iodopentyl acetate (**68c**)

5-Chloropentyl acetate (10 g, 60 mmol) in acetone (500 ml) was treated with NaI (45 g, 0.3 mol) and heated under reflux for 32 h.[98] The mixture was evaporated to half of its volume, partioned between H_2O and $CHCl_3$, organic fraction was washed with H_2O, saturated $Na_2S_2O_3$ and H_2O, dried over $MgSO_4$ and evaporated. Pale yellow oil, yield 11.4 g (73%). ^{1}H NMR (DMSO-*d*$_6$): δ = 3.99 (t, 2H, *J*(1,2) = 6.57, H-1), 3.28 (t, 2H, *J*(5,4) = 6.88, H-5), 1.99 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.38 (m, 2H, CH_2). MS (ESI): m/z (%) = 279.0 (26) $[MNa]^+$. HR MS (ESI) calcd. for $C_7H_{13}INaO_2$ $[MNa]^+$ 278.9858, found 278.9852.

6-Iodohexyl acetate (**68d**)

Acetanhydride (10 ml) was added dropwise to 6-chlorohexanol (10 ml, 71.5 mmol) in pyridine (30 ml) at 0 °C, the mixture was slowly warmed to r.t. and stirred for 4 h. EtOH (20 ml) was added and the mixture was evaporated, the residue was codistilled with EtOH, diluted with $CHCl_3$ and washed with HCl (1 M), saturated $NaHCO_3$ and H_2O. The organic fraction was dried over $MgSO_4$ and evaporated to give 6-chlorohexyl acetate, yield 12.8 g (99%). ^{1}H NMR ($CDCl_3$): δ = 4.06 (t, 2H, *J*(1,2) = 6.67, H-1), 3.53 (t, 2H, *J*(6,5) = 6.67, H-6), 2.04 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.64 (m, 2H, CH_2), 1.47 (m, 2H, CH_2), 1.38 (m, 2H, CH_2) ppm. MS (ESI): m/z (%) = 179.0 (15) $[MH]^+$.

6-Chlorohexyl acetate was converted to 6-iodo congener by the method described for **68c**, pale yellow oil, yield 90%. ^{1}H NMR ($CDCl_3$): δ = 4.05 (t, 2H, *J*(1,2) = 6.67, H-1), 3.18 (t, 2H, *J*(6,5) = 6.97, H-6), 2.04 (s, 3H, CH_3), 1.84 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.46–1.32 (m, 4H, 2 × CH_2) ppm. MS (ESI): m/z (%) = 271 (85) $[MH]^+$. HR MS (ESI) calcd. for $C_8H_{15}INaO_2$ $[MNa]^+$ 293.0977, found 293.0976.

2-(2-Iodoethoxy)ethyl acetate (**68e**)

Prepared from 2-(chloroethoxy)ethanol by the procedure described for **68d**.

2-(2-Chloroethoxy)ethyl acetate: colorless oil, yield 88%. ^{1}H NMR ($CDCl_3$): δ = 4.36 (m, 2H, H-1), 3.88 (t, 2H, *J*(3,4) = 5.76, H-3), 3.85 (m, 2H, H-2), 3.76 (t, 2H, *J*(4,3) = 5.76, H-4), 2.08 (s, 3H, CH_3) ppm. MS (ESI), m/z (%) = 189.0 (94) $[MNa]^+$. 2-(2-Iodoethoxy)ethyl acetate (**68e**): pale yellow oil, yield 65%. ^{1}H NMR ($CDCl_3$): δ = 4.22 (m, 2H, H-1), 3.74 (m, 2H, H-3), 3.69 (m, 2H, H-2), 3.25 (m, 2H, H-4), 2.08 (s, 3H, CH_3) ppm. MS (ESI), m/z (%) = 280.9 (65) $[MNa]^+$. HR MS (ESI) calcd. for $C_6H_{11}INaO_3$ $[MNa]^+$ 280.9645, found 280.9644.

Diisopropyl [2-hydroxyethoxymethyl(isopropoxy)phosphoryl]methylphosphonate (**69a**)

GP9, colorless oil, yield 41%. ^{1}H NMR ($CDCl_3$): δ = 4.63–4.77 (m, 3H, CHipr.), 3.97 (dd, *J*gem = 12.9, *J*(H,P) = 7.6, 1H) and 3.84 (dd, *J*gem = 12.9, *J*(H,P) = 8.7, 1H, OCH_2P), 3.57–3.72 (m, 4H, H-3, H-4), 2.30–2.51 (m, 2H, H-1), 1.25–1.32 (m, 18H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 74.83 (d, *J*(C,P) = 6.5) and 71.24 (d, *J*(C,P) = 6.6) and 70.56 (d, *J*(C,P) = 5.6, CHipr.), 66.42 (d, *J*(C,P) = 119.2, C-2), 60.39 (C-4), 27.11 (dd, *J*(C,P) = 137.0 and 83.3, PCH_2P), 23.9 (m, CH_3ipr.) ppm. MS (ESI): m/z (%) = 359 (100) $[M-H]^-$. HR MS (ESI) calcd. for $C_{13}H_{30}NaO_7P_2$ $[MNa]^+$ 383.1359, found 383.1359.

Diisopropyl [4-hydroxybutyl(isopropoxy)phosphoryl]methylphosphonate (**69b**)

GP9, colorless oil, yield 28%. ^{1}H NMR ($CDCl_3$): δ = 4.62–4.74 (m, 3H, CHipr.), 3.61 (t, *J*(4,3) = 5.9, 2H, H-4), 2.22–2.35 (m, 2H, PCH_2P), 1.85–1.98 (m, 2H, H-1), 1.74 (br s, 1H, OH), 1.66–1.73 (m, 2H, H-3), 1.53–1.64 (m, 2H, H-2), 1.26–1.29 (m, 18H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 71.73 (d, *J*(C,P) = 6.5, CHipr.), 71.27 (d, *J*(C,P) = 6.7, CHipr.), 69.70 (d, *J*(C,P) = 6.8, CHipr.), 60.30 (C-4), 32.58 (d, *J*(C,P) = 16.3, C-2), 28.74 (dd, $J(C,P_1)$ = 135.7, $J(C,P_2)$ = 77.6, PCH_2P), 28.42 (d, *J*(C,P) = 98.2, C-1), 24.44 (d, *J*(C,P) = 3.6, CH_3ipr.), 24.25 (d, *J*(C,P) = 4.2, CH_3ipr.), 24.05 (d, *J*(C,P) = 3.6, CH_3ipr.), 23.98 (d, *J*(C,P) = 4.6, 2 × CH_3ipr.), 23.84 (d, *J*(C,P) = 5.1, CH_3ipr.), 17.64 (d, *J*(C,P) = 3.6, C-3). MS (ESI): m/z (%) = 381 (100) $[MNa]^+$, 358.9 (52) $[MH]^+$. HR MS (ESI) calcd. for $C_{14}H_{32}NaO_6P_2$ $[MNa]^+$ 381.1566, found 381.1567.

Diisopropyl [5-hydroxypentyl(isopropoxy)phosphoryl]methylphosphonate (**69c**)

GP9, colorless oil, yield 30%. ^{1}H NMR ($CDCl_3$): δ = 4.68 (m, 3H, CHipr.), 3.58 (t, 2H, *J*(1,2) = 6.4, H-1), 2.27 (m, 2H, PCH_2P), 1.87 (m, 2H, H-5), 1.60 (m, 2H, H-4), 1.53 (m, 2H, H-2), 1.44 (m, 2H, H-3), 1.25 – 1.30 (m, 18H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 71.41 (d, *J*(C,P) = 6.7), 71.15 (d, *J*(C,P) = 6.5) and 69.66 (d, *J*(C,P) = 6.9, CHipr), 62.32 (C-1), 31.94 (C-2), 29.82 (d, *J*(C,P) = 98.5, C-5), 29.32 (dd, *J*(C,P) = 7.69 and 135.8, PCH_2P), 26.80 (d, *J*(C,P) = 16.3, C-3), 23.22–24.45 (m, CH_3ipr.), 21.28 (d, *J*(C,P) = 6.6, C-4) ppm. MS (ESI): m/z (%) = 395.1 (82) $[MNa]^+$. HR MS (ESI) calcd. for $C_{15}H_{34}NaO_6P_2$ $[MNa]^+$ 395.1723, found 395.1721.

Diisopropyl [6-hydroxyhexyl(isopropoxy)phosphoryl]methylphosphonate (**69d**)

GP9, colorless oil, yield 40%. ^{1}H NMR ($CDCl_3$): δ = 4.70–4.80 (m, 3H, CHipr.), 3.62 (t, *J*(1,2) = 6.5, 2H, H-1), 2.33 (m, 2H, PCH_2P), 1.86–2.0 (m, 2H, H-6), 1.65 (m, 2H, H-5), 1.57 (m, 2H, H-2), 1.37–1.48 (m, 4H, H-3, H-4), 1.32–1.36 (m, 18H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 71.32 (d, *J*(C,P) = 6.6), 71.09 (d, *J*(C,P) = 6.5) and 69.60 (d, *J*(C,P) = 6.8, CHipr.), 62.44 (C-1), 32.29 (C-2), 30.19 (d, *J*(4,P) = 16.4, C-4), 29.68 (d, *J*(6,P) = 98.5, C-6), 29.30 (dd, *J*(C,P) = 77.0 and 135.9, PCH_2P), 25.01 (C-3), 23.82–24.40 (m, CH_3ipr.), 21.42 (d, *J*(5,P) = 4.6, C-5) ppm. MS (ESI): m/z (%) = 387 (6) $[MH]^+$, 409.1 (55) $[MH]^+$. HR MS (ESI) calcd. for $C_{16}H_{36}NaO_6P_2$ $[MNa]^+$ 409.1879, found 409.1880.

Diisopropyl [2-hydroxyethoxyethyl(isopropoxy)phosphoryl]methylphosphonate (**69e**)

GP9, colorless oil, yield 37%. ^{1}H NMR ($CDCl_3$): δ = 4.76 (m, 3H, CHipr.), 3.85 (m, 2H, H-3), 3.71 (m, 2H, H-1), 3.59 (m, 2H, H-2), 2.51 (m, 2H,PCH_2P), 2.44 and 2.18 (m, 2H, H-4), 1.33 – 1.37 (m, 18H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 72.41 (C-2), 71.45 (d, *J*(C,P) = 6.6), 71.18 (d, *J*(C,P) = 6.6), 70.15 (d, *J*(C,P) = 7.0, CHipr.), 64.19 (d, *J*(C,P) = 5.9, C-3), 61.12 (C-1), 30.85 (dd, *J*(C,P) = 80.8 and 135.9, PCH_2P), 29.36 (d, *J*(C,P) = 98.2, C-4), 23.82–24.34 (m, CH_3ipr.) ppm. MS (ESI):

m/z (%) = 375 (48) $[MH]^+$; 397.1 (100) $[MNa]^+$. HR MS (ESI) calcd. for $C_{14}H_{32}NaO_7P_2$ $[MNa]^+$ 397.1515, found 397.1514.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}uracil (**71a**)

GP10, the crude product in dry MeOH (5 ml) was treated with MeONa/MeOH (1 M, 1 ml) at r.t. overnight, neutralized with acetic acid and evaporated. The product was purified by flash chromatography ($CHCl_3$/MeOH 0–10%) to give colorless oil (180 mg, 79%). ^{1}H NMR ($CDCl_3$): δ = 9.29 (br s, 1H, NH), 7.42 (d, *J*(6,5) = 7.9, H-6), 5.65 (dd, *J*(5,6) = 7.9, *J*(5,NH) = 1.9, H-5), 4.79 (octet, *J*(CH,CH_3) = *J*(CH,P) = 6.3, 1H, CHipr.), 4.78 (dh, *J*(CH,CH_3) = 6.2, *J*(CH,P) = 7.0, 1H, CHipr.), 4.74 (dh, *J*(CH,CH_3) = 6.2, *J*(CH,P) = 8.0, 1H, CHipr.), 4.01 (ddd, *J*gem = 14.5, *J*(1'a,2'a) = 5.8, *J*(1'a,2'b) = 3.2, 1H, H-1'a), 4.00 (dd, *J*gem = 13.3, *J*(H,P) = 6.9, 1H, OCH_2Pa), 3.92 (m, 2H, 1'b, OCH_2Pb), 3.84 (m, 2H, H-2'), 2.40 (m, 2H, PCH_2P), 1.36 (d, *J*(CH_3,CH) = 6.3, 3H, CH_3ipr.), 1.35 (d, *J*(CH_3,CH) = 6.3, 3H, CH_3ipr.), 1.35 (d, *J*(CH_3,CH) = 6.3, 3H, CH_3ipr.), 1.34 (d, *J*(CH_3,CH) = 6.3, 3H, CH_3ipr.), 1.31 (d, *J*(CH_3,CH) = 6.3, 3H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.68 (C-4), 150.79 (C-2), 145.80 (C-6), 101.50 (C-5), 71.83 (d, *J*(C,P) = 6.4, CHipr.) 71.36 (d, *J*(C,P) = 6.5, CHipr.), 71.16 (d, *J*(2',P) = 12.3, C-2'), 70.92 (d, *J*(C,P) = 6.7, CHipr.), 67.63 (d, *J*(C,P) = 117.3, OCH_2P), 48.11 (C-1'), 27.33 (dd, *J*(C,P) = 136.6 and 82.9, PCH_2P), 24.29 (d, *J*(C,P) = 3.8, CH_3ipr.), 24.19 (d, *J*(C,P) = 3.5, CH_3ipr.), 24.07 (d, *J*(C,P) = 3.1, CH_3ipr.), 23.97 (d, *J*(C,P) = 4.1, 2C, CH_3ipr.), 23.83 (d, *J*(C,P) = 5.0, CH_3ipr.) ppm. MS (ESI): m/z (%) = 477.0 (100) $[MNa]^+$, 454.9 (19) $[MH]^+$. For $C_{17}H_{32}N_2O_8P_2$ (454.39) calcd. C 44.94, H 7.10, N 6.17, O 28.17, P 13.63; found C 45.19, H 7.38, N 5.81, P 13.58.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}-thymine (**71b**)

GP10, the crude product in dry MeOH (5 ml) was treated with MeONa/MeOH (1 M, 1 ml) at r.t. overnight, neutralized with acetic acid and evaporated. The product was purified by flash chromatography ($CHCl_3$/MeOH 0–10%) to give colorless oil (122

mg, 52%). ^{1}H NMR ($CDCl_3$): δ = 8.16 (br s, 1H, NH), 7.18 (d, J(6,CH_3) = 1.20, H-6), 4.77 (m, 3H, CHipr.), 3.95 (m, 4H, H-1', H-2'), 3.80 (m, 2H, H-3'), 2.37 (m, 2H, PCH_2P), 1.36 (d, J(CH_3,CH) = 6.2, 3H, CH_3ipr.), 1.34 (d, J(CH_3,CH) = 6.2, 3H, CH_3ipr.), 1.33 (d, J(CH_3,CH) = 6.2, 3H, CH_3ipr.), 1.32 (d, J(CH_3,CH) = 6.2, 3H, CH_3ipr.), 1.31 (d, J(CH_3,CH) = 6.2, 3H, CH_3ipr.) ppm. MS (ESI): m/z (%) = 491.0 (100) $[MNa]^+$, 469.0 (35) $[MH]^+$. For $C_{18}H_{34}N_2O_8P_2$ (454.39) calcd. C 46.15, H 7.32, N 5.98, O 27.32, P 13.22; found C 45.98, H 7.41, N 5.86, P 13.41.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}uracil (**72a**)

GP11, white solid, yield 83%, m.p. 195 °C. ^{1}H NMR (D_2O): δ = 7.68 (d, J(6,5) = 7.9, 1H, H-6), 5.82 (d, 1H, J(5,6) = 7.8, H-5), 4.01 (m, 2H, H-1‘), 3.81 – 3.84 (m, 4H, H-2‘, OCH_2P), 2.40 (dd, J(H,P_1) = 20.3, J(H,P_2) = 17.0, 2H, PCH_2P) ppm. ^{13}C NMR (D_2O): δ = 167.54 (C-4), 152.87 (C-2), 148.64 (C-6), 101.77 (C-5), 71.07 (d, J(C,P) = 12.5, C-2‘), 68.40 (d, J(C,P) = 117.6, OCH_2P), 48.93 (C-1‘), 27.33 (dd, J(C,P_1) = 128.8, J(C,P_2) =80.5, PCH_2P) ppm. MS (ESI): m/z (%) = 351.1 (66) $[MNa]^+$. For $C_8H_{14}N_2O_8P_2$ (328.15) calcd. C 29.28, H 4.30, N 8.54, O 39.00, P 18.88; found C 29.62, H 4.44, N 8.23, P 18.73.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}thymine (**72b**)

GP11, freeze dried, white solid, yield 82%, m.p. 238 °C. ^{1}H NMR (D_2O): δ = 7.52 (q, J(6,CH_3) = 1.2, 1H, H-6), 3.97 (m, 2H, H-1‘), 3.81 – 3.83 (m, 4H, H-2‘, OCH_2P), 2.38 (dd, J(H,P) = 16.9, J(H,P) = 20.5, 2H, PCH_2P), 1.88 (d, J(CH_3,6) = 1.2, 3H, 5-CH_3) ppm. ^{13}C NMR (D_2O): δ = 167.68 (C-4), 152.90 (C-2), 144.49 (C-6), 110.91 (C-5), 71.20 (d, J(C,P) = 12.6, C-2‘), 68.38 (d, J(C,P) = 118.2, OCH_2P), 48.68 (C-1‘), 27.20 (dd, J(C,P) = 81.2, J(C,P) = 129.2, PCH_2P), 11.89 (5-CH_3) ppm. MS (ESI): m/z (%) = 341 (100) $[M-H]^-$. For $C_{10}H_{18}N_2O_7P_2$ (342.18) calcd. C 31.59, H 4.71, N 8.19, O 37.41, P 18.10; found C 31.39, H 4.72, N 7.96, P 18.41.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}-cytosine (**73**)

71a (310 mg, 0.68 mmol), Et_3N (0.3 ml) and TPSCl (0.63 g, 2.04 mmol) in CH_3CN (10 ml) was stirred at r.t. for 48 h. NH_4OH (25%, 5 ml) was added, the mixture was stirred for 24 h and evaporated. The residue in ethyl acetate was washed with brine, the aqueous fraction was than washed with 5 portions of $CHCl_3$; the organic fractions were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography to give pale yellow foam (240 mg, 78%). ^{1}H NMR (DMSO-d_6): δ = 7.60 d, 1H, *J*(6,5) = 7.25 (H-6); 7.49 br s, 1H (NHa); 7.23 br s, 1H (NHb); 5.67 d, 1H, *J*(5,6) = 7.25 (H-5); 4.60 m, 6H (CHipr.); 3.83 m, 4H (H-1', H-3'); 3.69 m, 2H (H-2'), 2.40 m, 2H (PCH_2P); 1.24 m, 9H (CH_3ipr.); 1.18 d, 3H, *J*(CH_3,CH) = 6.14 (CH_3ipr.); 1.16 d, 3H, *J*(CH_3,CH) = 6.19 (CH_3ipr.); 1.10 d, 3H, *J*(CH_3,CH) = 6.19 (CH_3ipr.) ppm. MS (ESI): m/z (%) = 454.0 (70) $[MH]^+$, 796.0 (84) $[MNa]^+$. For $C_{17}H_{33}N_3O_7P_2$ (453.41) calcd. C 45.03, H 7.34, N 9.27, O 24.70, P 13.66; found C 45.12, H 7.31, N 9.12, P 13.52.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}cytosine (**74**)

GP11, freeze dried, white foam, yield 69%. ^{1}H NMR (D_2O): δ = 7.90 (d, *J*(6,5) = 7.6, 1H, H-6), 6.17 (d, *J*(5,6) = 7.7, 1H, H-5), 4.08 (t, *J*(1‘,2‘) = 4.7, 2H, H-1‘), 3.83 (t, *J*(2‘,1‘) = 4.7, 2H, H-2‘), 3.78 (d, *J*(H,P) = 7.6, 2H, OCH_2P), 2.31 (dd, *J*(H,P) = 6.8 and 10.3, 2H, PCH_2P) ppm. ^{13}C NMR (D_2O): δ = 160.16 (C-4), 151.04 (C-6), 149.69 (C-2), 94.67 (C-5), 70.49 (d, *J*(2‘,P) = 13.1, C-2‘), 68.80 (d, *J*(C,P) = 119.9, OCH_2P), 49.87 (C-1‘), 27.79 (dd, *J*(C,P) = 79.1 and 125.9, PCH_2P) ppm. MS (ESI): m/z (%) = 326 (100) $[M-H]^-$. For $C_8H_{15}N_3O_7P_2$ (327.17) calcd. C 29.37, H 4.62, N 12.84, O 34.23, P 18.93; found C 29.31, H 4.82, N 12.83, P 18.78.

9-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}-adenine (**76**)

GP10, the crude product in dichloromethane (40 ml) was treated with HCl (6 M, 40 ml) and heated under reflux for 6 h. The pH of the aqueous phase was adjusted to 8

using a saturated aqueous solution of $NaHCO_3$. The mixture was then extracted with 5 portions of dichloromethane, the combined organic fractions were dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH). Crystallization from ethyl acetate – light petroleum gave white crystalline product (28%), m.p. 82 °C. ^{1}H NMR ($CDCl_3$): δ = 8.35 (s, 1H, H-2), 7.97 (s, 1H, H-8), 5.84 (br s, 2H, NH_2), 4.68–4.80 (m, 3H, CHipr.), 4.42 (m, 2H, H-1‘), 4.01 (dd, *J*gem = 13.3, *J*(H,P) = 6.4, OCH_2Pa), 3.98 (m, 2H, H-2‘), 3.90 (dd, *J*gem = 13.3, *J*(H,P) = 7.7, OCH_2Pb), 2.31–2.42 (m, 2H, PCH_2P), 1.31–1.35 (m, 15H, CH_3ipr.) and 1.24 (d, 3H, *J*(CH_3,CH) = 6.2, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 155.39 (C-6), 152.89 (C-2), 149.90 (C-4), 141.37 (C-8), 119.39 (C-5), 71.71 (d, *J*(C,P) = 6.5, CHipr.), 71.31 (d, *J*(2‘,P) = 12.1, C-2‘), 71.28 (d, *J*(C,P) = 7.0, CHipr.), 70.81 (d, *J*(C,P) = 6.8, CHipr.), 67.55 (d, *J*(C,P) = 116.8, OCH_2P), 43.31 (C-1‘), 27.22 (dd, *J*(C,P) = 136.4, *J*(C,P) = 83.1, PCH_2P), 23.99 (m, CH_3ipr.) ppm. MS (ESI): m/z (%) = 500.1 (100) $[MNa]^+$. For $C_{18}H_{33}N_5O_6P_2$ (477.43) calcd. C 45.28, H 6.97, N 14.67, O 20.11, P 12.98; found C 45.36, H 6.92, N 14.30, P 12.89.

9-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}adenine (**77**)

GP11, crystallized from water, white crystals, yield 78%, m.p. 196 °C. ^{1}H NMR (D_2O + NaOD): δ = 8.22 (s, 1H, H-8), 8.14 (s, 1H, H-2), 4.41 (t, 2H, *J*(1‘,2‘) = 5.1, H-1‘), 3.95 (t, 2H, *J*(2‘,1‘) = 5.1, H-2‘), 3.71 (d, 2H, *J*(H,P) = 6.5, PCH_2O), 1.96 (t, 2H, *J*(H,P) = 18.0, PCH_2P) ppm. ^{13}C NMR (D_2O + NaOD): δ = 155.91 (C-6), 152.75 (C-2), 149.27 (C-4), 143.63 (C-8), 118.76 (C-5), 71.15 (d, *J*(2‘,P) = 10.4, C-2‘), 69.99 (d, *J*(C,P) = 111.8, PCH_2O), 44.06 (C-1‘), 30.98 (dd, *J*(C,P) = 80.9, *J*(C,P) = 118.4), PCH_2P) ppm. MS (ESI): m/z (%) = 350.1 (100) $[M-H]^-$. For $C_9H_{15}N_5O_6P_2.H_2O$ (369.21) calcd. C 29.28, H 6.64, N 18.97, O 30.33, P 16.78; found C 29.60, H 4.65, N 18.98, P 16.06.

N^2-Triphenylphosphoranylidene 2-amino- 6-chloro-9-{2-[(diisopropoxyphosphoryl-methyl)(isopropoxy)phosphorylmethoxy]ethyl}purine (**79**)

GP10, separated by flash chromatography ($CHCl_3$/MeOH), white foam, yield 24%. ^{1}H NMR ($CDCl_3$): δ = 7.87 (m, 5H, arom.), 7.77 (s, 1H, H-8), 7.54 (m, 3H, arom.), 7.44 (m, 7H, arom.), 4.71 (m, 3H, CHipr.), 4.20 (m, 2H, H-2‘), 3.94 (dd, 1H, *J*gem = 13.3, *J*(H,P) = 6.2, PCH_2a), 3.79 (dd, 1H, *J*gem = 13.2, *J*(H,P) = 7.7, PCH_2b), 3.72 (m, 2H, H-1‘), 2.35 (m, 2H, PCH_2P), 1.31 (m, 15H, CH_3ipr.), 1.23 (d, *J*(CH_3,CH) = 6.2, CH_3ipr.) ppm. MS (ESI): m/z (%) = 772.2 (100) $[MH]^+$. MS (ESI): m/z (%) = 770.2 (100) $[M-H]^-$.

9-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}-guanine (**80**)

Compound **79** (350 mg, 0.45 mmol) in THF (10 ml) was treated with water (3 ml) and heated under reflux for 2 days. The mixture was evaporated and the crude product was treated with 75% TFA in water (10 ml) at r.t. overnight. The solvent was evaporated and the residue was codistilled with water. The product was purified by flash chromatography (CH_3Cl/MeOH) and crystallized from EtOH/Et_2O mixture to afford white crystals, yield (two steps) 167 mg (74 %), m.p. 108 °C. ^{1}H NMR (DMSO-d_6): δ = 10.56 (br s, 1H, NH), 7.65 (s, 1H, H-8), 6.44 (br s, 2H, NH_2), 4.53–4.63 (m, 3H, CHipr.), 4.12 (m, 2H, H-1‘), 3.85 (d, 2H, *J*(H,P) = 6.7, OCH_2P), 3.81 (m, 2H, H-2‘), 2.38–2.51 (m, 2H, PCH_2P), 1.23 (d, 6H), 1.22 (d, 6H), 1.20 (d, 3H) and 1.12 (d, 3H, CH_3ipr.) ppm. ^{13}C NMR (DMSO-d_6): 157.05 (C-6), 153.74 (C-2), 151.38 (C-4), 137.93 (C-8), 116.61 (C-5), 70.88 (d, *J*(2‘,P) = 11.3, C-2‘), 70.61 (d, *J*(C,P) = 6.2), 70.44 (d, *J*(C,P) = 6.2) and 69.80 (d, *J*(C,P) = 6.5, CHipr.), 66.85 (d, *J*(C,P) = 115.7, OCH_2P), 42.44 (C-1‘), 25.99 (dd, *J*(C,P) = 81.7 and 133.9), 23.78–24.24 (m, CH_3ipr.) ppm. MS (ESI): m/z (%) = 494.1 (34) $[MH]^+$; 516.1 (100) $[MNa]^+$. For $C_{18}H_{33}N_5O_7P_2$ (493.43) calcd. C 43.81, H 6.74, N 14.19, O 22.70, P 12.55; found C 43.87, H 6.77, N 13.76, P 12.59.

9-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}guanine (**81**)

GP11, DMF (1 ml) was added to the reaction mixture. White solid, yield 52%, m.p. 190 °C with dec. ^{1}H NMR (D_2O): δ = 7.88 (s, 1H, H-8), 4.25 (t, *J*(1‘,2‘) = 5.1, 2H, H-1‘), 3.92 (t, *J*(2‘,1‘) = 5.1, 2H, H-2‘), 3.71 (d, *J*(H,P) = 6.9, 2H, OCH_2P), 2.02 (dd, *J*(H,P) = 17.0 and 19.1, 2H, PCH_2P) ppm. ^{13}C NMR (D_2O): δ = 159.54 (C-6), 154.28 (C-2), 152.03 (C-4), 141.12 (C-8), 116.30 (C-5), 71.31 (d, *J*(2‘,P) = 10.9, C-2‘), 69.82 (d, *J*(C,P) = 112.9, OCH_2P), 43.70 (C-1‘), 30.33 (dd, *J*(C,P) = 80.1 and 119.6, PCH_2P) ppm. MS (ESI): m/z (%) = 366.0 (100) $[M-H]^-$. For $C_9H_{15}N_5O_7P_2.H_2O$ (385.20) calcd. C 28.06, H 4.45, N 18.18, O 33.23, P 16.08; found C 28.11, H 4.39, N 17.77, P 15.77.

1-{4-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl]butyl}uracil (**82a**)

GP10, the crude product in dry MeOH (5 ml) was treated with MeONa/MeOH (1 M, 1 ml) at r.t. overnight, neutralized with acetic acid and evaporated. Purification by flash chromatography ($CHCl_3$/MeOH 0–10%) afforded colorless oil (225 mg, 96%). ^{1}H NMR ($CDCl_3$): δ = 9.16 (d, *J*(NH,5) = 6.5, 1H, NH), 7.18 (d, *J*(6,5) = 7.9, 1H, H-6), 5.61 (dd, *J*(5,6) = 7.9, *J*(5,NH) = 2.0, 1H, H-5), 4.64–4.73 (m, 3H, CHipr.), 3.64–3.73 (m, 2H, H-1’a, 1’b), 2.22–2.36 (m, 2H, PCH_2P), 1.86–2.02 (m, 2H, H-4’), 1.74–1.79 (m, 2H, H-2‘), 1.60–1.66 (m, 2H, H-3‘), 1.25–1.29 (m, 18H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.68 (C-4), 150.71 (C-2), 144.57 (C-6), 102.06 (C-5), 71.57 (d, *J*(C,P) = 6.6, CHipr.), 71.19 (d, *J*(C,P) = 6.7, CHipr.), 69.93 (d, *J*(C,P) = 6.8, CHipr.), 48.41 (C-1‘), 29.63 (d, *J*(C,P) = 15.4, C-2‘), 29.43 (dd, *J*(C,P) = 135.3, *J*(C,P) = 177.5, PCH_2P), 29.07 (d, *J*(C,P) = 99.2, C-4‘), 24.42 (d, *J*(C,P) = 3.7, CH_3ipr.), 24.16 (d, *J*(C,P) = 4.3, CH_3ipr.), 24.05 (d, *J*(C,P) = 3.7, CH_3ipr.), 23.97 (d, *J*(C,P) = 4.5, 2 × CH_3ipr.), 23.86 (d, *J*(C,P) = 5.2, CH_3ipr.), 18.58 (d, *J*(C,P) = 4.4, C-3‘) ppm. MS (ESI): m/z (%) = 452.9 (100) $[MH]^+$, 475.1 (67) $[MNa]^+$. For $C_{18}H_{34}N_2O_7P_2$ (452.42) calcd. C 47.79, H 7.57, N 6.19, O 24.75, P 13.69; found C 47.71, H 7.53, N 5.92, P 13.60.

1-{5-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl]pentyl}uracil (**82b**)

GP10, the crude product in dry MeOH (5 ml) was treated with MeONa/MeOH (1 M, 1 ml) at r.t. overnight, neutralized with acetic acid and evaporated. The product was isolated by flash chromatography ($CHCl_3$/MeOH 0–10%) as colorless oil (186 mg, 79%). ^{1}H NMR ($CDCl_3$): δ = 9.15 (br s, 1H, NH), 7.21 (d, *J*(6,5) = 7.9, 1H, H-6), 5.68 (dd, *J*(5,6) = 7.9, *J*(5,NH) = 1.5, 1H. H-5), 4.70–4.80 (m, 3H, CHipr.), 3.73 (m, 2H, H-1‘), 2.35 (m, 2H, PCH_2P), 1.95 (m, 2H, H-5‘), 1.65–1.76 (m, 4H, H-2‘, H-4‘), 1.46 (m, 2H, H-3‘), 1.32–1.36 (m, 18H, CH_3ipr) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.65 (C-4), 150.71 (C-2), 144.49 (C-6), 102.04 (C-5), 71.46 (d, *J*(C,P) = 6.7), 71.13 (d, *J*(C,P) = 6.4) and 69.76 (d, *J*(C,P) = 7.0, CHipr.), 48.47 (C-1‘), 29.37 (dd, *J*(C,P) = 77.7 and 135.2, PCH_2P), 29.59 (d, *J*(C,P) = 99.0, C-5‘), 28.37 (C-2‘), 27.26 (d, *J*(3‘,P) = 16.5, C-3‘), 23.84–24.44 (m, CH_3ipr.), 21.10 (d, *J*(C,P) = 4.5, C-4‘) ppm. MS (ESI): m/z (%) = 489.1 $[MNa]^+$. HR MS (ESI) calcd. for $C_{19}H_{36}N_2NaO_7P_2$ $[MNa]^+$ 489.18954; found 489.18911. For $C_{19}H_{36}N_2O_7P_2$ (466.44) calcd. C 48.92, H 7.78, N 6.01, O 24.01, P 13.28; found C 48.81, H 7.72, N 5.83, P 13.11.

1-{6-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl]hexyl}uracil (**82c**)

GP10, the product in dry MeOH (5 ml) was treated with MeONa/MeOH (1 M, 1 ml) at r.t. overnight, neutralized with acetic acid and evaporated. Flash chromatography ($CHCl_3$/MeOH 0–10%) gave **82c** as colorless oil (187 mg, 78%). ^{1}H NMR ($CDCl_3$): δ = 9.45 (br s, 1H, NH), 7.19 (d, *J*(6,5) = 7.9, 1H, H-6), 5.70 (d, *J*(5,6) = 7.9, H-5), 4.70–4.82 (m, 3H, CHipr.), 3.72 (m, 2H, H-1‘), 2.36 (m, 2H, PCH_2P), 1.86–2.02 (m, 2H, H-6‘), 1.69 (m, 2H, H-2‘), 1.64 (m, 2H, H-5‘), 1.45 (m, 2H, H-4‘), 1.37 (m, 2H, H-3‘), 1.32–1.36 (m, 18H, CH_3ipr). ^{13}C NMR ($CDCl_3$): δ = 163.72 (C-4), 150.76 (C-2), 144.38 (C-6), 102.02 (C-5), 71.34 (d, *J*(C,P) = 6.6), 71.07 (d, *J*(C,P) = 6.5) and 69.65 (d, *J*(C,P) = 6.8, CHipr.), 48.61 (C-1‘), 30.04 (d, *J*(4‘,P) = 16.2, C-4‘), 29.70 (d, *J*(6‘,P) = 99.6, C-6‘), 29.32 (dd, *J*(C,P) = 77.8 and 134.3, PCH_2P), 28.68 (C-2‘), 25.83 (C-3‘), 23.83–24.43 (m, CH_3ipr.), 21.36 (d, *J*(5‘,P) = 4.6, C-5‘). MS (ESI): m/z (%) = 503 (100) $[MNa]^+$. HR MS (ESI) calcd. for $C_{20}H_{38}N_2NaO_7P_2$ $[MNa]^+$ 503.20519; found 503.20450. For $C_{20}H_{38}N_2O_7P_2$ (480.47) calcd. C 50.00, H 7.97, N 5.83, O 23.31, P 12.89; found C 50.26, H 7.99, N 5.68, P 12.79.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylethoxy]ethyl}uracil (**82d**)

GP10, the crude product in dry MeOH (5 ml) was treated with MeONa/MeOH (1 M, 1 ml) at r.t. overnight, neutralized with acetic acid and evaporated. The product was purified by flash chromatography ($CHCl_3$/MeOH 0–10%), colorless oil (171 mg, 73%). ^{1}H NMR ($CDCl_3$): δ = 9.26 (br s, 1H, NH), 7.43 (d, *J*(6,5) = 7.9, 1H, H-6), 5.66 (dd, *J*(5,6) = 7.9, *J*(5,NH) = 1.8, 1H, H-5),4.72–4.79 (m, 3H, CHipr.), 3.92 (m, 2H, H-1‘), 3.66–3.85 (m, 4H, H-2‘, H-3‘), 2.35–2.46 (m, 3H, PCH_2P, H-4a), 2.21 (m, 1H, H-4b), 1.32–1.36 (m, 18H, CH_3ipr.) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.76 (C-4), 150.85 (C-2), 146.06 (C-6), 101.48 (C-5), 71.48 (d, *J*(C,P) = 6.5), 71.26 (d, *J*(C,P) = 6.5) and 70.19 (d, *J*(C,P) = 6.9, CHipr.), 68.63 (C-2‘), 64.90 (d, *J*(C,P) = 2.4, C-3‘), 48.21 (C-1‘), 30.57 (dd, *J*(C,P) = 80.3 and 135.4, PCH_2P), 30.51 (d, *J*(C,P) = 97.5, C-4‘), 23.84–24.37 (m, CH_3ipr.) ppm. MS (ESI): m/z (%) = 491.1 (100) $[MNa]^+$. HRMS (ESI): found 491.16816, calculated for $C_{18}H_{34}N_2NaO_8P_2$: 491.16881.

1-{4-[(Hydroxy)(phosphonomethyl)phosphoryl]butyl}uracil (**83a**)

GP11, white solid, yield 74%, 182 °C. ^{1}H NMR (D_2O): δ = 7.65 (d, *J*(6,5) = 7.9, 1H, H-6), 5.82 (d, *J*(5,6) = 7.8, 1H, H-5), 3.81 (t, *J*(1‘,2‘) = 7.2, 2H, H-1‘), 2.47 (dd, *J*(H,P) = 16.8, *J*(H,P) = 20.4, 2H, PCH_2P), 1.94 – 1.99 (m, 2H, H-4’a, 4’b), 1.78 – 1.83 (m, 2H, H-2’a, 2’b), 1.58–1.65 (m, 2H, H-3’a, 3’b) ppm. ^{13}C NMR (D_2O): δ = 167.55 (C-4), 152.98 (C-2), 147.98 (C-6), 102.09 (C-5), 48.96 (C-1‘), 29.63 (d, *J*(C,P) = 16.5, C-2‘), 29.32 (d, *J*(C,P) = 96.4, C-4‘), 29.25 (dd, *J*(C,P_1) = 80.0, *J*(C,P2) = 127.0, PCH_2P), 18.71 (d, *J*(C,P) = 4.1, C-3‘) ppm. MS (ESI): m/z (%) = 325 (100) $[M-H]^-$. For $C_9H_{16}N_2O_7P_2$.1/2 H_2O (335.19) calcd. C 32.25, H 5.11, N 8.36, O 35.80, P 18.48; found C 32.07, H 5.29, N 8.02, P 18.37.

1-{5-[(Hydroxy)(phosphonomethyl)phosphoryl]pentyl}uracil (**83b**)

GP11, white solid, yield 90%, m.p. 198–200 °C. ^{1}H NMR (D_2O): δ = 7.64 (d, *J*(6,5) = 7.9, 1H, H-6), 5.81 (d, *J*(5,6) = 7.8, 1H, H-5), 3.79 (t, *J*(1‘,2‘) = 7.2, 2H, H-1‘), 2.46 (dd, *J*(H,P) = 16.7 and 20.3, 2H, PCH_2P), 1.92 (m, 2H, H-5‘), 1.72 (m, 2H, H-

2‘), 1.62 (m, 2H, H-4‘), 1.43 (m, 2H, H-3‘) ppm. ^{13}C NMR (D_2O): δ = 167.57 (C-4), 153.01 (C-2), 148.09 (C-6), 101.98 (C-5), 49.37 (C-1‘), 29.54 (d, *J*(C,P) = 95.8, C-5‘), 29.25 (dd, *J*(C,P) = 79.6 and 126.6, PCH_2P), 28.13 (C-2‘), 27.24 (d, *J*(3‘,P) = 16.7, C-3‘), 21.24 (d, *J*(4‘,P) = 4.1, C-4‘) ppm. MS (ESI): m/z (%) = 339 (100) [M-H]$^-$. For $C_{10}H_{18}N_2O_7P_2$ (340.21) calcd. C 35.30, H 5.33, N 8.23, O 32.92, P 18.21; found C 35.36, H 5.39, N 8.08, P 18.29.

1-{6-[(Hydroxy)(phosphonomethyl)phosphoryl]hexyl}uracil (**83c**)

GP11, white solid, yield 73%, m.p. 185–186 °C. ^{1}H NMR (D_2O): δ = 7.64 (d, *J*(6,5) = 7.8, 1H, H-6), 5.81 (d, *J*(5,6) = 7.8, 1H, H-5), 3.77 (t, 2H, H-1‘), 2.33 (dd, *J*(H,P) = 16.7 and 19.8, 2H, PCH_2P), 1.89 (m, 2H, H-6‘), 1.69 (m, 2H, H-2‘), 1.56 (m, 2H, H-5‘), 1.43 (m, 2H, H-4‘), 1.35 (m, 2H, H-3‘) ppm. ^{13}C NMR (D_2O): δ = 167.54 (C-4), 152.98 (C-2), 148.13 (C-6), 101.92 (C-5), 49.57 (C-1‘), 30.09 (d, *J*(4‘,P) = 16.4, C-4‘), 29.81 (dd, *J*(C,P) = 78.1 and 123.4, PCH_2P), 29.70 (d, *J*(5‘,P) = 95.7, C-6‘), 28.42 (C-2‘), 25.67 (C-3‘), 21.51 (d, *J*(5‘,P) = 4.3, C-5‘) ppm. ^{31}P NMR (D_2O): δ = 50.53 (d, *J*(Pβ,Pα) = 7.6, P-β), 14.82 (d, *J*(Pα,Pβ) = 7.6, P-α) ppm. MS (ESI): m/z (%) = 353 (100) [M-H]$^-$. For $C_{11}H_{20}N_2O_7P_2$ (354.23) calcd. C 37.30, H 5.69, N 7.91, O 31.62, P 17.49; found C 37.69, H 5.71, N 7.96, P 17.20.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylethoxy]ethyl}uracil (**83d**)

GP11, white solid, yield 72%, m.p. 195 °C. ^{1}H NMR (D_2O): δ = 7.65 (d, *J*(6,5) = 7.9, 1H, H-6), 5.81 (d, *J*(5,6) = 7.9, 1H, H-5), 3.99 (m, 2H, H-1‘), 3.80 (dt, *J*(3‘,4‘) = 6.9, *J*(3‘,P) = 15.5, 2H, H-3‘), 3.76 (m, 2H, H-2‘), 2.45 (dd, *J*(H,P) = 17.1 and 20.3, 2H, PCH_2P), 2.23 (dt, *J*(H,P) = 14.7, *J*(4‘,3‘) = 6.9, 2H, H-4‘) ppm. ^{13}C NMR (D_2O): δ = 167.53 (C-4), 152.92 (C-2), 148.45 (C-6), 101.87 (C-5), 68.40 (C-2‘), 65.17 (d, *J*(C,P) = 2.7, C-3‘), 48.84 (C-1‘), 30.68 (d, *J*(C,P) = 96.3, C-4‘), 30.42 (dd, *J*(C,P) = 81.1 and 126.6, PCH_2P) ppm. MS (ESI): m/z (%) = 341 (100) [M-H]$^-$. For $C_9H_{16}N_2O_8P_2$ (342.18) calcd. C 31.59, H 4.71, N 8.19, O 37.41, P 18.10; found C 31.48, H 4.83, N 8.15, P 17.82.

Synthesis of phosphonomethylphosphinate phosphates

Phosphonylphosphinate **72a** or **83a–d** (0.061 mmol) in MeOH (5 ml) was treated with tri-*n*-butylamine (29 μl, 0.122mmol) and the mixture was heated until clear solution was obtained. The solvent was evaporated and the residue was dried over P_2O_5 in vacuo. To the solution of prepared bis(tributylammonium) salt in DMF (1 ml) CDI (49 mg, 0.305 mmol) in DMF (1 ml) was added dropwise at 0°C under argon atmosphere and the resulting mixture was stirred at r.t. for 3 h. Methanol (10 μl, 0.244 mmol) was added and after 1 h of stirring tri-*n*-butylammonium phosphate (0.5 M in DMF) was added and the reaction mixture was stirred for 6–7 h at r.t. The reaction solution was diluted with TEAB (2 ml, 0.025 M), applied onto column of Poros and eluted with linear gradient of TEAB (0–0.4 M). The fractions containing product were evaporated and the residue was applied onto DOWEX 50 × 8 (Na^+ form), eluted with water and freeze dried.

84: white powder, yield 5%. 1H NMR (D_2O): δ = 7.84 (d, *J*(6,5) = 7.9, 1H, H-6), 5.93 (d, 1H, *J*(5,6) = 7.8, H-5), 4.11 (t, *J*(1',2') = 5.0, 2H, H-1'), 3.93 (t, *J*(2',1') = 5.0, 2H, H-2'), 3.83 (d, *J*(CH_2,P) = 6.8, 2H, PCH_2), 2.37 (dd, *J*(H,P_1) = 20.3, *J*(H,P_2) = 17.2, 2H, PCH_2P) ppm.

85a: white powder, yield 16%. ^{1}H NMR (D_2O): δ = 7.8 (d, *J*(6,5) = 7.8, 1H, H-6), 5.93 (d, *J*(5,6) = 7.8, 1H, H-5), 3.9 (t, *J*(1',2') = 7.3, 2H, H-1'), 2.6 (m, 2H, PCH_2P), 2.25–2.18 (m, 2H, H-4'), 1.96–1.88 (m, 2H, H-2'), 1.81–1.75 (m, 2H, H-3').

85b: white powder, yield 22%. ^{1}H NMR (D_2O): δ = 7.74 (d, *J*(6,5) = 7.7, 1H, H-6), 5.9 (d, *J*(5,6) = 7.7, 1H, H-5), 3.89 (t, *J*(1',2') = 7.2, 2H, H-1'), 2.38 (dd, $J_1 = J_2$ = 17.7, PCH_2P), 2.17 (m, 2H, H-5'), 1.84–1.82 (m, 4H, H-2', H-4'), 1.54 (m, 2H, H-3') ppm.

85c: white powder, yield 19%. ^{1}H NMR (D_2O): δ = 7.72 (d, *J*(6,5) = 7.7, 1H, H-6), 5.9 (d, *J*(5,6) = 7.7, 1H, H-5), 3.87 (t, *J*(1',2') = 6.6, 2H, H-1'), 2.38 (dd, $J_1 = J_2$ = 17.7, 2H, PCH_2P), 2.16 (m, 2H, H-6'), 1.81–1.78 (m, 4H, H-2', H-5'), 1.54–1.44 (m, 4H, H-4', H-3').

85d: white powder, yield 28%. ^{1}H NMR (D_2O): δ = 7.81 (d, *J*(6,5) = 7.9, 1H, H-6), 5.94 (d, *J*(5,6) = 7.9, 1H, H-5), 4.11 (m, 2H, H-1'), 4.0–3.95 (m, 2H, H-3'), 3.9 (m, 2H, H-2'), 2.45 (dd, *J*(H,P) = 18.2 and 19.0, 2H, PCH_2P), 2.23 (m, 2H, H-4') ppm.

86: white powder, yield 13%. ^{1}H NMR (D_2O): δ = 7.84 (d, *J*(6,5) = 7.9, 1H, H-6), 5.93 (d, 1H, *J*(5,6) = 7.8, H-5), 4.04 (t, *J*(1',2') = 4.8, 2H, H-1'), 3.91 (t, *J*(2',1') = 4.88, H-2'), 3.66 (d, *J*(CH_2,P) = 7.0, 2H, PCH_2O), 2.56 (dd, *J*(H,P_1) = 19.7, *J*(H,P_2) = 17.8, 2H, PCH_2P) ppm.

pKa determination: Compound **72a** (10 mg) was dissolved in acetate buffer (0.025 M, 0.5 mL) and acidified with HCl (2 M), pH was measured and then ^{31}P NMR spectrum was acquired. Then a drop of NaOH (0.1 M) was added repeatedly, the sample was shaken and pH and ^{31}P NMR spectrum was measured. The pH dependence of ^{31}P chemical shifts was plotted and pKa was estimated to be at the pH, where phosphorus chemical shift is just in the middle between chemical shifts of protonated and nonprotonated forms.

Method of calculation: Gas phase geometries and energies of the studied molecules were obtained using RI-MP2/cc-pVDZ.[99] These calculations were performed with Turbomole5.8.[100] Hydration free energies were calculated using the C-PCM implicit solvent model[101] implemented in the Gaussian03 code.[102] The recommended HF/6-31G* level combined with the united atom radii (UAHF) model was used.

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).[103]

The methodology of the antiviral activity assays followed previously described procedures.[104,20a]

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Synthesis and Properties of Chiral Acyclic Nucleoside Bisphosphonates and Phosphonomethylphosphinates

Doctoral Thesis

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Declaration of the author

I declare that I wrote this thesis myself and that it represents the results of my own work, unless otherwise stated in the text. All books, articles, internet sites, and other sources of information used are properly cited in the References section. Neither the thesis nor any of its parts were used previously for obtaining any academic degree.

Petra Doláková

Table of Contents

1. Introduction 1
1.1. Acyclic nucleoside phosphonates 1
1.1.1. Overview of acyclic nucleoside phosphonates 1
1.1.2. Synthesis of acyclic nucleoside phosphonates 5
1.2. Bisphosphonates 6
1.2.1. Acyclic nucleoside bisphosphonates 6
1.2.2. Other bisphosphonates and bisphosphates 8
1.3. Phosphonomethylphosphinates 10
1.4. dUTPase 13
2. Aims of the work 15
3. Results and Discussion 16
3.1. Acyclic nucleoside bisphosphonates 16
3.1.1. Synthesis of bisphosphonates from 2-amino-4,6-dichloro-pyrimidine… 16
3.1.1.1. Synthesis of bis-HPMPO derivatives 16
3.1.1.2. Synthesis of bis-PMPO derivatives 18
3.1.1.3. Comparison of reactivity of chloro- and fluoropyrimidines toward S_NAr 20
3.1.2. Synthesis of bisphosphonates from 4,6-dihydroxy-2-(methylsulfanyl)-pyrimidine 21
3.1.2.1. Synthesis of bisphosphonates 22
3.1.2.2. Synthesis of 2-substituted bisphosphonates 24
3.1.3. Bisphosphonates derived from 2-amino-4,6-disulfanyl-pyrimidine…… 25
3.1.4. Alkoxyalkyl esters of bisphosphonates 26
3.1.5. Properties of bisphosphonates 27
3.1.5.1. Capillary zone electrophoresis 27
3.1.5.2. Biological activity 28
3.1.6. Conclusion 29
3.2. Acyclic nucleoside phosphonomethylphosphinates 31

3.2.1. Acyclic nucleoside phosphonomethylphosphinates with natural heterocyclic bases 31
3.2.2. Analogues of dUDP and dUTP 33
3.2.3. Conclusion 38
4. Summary 40
5. List of publications of the author related to the thesis 42
6. Experimental part 43
6.1. General procedures 43
6.2. Bisphosphonates 48
6.3. Phosphonomethylphosphinates 85
7. References 99

List of Abbreviations

A	adenine
AIDS	acquired immunodeficiency syndrome
AMP	adenosine 5'-monophosphate
ANP	acyclic nucleoside phosphonate
ANPp	acyclic nucleoside phosphonate phosphate
BGE	background electrolyte
Boc	*t*-butyloxycarbonyl
BP	bisphosphonate
C	cytosine
CDI	1,1'-carbonyldiimidazole
CMV	cytomegalovirus
CZE	capillary zone electrophoresis
DBU	1,8-diazabicyklo[5.4.0]undec-7-en (1,5-5)
(*S*)-DHPA	9-[2,3-(dihydroxy)propyl]adenine
DMF	*N*,*N*-dimethylformamide
DMAP	4-dimethylaminopyridine
DMSO	dimethyl sulfoxide
DMTrCl	4,4´-dimethoxytrityl chloride
DNA	2'-deoxyribonucleic acid
dTTP	2'-deoxythymidine 5'-triphosphate
dUDP	2'-deoxyuridine 5'-diphosphate
dUMP	2'-deoxyuridine 5'-monophosphate
dUTP	2'-deoxyuridine 5'-triphosphate
dUTPase	deoxyuridine nucleotidohydrolyase
EI MS	electron impact mass spectrometry
eof	electroosmotic flow marker
FAB MS	fast atom bombardment mass spectrometry
FPP	farnesyl pyrophosphate
G	guanine
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography

HPMP	3-hydroxy-2-(phosphonomethoxy)propyl
HPMPC	1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine
HPV	human papillomavirus
HSV	herpes simplex virus
iPr	isopropyl
m-CPBA	3-chloroperbenzoic acid
Me	methyl
MeOH	methyl alcohol
PME	2-(phosphonomethoxy)ethyl
PMEA	9-[2-(phosphonomethoxy)ethyl]adenine
PMEG	9-[2-(phosphonomethoxy)ethyl]guanine
PMP	2-(phosphonomethoxy)propyl
(*R*)-PMPA	(*R*)-9-[2-(phosphonomethoxy)propyl]adenine
POM	pivaloyloxymethyl
py	pyridine
RNA	ribonucleic acid
SAH	(*S*)-adenosylhomocystein hydrolase
SAR	structure-activity relationship
T	thymine
TEAB	triethylammonium hydrogen carbonate
THF	tetrahydrofuran
TPSCl	2,4,6-triisopropylbenzenesulfonyl chloride
U	uracil
VZV	varicella zoster virus