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Synthesis of Acyclic Nucleoside Bisphosphonates

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Mgr. Silvie Vrbková

To Luboš

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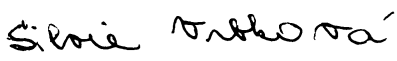
And last but not least, big thanks go to my colleagues from the Institute of Organic Chemistry and Biochemistry for creating a harmonious (not only) working atmosphere.

I solemnly swear that I wrote this thesis myself and that it represents the results of my own work, unless stated otherwise 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 have been used previously for obtaining any academic degree.

Silvie Vrbková



Contents

List of Figures

List of Abbreviations

ADP	adenosine diphosphate
AHPA	3-(6-amino-9*H*-purin-9-yl)-2-hydroxypropanoic acid
AIDS	acquired immunodeficiency syndrome
AMP	adenosine monophosphate
ANbP	acyclic nucleoside bisphosphonate
ANP	acyclic nucleoside phosphonate
ATP	adenosine triphosphate
AZT	3'-azido-2'-deoxythymidine
BGE	background electrolyte
BPTI	bovine pancreatic trypsin inhibitor
BSA	bovine serum albumin
CD	circular dichroism
CMV	cytomegalovirus
cypr	cyclopropylamino
CZE	capillary zone electrophoresis
DHPA	3-(6-amino-9*H*-purin-9-yl)propane-1,2-diol
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	2'-deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTPMP	diethylene triamine pentamethylene phosphonate
EA	ethyl acetate
EDTMP	ethylene diamine tetramethylene phosphonate
EI MS	electron impact-mass spectrometry
FABMS	fast atom bombardment mass spectrometry
FIV	feline immunodeficiency virus
FTIR	Fourier transform infrared spectroscopy
FTNMR	Fourier transform nuclear magnetic resonance
FPPS	farnesyl pyrophosphate synthase
$GABA_B$	γ-aminobutyric acid metabotropic receptor

gBP	geminal bisphosphonate
HBV	hepatitis B virus
HE	hexane
HHV	human herpesvirus
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HMBC	heteronuclear multiple bond correlation
HPMPC	9-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine
HSQC	heteronuclear single quantum correlation
HSV	herpes simplex virus
IR	infrared radiation spectroscopy
MS	mass spectrometry
MSV	Moloney sarcoma virus
NMR	nuclear magnetic resonance
NtRTI	nucleotide reverse transcriptase inhibitor
PBS	Dublecco's phosphate buffered saline
PMEA	9-[2-(phosphonomethoxy)ethyl]adenine
PMEC	1-[2-(phosphonomethoxy)ethyl]cytosine
PMEG	9-[2-(phosphonomethoxy)ethyl]guanine
PMPA	9-[2-(phosphonomethoxy)propyl]adenine
PP	phosphate-phosphonate
QSAR	quantitative structure-activity relationships
RNA	ribonucleic acid
RT	room temperature
SAH	*S*-adenosyl-L-homocysteine
SAR	structure activity relationship
TCA	trichloroacetic acid
TEAB	triethylammonium bicarbonate
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSBr	bromotrimethylsilane
TMSI	iodotrimethylsilane
VZV	varicella zoster virus

Preface

Contemporary medicine is largery based on modern and specifically targeted pharmaceuticals. Medicinal or pharmaceutical chemistry is a scientific discipline at the intersection of chemistry and pharmacy involved in design, synthesis and development of pharmaceutical drugs. Medicinal chemistry involves identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties, and their quantitative structure-activity relationships (QSAR). Pharmaceutical chemistry is focused on quality aspects of medicine and aims at assuring fitness for the purpose of medicinal products.

Antiviral drugs are a class of medication used specifically for treatment of viral infections. Like antibiotics, specific antivirals are used for specific viruses. Antiviral drugs are one class of antimicrobials, a larger group which also includes antibiotics, anti-fungal and anti-parasitic drugs. Most of the antivirals now available are designed to help to deal with HIV, herpesvirus, and the hepatitis B and C viruses, which can cause liver cancer. The emergence of antivirals is the product of a greatly expanded knowledge of the genetic and molecular function of organisms. This allows for better understanding of the structure and function of viruses and for major advances in the techniques for finding new drugs. Of particular interest is the research dealing with the human immunodeficiency virus (HIV), the cause of the deadly acquired immunodeficiency syndrome (AIDS) pandemic.

The first step of drug discovery involves the identification of new active compounds, which are typically found by screening of many compounds for the desired biological properties. They can come from synthetic sources and combinatorial chemistry.

This work focused on synthesis of novel types of acyclic nucleoside bisphosphonates, *i.e.*, analogues of basic building blocks of DNA and RNA – nucleotides. Structurally similar compounds, acyclic nucleoside phosphonates, have shown antiviral activity against wide range of viruses. Our effort was to look for other substances that might act similarly. By

introducing second phosphonate group into the nucleoside moiety we tried to explore its potential effect on antiviral properties of the studied compounds.

Chapters 1 and 2 give brief overview of the history and present state of the chemistry of nucleic acid analogues, and summary of the aims of this work, respectively. The synthesis of symmetrical acyclic nucleoside bisphosphonates (ANbPs), as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor, are discussed in Chapters 3 and 4. Chapter 5 is dealing with the synthesis and characterization of lipophilic ANPs and ANbPs. The synthesis and characterization of chiral ANbPs is reported in Chapter 6. Chapter 7 deals with synthesis of alendronate-like BPs and PPs as new potential substances for osteoporosis treatment. Results of the cytostatic pre-screening of some of the synthesized compounds are reported in Chapter 8. The summary and conclusions are given in Chapter 9.

The work described in this thesis resulted in four publications in international peer-reviewed journals.[1–4] Due to copyright issuess the manuscripts are not attached. The reprints are, however, available upon request.

Chapter 1

Introduction

Studies of nucleic acid analogues, particularly nucleosides and nucleotides, have been the target of biochemical, biological and pharmacological research for many years. These investigations have importance for the knowledge of relationship between the structure of compounds and their biological activity. The aim of these studies was the systematic exploration and the synthesis of such substances, that are structurally similar to natural nucleosides and nucleotides, and that are able to substitute the natural compounds in methabolic pathways.[5]

Incorporation of these analogues into newly synthesized DNA or RNA chains, together with natural nucleotides, may influence the transport of genetic information. Perhaps the best known synthesized analogue belonging to this group is 3'-azido-2'-deoxythymidine (AZT) used for AIDS treatment.[5] Oxetanocin A[6,7] and Aristeromycin,[8] both isolated from the natural material, are other representatives of this group. All three substances possess antiviral properties. Their structures are displayed in Figure 1.1.

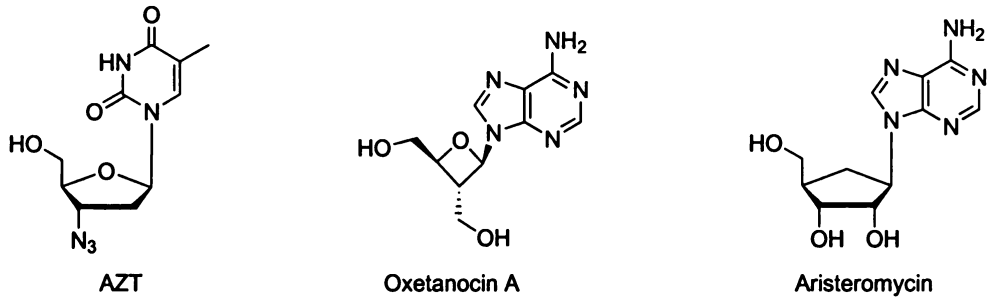


Figure 1.1: Structures of AZT, Oxetanocin A, and Aristeromycin – nucleoside analogues used in medicinal practice.

1.1 Overview of nucleic acid analogues research

Various biologically active compounds have been discovered in the chemistry of nucleic acids during the past decades. The effort to obtain new cytostatics and compounds for the therapy of metabolic diseases was the main motivation. Considerable amount of interest was directed towards the discovery of antivirals for AIDS treatment (*i.e.*, compounds effective against retroviruses HIV-1 and HIV-2).

Early discovered analogues, belonging to antimetabolite group, act by entering the metabolic pathway of the nucleic acids. Interactions with appropriate enzymes as their alternative substrate, inhibitor or inactivator cause virostatic, cytostatic, or another biological activity of such compound.

The closest structural similarity with the natural metabolites was the aim in the group of primary generation of antimetabolites.[9–11] Instability in the organism arising from their similarity to the natural metabolites was a big disadvantage of the first generation of nucleic acid analogues.

This problem led to the discovery of second generation of antimetabolites, *e.g.*, C-nucleosides,[12] carbocyclic nucleosides,[13] or acyclic nucleosides (see Figure 1.2), where the sugar moiety was replaced by aliphatic chain linked to the N^9 nitrogen of purines (*e.g.*, acyclovir,[14,15] ganciclovir,[16,17] 3-(6-amino-9*H*-purin-9-yl)-2-hydroxypropanoic acid (AHPA),[18] or (*S*)-3-(6-amino-9*H*-purin-9-yl)propane-1,2-diol ((*S*)-DHPA).[19]

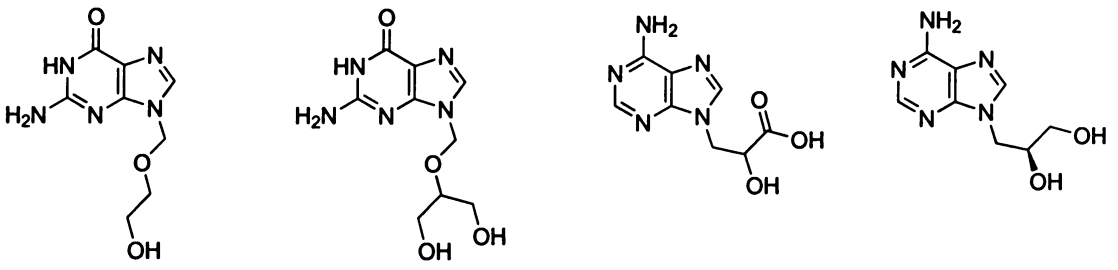


Figure 1.2: Second generation of antimetabolites – acyclic nucleosides.

Systematic studies showed that acyclovir and ganciclovir were phosphorylated to their 5'-nucleotides *in vivo*, whereas AHPA and DHPA act as SAH-hydrolase inhibitors without being phosphorylated. After the undergoing deconversion to triphosphate, they inhibit

viral DNA-polymerase. However, their direct application failed because of the lability of the phosphomonoester bond. Dephosphorylation occurred in blood plasma or during the transport through the cell membrane.[20] This complication resulted in the development of new isopolar and isosteric phosphate analogues resistant against the enzymatic reactions. One of the simplest solutions fulfilling these requirements was replacement of phosphate by enzymatically stable phosphonate group (change of -C-O-P- to -C-O-C-P-). Very interesting in this respect are phosphonomethyl ethers.[21]

Structure activity relationship (SAR) investigations in the series of acyclic nucleotide analogues bearing a modified phosphonic acid residue in the side chain revealed several biologically active acyclic nucleoside phosphonates (ANPs) so far. Some of them are nowadays used in clinical practice[22,23] and will be briefly discussed in the following text.

9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA, Adefovir, see Figure 1.3) was originally intended as an anti-HIV drug, but due to the side effects at the therapeutic dosage, the clinical trials were abandoned.[24] However, very promising results were found in the tests against hepatitis B virus (HBV). In 2002 Adefovir (formulated as the orally available prodrug adefovir dipivoxil,[25] Hepsera™) was approved as an orally administered nucleotide reverse transcriptase inhibitor (NtRTI) used for treatment of hepatitis B, particularly for patients resistant to Lamivudine.[26,27] PMEA is used against human herpesvirus 6 (HHV-6), cytomegalovirus (CMV), and duck hepatitis virus,[28] and also against retroviruses – Moloney sarcoma virus (MSV), HIV (human immunodeficiency virus), feline immunodeficiency virus (FIV), and Visna.

9-(*R*)-[2-(Phosphonomethoxy)propyl]adenine ((*R*)-PMPA, Tenofovir, see Figure 1.3) is highly active against retroviruses. After the withdrawal of adefovir dipivoxil from clinical trials in AIDS patients, it turned to be the most promising ANP type candidate for anti-AIDS drug. These substances block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people.[29–31] In 2001 it was approved for the treatment of HIV infections (AIDS) in the form of tenofovir disoproxil fumarate (Viread™).[32] Tenofovir is also available in a combination with Emtricitabine in a product with the brand name Truvada®. Atripla®, a triple combination of Tenofovir, Emtricitabine and Efavirenz is now also available for treatment of HIV.

(*S*)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine ((*S*)-HPMPC, Cidofovir, see Figure 1.3) is the most studied compound of the ANP series.[33,34] Cidofovir has been originally approved for intravenous treatment of cytomegalovirus retinitis in AIDS patients

(Vistide^{TM}).[35–37] It exhibits strong activity against herpesviruses and adenoviruses and it was successfully used for therapy of mucocutaneous herpes simplex virus infectios (local application),[38] and of genital herpes.[39] Its main application lies probably in its high anti-poxvirus activity,[40] and most importantly, variola virus (causative agent of smallpox), and the monkeypox virus.[41] The last two viruses are suspected as biological weapons, directed against densely populated areas, where they could cause an epidemy with high morbidity and mortality. As there is not enough population vaccinated against this virus and there is no knowledge how high the corresponding antibody level must be in the population, there is an iminent danger of such a virus being spread. Cidofovir is further explored in design and development of lipophilic prodrugs, in order to improve efficiency.[42,43]

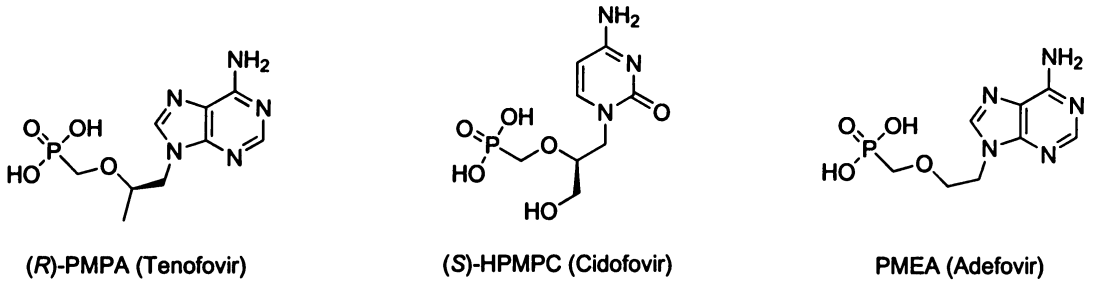


Figure 1.3: Acyclic nucleoside phosphonates (ANPs) used in medicine.

Recently, the attention in our laboratory was directed towards the synthesis of new types of ANPs originating from 4-substituted 2-amino-6-hydroxypyrimidines.[44] During these investigations, significant potential activity of 6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives, compounds derived from 2,4-diaminopyrimidine (**1a**, **1c**, **1d**)[45] and 2-amino-4-hydroxypyrimidine (**1b**),[45] and their C5-substituted congeners **2**[46,47] were discovered (see Figure 1.4).

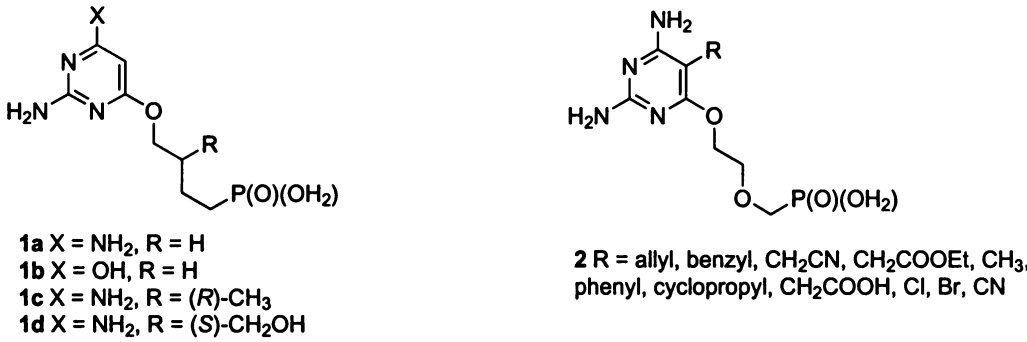
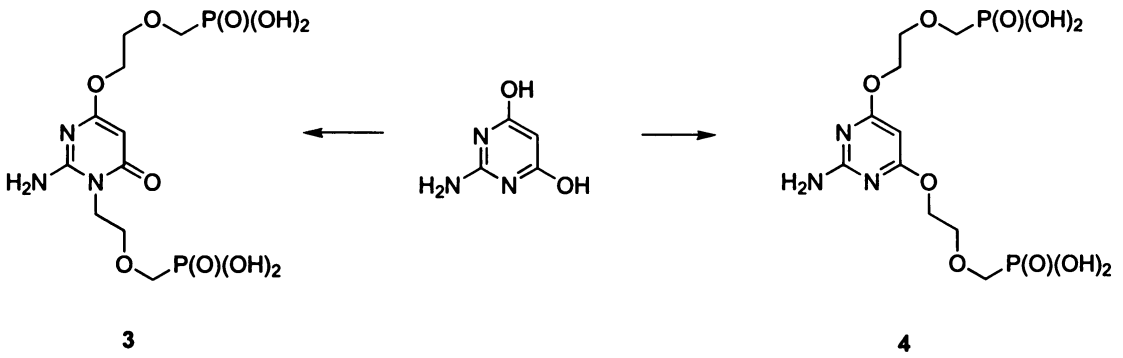


Figure 1.4: 6-*O*-Substituted ANPs and their C5'–substituted congeners.

Among the products isolated in these studies, bisphosphonates **3** and **4** were also identified[44] (see Figure 1.5). Despite the fact that these compounds constitute a new class of potential antiviral agents, they have not yet received much attention.

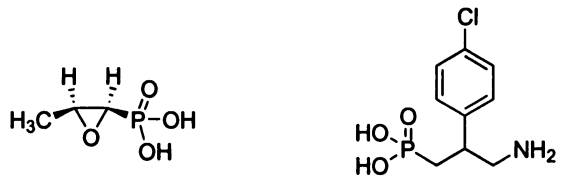


3 4

Figure 1.5: First isolated acyclic nucleoside bisphosphonates (ANbPs).

1.2 Phosphonates

Phosphonates or phosphonic acids are organic compounds containing one C-$PO(OH)_2$ or C-$PO(OR)_2$ groups with R being alkyl or aryl. The first natural phosphonate, 2-aminoethylphosphonic acid, was identified in 1959. It occurs in plants and many animals, mostly in cellular membranes. Phosphonates are quite common among various organisms, ranging from prokaryotes to eubacteria, fungi, mollusks, or insects. Their biological role is still poorly understood, however, some of the natural phosphonates possess significant biological activity. For example, Fosfomycin (Monurol®) is used in clinical practice as bactericidal antibiotic,[48] and Phaclofen is selective γ-aminobutyric acid metabotropic receptor ($GABA_B$) antagonist (see Figure 1.6 for their respective structures).[49]



Fosfomycin Phaclofen

Figure 1.6: Natural phosphonates used in medicine.

Phosphonates possess three distinctive properties. Since the work of Schwarzenbach[50] in 1949, phosphonic acids have been known as effective chelating agents for two- and trivalent metal ions.[51] Introduction of an amine group into the molecule (resulting in -NH_2-C-$PO(OH)_2$ arrangement) increases the metal binding ability of the phosphonate. Examples for such compounds are ethylene diamine tetramethylene phosphonate (EDTMP) and diethylene triamine pentamethylene phosphonate (DTPMP), displayed in Figure 1.7.

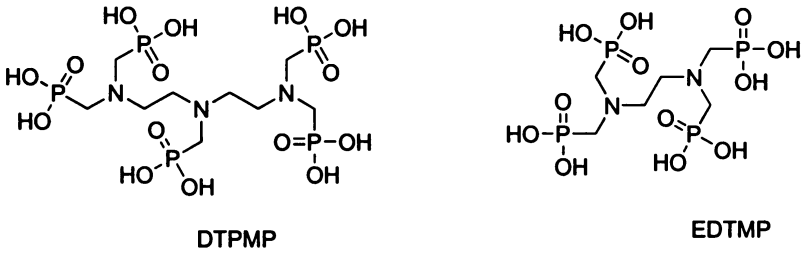


Figure 1.7: Phosphonates with increased chelating ability.

An important industrial use of phosphonates is in cooling waters, desalination systems, and in oil fields to inhibit scale formation. In pulp and paper manufacturing and in textile industry, they are used as peroxide bleach stabilizers. This effect comes from their chelating ability for metals that could inactivate the peroxide. In detergents, they are used in a combination of chelating agent, scale inhibitor, and bleach stabilizer. Phosphonates are also used in medicine for treatment of various bone and calcium metabolism diseases and as carriers for radionuclides in bone cancer treatments.[52]

1.3 Bisphosphonates

1.3.1 Geminal bisphosphonates

The first geminal bisphosphonates (gBPs) were synthesized in 1865.[53] They were initially used mainly as antiscalling and anticorosive agents but also as complexing agents in the textile, fertilizer, and oil industry, similarly to phosphonates.[54] However, their potential for the treatment of various diseases of bone mineral metabolism has been recognized only in late 1960s.

Geminal bisphosphonates are isosteric analogues of the naturally occuring inorganic pyrophosphates in which the oxygen in the P-O-P group is replaced by a carbon, resulting

in a metabolically stable P-C-P structure. Unlike pyrophosphates, gBPs possess better metabolic stability because they are not recognized by pyrophosphatases. They are also resistant against acidic hydrolysis. Similarly to pyrophosphates, gBPs have high affinity to bone minerals. Importantly, the replacement of the oxygen atom between the two phosphonic acid moieties of pyrophosphate by a carbon atom opened up the possibility of attaching side chains (see Figure 1.8).

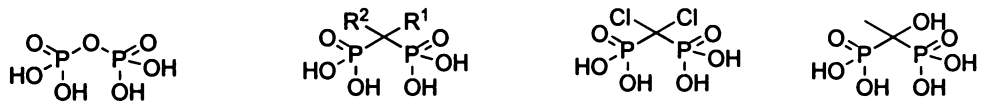


Figure 1.8: Pyrophosphate and the first generation of geminal bisphosphonates used in medicine.

Substitution by hydroxyl group at the R^1 position of the gBP increases the affinity for calcium even further when compared to phosphonates. Such derivatives are then able to act as tridentate ligands. The nature of R^2 is a key to the optimization of gBPs as potent inhibitors of osteoclastic bone resorption. The first generation gBPs had either a single atom or a simple alkyl side chain at R^2 (*e.g.*, clodronic and etidronic acid) and was a relatively weak inhibitor of bone resorption.

Antiresorptive potency was markedly increased in the second generation of gBP compounds (*e.g.*, pamidronate) with a basic aminoalkyl group at R^2. The most potent third generation gBPs were found in the series containing heteroaromatic moiety with at least one nitrogen atom, linked via a single methylene group to the geminal BP unit.

Nowadays, geminal bisphosphonates (also called diphosphonates) are used in the treatment of diseases of bone and calcium metabolism with osteoporosis being the most common form (see table in Figure 1.9 for substances used in medicinal practice).[55] Their uses include the prevention and treatment of osteoporosis, osteitis deformans (*Paget's disease of bone*), bone metastasis (with or without hypercalcemia), multiple myeloma and other conditions accompanied by bone fragility.[56]

Recently, there has been a lot of speculation about the exact mechanism of gBPs action. It has been found that the molecular target of nitrogen containing gBPs (*e.g.*, risedronate, zoledronate) is farnesyl pyrophosphate synthase (FPPS), an enzyme responsible for the synthesis of the FPP used in protein prenylation, and in cholesterol, ergosterol, heme A,

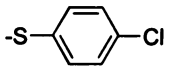
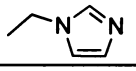
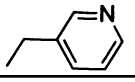
Agent	R^1	R^2	Agent	R^1	R^2
Etidronate	-OH	$-CH_3$	Neridronate	-OH	$-(CH_2)_5NH_2$
Clodronate	-OH	-Cl	Olpadronate	-OH	$-(CH_2)_2N(CH_3)_2$
Tiludronate	-H		Alendronate	-OH	$-(CH_2)_3NH_2$
Pamidronate	-OH	$-CH_2CH_2NH_2$	Ibandronate	-OH	$-CH_2CH_2N(CH_3)(CH_2)_4CH_3$
Zolendronate	-OH		Risendronate	-OH	

Figure 1.9: Geminal bisphosphonates used in medicine.

and ubiquinone production. However, the exact mechanism of the BP function is still not completely clear.

1.3.2 Other bisphosphonates and bisphosphates

ATP and other purine and pyrimidine nucleotides act as extracellular signaling molecules through activation of P2 receptors. These receptors can be divided into two structurally unrelated categories: P2Y receptors, which are G protein-coupled receptors, and the P2X receptors, which are ligand-gated cation channels.[57,58] Numerous subtypes have been cloned within each family regulating a diverse range of functions in the central and peripheral nervous systems, the cardiovascular systems, the endocrine systems, lungs, intestines, muscle, and the immune systems.[57,59,60]

The P2Y receptors are activated by adenine and/or uracil nucleotides.[61] Agonist binding at $P2Y_1$ is known to result in activation of phospholipase C, which generates inositol phosphates and diacylglycerol from phosphatidylinositol-4,5-bisphosphate.[62] The $P2Y_1$ receptor is present in heart, skeletal and various smooth muscles, prostate, ovary, and brain. A $P2Y_1$ receptor in platelets is involved in ADP-promoted aggregation.[63] Thus, a selective $P2Y_1$ receptor antagonists may have potential as antithrombotic agents,[64] while a selective receptor agonist may have potential as an antihypertensive or antidiabetic agent.[65]

Various naturally occuring bisphosphates of adenosine (*e.g.*, 3',5'-bisphosphate and 2',5'-bisphosphate, see Figure 1.10) were reported to act as partial agonists or competitive antagonists of the $P2Y_1$ receptors.[66] Also, acyclic nucleoside analogues, containing mainly

symmetrical branched aliphatic chains, attached at the N^9-position of adenine, were shown to be $P2Y_1$ receptor antagonists.[67–69] The N^6-methyl and 2-chloro substitution of the adenine moiety was found to increase the potency and selectivity of the bisphosphates derivatives acting as competitive $P2Y_1$ antagonists.[70,71]

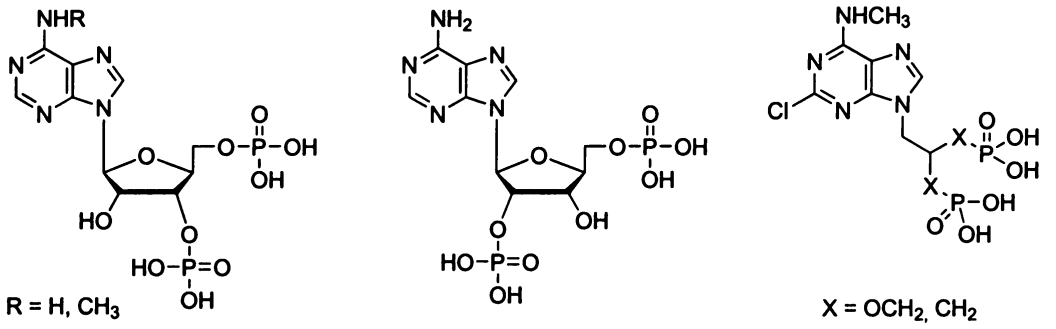


Figure 1.10: Structure of nucleotide derivatives that have been shown to act as inhibitors of $P2Y_1$ receptors.

Among ribose modifications, both rigidifying (*e.g.*, bicyclic carbocycles)[72] and adding flexibility (*e.g.*, acyclic modifications)[67] have led to effective $P2Y_1$ receptor ligands. A variety of modified cyclic and acyclic nucleotide analogues were synthesized and examined as antagonists of the $P2Y_1$ receptor on ADP-induced responses in human platelets and at recombinant human $P2Y_1$ receptors.[67–69,71,73] The synthetic study of pharmacological probes for the selective $P2Y_1$ agonists and antagonists with high affinity is an interesting subject not only for the discovery of new therapeutic drugs but also for the investigation of the role of this receptor *in vivo* from the points of view of medicinal chemistry.

Also, the bisphosphonate group was involved in the SAR to $P2Y_1$ receptor as a potentially more stable alternative to phosphates that would preserve the required negative charges. It was found[74] that a 5'-*O*-phosphonomethyl analogue of ribose interacts with $P2Y_1$ receptor. Despite its lower potency when compared to corresponding bisphosphates, the ratio of agonist/antagonist properties was the same. The present structural studies may, therefore, prove useful in the search for other phosphate group substitutes.

Chapter 2

Aims of the work

Acyclic nucleoside phosphonates (ANPs) are an interesting and important class of compounds exerting a wide range of biological activities.[21,23] This thesis is a part of the long-term exploration of the structure-activity relationship of modified ANPs, performed by our group.

The aims of this thesis can be described by the following terms.

- To synthesize a series of glycerol based acyclic nucleoside bisphosphonates whose structure follow up the known and biological active acyclic nucleoside phosphonates, phosphates or bisphosphates and evaluate them for biological activity (Chapters 3 and 4).
- To synthesize the lipophilic glycerol based acyclic nucleoside bisphosphonates and phosphonates and compare their biological activity with their free phosphonic acids (Chapter 5).
- To synthesize a series of the chiral acyclic nucleoside bisphosphonates with a very new structure in order to assess the biological activity (Chapter 6).
- To synthesize bisphosphonate and phosphate-phosphonate acyclic nucleosides based on alendronate and to assess their biological activity (not only limited to osteoporosis) (Chapter 7).

We wanted to develop new potential biological active compounds with high stability *in vivo*, more specifically ANPs bearing two phosphonate residues in the molecule, as a part

of the search for new antivirals and antineoplastics. Another goal was to obtain the data concerning the connection between the structure and antiviral or cytostatic effect of these substances.

2.1 Selected work-up methods

The general approach for the synthesis of acyclic nucleoside bisphosphonates was the alkylation of various nucleobases with appropriate bisphosphonate building block bearing a good living group (tosyl, mesyl, iodine, ...). Such prepared N^9 or N^7-alkylated purines and N^1 or O^2-alkylated pyrimidines were further converted to various base-modified derivatives.

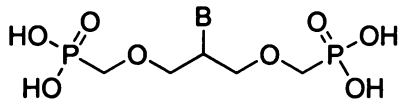
The progress of the reactions, as well as the purity of the intermediates and final products were monitored by TLC, electrophoresis and HPLC. Compounds were purified and isolated by column chromatography on silica gel, by thin-layer chromatography with UV active silica gel sorbent, by ion exchange chromatography and in some cases also by preparative HPLC with a reverse phase. NMR spectroscopy and mass spectrometry (MS) were chosed for the determination and characterization of the products. In case of optically active compounds, the CD spectra, capillary zone electrophoresis (CZE) and optical rotation were also used for analysis. The purity of final products was proved by elemental analysis or high resolution MS (HRMS).

The nomenclature of compounds used in this thesis corresponds to nomenclature used in the chemistry of nucleic acids. Almost in all cases, the bisphosphonate is treated as a side chain of the heterocyclic base.

Individual projects are written as publications, with introduction, results and discussion, conclusions, and experimental part.

Chapter 3

Synthesis of glycerol based ANbPs



B = purine or pyrimidine ring

3.1 Introduction

Acyclic nucleoside phosphonates (ANPs) deserve proper attention owing to their significant biological activity. Once transported into the cell, the liberated ANP undergo metabolic transformation to the α-modified triphosphate analogues – the active antimetabolites. These dNTP analogues then inhibit the DNA synthesis *de novo* acting as chain terminators (**2** and **3**) or as alternative substrates/inhibitors **1** capable of limited incorporation which is followed by appropriate consequences. Phosphonomethyl ether group is one of the characteristic features of the pharmacophore in biologically active ANP. To our knowledge, there are only two compounds described in the literature which bear two such groups in the molecule (Figure 3.1): the asymmetric 3-phosphonomethyl ether **5** of HPMPA, 9-[3-hydroxy-2-(phosphonomethoxy)propyl)adenine **4**,[75] and 2-amino-4,6-bis[2-(phosphonomethoxy)ethoxy]pyrimidine **7**, a symmetrical ANP from the novel class of open-ring compounds.[44] While compound **5** is devoid of any antiviral activity in contrast to its parent compound, the antiviral activity demonstrated by the parent pyrimidine derivative **6** remains preserved following the introduction of an additional 2-phosphonomethyl group in **7**.

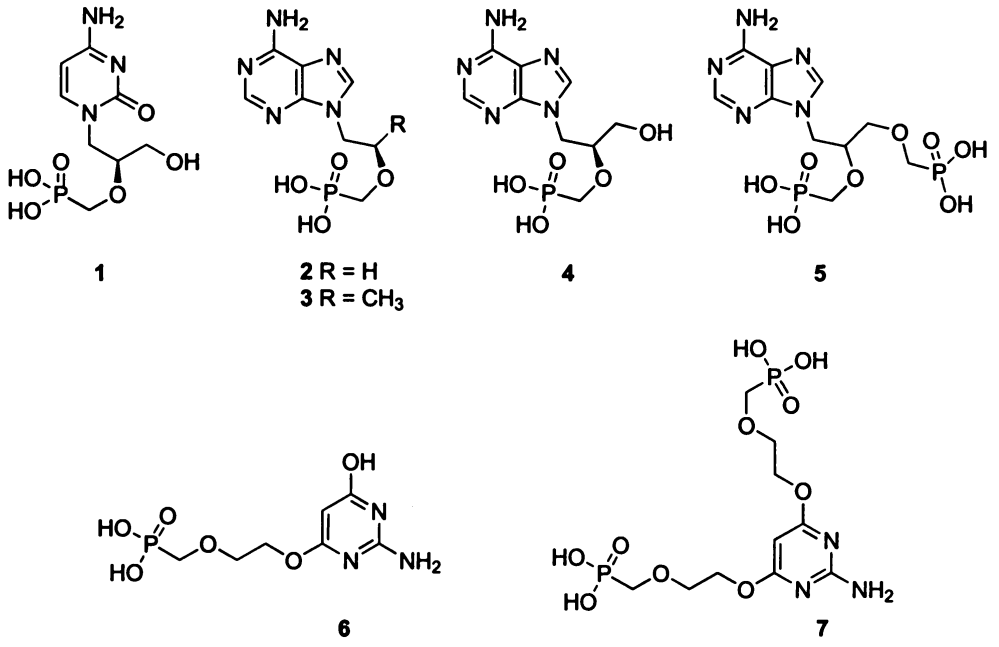


Figure 3.1: Model compounds containing phosphonomethyl ether group

Acyclic nucleoside phosphonates are compounds with broad spectrum of antiviral and cytostatic activity.[21,23] Among them, PMEA is active against DNA viruses and retroviruses, and was approved for hepatitis B therapy (adefovir dipivoxil).[25] As a part of the systematic study on the structure activity relatioships (SAR) in ANP series we intended to study the behaviour of ANP analogues bearing two phosphonate moieties in the molecule. Formally, these compounds could be also taken for double PME molecules. In addition to their potential biological activity, these compounds could be also considered as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor (Figure 3.2).[69]

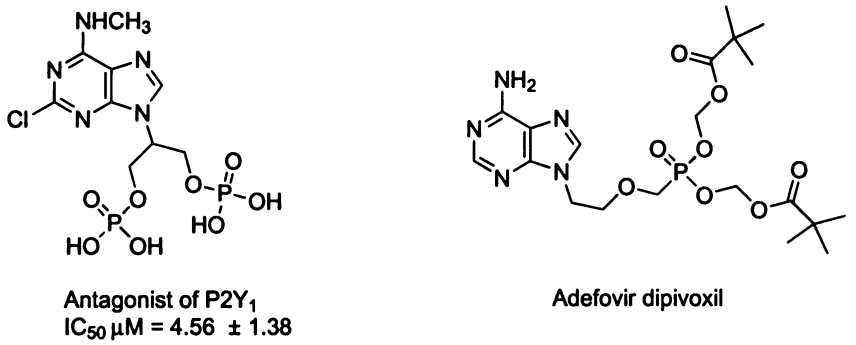
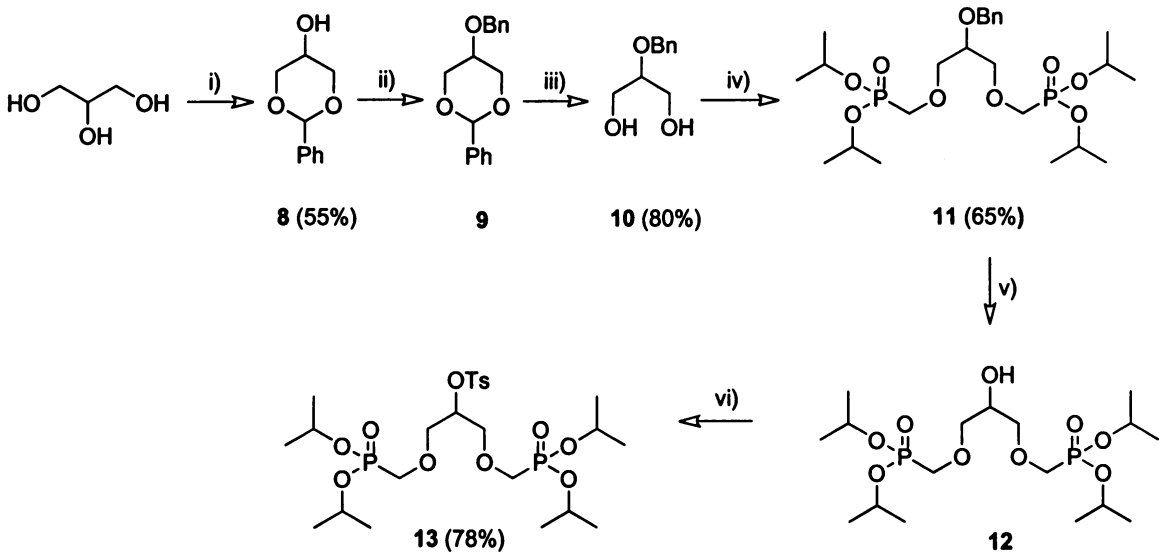


Figure 3.2: Biological active model compounds

3.2 Results and discussion

The synthesis of the bisphosphonate alkylating agent proceeded as depicted in Figure 3.3. 2-(Benzyloxy)propane-1,3-diol **10** can be synthesized by known methods,[76,77] however, it was used an alternative approach. 2-Phenyl-1,3-dioxan-5-ol **8** was prepared by the known procedure[78] and purified by crystallization from cyclohexane. It was subsequently converted to its *O*-benzyl derivative **9**. The benzylidene protecting group was removed by acid-catalyzed hydrolysis (Dowex 50 X 8, H^+ form) to provide 2-(benzyloxy)propane-1,3-diol **10**. This intermediate was further alkylated with (diiisopropoxyphosphoryl)methyl tosylate to form compound **11**. It was subsequently hydrogenated and the thus-obtained intermediate **12** was under standard conditions finally converted to 1,3-bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl tosylate **13**.



i) H_2SO_4, benzaldehyde, toluen, reflux; ii) NaH, BnBr, THF; iii) Dowex 50 x 8 (H^+ form), 80% MeOH, reflux; iv) $TsOCH_2P(O)(OiPr)_2$, NaH, DMF, 0 °C; v) H_2/10% Pd/C, MeOH, HCl, r.t.; vi) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C.

Figure 3.3: Synthesis of the bisphosphonate alkylating agent

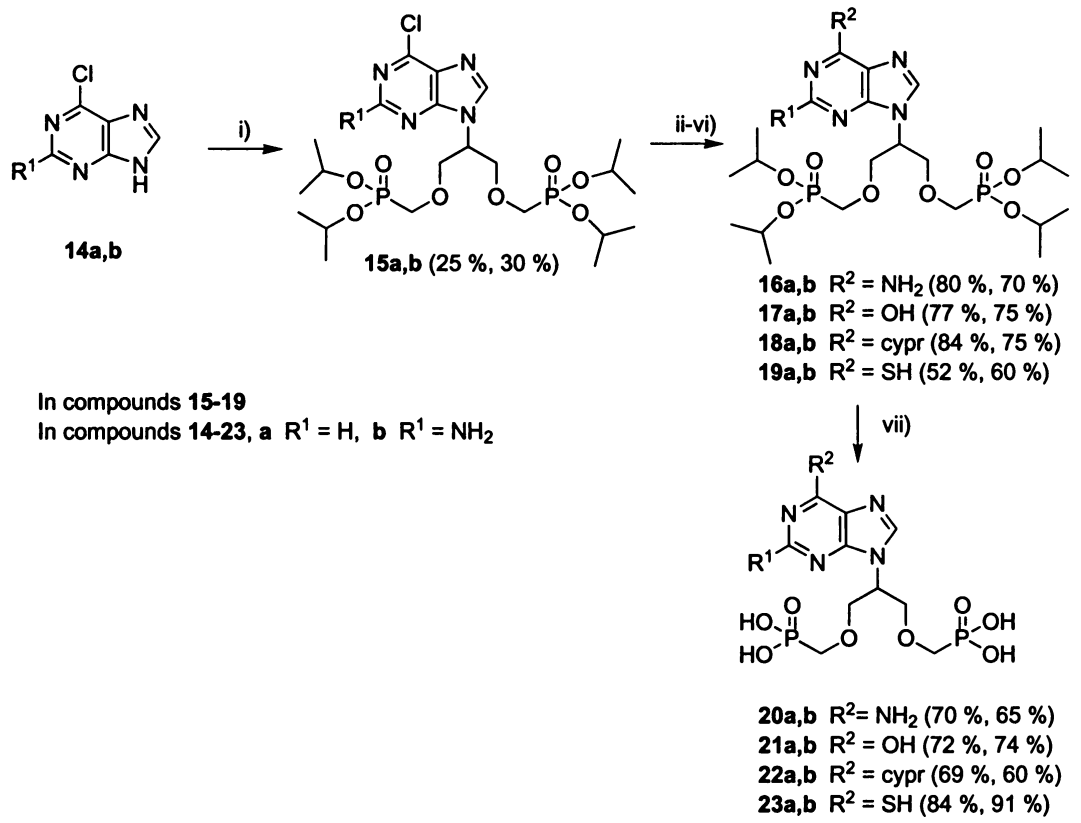
Alkylation of purines with compound **13** is depicted in Figure 3.4. The reaction of 6-chloropurine **14a** and 2-amino-6-chloropurine **14b** proceeded at N^9 position of the ring to give compounds **15a** and **15b** only. Essentially no N^7-regioisomer formation was observed. In all the discussed cases, NMR analysis was used to identify the position of substitution of the purine moiety. All signals of hydrogen and carbon atoms were assigned usig 2D-^{1}H,^{13}C

HSQC and 2D-$^{1}H,^{13}C$ HMBC experiments. In the case of N^{9}-isomers, protons on carbon atom next to the purine nitrogen have strong crosspeaks in HMBC spectra with carbons C-4 and C-8 of the purine moiety.

The intermediates **15** were further converted to other base-modified phosphonates: thus, adenine **16a** and 2,6-diaminopurine **16b** derivatives were prepared by the ammonolysis with methanolic ammonia; hypoxanthine derivative **17b** was obtained in reaction of **16a** with 3-methylbutyl nitrite in 80% acetic acid while acid hydrolysis of **15b** led to guanine derivative **17b**. Replacement of chlorine atom in the 6-chloro derivatives **15** by sulfanyl group in compounds **19a** and **19b** was achieved by the reaction with thiourea. It was also prepared the 6-(cyclopropyl)amino derivatives **18a** and **18b** by reaction of tetraester **15a** and **15b** with cyclopropylamine in dioxane. Cyclopropylamino group possesses very interesting feature. It is catabolized in the cells to 6-oxo derivative. 2-Amino-6-(cyclopropyl)aminopurines can therefore serve as guanine prodrugs.[79,80] Subsequent deprotection of compounds **16-19** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free bisphosphonic acids **20-23** that were ultimately purified as deionized crude materials by ion exchange chromatography.

The reaction of cytosine and 5-methylcytosine derivatives **24a** and **24b** with bisphosphonate **13** afforded a mixture of O^{2}-regioisomer (**25**; yield 7 %,) and N^{1}-regioisomer (**26**; yield 14 %) in the ratio 1:2, which were further converted to the corresponding free bisphosphonic acids **27** as shown in Figure 3.5. O^{2}- and N^{1}-isomers in the pyrimidine series can be readily distinguished by NMR spectroscopy. The carbon atom chain linked to the oxygen shows $\delta = 72$ ppm; when bonded to nitrogen its chemical shift was $\delta = 54$ ppm (cf. Experimental). Furthermore, the protons bonded to this carbon have crosspeaks in 2D-$^{1}H,^{13}C$-HMBC spectra to carbon atoms C-2 and C-6 (in the case of N^{1}-isomers) or only to C-2 (in the case of O^{2} isomer).

Figure 3.6 shows the course of alkylation of uracil **29a** and thymine **29b**. Of the three products formed in both cases (O^{2}- **30a,b**, N^{1}- **31a,b** and N^{1},N^{3}-isomer **32a,b**) the N^{1}-isomer **31a,b** was always the major product. It was isolated in about 15% yield while the O^{2}-isomer and N^{1},N^{3}-alkylated compound were obtained only in 1.1% and 0.5% yield, respectively. The subsequent deprotection of tetraester **31a,b** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free bisphosphonic acids **33a,b** that were isolated from the deionized product by ion exchange chromatography. Isolated isomers could again be distinguished by NMR spectroscopy. All signals of hydrogen and carbon atoms were assigned using 1D and 2D NMR experiments. The position of the



i) **8**, Cs_2CO_3, DMF, 90 °C; ii) for compounds 16: **15**, methanolic ammonia, 100 °C; iii) for 17a: **16a**, 3-methylbutyl nitrite, 80% CH_3COOH, r.t.; iv) for 17b: **16b**, 80% CH_3COOH, reflux; v) for 18: **15**, cyclopropylamine, dioxane, reflux; vi) for 19: **15**, thiourea, ethanol, reflux; vii) for 20 - 23: TMSBr, acetonitrile, r.t.

Figure 3.4: Alkylation of purines

substituent is clear from the chemical shift of the carbon atom bonded to the pyrimidine ring (72 ppm for the O^2 derivative, 54 ppm for the N^1 derivative, and 52 ppm and 44 ppm for the N^1,N^3derivative) and is confirmed also by 2D-HMBC spectra.

The results are therefore in agreement with the known fact that N^1 position of the thymine and uracil ring is the prefered alkylation target under given conditions.[81] Bis-N^1,N^3- and O-isomer are the minor products. To verify the general validity of this phenomenon it has been briefly examined the regiospecificity of alkylations of thymine with the tosyl derivatives of various primary and secondary alcohols. The results summarized in Figure 3.7 clearly demonstrate that – under the reaction conditions used – the major product of the reaction is the N^1-isomer both with the primary and secondary tosylates. All tosylates of primary alcohols (No. 1, 2, and 3) provide mixtures of N^1- and N^1,N^3 derivatives in the ratio 3:1. When tosylates of secondary alcohols is used (No. 4) N^1 derivative is preferentially formed.

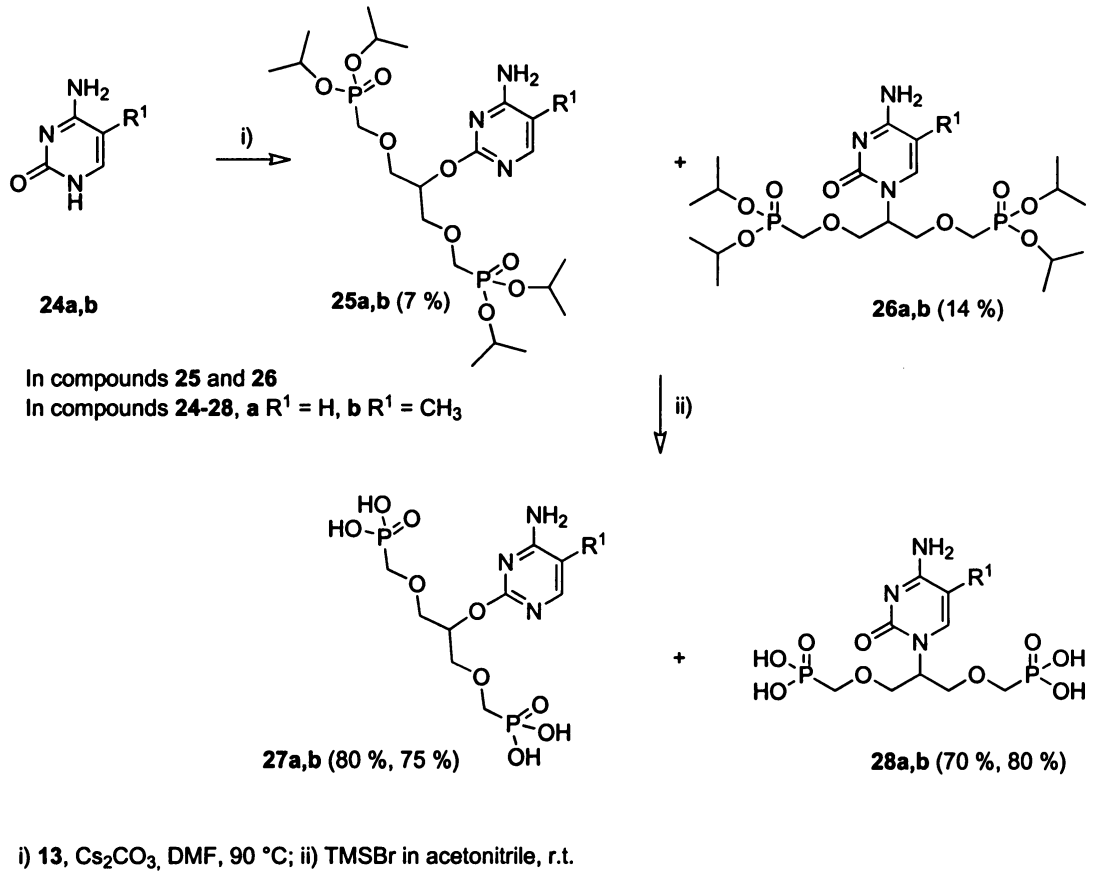


Figure 3.5: Alkylation of cytosine and 5-methylcytosine

Also, the reaction with "bis-phosphonate precursor" (No. 5) gives the same result. In all these cases O-derivative is formed as minor product.

3.3 Conclusions

In conclusion, it has been developed a general method for the synthesis of symmetrical bisphosphonates as a novel group of ANP. Selected purine and pyrimidine derivatives were prepared and characterized. Biological activity of the bisphosphonates both in the purine and pyrimidine series is limited by their high polarity. They are inactive both in the antiviral assays of DNA viruses, RNA viruses and retroviruses, specifically against hepatitis B and C viruses and against HIV, against herpesviruses (HSV-1 and HSV-2, VZV and HCMV) and RNA virus models of human pathogens. All the target compounds were also subjected to the screening of inhibitory activity murine leukemia L1210 cells,

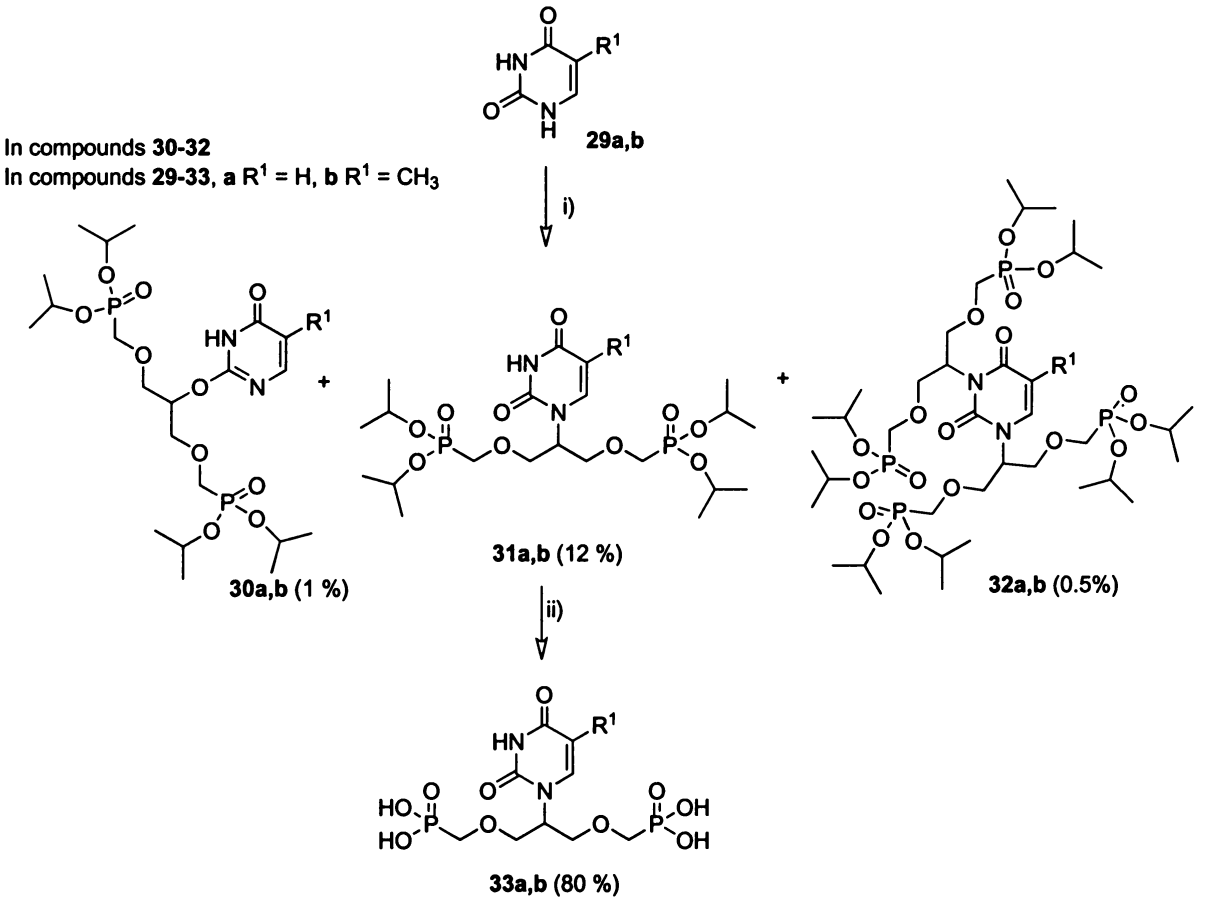
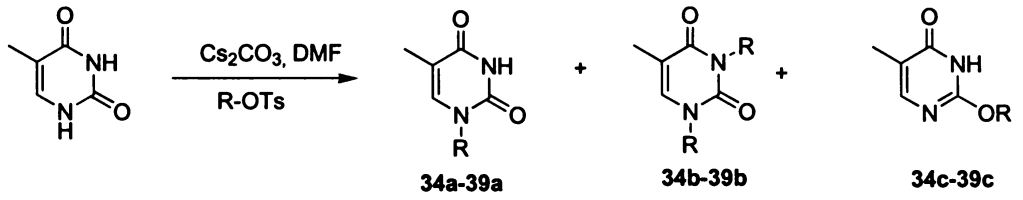


Figure 3.6: Alkylation of thymine and uracil

human promyelocytic leukemia HL60 cells, murine L929 cells, human cervix carcinoma HeLa S3 cells and human T-lymphoblastoid CCRF-CEM cell line. None of the compounds showed any significant cytostatic activity. Nonetheless, the data are not considered to be conclusive, since some tests are still in progress. Also, it is necessary to convert at least some of the compounds presented in this project to their lipophilic derivatives, *e.g.*, to mono- or diesters with long-chain aliphatic alcohols to enhance their transport across the cellular membrane. This study is discussed in Chapter 5.



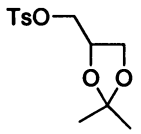
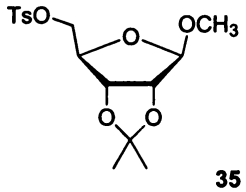
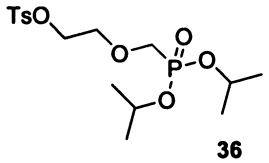
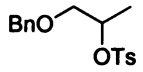
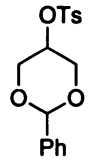
No.	R-OTs	N^1-derivative	bis-N^1,N^3 derivative	O-derivative
1.	 34	39% **34a**	10% **34b**	1% **34c**
2.	 35	31% **35a**	10% **35b**	1% **35c**
3.	 36	32% **36a**	10% **36b**	1% **36c**
4.	 37	25% **37a**	< 4% **37b**	1% **37c**
5.	 38	23% **38a**	< 1% **38b**	1% **38c**

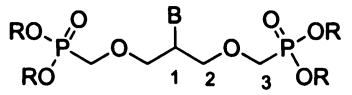
Figure 3.7: Alkylation of thymine with various tosylates

3.4 Experimental part

Unless otherwise stated, solvents were evaporated at 40 °C / 2 kPa and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. NMR spectra were measured on an FT NMR spectrometer Varian Unity 500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz frequency). Chemical shifts are in ppm and

coupling constants (J) in Hz. Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using FAB or EI. UV spectra were taken on a Beckman DU-65 spectrophotometer in aqueous solution. Elemental analyses were carried out on a Perkin Elmer CHN Analyser 2400, Series II Sys. Chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic). Dimethylformamide, acetonitrile and dichloromethane were distilled from P_2O_5 and stored over molecular sieves (4 Å). Tetrahydrofuran was distilled over sodium with benzophenone as indicator.

General structure for NMR numbering



B = purine or pyrimidine ring

2-Phenyl-1,3-dioxan-5-ol (8)

Concentrated H_2SO_4 (3 drops) was added to a solution of glycerol (113 g, 1227 mmol) and benzaldehyde (100 g, 942 mmol) in toluene (450 ml). The mixture was refluxed in modified Dean-Stark apparatus for 4 h (88 % of water separated). The reaction mixture was then cooled to room temperature and the solvent was evaporated. The residue was dissolved in ether (500 ml) and washed three times with water (500 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. Crystallization from n-hexane provided compound **8** as a white solid (yield 92 g, 55 %). Mp 61.9–63.3 °C. For $C_{10}H_{12}O_3$ (180.20) calcd: C, 66.65; H, 6.71. Found: C, 66.44; H, 6.63. FABMS: 181.1 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6 + DAc): 7.40-7.30 (m, 10H, $H_{arom.}$); 5.53 and 5.41 (2 × s, 2 × 1H, 2 × O-CH-O); 5.24 and 5.01 (2 × d, 2 × 1H, J = 2 × 5.2, 2 × OH); 4.13 (dd, 2H, J = 5.1 and 11.0, O-CH_2); 3.48 (t, 2H, J = 10.6 and 10.6, O-CH_2); 4.04 (dd, 2H, J = 1.5 and 11.8, O-CH_2); 3.95 (dd, 2H, J = 1.2 and 11.8, O-CH_2); 3.72 (tq, 1H, J = 3 × 5.2 and 2 × 10.3, O-CH_{ax}); 3.50 (m, 1H, O-CH_{eq}). ^{13}C NMR (125.7 MHz, DMSO-d_6 + DAc): 139.25; 138.57; 129.01; 128.92; 128.35 (2C); 128.28 (2C); 126.60 (2C); 126.54 (2C); 100.64 and 100.60 (2 × O-CH-O); 72.02 (2C, O-CH_2), 71.76 (2C, O-CH_2); 62.76 and 60.57 (O-CH).

5-(Benzyloxy)-2-phenyl-1,3-dioxane (9)

Under argon atmosphere **8** (90 g, 499 mmol)) was added at 0 °C to a suspension of NaH (20 g of 60% suspension in mineral oil, 499 mmol, prewashed with n-hexane) in dry THF (550 ml). The reaction mixture was then cooled to −10 °C and benzyl bromide (94 g, 549 mmol) in THF (200 ml) was added dropwise during 1 h. The mixture was stirred for 30 min at −10 °C and at room temperature overnight under argon. When the reaction was complete (TLC), methanolic ammonia (30 ml) was added. After stirring the solution for 1 h, the solvent was evaporated. The residue was dissolved in chloroform (500 ml) and washed with water (500 ml). The organic layer was dried with anhydrous $MgSO_4$, filtered and evaporated to yield crude **9**, which was used without further purification.

2-(Benzyloxy)propane-1,3-diol (10)

A solution of crude **9** in 80% methanol (500 ml) was refluxed with Dowex 50 X 8 (H^+ form, 10 g) for 4 h.

The resin was filtered off, the solution neutralized with aqueous ammonia and the solvent was evaporated. The crude product was purified by silica gel column chromatography, using chloroform – methanol gradient 0-5 %, to yield 74 g (80 %) of pure **10** as colorless solid. Mp 38.1-39.6 °C. For $C_{10}H_{14}O_3$ (182.22) calcd: C, 65.91; H, 7.74. Found: C, 65.87; H, 7.68. MS (EI) m/z: 182.2 (M^+). ^{1}H NMR (500 MHz, DMSO-d_6): 7.34 (m, 5H, $H_{arom.}$); 4.61 (s, 2H, CH_2-benzyl); 4.58 (t, 2H, $J_{OH,CH_2}=5.6$, OH); 3.52 (dt, 2H, $J_{CH_2,CH} \sim J_{CH_2,OH}=5.4$, O-$CH_2$); 3.45 (dt, 2H, $J_{gem}=11.2$, O-CH_2); 3.38 (q, 2H, $J=5.2$, O-CH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 139.47; 128.27 (2C); 127.61 (2C); 127.35; 81.12 (O-CH); 70.96 (CH_2-O-Ph); 61.05 (O-CH_2).

2-(Benzyloxy)-1,3-bis(diisopropoxyphosphorylmethoxy)propane (11)

A solution of **10** (20 g, 110 mmol) in dry DMF (50 ml) was added dropwise at 0 °C to a stirred suspension of NaH (11 g of 60% suspension in mineral oil, 274 mmol, prewashed with n-hexane) in dry DMF (450 ml) under a $CaCl_2$ protecting tube. (Diisopropoxyphosphoryl)methyl tosylate (88 g, 252 mmol) was added dropwise and the mixture was stirred at room temperature for 8 h. The reaction mixture was neutralized with 4.5 M HCl in DMF, the solvent was evaporated, the residue was co-evaporated with toluene, dissolved in ethyl acetate (300 ml) and washed three times with water (300 ml). The organic layer was dried with anhydrous $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography, using chloroform – methanol gradient 0-3 %, to yield 37 g (65 %) of pure **11** as yellowish oil. For $C_{24}H_{44}O_9P_2$ (538.55) calcd: C, 53.52; H, 8.23; P, 11.50. Found: C, 53.23; H, 8.39; P, 11.75. FABMS: 539.2 (MH^+) (40). ^{1}H NMR (500 MHz, $CDCl_3$): 7.37-7.30 (m, 5H, $H_{arom.}$); 4.74 (m, 4H, $J_{H,P}=7.7$, $J_{vic}=6.2$, CH-iPr); 4.69 (s, 2H, CH_2-O-Ph); 3.78 (tt, 1H, $J_{1,2}=5.7$ and 4.8, H-1); 3.78 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.3$, H-3); 3.68 (dd, 2H, $J_{gem}=10.3$, $J_{2a,1}=5.7$, H-2a); 3.72 (dd, 2H, $J_{gem}=10.3$, $J_{2b,1}=4.8$, H-2b); 1.34, 1.32, 1.31 and 1.30 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, $CDCl_3$): 128.29; 127.71; 127.55; 76.94 (CH-1); 73.31 (d, $J_{2,P}=11.0$, CH_2-2); 72.25 (CH_2-benzyl); 70.97 (d, $J_{C,P}=7.0$, CH-iPr); 66.36 (d, $J_{3,P}=167.0$, CH_2-3); 24.10 and 24.09 (d, $J_{C,P}=4.0$, CH_3-iPr).

1,3-Bis[(diisopropoxyphosphoryl)methoxy]propan-2-ol (12)

Palladium on activated charcoal (10% Pd, 1 g) and conc. HCl (1 ml) was added to a solution of **11** (34 g, 63 mmol) in methanol (300 ml). The reaction mixture was hydrogenated at atmospheric pressure and room temperature overnight. The catalyst was filtered off through a Celite pad, the filtrate was neutralized with Et_3N and evaporated to give crude **12**, which was directly used in the subsequent reaction. FABMS: 449.1 (MH^+) (60). ^{1}H NMR (500 MHz, DMSO-d_6): 4.93 (bs, 1H, OH); 4.59 (tt, 1H, $J_{1,2}=7.4$ and 4.5, H-1); 4.50 (m, 4H, CH-iPr); 3.78 and 3.76 (2 × dd, 2 × 2H, $J_{gem}=13.8$, $J_{3,P}=8.1$, H-3); 3.48 (dd, 2H, $J_{gem}=10.0$, $J_{2a,1}=7.6$, H-2a); 3.43 (dd, 2H, $J_{gem}=10.0$, $J_{2b,1}=4.8$, H-2b); 1.24, 1.23, 1.22 and 1.21 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 74.55 (d, $J_{2,P}=10.7$, CH_2-2); 70.26 (d, $J_{C,P}=6.4$, CH-iPr); 68.47 (CH-1); 65.56 (d, $J_{3,P}=164.6$, CH_2-3); 24.01 and 23.90 (d, $J_{C,P}=4.0$, CH_3-iPr).

1,3-Bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl tosylate (13)

A mixture of crude **12**, DMAP (0.7 g) and Et_3N (8 g) in dry dichloromethane (200 ml) was stirred at 0 °C with a $CaCl_2$ protecting tube. Tosyl chloride (15 g) in dichloromethane (100 ml) was added dropwise. The mixture was stirred for 1 h at 0 °C and then kept in refrigerator overnight. The organic solution was diluted with ice water (300 ml) and the layers separated. The organic layer was dried with anhydrous $MgSO_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica

gel, using chloroform – methanol gradient 0-2 %, to yield 30 g (78 %) of pure **13** as yellowish oil. For $C_{24}H_{44}O_{11}P_2S$ (602.61) calcd: C, 47.83; H, 7.36; P, 10.28; S, 5.32. Found: C, 47.50; H, 7.38; P, 10.53; S, 5.57. FABMS: 602.9 (MH^+) (75). ^{1}H NMR (500 MHz, DMSO-d_6): 7.80 and 7.46 (d, 2 × 2H, $H_{arom.}$); 4.70 (tt, 1H, $J_{1,2}$ = 7.4 and 4.5, H-1); 4.56 (m, 4H, CH-iPr); 3.79 and 3.75 (2 × dd, 2 × 2H, J_{gem} = 13.8, $J_{3,P}$ = 8.1, H-3); 3.67 (dd, 2H, J_{gem} = 10.3, $J_{2a,1}$ = 7.4, H-2a); 3.64 (dd, 2H, J_{gem} = 10.3, $J_{2b,1}$ = 4.5, H-2b); 2.41 (s, 3H, CH_3-tosyl); 1.23, 1.22, 1.21 and 1.20 (4 × d, 24H, J_{vic} = 6.2, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 144.94; 133.48; 130.18 (2C); 127.79 (2C); 79.32 (CH-1); 70.93 (d, $J_{2,P}$ = 10.2, CH_2-2); 70.37 (d, $J_{C,P}$ = 5.9, CH-iPr); 65.40 (d, $J_{3,P}$ = 164.1,CH_2-3); 23.97 and 23.85 (d, $J_{C,P}$ = 3.6, CH_3-iPr); 21.27 (CH_3).

3.4.1 General procedure for alkylation of nucleobases

A solution of an appropriate nucleobase (**14a,b**; **24a,b**; **29a,b**; 7-10 mmol) in dry DMF (50 ml) was treated with Cs_2CO_3 (0.5 equiv.) at 60 °C under a $CaCl_2$ protecting tube for 1 h, then bisphosphonate **13** (1.0 equiv.) was added. The mixture was stirred at 90 °C for 24 h. Solvent was evaporated and the residue was co-evaporated with toluene. The residue in chloroform was filtered through a Celite pad, evaporated and purified by silica gel column chromatography.

6-Chloro-9-{1,3-bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}purine (15a)
Column chromatography (silica gel, chloroform – methanol gradient 0-6 %) afforded **15a** as yellowish oil (yield 25 %). FABMS: 585.9 (MH^+) (70). ^{1}H NMR (500 MHz, DMSO-d_6): 8.79 (s, 1H, Pu-2); 8.76 (s, 1H, Pu-8); 5.14 (tt, 1H, $J_{1,2}$ = 8.1 and 4.3, H-1); 4.41 (m, 4H, CH-iPr); 4.16 (dd, 2H, J_{gem} = 10.5, $J_{2a,1}$ = 8.1, H-2a); 3.95 (dd, 2H, J_{gem} = 10.5, $J_{2b,1}$ = 4.3, H-2b); 3.80 and 3.73 (2 × dd, 2 × 2H, J_{gem} = 14.0, $J_{3,P}$ = 8.3, H-3); 1.13, 1.12, 1.10 and 1.05 (4 × d, 24H, J_{vic} = 6.2, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 152.35 (Pu-2); 151.59 (Pu-6); 149.20 (Pu-4); 146.76 (Pu-8); 130.99 (Pu-5); 70.45 (d, $J_{2,P}$ = 11.0, CH_2-2); 70.30 and 70.26 (d, $J_{C,P}$ = 6.0, CH-iPr); 64.98 (d, $J_{3,P}$ = 164.0, CH_2-3); 54.85 (CH-1); 23.87, 23.75, 23.64 and 23.62 (d, $J_{C,P}$ = 4.0, CH_3-iPr).

2-Amino-6-chloro-9-{1,3-bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}purine (15b)
Column chromatography (silica gel, chloroform – methanol gradient 0-6 %) afforded **15b** as yellowish foam (yield 30 %). FABMS: 600.5 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 8.17 (s, 1H, Pu-8); 6.88 (bs, 2H, NH_2); 4.80 (tt, 1H, $J_{1,2}$ = 7.9 and 4.4, H-1); 4.46 (m, 4H, CH-iPr); 4.05 (dd, 2H, J_{gem} = 10.3, $J_{2a,1}$ = 7.9, H-2a); 3.87 (dd, 2H, J_{gem} = 10.3, $J_{2b,1}$ = 4.4, H-2b); 3.79 and 3.72 (2 × dd, 2 × 2H, J_{gem} = 13.9, $J_{3,P}$ = 8.2, H-3); 1.10, 1.09, 1.07 and 1.06 (4 × d, 24H, J_{vic} = 6.2, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 159.81 (Pu-2); 154.49 (Pu-6); 149.44 (Pu-4); 142.17 (Pu-8); 123.43 (Pu-5); 70.67 (d, $J_{2,P}$ = 12.0, CH_2-2); 70.38 and 70.27 (d, $J_{C,P}$ = 6.0, CH-iPr); 65.05 (d, $J_{3,P}$ = 164.0, CH_2-3); 53.70 (CH-1); 23.87, 23.82, 23.75 and 23.70 (d, $J_{C,P}$ = 4.0, CH_3-iPr).

2-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yloxy}cytosine (25a)
Column chromatography (silica gel, chloroform – methanol gradient 0-12 %) afforded **25a** as yellowish oil

(7 %). FABMS: 542.4 (MH^+) (40). ^1H NMR (500 MHz, DMSO-d_6): 7.83 (d, 1H, $J_{6,5}=5.8$, Py-6); 6.82 (bs, 2H, NH_2); 6.06 (d, 1H, $J_{5,6}=5.8$, Py-5); 5.28 (tt, 1H, $J_{1,2}=8.2$ and 4.8, H-1); 4.58 (m, 4H, CH-iPr); 3.78 (dd, 2H, $J_{gem}=13.9$, $J_{2a,1}=8.2$, H-2a); 3.74 (dd, 2H, $J_{gem}=13.9$, $J_{2b,1}=4.8$, H-2b); 3.71 and 3.70 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.5$, H-3); 1.22, 1.21, 1.20 and 1.19 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 165.60 (Py-4); 164.38 (Py-2); 156.29 (Py-6); 99.69 (Py-5); 72.40 (CH-1); 71.55 (d, $J_{2,P}=11.7$, CH_2-2); 70.46 and 70.35 (d, $J_{C,P}=6.3$, CH-iPr); 65.48 (d, $J_{3,P}=164.1$, CH_2-3); 23.96, 23.94, 23.85 and 23.81 (d, $J_{C,P}=4.0$, CH_3-iPr).

1-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yl}cytosine (26a)
Column chromatography (silica gel, chloroform – methanol gradient 0-12 %) afforded **26a** as yellowish oil (14 %). FABMS: 542.3 (MH^+) (40). ^1H NMR (500 MHz, DMSO-d_6): 7.53 (d, 1H, $J_{6,5}=7.3$, Py-6); 6.97 (bs, 2H, NH_2); 5.63 (d, 1H, $J_{5,6}=7.3$, Py-5); 4.70 (tt, 1H, $J_{1,2}=7.4$ and 4.8, H-1); 4.54 (m, 4H, CH-iPr); 3.84 (dd, 2H, $J_{gem}=10.5$, $J_{2a,1}=7.4$, H-2a); 3.68 (dd, 2H, $J_{gem}=10.5$, $J_{2b,1}=4.8$, H-2b); 3.75 and 3.70 (2 × dd, 2 × 2H, $J_{gem}=14.0$, $J_{3,P}=8.2$, H-3); 1.22, 1.21, 1.20 and 1.19 (4 × d, 24H, $J_{vic}=6.1$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 165.54 (Py-4); 155.93 (Py-2); 142.70 (Py-6); 93.32 (Py-5); 70.67 (d, $J_{2,P}=11.2$, CH_2-2); 70.37 and 70.46 (d, $J_{C,P}=6.3$, CH-iPr); 65.00 (d, $J_{3,P}=163.6$, CH_2-3); 54.50 (CH-1); 23.96, 23.94, 23.86 and 23.81(d, $J_{C,P}=4.3$, CH_3-iPr).

2-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yloxy}5-methylcytosine (25b)
Column chromatography (silica gel, chloroform – methanol gradient 0-12 % afforded **25b** as yellowish oil (7 %). FABMS: 556.0 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 7.74 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 6.65 (bs, 1H, NH_2); 5.36 (tt, 1H, $J_{1,2}=8.2$ and 4.9, H-1); 4.56 (m, 4H, CH-iPr); 3.78 (dd, 2H, $J_{gem}=13.9$, $J_{2a,1}=8.2$, H-2a); 3.73 (dd, 2H, $J_{gem}=13.9$, $J_{2b,1}=4.9$, H-2b); 3.53 and 3.49 (2 × dd, 2 × 2H, $J_{gem}=12.8$, $J_{3,P}=8.4$, H-3); 1.80 (d, 3H, $J_{CH_3,6}=1.0$, CH_3); 1.22, 1.21, 1.20 and 1.19 (4 × d, 24H, $J_{vic}=6.1$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 164.33 (Py-4); 163.10 (Py-2); 154.91 (Py-6); 107.02 (Py-5); 72.31 (CH-1); 71.61 (d, $J_{2,P}=11.7$, CH_2-2); 70.36 and 70.30 (d, $J_{C,P}=6.3$, CH-iPr); 65.48 (d, $J_{3,P}=164.5$, CH_2-3); 23.86, 23.85, 23.83 and 23.81 (d, $J_{C,P}=4.2$, CH_3-iPr); 13.13 (CH_3).

1-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yl}5-methylcytosine (26b)
Column chromatography (silica gel, chloroform – methanol gradient 0-15 %) afforded **26b** as yellowish oil (14 %). FABMS: 556.1 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 7.43 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 6.87 (bs, 2H, NH_2); 4.80 (tt, 1H, $J_{1,2}=7.7$ and 4.8, H-1); 4.53 (m, 4H, CH-iPr); 3.84 (dd, 2H, $J_{gem}=10.5$, $J_{2a,1}=7.7$, H-2a); 3.75 and 3.70 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.5$, H-3); 3.68 (dd, 2H, $J_{gem}=10.5$, $J_{2b,1}=4.8$, H-2b); 1.82 (d, 3H, $J_{CH_3,6}=1.0$, CH_3); 1.21, 1.20, 1.19 and 1.18 (4 × d, 24H, $J_{vic}=6.1$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 164.78 (Py-4); 155.55 (Py-2); 142.70 (Py-6); 100.66 (Py-5); 70.65 (d, $J_{2,P}=11.2$, CH_2-2); 70.47 and 70.46 (d, $J_{C,P}=6.3$, CH-iPr); 64.98 (d, $J_{3,P}=164.1$, CH_2-3); 53.88 (CH-1); 24.01, 23.98, 23.86 and 23.82 (d, $J_{C,P}=4.1$, CH_3-iPr); 13.35 (CH_3).

2-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yloxy}uracil (30a)
Column chromatography (silica gel, chloroform – methanol gradient 0-10 %) afforded **30a** as yellowish oil (1 %). FABMS: 543.2 (MH^+) (60). ^1H NMR (500 MHz, DMSO-d_6): 7.75 (d, 1H, $J_{6,5}=8.0$, Py-6); 5.64 (d, 1H, $J_{5,6}=8.0$, Py-5); 5.01 (tt, 1H, $J_{1,2}=8.1$ and 4.5, H-1); 4.55 (m, 4H, CH-iPr); 3.78 (dd, 2H, $J_{gem}=10.7$, $J_{2a,1}=8.1$, H-2a); 3.74 (dd, 2H, $J_{gem}=10.7$, $J_{2b,1}=4.5$, H-2b); 3.72 and 3.70 (2 × dd, 2 × 2H, $J_{gem}=14.0$, $J_{3,P}=8.3$, H-3); 1.22, 1.21, 1.20 and 1.19 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C

NMR (125.7 MHz, DMSO-d_6): 163.40 (Py-4); 162.46 (Py-2); 155.32 (Py-6); 106.19 (Py-5); 72.62 (CH-1); 71.25 (d, $J_{2,P}=12.00$, CH_2-2); 70.44 and 70.31 (d, $J_{C,P}=6.0$, CH-iPr); 65.42 (d, $J_{3,P}=164.0$, CH_2-3); 23.95, 23.93, 23.86 and 23.89 (d, $J_{C,P}=4.1$, CH_3-iPr).

1-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yl}uracil (31a)
Column chromatography (silica gel, chloroform – methanol gradient 0-8 %) afforded **31a** as yellowish oil. Crystallization from ethyl acetate/petroleum ether gave white solid (12 %). FABMS: 543.2 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 11.28 (bs, 1H, NH); 7.64 (d, 1H, $J_{6,5}=8.0$, Py-6); 5.56 (d, 1H, $J_{5,6}=8.0$, Py-5); 4.80 (tt, 1H, $J_{1,2}=8.1$ and 4.5, H-1); 4.57 (m, 4H, CH-iPr); 3.86 (dd, 2H, $J_{gem}=10.7$, $J_{2a,1}=8.1$, H-2a); 3.78 (dd, 2H, $J_{gem}=10.7$, $J_{2b,1}=4.5$, H-2b); 3.74 and 3.70 (2 × dd, 2 × 2H, $J_{gem}=14.0$, $J_{3,P}=8.3$, H-3); 1.22, 1.21, 1.20 and 1.19 (3 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 163.29 (Py-4); 151.46 (Py-2); 143.13 (Py-6); 101.11 (Py-5); 70.42 (d, $J_{2,P}=12.0$, CH_2-2); 70.40 and 70.21 (d, $J_{C,P}=6.0$, CH-iPr); 64.98 (d, $J_{3,P}=164.0$, CH_2-3); 54.12 (CH-1); 23.95, 23.93, 23.85 and 23.89 (d, $J_{C,P}=4.1$, CH_3-iPr).

1,3-Bis-{[1,3-bis(diisopropoxyphosphoryl)methoxy]propan-2-yl}uracil (32a)
Column chromatography (silica gel, chloroform – methanol gradient 0-4 %) afforded **32a** as yellowish oil (0.5 %). FABMS: 973.2 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 8.28 (d, 1H, $J_{6,5}=5.7$, Py-6); 6.52 (d, 1H, $J_{5,6}=5.7$, Py-5); 5.42 and 5.34 (2 × tt, 2 × 1H, $J_{1,2}=6.1$ and 5.0, H-1); 4.57 (m, 8H, CH-iPr); 3.87 and 3.85 (2 × dd, 2H, $J_{gem}=10.1$, $J_{2a,1}=6.1$, H-2a); 3.78 and 3.76 (2 × dd, 2H, $J_{gem}=10.7$, $J_{2b,1}=5.0$, H-2b); 3.48 and 3.43 (4 × dd, 4 × 2H, $J_{gem}=14.0$, $J_{3,P}=8.4$, H-3); 1.22, 1.21, 1.20 and 1.19 (8 × d, 48H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 163.26 (Py-4); 151.97 (Py-2); 143.65 (Py-6); 101.15 (Py-5); 70.23 and 70.19 (d, $J_{2,P}=12.0$, CH_2-2); 70.41 and 70.21 (d, $J_{C,P}=6.3$, CH-iPr); 64.83 and 64.79 (d, $J_{3,P}=164.6$, CH_2-3); 53.11 and 45.46 (CH-1); 23.95, 23.93, 23.91 and 23.89 (d, $J_{C,P}=4.1$, CH_3-iPr).

2-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yloxy}thymine (30b)
Column chromatography (silica gel, in chloroform – methanol gradient 0-9 %) afforded **30b** as yellowish oil (1.1 %). FABMS: 557.1 (MH^+) (60). ^{1}H NMR (500 MHz, DMSO-d_6): 7.53 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.90 (tt, 1H, $J_{1,2}=8.2$ and 4.6, H-1); 4.53 (m, 4H, CH-iPr); 3.85 (dd, 2H, $J_{gem}=10.7$, $J_{2a,1}=8.2$, H-2a); 3.71 (dd, 2H, $J_{gem}=10.7$, $J_{2b,1}=4.6$, H-2b); 3.72 and 3.70 (2 × dd, 2 × 2H, $J_{gem}=14.0$, $J_{3,P}=8.2$, H-3); 1.82 (d, 3H, $J_{CH_3,6}=1.0$, CH_3); 1.22, 1.21, 1.20 and 1.19 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 163.91 (Py-4); 162.46 (Py-2); 139.01 (Py-6); 108.19 (Py-5); 71.62 (CH-1); 70.45 (d, $J_{2,P}=12.0$, CH_2-2); 70.38 and 70.31 (d, $J_{C,P}=6.0$, CH-iPr); 65.22 (d, $J_{3,P}=164.0$, CH_2-3); 23.95, 23.93, 23.85 and 23.89 (d, $J_{C,P}=4.1$, CH_3-iPr).

1-{[1,3-Bis(diisopropoxyphosphoryl)methoxy]propan-2-yl}thymine (31b)
Column chromatography (silica gel, chloroform – methanol gradient 0-7 %) afforded **31b** as yellowish oil (12 %). FABMS: 557.0 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 11.20 (bs, 1H, NH); 7.52 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.80 (tt, 1H, $J_{1,2}=8.3$ and 4.6, H-1); 4.53 (m, 4H, CH-iPr); 3.86 (dd, 2H, $J_{gem}=10.5$, $J_{2a,1}=8.3$, H-2a); 3.69 (dd, 2H, $J_{gem}=10.5$, $J_{2b,1}=4.6$, H-2b); 3.77 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=14.0$, $J_{3,P}=8.3$, H-3); 1.82 (d, 3H, $J_{CH_3,6}=1.0$, CH_3); 1.22, 1.21, 1.20 and 1.19 (4 × d, 24H, $J_{vic}=6.1$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 163.89 (Py-4); 151.46 (Py-2); 138.72 (Py-6); 108.63 (Py-5); 70.41 (d, $J_{2,P}=11.2$, CH_2-2); 70.38 and 70.09 (d, $J_{C,P}=6.4$, CH-iPr); 64.95 (d, $J_{3,P}=164.1$, CH_2-3); 53.74

(CH-1); 23.95, 23.90, 23.86 and 23.85 (d, $J_{C,P}=4.1$, CH_3-iPr); 23.76 (CH_3).

1,3-Bis-{[1,3-bis(diisopropoxyphosphoryl)methoxy]propan-2-yl}thymine (32b)
Column chromatography (silica gel, chloroform – methanol gradient 0-4 %) afforded **32b** as yellowish oil (0.5 %). FABMS: 987.2 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 7.43 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 5.40 and 5.31 (2 × tt, 2 × 1H, $J_{1,2}=6.1$ and 5.0, H-1); 4.56 (m, 8H, CH-iPr); 3.87 and 3.85 (2 × dd, 2H, $J_{gem}=10.1$, $J_{2a,1}=6.1$, H-2a); 3.78 and 3.72 (2 × dd, 2H, $J_{gem}=10.7$, $J_{2b,1}=5.0$, H-2b); 3.48 and 3.43 (4 × dd, 4 × 2H, $J_{gem}=14.0$, $J_{3,P}=8.4$, H-3); 1.81 (d, 3H, $J_{CH_3,6}=1.0$, CH_3) 1.22, 1.21, 1.20 and 1.19 (8 × d, 48H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 163.91 (Py-4); 151.45 (Py-2); 139.23 (Py-6); 108.15 (Py-5); 70.23 and 70.19 (d, $J_{2,P}=12.0$, CH_2-2); 70.41 and 70.21 (d, $J_{C,P}=6.3$, CH-iPr); 64.83 and 64.56 (d, $J_{3,P}=164.6$, CH_2-3); 51.17 and 43.56 (CH-1); 23.95, 23.93, 23.91 and 23.89 (d, $J_{C,P}=4.1$, CH_3-iPr).

9-{1,3-Bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}adenine (16a)
A solution of **15a** (940 mg, 1.6 mmol) in methanolic ammonia (50 ml) was stirred and heated (100 °C) in autoclave for 3 days. The solvent was evaporated and the residue was purified by column chromatography on silica gel with chloroform – methanol gradient 0-6 % to yield **16a** as yellowish oil (80 %). FABMS: 566.5 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 8.16 (s, 1H, Pu-2); 8.11 (s, 1H, Pu-8); 7.20 (bs, 2H, NH_2); 4.93 (tt, 1H, $J_{1,2}=7.4$ and 4.7, H-1); 4.45 (m, 4H, CH-iPr); 4.09 (dd, 2H, $J_{gem}=10.3$, $J_{2a,1}=7.4$, H-2a); 3.90 (dd, 2H, $J_{gem}=10.3$, $J_{2b,1}=4.7$, H-2b); 3.79 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.2$, H-3); 1.15, 1.13, 1.10 and 1.09 (4 × d, 24H $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 156.15 (Pu-6); 152.36 (Pu-2); 149.82 (Pu-4); 139.99 (Pu-8); 118.88 (Pu-5); 70.95 (d, $J_{2,P}=12.0$, CH_2-2), 70.36 and 70.35 (d, $J_{C,P}=6.3$, CH-iPr); 65.02 (d, $J_{3,P}=163.6$, CH_2-3); 53.64 (CH-1); 23.89, 23.85, 23.78 and 23.72 (d, $J_{C,P}=3.7$, CH_3-iPr).

2,6-Diamino-9-{1,3-bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}purine (16b)
A solution of **15b** (980 mg, 1.6 mmol) in methanolic ammonia (50 ml) was stirred and heated (100 °C) in autoclave for 3 days. The solvent was evaporated and the residue was purified by column chromatography on silica gel with chloroform – methanol gradient 0-10 % to yield **16b** as yellowish foam (70 %). FABMS: 581.3 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 7.73 (s, 1H, Pu-8); 6.63 (bs, 2H, NH_2); 5.71 (bs, 2H, NH_2); 4.68 (tt, 1H, $J_{1,2}=7.3$ and 4.8, H-1); 4.50 (m, 4H, CH-iPr); 4.00 (dd, 2H, $J_{gem}=10.3$, $J_{2a,1}=7.3$, H-2a); 3.84 (dd, 2H, $J_{gem}=10.3$, $J_{2b,1}=4.8$, H-2b); 3.78 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.3$, H-3); 1.19, 1.17, 1.15 and 1.14 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 160.26 (Pu-2); 156.26 (Pu-6); 152.01 (Pu-4); 136.38 (Pu-8); 113.24 (Pu-5); 71.12 (d, $J_{2,P}=12.0$, CH_2-2); 70.43 and 70.40 (d, $J_{C,P}=6.0$, CH-iPr); 65.10 (d, $J_{3,P}=164.0$, CH_2-3); 52.79 (CH-1); 23.95, 23.81, 23.74 and 23.79 (d, $J_{C,P}=4.0$, CH_3-iPr).

9-{1,3-Bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}hypoxanthine (17a)
3-Methylbutyl nitrite (10 equiv.) was added to a solution of compound **16a** (450 mg, 0.8 mmol) in 80% acetic acid (20 ml) and the mixture was stirred at room temperature overnight and then at 70 °C for 2h. After evaporation of volatiles the residue was codistilled with water and purified by column chromatography on silica gel with chloroform – methanol gradient 0-12 % to yield **17a** as yellowish foam (77 %). FABMS: 567.4 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 12.25 (bs, 1H, NH); 8.13 (s, 1H, Pu-2); 8.04 (s, 1H, Pu-8); 4.92 (tt, 1H, $J_{1,2}=7.8$ and 4.3, H-1); 4.46 (m, 4H, $J_{H,P}=7.8$, $J_{vic}=6.2$, CH-iPr); 4.06 (dd,

2H, $J_{gem}=10.4$, $J_{2a,1}=7.8$, H-2a); 3.88 (dd, 2H, $J_{gem}=10.4$, $J_{2b,1}=4.3$, H-2b); 3.79 and 3.73 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.3$, H-3); 1.17, 1.16, 1.15 and 1.13 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 156.80 (Pu-6); 148.70 (Pu-4); 145.47 (Pu-2); 139.41 (Pu-8); 124.03 (Pu-5); 71.00 (d, $J_{2,P}=11.2$, CH_2-2); 70.38 and 70.35 (d, $J_{C,P}=6.3$, CH-iPr); 65.02 (d, $J_{3,P}=164.1$, CH_2-3); 54.02 (CH-1); 23.89, 23.87, 23.78 and 23.72 (d, $J_{C,P}=4.0$, CH_3-iPr).

9-{1,3-Bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}guanine (17b)

A solution of **15b** (620 mg, 1.0 mmol) in 80% acetic acid (20 ml) was refluxed for 6 h. The excess of acetic acid was evaporated. The residue was co-evaporated with toluene and ethanol and purified by column chromatography on silica gel chloroform – methanol gradient 0-15 % afforded **17b** as yellowish foam (75 %). FABMS: 582.4 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 10.56 (bs, 1H, NH); 7.73 (s, 1H, Pu-8); 6.42 (bs, 2H, NH_2); 4.66 (tt, 1H, $J_{1,2}=7.4$, 4.7, H-1); 4.50 (m, 4H, $J_{H,P}=7.7$, $J_{vic}=6.2$, CH-iPr); 3.97 (dd, 2H, $J_{gem}=10.3$, $J_{2a,1}=7.4$, H-2a); 3.82 (dd, 2H, $J_{gem}=10.3$, $J_{2b,1}=4.7$, H-2b); 3.78 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.3$, H-3); 1.19, 1.17, 1.15 and 1.14 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 156.96 (Pu-6); 153.56 (Pu-2); 151.45 (Pu-4); 136.28 (Pu-8); 116.54 (Pu-5); 71.11 (d, $J_{2,P}=12.0$, CH_2-2); 70.44 and 70.40 (d, $J_{C,P}=6.0$, CH-iPr); 65.10 (d, $J_{3,P}=164.0$, CH_2-3); 53.07 (CH-1); 23.93, 23.89, 23.78 and 23.76 (d, $J_{C,P}=4.0$, CH_3-iPr).

6-(Cyclopropyl)amino-9-{1,3-bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}purine (18a)

A solution of **15a** (430 mg, 0.7 mmol) and cyclopropylamine (7 equiv.) in dioxane (15 ml) was refluxed for 7 h. The solvent and excess of amine was then evaporated to dryness and codistilled with toluene. The residue was purified by column chromatography on silica gel with chloroform – methanol gradient 0-6 % to yield **18a** as yellowish oil (84 %). FABMS: 606.3 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 8.21 (s, 1H, Pu-2); 8.16 (s, 1H, Pu-8); 7.85 (bs, 1H, NH); 4.95 (tt, 1H, $J_{1,2}=7.8$ and 4.5, H-1); 4.45 (m, 4H, CH-iPr); 4.09 (dd, 2H, $J_{gem}=10.4$, $J_{2a,1}=7.8$, H-2a); 3.90 (dd, 2H, $J_{gem}=10.4$, $J_{2b,1}=4.6$, H-2b); 3.79 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.2$, H-3); 3.04 (bm, 1H, $CH_{cycloprop.}$); 1.16, 1.15, 1.12 and 1.10 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr); 0.71 and 0.59 (2 × m, 2 × 2H, $CH_{2cycloprop.}$. ^{13}C NMR (125.7 MHz, DMSO-d_6): 155.75 (Pu-6); 152.27 (Pu-2); 149.61 (Pu-4); 139.84 (Pu-8); 119.26 (Pu-5); 70.94 (d, $J_{2,P}=12.0$, CH_2-2); 70.37 and 70.32 (d, $J_{C,P}=6.0$, CH-iPr); 65.01 (d, $J_{3,P}=164.0$, CH_2-3); 53.61 (CH-1); 23.88, 23.81, 23.73 and 23.71 (d, $J_{C,P}=4.0$, CH_3-iPr and $CH_{cycloprop.}$); 6.56 ($CH_{2cycloprop.}$).

2-Amino-6-(cyclopropyl)amino-9-{1,3-bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}purine (18b)

A solution of **15a** (490 mg, 0.8 mmol) and cyclopropylamine (7 equiv.) in dioxane (15 ml) was refluxed for 7 h and worked up as described for compound **18a**. Purification by silica gel chromatography yielded **18b** as yellowish oil (75 %). FABMS: 621.5 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 7.73 (s, 1H, Pu-8); 7.60 (bs, 1H, NH); 5.79 (bs, 2H, NH_2); 4.70 (tt, 1H, $J_{1,2}=7.3$ and 4.6, H-1); 4.48 (m, 4H, CH-iPr); 4.00 (dd, 2H, $J_{gem}=10.3$, $J_{2a,1}=7.3$, H-2a); 3.84 (dd, 2H, $J_{gem}=10.3$, $J_{2b,1}=4.6$, H-2b); 3.78 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.3$, H-3); 3.05 (bm, 1H, $CH_{cycloprop.}$); 1.18, 1.16, 1.14 and 1.13 (4 × d, 24H, $J_{vic}=6.1$, CH_3-iPr); 0.65 and 0.57 (2 × m, 2 × 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, DMSO-d_6): 160.19 (Pu-2); 150.07 (Pu-6); 149.92 (Pu-4); 136.14 (Pu-8); 113.51 (Pu-5); 71.12 (d, $J_{2,P}=11.7$, CH_2-2); 70.42 and 70.39 (d, $J_{C,P}=6.3$, CH-iPr); 65.10 (d, $J_{3,P}=163.6$, CH_2-3); 52.74 (CH-1); 23.94, 23.92, 23.88 and 23.80 (d, $J_{C,P}=3.9$, CH_3-iPr and $CH_{cycloprop.}$); 6.56 ($CH_{2cycloprop.}$).

9-{1,3-Bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}6-thiopurine (19a)
A solution of compound **15a** (350 mg, 0.6 mmol) and thiourea (3 equiv.) in ethanol (20 ml) was refluxed for 3 h, cooled and made alkaline with triethylamine. The mixture was evaporated, refluxed with chloroform, and filtered while hot. The filtrate was evaporated and purified on of silica gel column with chloroform – methanol gradient 0-10 % to yield **19a** as yellowish foam (52 %). FABMS: 583.0 (MH^+) (80). ^{1}H NMR (500 MHz, DMSO-d_6): 13.70 (bs, 1H, NH); 8.33 (s, 1H, Pu-2); 8.32 (s, 1H, Pu-8); 4.95 (tt, 1H, $J_{1,2}=7.8$ and 4.3, H-1); 4.45 (m, 4H, $J_{H,P}=7.8$, $J_{vic}=6.2$, CH-iPr); 4.07 (dd, 2H, $J_{gem}=10.5$, $J_{2a,1}=7.8$, H-2a); 3.90 (dd, 2H, $J_{gem}=10.5$, $J_{2b,1}=4.3$, H-2b); 3.79 and 3.73 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.3$, H-3); 1.19, 1.15, 1.11 and 1.10 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 176.12 (Pu-6); 144.94 (Pu–2); 144.49 (Pu-4); 142.06 (Pu-8); 135.11 (Pu-5); 70.80 (d, $J_{2,P}=11.7$, CH_2-2); 70.36 and 70.30 (d, $J_{C,P}=6.3$, CH-iPr); 65.10 (d, $J_{3,P}=164.1$, CH_2-3); 54.21 (CH-1); 23.89, 23.88, 23.79 and 23.73 (d, $J_{C,P}=4.0$, CH_3-iPr).

2-Amino-9-{1,3-bis[(diisopropoxyphosphoryl)methoxy]propan-2-yl}-6-thiopurine (19b)
A solution of compound **15b** (360 mg, 0.6 mmol) and thiourea (3 equiv.) in ethanol (20 ml) was refluxed for 3 h and worked up as described for compound **19a**. Purification by silica gel chromatography yielded yellowish oil (60 %). FABMS: 598.0 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 11.70 (bs, 1H, NH); 7.93 (s, 1H, Pu-8); 6.80 (bs, 2H, NH_2); 4.67 (tt, 1H, $J_{1,2}=7.8$ and 4.3, H-1); 4.48 (m, 4H, $J_{H,P}=7.8$, $J_{vic}=6.2$, CH-iPr); 3.99 (dd, 2H, $J_{gem}=10.4$, $J_{2a,1}=7.8$, H-2a); 3.84 (dd, 2H, $J_{gem}=10.4$, $J_{2b,1}=4.3$, H-2b); 3.78 and 3.72 (2 × dd, 2 × 2H, $J_{gem}=13.9$, $J_{3,P}=8.3$, H-3); 1.18, 1.16, 1.14 and 1.13 (4 × d, 24H, $J_{vic}=6.2$, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 175.07 (Pu-6); 152.99 (Pu-2); 148.20 (Pu-4); 139.33 (Pu-8); 128.28 (Pu-5); 70.89 (d, $J_{2,P}=11.7$, CH_2-2); 70.42 and 70.40 (d, $J_{C,P}=6.3$, CH-iPr); 65.10 (d, $J_{3,P}=164.1$, CH_2-3); 53.28 (CH-1); 23.92, 23.91, 23.82 and 23.76 (d, $J_{C,P}=4.1$, CH_3-iPr).

3.4.2 General procedure for preparation of free phosphonic acids

The starting 1,3-bis(diisopropoxyphosphoryl)methoxy]propan-2-yl derivatives (**16-19**, **25-26**, and **31**, 1 mmol, co-distilled with acetonitrile), acetonitrile (20 ml) and $BrSiMe_3$ (3 ml) were stirred at room temperature overnight. After evaporation and co-distillation with acetonitrile, the residue was treated with water and aqueous ammonia. The mixture was evaporated to dryness, and the residue dissolved in water was applied onto a column of Dowex 50 X 8 (H^+ form). Elution with water and evaporation in vacuo afforded the product as the free phosphonic acid (**20-23**, **27-28**, and **33**).

9-[1,3-Bis(phosphonomethoxy)propan-2-yl]adenine (20a)
White solid (yield 70 %), mp 256-257 °C (D). For $C_{10}H_{17}N_5O_8P_2 \cdot H_2O$ (415.23) calcd: C, 28.93; H, 4.61; N, 16.87; P, 14.92. Found: C, 29.14; H, 4.44; N, 16.71; P, 15.01. FABMS: 398.3 (MH^+) (100). ^{1}H NMR (500 MHz, D_2O + NaOD, ref. dioxane = 3.75 ppm): 8.48 (s, 1H, Pu-8); 8.23 (s, 1H, Pu-2); 5.04 (tt, 1H, $J_{1,2}=7.4$ and 4.4, H-1); 4.13 (dd, 2H, $J_{gem}=11.0$, $J_{2a,1}=7.4$, H-2a); 4.04 (dd, 2H, $J_{gem}=11.0$, $J_{2b,1}=4.4$, H-2b); 3.47 and 3.45 (2 × dd, 2 × 2H, $J_{gem}=12.6$, $J_{3,P}=8.5$, H-3). ^{13}C NMR (125.7 MHz, D_2O + NaOD, ref. dioxane = 69.3 ppm): 158.31 (Pu-6); 155.17 (Pu-2); 152.00 (Pu-4); 144.86 (Pu-8);

121.12 (Pu-5); 73.73 (d, $J_{2,P}=10.0$, CH_2-2); 72.12 (d, $J_{3,P}=150.0$, CH_2-3); 57.48 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max}=259$ nm ($\epsilon_{max}=14302$); (H_2O) $\lambda_{max}=260$ nm ($\epsilon_{max}=13756$); (0.01 M NaOH) $\lambda_{max}=261$ nm ($\epsilon_{max}=13837$).

2,6-Diamino-9-[1,3-bis(phosphonomethoxy)propan-2-yl]purine (20b)
White solid (yield 65 %), mp 237-238 °C (D). For $C_{10}H_{18}N_6O_8P_2 \cdot \frac{1}{2} H_2O$ (421.23) calcd: C, 28.51; H, 4.55; N, 19.95; P, 14.71. Found: C, 28.55; H, 4.58; N, 19.70; P, 14.81. FABMS: 413.1 (MH^+) (90). ^1H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 8.17 (s, 1H, Pu-8); 4.84 (tt, 1H, $J_{1,2}=7.2$ and 4.6, H-1); 4.04 (dd, 2H, $J_{gem}=11.0$, $J_{2a,1}=7.2$, H-2a); 3.98 (dd, 2H, $J_{gem}=11.0$, $J_{2b,1}=4.6$, H-2b); 3.49 and 3.44 (2 × dd, 2 × 2H, $J_{gem}=13.7$, $J_{2,P}=8.6$, H-3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 156.28 (Pu-2); 155.17 (Pu-6); 151.55 (Pu-4); 139.52 (Pu-8); 112.93 (Pu-5); 71.22 (d, $J_{2,P}=10.8$, CH_2-2); 69.56 (d, $J_{3,P}=149.9$, CH_2-3); 54.03 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max}=288$ nm ($\epsilon_{max}=9070$); (H_2O) $\lambda_{max}=282$ nm ($\epsilon_{max}=8807$); (0.01 M NaOH) $\lambda_{max}=281$ nm ($\epsilon_{max}=10059$).

9-[1,3-Bis(phosphonomethoxy)propan-2-yl]hypoxanthine (21a)
White solid (72 %), mp 107-109 °C (D). For $C_{10}H_{16}N_4O_9P_2 \cdot \frac{3}{2} H_2O$ (425.20) calcd: C, 28.25; H, 4.50 N, 13.18; P, 14.57 Found: C, 28.46; H, 4.45; N, 13.23; P, 14.71. FABMS: 399.2 (MH^+) (10). ^1H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 8.32 (s, 1H, Pu-8); 8.14 (s, 1H, Pu-2); 4.99 (tt, 1H, $J_{1,2}=7.3$ and 4.6, H-1); 4.09 (dd, 2H, $J_{gem}=11.0$, $J_{2a,1}=7.3$, H-2a); 4.02 (dd, 2H, $J_{gem}=11.0$, $J_{2b,1}=4.6$, H-2b); 3.46 and 3.43 (2 × dd, 2 × 2H, $J_{gem}=12.7$, $J_{3,P}=8.6$, H-3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 165.59 (Pu-6); 151.68 (Pu-4); 149.84 (Pu-2); 140.46 (Pu-8); 123.08 (Pu-5); 71.15 (d, $J_{2,P}=10.3$, CH_2-2); 69.45 (d, $J_{3,P}=149.9$, CH_2-3); 54.70 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max}=250$ nm ($\epsilon_{max}=9797$); (H_2O) $\lambda_{max}=249$ nm ($\epsilon_{max}=9555$); (0.01 M NaOH) $\lambda_{max}=254$ nm ($\epsilon_{max}=10080$).

9-[1,3-Bis(phosphonomethoxy)propan-2-yl]guanine (21b)
White solid (yield 74 %), mp 220-221 °C (D). For $C_{10}H_{17}N_5O_9P_2 \cdot \frac{1}{2} H_2O$ (422.22) calcd: C, 28.45; H, 4.30; N, 16.59; P, 14.67. Found: C, 28.73; H, 4.55; N, 16.68; P, 14.79. FABMS: 414.1 (MH^+) (90). ^1H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 8.98 (s, 1H, Pu-8); 5.14 (tt, 1H, $J_{1,2}=6.5$ and 4.0, H-1); 4.21 (dd, 2H, $J_{gem}=11.0$, $J_{2a,1}=6.5$, H-2a); 4.08 (dd, 2H, $J_{gem}=11.0$, $J_{2b,1}=4.0$, H-2b); 3.47 and 3.45 (2 × dd, 2 × 2H, $J_{gem}=13.4$, $J_{3,P}=8.8$, H-3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 155.17 (Pu-6); 155.28 (Pu-2); 150.25 (Pu-4); 137.34 (Pu-8); 107.44 (Pu-5); 70.16 (d, $J_{2,P}=12.2$, CH_2-2); 66.76 (d, $J_{3,P}=157.7$, CH_2-3); 55.31 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max}=255$ nm ($\epsilon_{max}=11352$); (H_2O) $\lambda_{max}=251$ nm ($\epsilon_{max}=11635$); (0.01 M NaOH) $\lambda_{max}=267$ nm ($\epsilon_{max}=10464$).

6-(Cyclopropyl)amino-9-[1,3-bis(phosphonomethoxy)propan-2-yl]purine (22a)
Yellowish hygroskopic solid (yield 69 %), mp 142-144 °C (D). For $C_{13}H_{21}N_5O_8P_2 \cdot H_2O$ (455.28) calcd: C, 34.29; H, 5.09; N, 15.38; P, 13.61 Found: C, 34.52; H, 5.25; N, 15.29; P, 13.72. FABMS: 438.0 (MH^+) (80). ^1H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 8.55 and 8.46 (2 × s, 2 × 1H, Pu-2 and Pu-8); 5.16 (tt, 1H, $J_{1,2}=7.3$ and 4.3, H-1); 4.18 (dd, 2H, $J_{gem}=11.0$, $J_{2a,1}=7.3$, H-2a); 4.06 (dd, 2H, $J_{gem}=11.0$, $J_{2b,1}=4.3$, H-2b); 3.70 and 3.65 (2 × dd, 2 × 2H, $J_{gem}=13.4$, $J_3=8.7$, H-3); 2.89 (bm, 1H, $CH_{cycloprop.}$); 1.09 and 0.88 (2 × m, 2 × 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 152.66 (Pu-6); 150.49 (Pu-4); 146.66 (Pu-2 and Pu-8); 120.95 (Pu-5); 73.55 (d, $J_{2,P}=12$, CH_2-2); 69.50 (d, $J_{3,P}=158$, CH_2-3); 57.69 (CH-1); 25.45 ($CH_{cycloprop.}$); 9.35 ($CH_{2cycloprop.}$). UV spectrum: (0.01 M HCl) $\lambda_{max}=268$ nm ($\epsilon_{max}=17655$); (H_2O) $\lambda_{max}=269$ nm ($\epsilon_{max}=16968$); (0.01 M NaOH) $\lambda_{max}=271$

nm ($\epsilon_{max} = 18301$).

2-Amino-6-(cyclopropyl)amino-9-[1,3-bis(phosphonomethoxy)propan-2-yl]purine (22b)
White solid (60 %), mp 164-166 °C (D). For $C_{13}H_{22}N_6O_8P_2 \cdot H_2O$ (470.30) calcd: C, 33.20; H, 5.14; N, 17.87; P, 13.17. Found: C, 33.18; H, 5.24; N, 17.57; P, 13.32. FABMS: 453.3 (MH^+) (100). ^{1}H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 8.21 (s, 1H, Pu-8); 4.92 (tt, 1H, $J_{1,2} = 7.3$ and 4.1, H-1); 4.10 (dd, 2H, $J_{gem} = 11.0$, $J_{2a,1} = 7.3$, H-2a); 4.0 (dd, 2H, $J_{gem} = 11.0$, $J_{2b,1} = 4.1$, H-2b); 3.71 and 3.65 (2 × dd, 2 × 2H, $J_{gem} = 13.3$, $J_{3,P} = 8.7$, H-3); 2.82 (bm, 1H, $CH_{cycloprop.}$); 1.03 and 0.83 (2 × m, 2 × 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 157.40 (Pu-2); 152.59 (Pu-6); 150.52 (Pu-4); 140.95 (Pu-8); 114.35 (Pu-5); 70.77 (d, $J_{2,P} = 12.2$, CH_2-2); 66.88 (d, $J_{3,P} = 157.2$, CH_2-3); 54.50 (CH-1); 22.71 ($CH_{cycloprop.}$); 6.75 ($CH_{2cycloprop.}$). UV spectrum: (0.01 M HCl) λ_{max}=294 nm (ϵ_{max}=12584.6); (H_2O) λ_{max}=291 nm (ϵ_{max}=12059.4); (0.01 M NaOH) λ_{max}=284 nm (ϵ_{max}=14119.8).

9-[1,3-Bis(phosphonomethoxy)propan-2-yl]-6-thiopurine (23a)
Yellow solid (84 %), mp 109-110 °C (D). For $C_{10}H_{16}N_4O_8P_2S \cdot \frac{3}{2} H_2O$ (441.27) calcd: C, 27.22; H, 4.34; N, 12.70; P, 14.04; S, 7.27. Found: C, 27.44; H, 4.18; N, 12.41; P, 14.15; S, 7.42. FABMS: 415.0 (MH^+) (10). ^{1}H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 8.46 (s, 1H, Pu-8); 8.30 (s, 1H, Pu-2); 5.02 (tt, 1H, $J_{1,2} = 7.2$ and 4.5, H-1); 4.10 (dd, 2H, $J_{gem} = 11.0$, $J_{2a,1} = 7.2$, H-2a); 4.03 (dd, 2H, $J_{gem} = 11.0$, $J_{2b,1} = 4.5$, H-2b); 3.47 and 3.43 (2 × dd, 2 × 2H, $J_{gem} = 12.8$, $J_{3,P} = 8.5$, H-3); ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 177.16 (Pu-6); 150.77 (Pu-2); 146.17 (Pu-4); 142.37 (Pu-8); 135.26 (Pu-5); 71.03 (d, $J_{2,P} = 10.2$, CH_2-2); 69.45 (d, $J_{3,P} = 149.9$, CH_2-3); 54.58 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max} = 323$ nm ($\epsilon_{max} = 18927$); (H_2O) $\lambda_{max} = 322$ nm ($\epsilon_{max} = 20321$); (0.01 M NaOH) $\lambda_{max} = 310$ nm ($\epsilon_{max} = 18099$).

2-Amino-9-[1,3-bis(phosphonomethoxy)propan-2-yl]-6-thiopurine (23b)
Yellowish solid (91 %), mp 183-184 °C (D). For $C_{10}H_{17}N_5O_8P_2S \cdot \frac{3}{2} H_2O$ (456.28) calcd: C, 26.32; H, 4.42; N, 15.35; P, 13.58; S, 7.03. Found: C, 26.08; H, 4.35; N, 15.19; P, 13.62; S, 7.23. FABMS: 430.0 (MH^+) (40). ^{1}H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 9.15 (s, 1H, Pu-8); 5.09 (tt, 1H, $J_{1,2} = 6.5$ and 4.0, H-1); 4.16 (dd, 2H, $J_{gem} = 11.0$, $J_{2a,1} = 6.7$, H-2a); 4.03 (dd, 2H, $J_{gem} = 11.0$, $J_{2b,1} = 4.0$, H-2b); 3.72 and 3.685 (2 × dd, 2 × 2H, $J_{gem} = 13.3$, $J_{3,P} = 8.8$, H-3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 174.72 (Pu-6); 154.69 (Pu-2); 146.31 (Pu-4); 139.96 (Pu-8); 119.97 (Pu-5); 70.16 (d, $J_{2,P} = 12.2$, CH_2-2); 67.00 (d, $J_{3,P}$ =157.2, CH_2-3); 55.28 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max} = 345$ nm ($\epsilon_{max} = 19008$); (H_2O) $\lambda_{max} = 341$ nm ($\epsilon_{max} = 22280.6$); (0.01 M NaOH) $\lambda_{max} = 319$ nm ($\epsilon_{max} = 17654.8$).

2-[1,3-Bis(phosphonomethoxy)propan-2-yloxy]cytosine (27a)
White hygroscopic solid (80 %), mp 118-119 °C (D). For $C_9H_{17}N_3O_9P_2 \cdot H_2O$ (391.21) calcd: C, 27.63; H, 4.90; N, 10.74; P, 15.83. Found: C, 27.50; H, 5.10; N, 10.54; P, 15.95. FABMS: 374.1 (MH^+) (30). ^{1}H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 7.83 (d, 1H, $J_{6,5} = 7.2$, Py-6); 6.43 (d, 1H, $J_{5,6}$ = 7.2, Py-5); 5.68 (tt, 1H, $J_{1,2} = 6.0$ and 4.0, H-1); 3.96 (dd, 2H, $J_{gem} = 11.8$, $J_{2a,1} = 6.0$, H-2a); 3.93 (dd, 2H, $J_{gem} = 11.8$, $J_{2b,1} = 4.0$, H-2b); 3.83 and 3.74 (2 × dd, 2 × 2H, $J_{gem} = 13.6$, $J_{3,P} = 8.6$, H-3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 166.45 (Py-2); 157.68 (Py-4); 142.93 (Py-6); 100.07 (Py-5); 76.33 (CH-1); 70.66 (d, $J_{2,P} = 11.2$, CH_2-2); 66.70 (d, $J_{3,P} = 157.7$, CH_2-3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 259$ nm ($\epsilon_{max} = 8282$); (H_2O) $\lambda_{max} = 260$ nm ($\epsilon_{max} = 8181$); (0.01 M NaOH) $\lambda_{max} = 271$ nm ($\epsilon_{max} = 6969$).

1-[1,3-Bis(phosphonomethoxy)propan-2-yl]cytosine (28a)

White solid (70 %), mp 130-131 °C (D). For $C_9H_{17}N_3O_9P_2 \cdot H_2O$ (391.21) calcd: C, 27.63; H, 4.90; N, 10.74; P, 15.84. Found: C, 27.80; H, 5.12; N, 10.60; P, 15.92. FABMS: 374.1 (MH^+) (30). ^1H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 8.08 (d, 1H, $J_{6,5} = 7.8$, Py-6); 6.20 (d, 1H, $J_{5,6} = 7.2$, Py-5); 5.02 (tt, 1H, $J_{1,2} = 7.2$ and 4.2, H-1); 3.99 (dd, 2H, $J_{gem} = 11.2$, $J_{2a,1} = 7.2$, H-2a); 3.52 (dd, 2H, $J_{gem} = 11.2$, $J_{2b,1} = 4.2$, H-2b); 3.68 and 3.64 (2 × dd, 2 × 2H, $J_{gem} = 13.4$, $J_{3,P} = 8.8$, H-3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 158.94 (Py-4); 149.41 (Py-2); 147.53 (Py-6); 94.61 (Py-5); 69.83 (d, $J_{2,P} = 12.2$, CH_2-2); 66.82 (d, $J_{3,P} = 157.7$, CH_2-3); 52.96 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max} = 282$ nm ($\epsilon_{max} = 15473$); (H_2O) $\lambda_{max} = 280$ nm ($\epsilon_{max} = 10484$); (0.01 M NaOH) $\lambda_{max} = 274$ nm ($\epsilon_{max} = 8201$).

5-Methyl-2-[1,3-bis(phosphonomethoxy)propan-2-yloxy]cytosine (27b)

White hygroscopic solid (75 %), mp 154-155 °C (D). For $C_{10}H_{19}N_3O_9P_2 \cdot H_2O$ (405.22) calcd: C, 29.64; H, 5.22; N, 10.37; P, 15.29. Found: C, 29.42; H, 5.32; N, 10.46; P, 15.17. FABMS: 388.2 (MH^+) (100). ^1H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 7.77 (q, 1H, $J_{6,CH_3} = 1.0$, Py-6); 6.34 (tt, 1H, $J_{1,2} = 6.2$ and 4.9, H-1); 3.86 (dd, 2H, $J_{gem} = 10.5$, $J_{2a,1} = 6.2$, H-2a); 3.83 (dd, 2H, $J_{gem} = 10.5$, $J_{2b,1} = 4.9$, H-2b); 3.53 and 3.49 (2 × dd, 2 × 2H, $J_{gem} = 12.8$, $J_{3,P} = 8.4$, H-3); 2.10 (d, $J_{CH_3,6} = 1.0$, 3H, CH_3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 164.99 (Py-2); 164.68 (Py-4); 154.55 (Py-6); 108.92 (Py-5); 74.29 (CH-1); 71.61 (d, $J_{2,P} = 11.2$, CH_2-2); 69.70 (d, $J_{3,P} = 150.4$, CH_2-3); 12.14 (CH_3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 264$ nm ($\epsilon_{max} = 7918$); (H_2O) $\lambda_{max} = 264$ nm ($\epsilon_{max} = 8100$); (0.01 M NaOH) $\lambda_{max} = 275$ nm ($\epsilon_{max} = 7009$).

5-Methyl-1-[1,3-bis(phosphonomethoxy)propan-2-yl]cytosine (28b)

White solid (80 %), mp 124-126 °C (D). For $C_{10}H_{19}N_3O_9P_2 \cdot H_2O$ (405.22) calcd: C, 29.64; H, 5.22; N, 10.37; P, 15.29. Found: C, 29.38; H, 5.30; N, 10.54; P, 15.27. FABMS: 388.3 (MH^+) (100). ^1H NMR (500 MHz, D_2O, ref. dioxane = 3.75 ppm): 7.70 (q, 1H, $J_{6,CH_3} = 1.0$, Py-6); 5.06 (tt, 1H, $J_{1,2} = 7.3$ and 4.3 H-1); 3.87 (dd, 2H, $J_{gem} = 11.2$, $J_{2a,1} = 7.3$, H-2a); 3.52 (dd, 2H, $J_{gem} = 11.2$, $J_{2b,1} = 4.3$, H-2b); 3.50 and 3.49 (2 × dd, 2 × 2H, $J_{gem} = 12.8$, $J_{3,P} = 8.4$, H-3); 1.99 (d, JCH_3,H6 = 1.0, 3H, CH_3). ^{13}C NMR (125.7 MHz, D_2O, ref. dioxane = 69.3 ppm): 160.29 (Py-4); 156.50 (Py-2); 138.93 (Py-6); 104.53 (Py-5); 71.25 (d, $J_{2,P} = 10.2$, CH_2-2); 69.49 (d, $J_{3,P} = 148.9$, CH_2-3); 53.96 (CH-1); 12.14 (CH_3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 289$ nm ($\epsilon_{max} = 14241$); (H_2O) $\lambda_{max} = 288$ nm ($\epsilon_{max} = 9837$); (0.01 M NaOH) $\lambda_{max} = 280$ nm ($\epsilon_{max} = 7898$).

1-[1,3-Bis(phosphonomethoxy)propan-2-yl]uracil (33a)

White solid (80 %), mp 105-106 °C (D). For $C_9H_{16}N_2O_{10}P_2 \cdot H_2O$ (392.19) calcd: C, 27.56; H, 4.63; N, 7.14; P, 15.80. Found: C, 27.30; H, 4.58; N, 6.98; P, 15.53. FABMS: 375.0 (MH^+) (10). ^1H NMR (500 MHz, D_2O + NaOD, ref. dioxane = 3.75 ppm): 7.55 (d, 1H, $J_{6,5} = 7.5$, Py-6); 5.82 (d, 1H, $J_{5,6} = 7.5$, Py-5); 5.03 (tt, 1H, $J_{1,2} = 7.3$ and 5.1, H-1); 3.87 (dd, 2H, $J_{gem} = 11.0$, $J_{2a,1} = 7.3$, H-2a); 3.84 (dd, 2H, $J_{gem} = 11.0$, $J_{2b,1} = 5.1$, H-2b); 3.47 and 3.44 (2 × dd, 2 × 2H, $J_{gem} = 12.7$, $J_{3,P} = 8.5$, H-3). ^{13}C NMR (125.7 MHz, D_2O + NaOD, ref. dioxane = 69.3 ppm): 162.64 (Py-4); 155.60 (Py-2); 144.96 (Py-6); 104.38 (Py-5); 73.07 (d, $J_{2,P} = 10.7$, CH_2-2); 71.45 (d, $J_{3,P} = 149.9$, CH_2-3); 56.93 (CH-1). UV spectrum: (0.01 M HCl) $\lambda_{max} = 266$ nm ($\epsilon_{max} = 8484$); (H_2O) $\lambda_{max} = 264$ nm ($\epsilon_{max} = 8161$); (0.01 M NaOH) $\lambda_{max} = 265$ nm ($\epsilon_{max} = 8444$).

1-[1,3-Bis(phosphonomethoxy)propan-2-yl]thymine (33b)
Yellowish solid (80 %), mp 96-97 °C (D). For $C_{10}H_{18}N_2O_{10}P_2 \cdot H_2O$ (406.20) calcd: C, 29.57; H, 4.96; N, 6.90; P, 15.25. Found: C, 29.30; H, 4.72; N, 6.85; P, 15.59. FABMS: 389.0 (MH^+) (10). ^{1}H NMR (500 MHz, D_2O + NaOD, ref. dioxane = 3.75 ppm): 7.77 (q, 1H, $J_{6,CH_3} = 1.0$, Py-6); 5.14 (tt, 1H, $J_{1,2} = 7.3$ and 5.1, H-1); 3.96 (dd, 2H, $J_{gem} = 11.0$, $J_{2a,1} = 7.3$, H-2a); 3.84 (dd, 2H, $J_{gem} = 11.0$, $J_{2b,1} = 5.1$, H-2b); 3.47 and 3.44 (2 × dd, 2 × 2H, $J_{gem} = 12.7$, $J_{3,P} = 8.6$, H-3); 1.19 (d, 3H, $J_{CH_3,6} = 6.1$, CH_3). ^{13}C NMR (125.7 MHz, D_2O + NaOD, ref. dioxane = 69.3 ppm): 156.80 (Py-4); 154.90 (Py-2); 139.46 (Py-6); 111.34 (Py-5); 70.57 (d, $J_{2,P} = 11.2$, CH_2-2); 69.34 (d, $J_{3,P} = 149.9$, CH_2-3); 54.90 (CH-1), 12.08 (CH_3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 270$ nm ($\epsilon_{max} = 8444$); (H_2O) $\lambda_{max} = 271$ nm ($\epsilon_{max} = 8150$); (0.01 M NaOH) $\lambda_{max} = 271$ nm ($\epsilon_{max} = 8320$).

3.4.3 General procedure for alkylation of thymine with primary and secondary alkyl tosylates

A solution of thymine (0.5 g) in dry DMF (20 ml) was treated with Cs_2CO_3 (0.5 equiv.) at room temperature under a $CaCl_2$ protecting tube for 1 h. The reaction mixture was then heated at 60 °C and synthon (**34-39**, 1.0 equiv.) was added. The mixture was then stirred at 90 °C for 24 h. Solvent was evaporated and the residue was co-evaporated with toluene. The residue in chloroform was filtered through a Celite pad, evaporated and purified on a preparative thin layer chromatography (silica gel).

1-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl]thymine (34a)
Preparative TLC (chloroform – 8 % methanol) afforded **34a** as white solid (39 %). FABMS: 241.09 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 11.30 (bs, 1H, NH); 7.45 (q, 1H, $J_{6,CH_3} = 1.0$, Py-6); 4.28 (m, 1H, OCH-2); 3.99 (dd, 1H, $J_{3a,2} = 6.6$, $J_{gem} = 8.7$, CH-3a); 3.79 (dd, 1H, $J_{1a,2} = 4.3$, $J_{gem} = 14.0$, NCH-1a); 3.72 (dd, 1H, $J_{1b,2} = 6.6$, $J_{gem} = 14.0$, NCH-1b); 3.66 (dd, 1H, $J_{3b,2} = 5.6$, $J_{gem} = 8.7$, CH-3b); 2.01 (d, 3H, $J_{CH_3,6} = 1.0$, CH_3); 1.32 and 1.24 (2 × s, 6H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 164.44 (Py-4); 151.36 (Py-2); 142.42 (Py-6); 109.00 (C-iPr); 108.27 (Py-5); 73.57 (C-2); 66.13 (C-3); 49.54 (C-1); 26.68 and 25.35 (CH_3-iPr); 12.11(CH_3).

1,3-Bis[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]thymine (34b)
Preparative TLC (hexane - ethylacetate = 1:1) afforded **34b** as yellowish oil (10 %). FABMS: 355.12 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 8.13 (q, 1H, $J_{6,CH_3} = 1.0$, Py-6); 4.40 and 4.38 (m, 2H, OCH-2); 4.29 and 4.28 (2 × dd, 2H, $J_{1a,2} = 1.6$, $J_{gem} = 11.2$, NCH-1a); 4.23 and 4.22 (2 × dd, 2H, $J_{1b,2} = 2.3$, $J_{gem} = 11.2$, NCH-1b); 4.07 and 4.06 (2 × dd, 2H, $J_{3a,2} = 6.6$, $J_{gem} = 8.4$, CH-3a); 3.79 and 3.73 (dd, 2H, $J_{3b,2} = 6.2$,$J_{gem} = 8.4$, CH-3b); 2.01 (d, 3H, $J_{CH_3,6} = 1.0$, CH_3); 1.34, 1.29 and 1.28 (s, 12H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 168.65 (Py-4); 162.93 (Py-2); 157.78 (Py-6); 110.93 (Py-5); 109.03 and 108.96 (C-iPr); 73.47 (2C, C-2); 67.65 and 66.61 (C-3); 65.94 and 65.61 (C-1); 26.82, 26.68, 25.55 and 11.58 (CH_3-iPr); 12.11(CH_3).

2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]thymine (34c)
Preparative TLC (chloroform – 10 % methanol) afforded **34c** as yellowish oil (1 %). FABMS: 241.11 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 7.65 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.28 (m, 1H, OCH-2); 4.25 (dd, 1H, $J_{1a,2}=4.3$, $J_{gem}=14.0$, NCH-1a); 3.86 (dd, 1H, $J_{3a,2}=6.6$, $J_{gem}=8.7$, CH-3a); 3.72 (dd, 1H, $J_{1b,2}=4.3$, $J_{gem}=14.0$, NCH-1b); 3.66 (dd, 1H, $J_{3b,2}=5.6$, $J_{gem}=8.7$, CH-3b); 1.95 (d, 3H, $J_{CH_3,6}=1.0$, CH_3); 1.32 and 1.24 (2 × s, 6H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 164.44 (Py-4); 151.36 (Py-2); 142.42 (Py-6); 109.00 (C-iPr); 108.27 (Py-5); 73.57 (C-2); 70.21 (C-1); 66.13 (C-3); 26.68 and 25.35 (CH_3-iPr); 12.11 (CH_3).

1-[(Tetrahydro-4-methoxy-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)methyl]thymine (35a)
Preparative TLC (chloroform – 8 % methanol) afforded **35a** as white solid (31 %). FABMS: 313.35 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 11.34 (bs, 1H, NH); 7.51 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.95 (s, 1H, CH-1); 4.72 (bd, 1H, $J_{3,4}=1.0$ and $J_{3,2}=6.0$, OCH-3); 4.62 (d, 1H, $J_{2,3}=6.0$, OCH-2); 4.32 (bt, 1H, $J_{4,3}=1.0$ and $J_{4,5}=7.3$, CH-4); 3.87 (dd, 1H, $J_{5a,4}=7.6$, $J_{gem}=13.9$, NCH-5a); 3.55 (dd, 1H, $J_{5b,4}=7.2$, $J_{gem}=13.9$, NCH-5b); 3.28 (s, 3H, OCH_3); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.36 and 1.24 (2 × s, 6H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 164.35 (Py-4); 151.21 (Py-2); 141.93 (Py-6); 111.89 (C-iPr); 109.35 (C-1); 108.79 (Py-5); 84.76 (C-4); 83.69 (C-2); 81.31 (C-3); 55.09 (OCH_3); 50.25 (C-5); 26.44 and 24.91 (CH_3-iPr); 12.11(CH_3).

1,3-Bis[(tetrahydro-4-methoxy-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)methyl]thymine (35b)
Preparative TLC (hexane - ethylacetate = 1:1) afforded **35b** as colorless oil (10 %). FABMS: 499.32 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 7.62 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.95 and 4.94 (2 × s, 2H, CH-1); 4.74 and 4.68 (2 × bd, 2H, $J_{3,4}=1.0$, OCH-3); 4.63 and 4.60 (2 × d, 2H, $J_{2,3}=6.0$, OCH-2); 4.37 and 4.23 (2 × dd, 2H, $J_{4,5}=7.3$, 4.8 and 9.6, CH-4); 4.07 and 3.96 (2 × dd, 2H, $J_{5a,4}=9.6$ and 7.6, NCH-5a); 3.65 and 3.48 (2 × dd, 2H, $J_{5b,4}=7.2$ and 4.8, $J_{gem}=$ 13.9 and 13.0, NCH-5b); 3.28 and 3.27 (2 × s, 6H, OCH_3); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.36, 1.33, 1.24, and 1.20 (4 × s, 12H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 163.30 (Py-4); 151.44 (Py-2); 140.95 (Py-6); 111.85 and 111.69 (C-iPr); 109.49 (Py-5); 108.06 and 108.85 (C-1); 84.88 and 84.75 (C-4); 83.52 and 83.51 (C-2); 81.84 and 81.34 (C-3); 55.16 and 54.71 (OCH_3); 51.39 and 43.41 (C-5); 26.47, 26.43, 24.92 and 24.91 (CH_3-iPr); 12.72 (CH_3).

2-[(Tetrahydro-4-methoxy-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)methoxy]thymine (35c)
Preparative TLC (chloroform – 8 % methanol) afforded **35c** as colorless oil (1 %). FABMS: 313.21 (MH^+) (35). ^{1}H NMR (500 MHz, DMSO-d_6): 7.51 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.98 (s, 1H, CH-1); 4.74 (bd, 1H, $J_{3,4}=1.0$ and $J_{3,2}=6.0$, OCH-3); 4.62 (d, 1H, $J_{2,3}=6.0$, OCH-2); 4.35 (bt, 1H, $J_{4,3}=1.0$ and $J_{4,5}=7.3$, CH-4); 3.85 (dd, 1H, $J_{5a,4}=7.6$, $J_{gem}=13.9$, NCH-5a); 3.65 (dd, 1H, $J_{5b,4}=7.2$, $J_{gem}=13.9$, NCH-5b); 3.25 (s, 3H, OCH_3); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.36 and 1.24 (2 × s, 6H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 164.35 (Py-4); 151.21 (Py-2); 141.93 (Py-6); 111.89 (C-iPr); 109.35 (C-1); 108.79 (Py-5); 84.76 (C-4); 83.69 (C-2); 81.31 (C-3); 71.13 (C-5), 55.09 (OCH_3); 26.44 and 24.91 (CH_3-iPr); 12.11 (CH_3).

1-{[(Diisopropoxyphosphoryl)methoxy]ethyl}thymine (36a)
Preparative TLC (chloroform – 10 % methanol) afforded **36a** as white solid (32 %). FABMS: 349.25 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 11.25 (bs, 1H, NH); 7.43 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.55 (m, 2H, CH-iPr); 3.81 (t, 2H, $J=5.0$, NCH); 3.76 (d, 2H, $J=8.4$, PCH_2O); 3.68 (t, 2H, $J=5.0$, OCH_2); 1.75 (d,

3H, $J_{CH_3,6}=1.2$, CH_3); 1.21 and 1.19 (2 × d, 12H, $J=6.2$, CH_3-iPr).^{13}C NMR (125.7 MHz, DMSO-d_6): 164.49 (Py-4); 151.05 (Py-2); 142.25 (Py-6); 108.04 (Py-5); 70.34 (d, $J=6.4$, CH-iPr); 70.14 (d, $J=11.7$, OCH_2); 64.84 (d, $J=164.1$, PCH_2O); 46.97 (NCH), 23.97 and 23.83 (CH_3-iPr); 12.15 (CH_3).

1,3-Bis{[(diisopropoxyphosphoryl)methoxy]ethyl}thymine (36b)
Preparative TLC (chloroform – 10 % methanol) afforded **36b** as colorless oil (10 %). FABMS: 571.23 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 7.51 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.55 (m, 4H, CH-iPr); 4.07 and 3.81 (2 × t, 4H, $J=6.0$ and 5.0, NCH); 3.76 and 3.74 (2 × d, 4H, $J=8.4$, PCH_2O); 3.72 and 3.68 (2 × t, 4H, $J=6.0$ and 5.0, OCH_2); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.22, 1.21, 1.20 and 1.19 (4 × d, 24H, $J=6.1$, CH_3-iPr).^{13}C NMR (125.7 MHz, DMSO-d_6): 163.26 (Py-4); 150.99 (Py-2); 141.02 (Py-6); 107.24 (Py-5); 70.34 (4C, $J=6.3$, CH-iPr); 70.00 and 68.97 (d, $J=12.2$ and 11.7, OCH_2); 64.83 and 64.68 (d, $J=164.6$, PCH_2O); 48.11 and 39.46 (NCH); 23.97, 23.94, 23.83 and 23.80 (CH_3-iPr); 12.75 (CH_3).

2-{[(Diisopropoxyphosphoryl)methoxy]ethoxy}thymine (36c)
Preparative TLC (chloroform – 10 % methanol) afforded **36c** as yellowish oil (1 %). FABMS: 349.12 (MH^+) (25). ^{1}H NMR (500 MHz, DMSO-d_6): 7.43 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 4.55 (m, 2H, CH-iPr); 3.79 (t, 2H, $J=5.0$, OCH); 3.75 (d, 2H, $J=8.4$, PCH_2O); 3.66 (t, 2H, $J=5.0$, OCH_2); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.21 and 1.19 (2 × d, 6H, $J=6.2$, CH_3-iPr).^{13}C NMR (125.7 MHz, DMSO-d_6): 164.49 (Py-4); 151.05 (Py-2); 142.25 (Py-6); 108.04 (Py-5); 70.34 (2C, $J=6.4$, CH-iPr); 70.14 (d, $J=11.7$, OCH_2); 65.13 (OCH); 64.84 (d, $J=164.1$, PCH_2O); 23.97, 26.43, 23.83, 23.54 (CH_3-iPr); 12.15 (CH_3).

1-[1-(Benzyloxy)propan-2-yl]thymine (37a)
Preparative TLC (chloroform – 5 % methanol) afforded **37a** as white solid (25 %). FABMS: 275.21 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 11.20 (bs, 1H, NH); 7.54 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 7.30-7.24 (m, 5H, $H_{arom.}$); 4.75 (m, 1H, NCH); 4.50 and 4.42 (2 × d, 2H, $J_{gem}=12.2$, $BnOCH_2$); 3.62 and 3.51 (2 × dd, 2H, $J=10.5$ and 8.2, 10.5 and 4.6, OCH_2); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.21 (d, 3H, $J=7.1$, CH_3).^{13}C NMR (125.7 MHz, DMSO-d_6): 163.95 (Py-4); 151.29 (Py-2); 138.30; 138.12 (Py-6); 128.45 (2C); 127.69; 127.58 (2C); 108.83 (Py-5); 71.98 and 70.94 (OCH_2); 49.89 (NCH); 15.66 (CH_3); 12.27 (CH_3).

1,3-Bis[1-(benzyloxy)propan-2-yl]thymine (37b)
Preparative TLC (hexane - ethylacetate = 7:3) afforded **37b** as white solid (3.5 %). FABMS: 423.52 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 7.59 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 7.30-7.24 (m, 10H, $H_{arom.}$); 5.20 and 4.81 (m, 2H, NCH); 4.48, 4.45, 4.40 and 4.37 (4 × d, 4H, $J=12.2$, $BnOCH_2$); 3.93 and 3.64 (2 × dd, 2H, $J=9.5$ and 8.3, 9.5 and 6.1, OCH_2); 3.62 and 3.53 (2 × dd, 2H, $J=10.5$ and 8.1, 10.5 and 4.8, OCH_2); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.30 and 1.21 (d, 6H, $J=7.1$, CH_3).^{13}C NMR (125.7 MHz, DMSO-d_6): 163.95 (Py-4); 151.29 (Py-2); 138.61; 136.84 (Py-6); 136.52, 138.26, 129.66, 129.33 and 128.35 (4C); 127.64 (2C); 127.52 (2C); 127.58 (2C); 109.0 (Py-5); 71.98, 71.90, 70.92, 70.54 (OCH_2); 50.94 and 50.90 (NCH); 15.58, 14.71 and 12.97 (CH_3).

2-[1-(Benzyloxy)propan-2-yloxy]thymine (37c)
Preparative TLC (chloroform – 5 % methanol) afforded **37c** as white solid (1 %). FABMS: 275.31 (MH^+) (55). ^{1}H NMR (500 MHz, DMSO-d_6): 7.54 (q, 1H, $J_{6,CH_3}=1.0$, Py-6); 7.30-7.24 (m, 5H, $H_{arom.}$); 4.75 (m, 1H, OCH); 4.52 and 4.42 (2 × d, 2H, $J=12.2$, $BnOCH_2$); 3.61 and 3.51 (2 × dd, 2H, $J=10.5$ and 8.2, 10.5 and 4.6, OCH_2); 1.75 (d, 3H, $J_{CH_3,6}=1.2$, CH_3); 1.21 (d, 3H, $J=7.1$, CH_3).^{13}C NMR (125.7 MHz, DMSO-d_6): 163.95 (Py-4); 151.29 (Py-2); 138.30 (C); 138.12 (Py-6); 128.45 (2C); 127.69 (C); 127.58 (2C);

108.83 (Py-5); 71.98 and 71.94 (OCH_2); 65.85 (OCH); 15.66 (CH_3); 12.27 (CH_3).

1-(2-Phenyl-1,3-dioxan-5-yl)thymine (38a)

Preparative TLC (hexane - ethylacetate = 7:3) afforded **38a** as white solid (23 %). FABMS: 335.12 (MH^+) (65). ^{1}H NMR (500 MHz, DMSO-d_6): 11.38 (bs, 1H, NH); 8.16 (q, 1H, $J_{6,CH_3} = 1.0$, Py-6); 7.42-7.24 (m, 5H, $H_{arom.}$); 5.73 (s, 1H, O-CH-O); 4.42 and 4.25 (bd, 4H, $J_{gem} = 12.6$, OCH_2); 4.37 (bt, 1H, $J_{CH,CH_2} = 2.0$ NCH); 1.80 (d, 3H, $J_{CH_3,6} = 1.2$, CH_3).^{13}C NMR (125.7 MHz, DMSO-d_6): 163.95 (Py-4); 151.29 (Py-2); 139.37 (Py-6); 138.19; 129.28; 128.51 (2C); 126.25 (2C); 108.28 (Py-5); 101.04 (O-CH-O); 68.86 (2C, OCH_2); 47.53 (NCH); 12.73 (CH_3).

1,3-Bis(2-phenyl-1,3-dioxan-5-yl)thymine (38b)

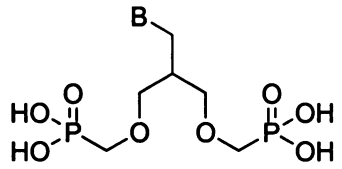
Preparative TLC (hexane - ethylacetate = 7:3) afforded **38b** as white solid (1 %). FABMS: 451.23 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 8.16 (q, 1H, $J_{6,CH} = 1.0$, Py-6); 7.50-7.30 (m, 10H, $H_{arom.}$); 5.67 and 5.66 (s, 2H, O-CH-O); 5.10 and 4.90 (p, 2H, $J_{CH,CH_2} = 1.6$, NCH); 4.28 (m, 8H, OCH_2); 2.10 (d, 3H, $J_{CH_3,6} = 1.2$, CH_3).^{13}C NMR (125.7 MHz, DMSO-d_6): 168.36 (Py-4); 162.55 (Py-2); 157.99 (Py-6); 138.68; 138.60, 128.96; 128.84, 128.22 (2C); 128.18 (2C); 126.31 (2C); 126.18 (2C); 111.29 (Py-5); 100.32 and 100.13 (O-CH-O); 68.54 and 68.38 (OCH_2); 48.17 and 47.88 (NCH); 11.68 (CH_3).

2-(2-Phenyl-1,3-dioxan-5-yloxy)thymine (38c)

Preparative TLC (hexane - ethylacetate = 7:3) afforded **38c** as colorless oil (1 %). FABMS: 335.13 (MH^+) (45). ^{1}H NMR (500 MHz, DMSO-d_6): 8.16 (q, 1H, $J_{6,CH_3} = 1.0$, Py-6); 7.42-7.24 (m, 5H, $H_{arom.}$); 5.69 (s, 1H, O-CH-O); 4.37 (bt, 1H, $J_{CH,CH_2} = 2.0$, OCH); 4.32 and 4.25 (bd, 4H, $J_{gem} = 12.6$, OCH_2); 1.80 (d, 3H, $J_{CH_3,6} = 1.2$, CH_3).^{13}C NMR (125.7 MHz, DMSO-d_6): 163.95 (Py-4); 151.29 (Py-2); 139.37 (Py-6); 138.19; 129.28; 128.51 (2C); 126.25 (2C); 108.28 (Py-5); 101.04 (O-CH-O); 69.12 (OCH); 68.86 (2C, OCH_2); 12.73 (CH_3).

Chapter 4

Synthesis of extended glycerol based ANbPs



B = purine or pyrimidine ring

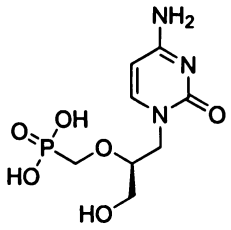
4.1 Introduction

Analogues of nucleic acids components belong to antimetabolite group. Their ability to interfere with the metabolic pathways of nucleic acids synthesis *de novo* may cause antiviral, antineoplastic or other biological activity. First generation of nucleic acid analogues aimed at their close structural similarity to natural metabolites,[9–11] however, led to their limited stability in the organism due to their easy degradation in the metabolic pathways.

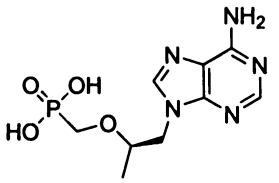
The second generation of antimetabolites, acyclic nucleosides, has overcome this limitation by replacing the sugar moiety with aliphatic chain bound to the N^9 nitrogen of purines (*e.g.*, acyclovir,[14,15] ganciclovir,[16,17] AHPA,[18] or (*S*)-DHPA[19]). These compounds are *in vivo* phosphorylated to their 5'-nucleotide. However, the direct application of such nucleotides is not possible due to the lability of the phosphomonoester bond in blood plasma and during the transmembrane transport.[20] Therefore, there is a need for new isopolar and

isosteric phosphate analogues resistant to enzymatic reactions. One of the solutions was replacement of phosphate by enzymatically stable phosphonate group. Very interesting in this respect are phosphonomethyl ethers.[21]

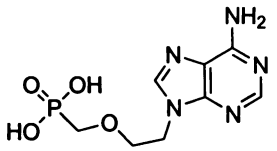
Structure activity relationship investigations in the series of acyclic nucleotide analogues bearing a modified phosphoric acid residue in the side chain has revealed so far several biologically active acyclic nucleoside phosphonates (ANPs). Some of them, are currently used in clinical practice.[22,23] Cidofovir or (*S*)-HPMPC **1** (Vistide® a injectable form of Cidofovir) is an antiviral medication for the treatment of cytomegalovirus (CMV) retinitis in patients with AIDS.[37] Tenofovir or (*R*)-PMPA **2**, and its prodrug Viread® belongs to nucleotide reverse transcriptase inhibitors (NtRTIs) which block an enzyme crucial to viral production in HIV-infected people.[29] Adefovir or PMEA **3**, and its prodrug Hepsera® is an orally-administered NtRTI used for treatment of hepatitis B.[27]



(*S*)-HPMPC (Cidofovir)
1



(*R*)-PMPA (Tenofovir)
2

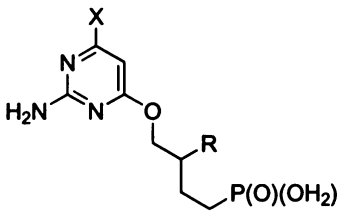


PMEA (Adefovir)
3

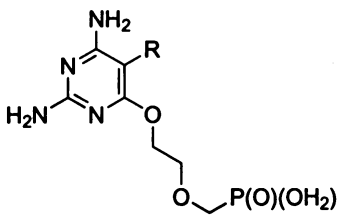
Figure 4.1: First generation of acyclic nucleoside phosphonates

Recently, attention was turned to the synthesis of a new type of ANPs originating from 2-substituted 4-amino-6-hydroxypyrimidines.[44] In these investigations, a significant potential activity of 6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives, compounds derived from 2,4-diaminopyrimidine (**4a**, **4c**, **4d**)[45] and 2-amino-4-hydroxypyrimidine (**4b**),[45] and their C5-substituted congeners **5**[46,47] was discovered (Figure 4.2).

Among the products isolated in these studies bisphosphonates **6** and **7** were also identified (Figure 4.3).[44] Despite the fact that these compounds constitute a new class of possible antiviral agents, they have not yet received much attention. The aim of this work was to explore the potential biological activity of such substances, which could be also considered as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor.[69]

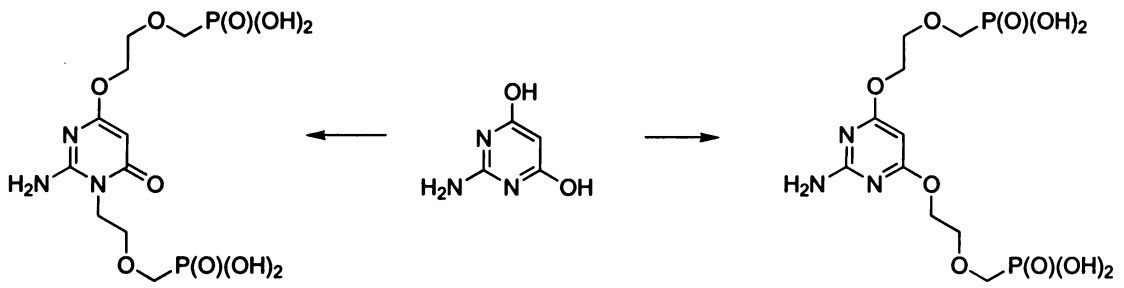


4a X = NH_2, R = H
4b X = OH, R = H
4c X = NH_2, R = (*R*)-CH_3
4d X = NH_2, R = (*S*)-CH_2OH



5 R = allyl, benzyl, CH_2CN, CH_2COOEt, CH_3, phenyl, cyclopropyl, CH_2COOH, Cl, Br, CN

Figure 4.2: ANPs originating from 2-substituted 4-amino-6-hydroxypyrimidines



6 **7**

Figure 4.3: Fist acyclic nucleoside bisphosphonates isolated in our group

4.2 Results and discussion

In the previous project of this series we have described the synthesis of symmetrical 1,3-bis(phosphonomethoxy)propan-2-yl compounds **8**, derived from the 2-(phosphonomethoxy)-ethyl (PME) chain, where an additional (phosphonomethoxy)methyl group is attached at the C1 position of the ethyl chain of the parent PME compound **9** (Figure 4.4). In this project it is discussed the synthesis of another symmetrical acyclic nucleoside bisphosphonate (ANbP) **10** extended by one carbon atom.

The strategy chosen for the synthesis of ANbP **10**, was based on alkylation of an appropriate heterocyclic base with reagent **19** which contains a good leaving group on an alkyl chain bearing an esterified bisphosphonate grouping linked through an ether bridge. This approach in most cases does not require any protection of the nucleobase and can be also applied to sensitive heterocyclic systems bearing reactive substituents (*e.g.*, 6-chloropurines).

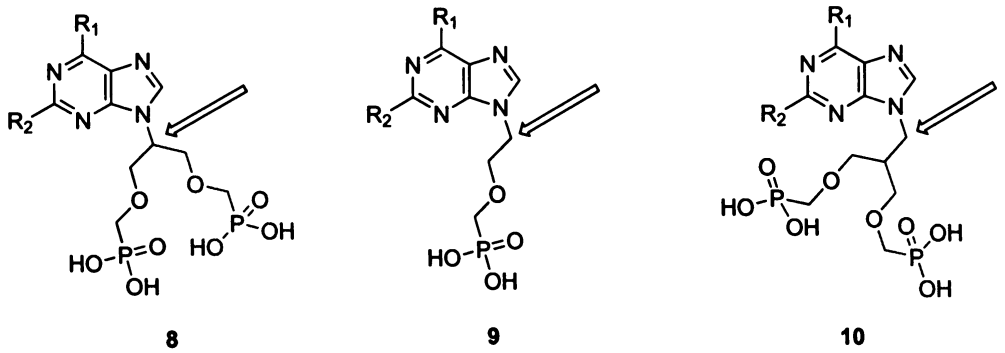
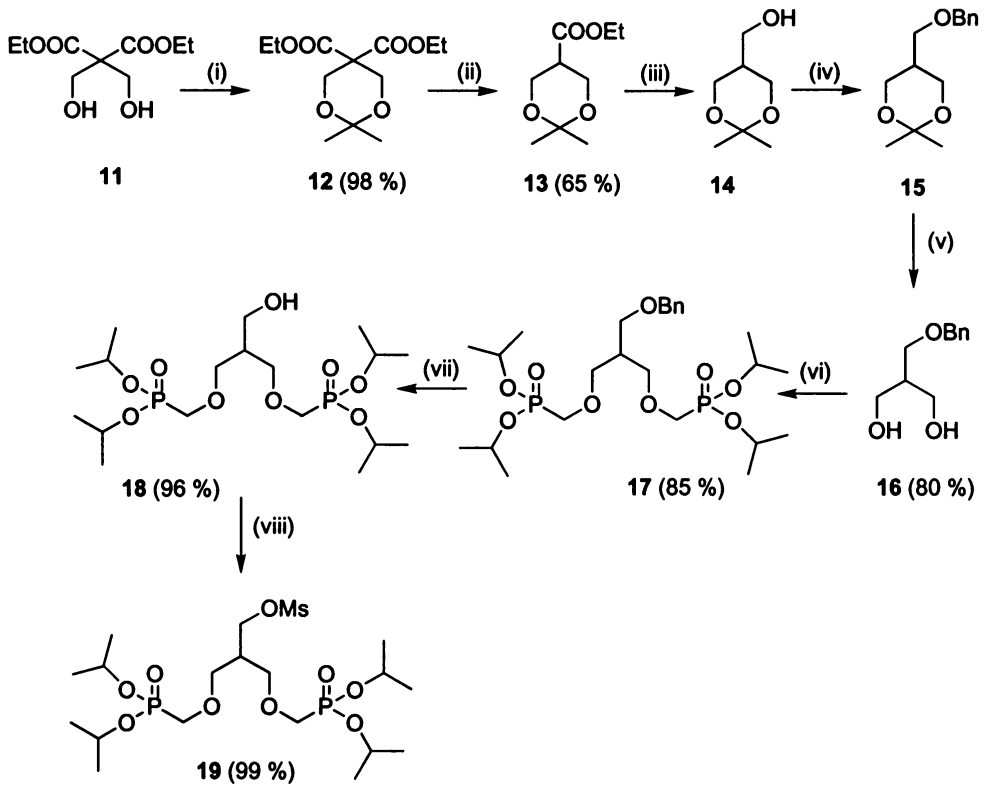


Figure 4.4: Extended glycerol based ANbPs

As shown in the Figure 4.5, the synthetis of the corresponding bisphosphonate alkylating agent **19**, started from commercially available diethyl 2,2-bis(hydroxymethyl)malonate **11**, which was transformed to an acetonide **12**.[82] The mono ester **13** was obtained by decarbethoxylation of **12** in the presence of NaCl and DMSO according to the procedure described in the literature.[83–85] Ester **13** was reduced to primary alcohol **14** with $LiAlH_4$ in ether and benzylated with benzyl bromide to afford benzyloxy derivative **15**. Acid hydrolysis of the isopropylidene protecting group of **15** gave 2-[(benzyloxy)methyl]propane-1,3-diol **16**. Chloromethylation of **16** with paraformaldehyde and gaseous HCl followed by the Arbuzov reaction with triisopropyl phosphite gave the bisphosphonate **17**. The target bisphosphonate alkylating agent **19** was then prepared by hydrogenolysis of **17** followed by mesylation of **18**.

The bisphosphonate **19** was used in alkylations of various nucleobases; 2-amino-6-chloropurine **20**, adenine **21**, 6-(cyclopropyl)aminopurine **22**, cytosine **31**, uracil **33**, 4-methoxy-5-methylpyrimidin-2(1*H*)-one **35**. All reactions were performed at 100 °C in the presence of Cs_2CO_3 in DMF.

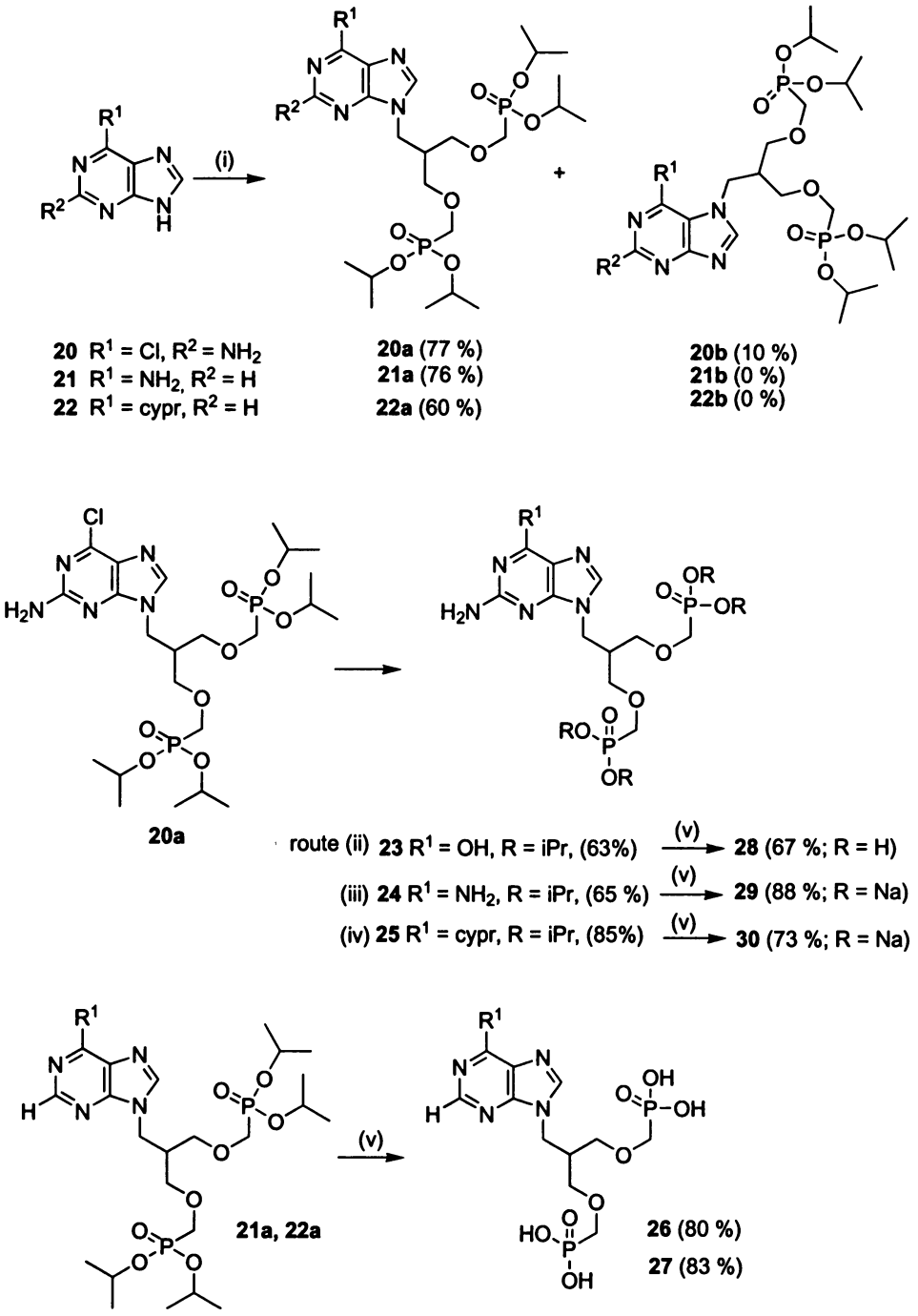
Alkylation of purines is depicted in Figure 4.6. The reaction of 2-amino-6-chloropurine **20** easily proceeded at N^9 and also at N^7 positions to give regioisomers **20a** and **20b**. These isomers were easily separated by silica gel column chromatography. The intermediate **20a** was further converted to other base-modified bisphosphonates: thus, acid hydrolysis led to the guanine derivative **23**; the 2,6-diaminopurine derivative **24** was prepared by ammonolysis with methanolic ammonia; and replacement of chlorine with the cyclopropylamino group in the reaction with cyclopropylamine in dioxane gave 2-amino-6-(cyclopropyl)amino derivative **25**.



(i) 2,2-dimethoxypropane, H_2SO_4, acetone; (ii) H_2O, NaCl, DMSO, 190-195 °C; (iii) $LiAlH_4$, ether, 0 °C; (iv) BnBr, NaH, THF; (v) Dowex 50 (H^+ form), 80% methanol, reflux; (vi) $(CH_2O)_n$, (g) HCl, $CaCl_2$, DCM, 0 °C, then $P(OiPr)_3$, 120 °C; (vii) H_2/Pd/C, conc. HCl, MeOH; (viii) MsCl, Et_3N, DCM.

Figure 4.5: Synthesis of bisphosphonate alkylating agent

The alkylation of adenine **21** and 6-(cyclopropyl)aminopurine **22** gave exclusively the N^9-isomers **21a** and **22a**. In contrast to the analogous alkylation of **20**, essentially no N^7-regioisomer formation was observed in alkylation of compounds **21** and **22**. In all the discussed cases, NMR analysis was used to identify the position of substitution of the purine moiety. All signals of hydrogen and carbon atoms were assigned usig 2D-^{1}H,^{13}C HSQC and 2D-^{1}H,^{13}C HMBC experiments. In the case of N^9-isomers, protons on carbon atom next to the purine nitrogen have strong crosspeaks in HMBC spectra with carbons C-4 and C-8 of the purine moiety. In N^7-isomers these protons correlate with C-5 and C-8 atoms. Subsequent deprotection of compounds **21a**, **22a**, and **23** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free bisphosphonic acids **26-28**, which were ultimately purified by ion exchange chromatography. The behaviour of compounds **24** and **25** differed from the above-mentioned substances. The deprotected



(i) **19**, Cs_2CO_3, DMF, 100 °C; (ii) 80% CH_3COOH, reflux; (iii) methanolic ammonia, MeOH, 100 °C; (iv) cyclopropylamine, dioxane, reflux; (v) TMSBr, CH_3CN, RT.

Figure 4.6: Alkylation of purines

and hydrolyzed products were applied onto Dowex 50 X 8 (H^+ form) as well. However, they adsorbed on the stationary phase and had to be eluted with dilute ammonia solution. Thus, obtained ammonia salts were finally transformed into sodium salts of bisphosphonic acids **29** and **30** by Dowex 50 X 8 (Na^+ form) chromatography.

The reaction of cytosine **31** with bisphosphonate **19** under the above conditions afforded a mixture of N^1-regioisomer (**31a**; yield 49 %) and O^2-regioisomer (**31b**; yield 15 %). Only the N^1 derivative was further converted to the corresponding free bisphosphonic acid **32** as shown in the Figure 4.7. O^2- and N^1-isomers in the pyrimidine series could be readily distinguished by NMR spectroscopy. The carbon atom chain linked to the oxygen shows $\delta = 64$ ppm; when bonded to nitrogen its chemical shift was $\delta = 48$ ppm (cf. Experimental). Furthermore, the protons bonded to this carbon have crosspeaks in 2D-^{1}H,^{13}C-HMBC spectra to carbon atoms C-2 and C-6 (in the case of N^1-isomers) or only to C-2 (in the case of O^2 isomer).

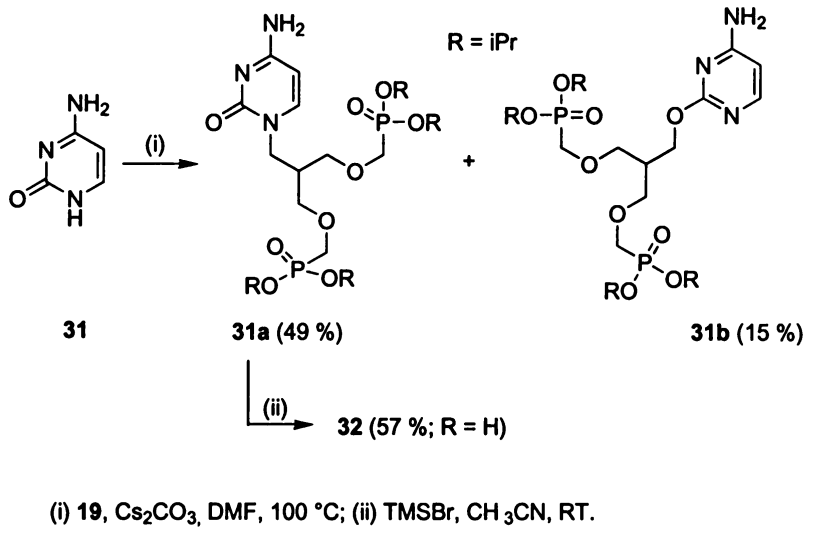


Figure 4.7: Alkylation of cytosine

Figure 4.8 shows the course of alkylation of uracil **33** with compound **19** under standard conditions. Of the two products formed in the reaction, N^1-alkylate **33a** and N^1,N^3-bisalkylate **33b**, required **33a** was the major product. It was isolated in about 54% yield while the bisalkylate was obtained in 15% yield. Subsequent deprotection of the tetraester **33a** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free bisphosphonic acid **34** which was isolated from the deionized product by ion exchange chromatography. The structure of all products was identified using NMR

spectroscopy. In ^{1}H NMR spectra of bisalkylate **33b** the NH signal is missing. In ^{13}C NMR, there are two signals of carbons bonded to the pyrimidine ring with $\delta = 48$ ppm and 40 ppm for N^1 and N^3, respectively. Full assignment of all carbon and hydrogen atoms was done using 2D heterocorrelated experiments.

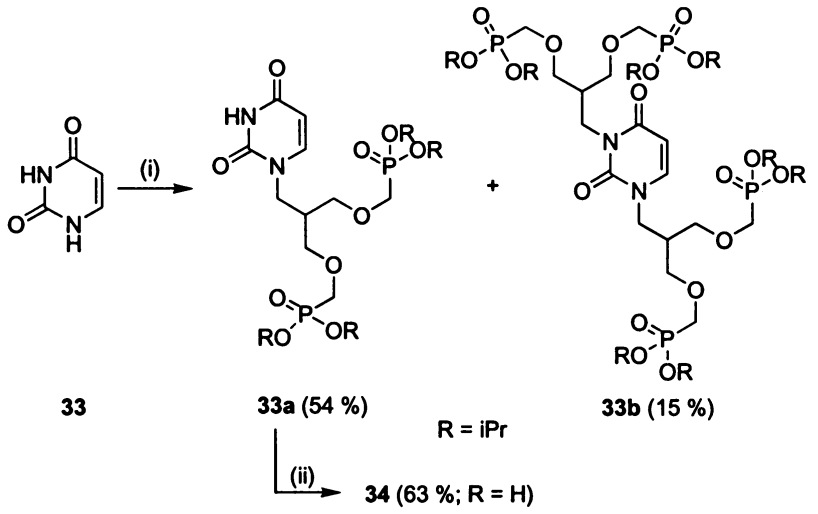


Figure 4.8: Alkylation of uracil

Analogously to cytosine, also the alkylation of 4-methoxy-5-methylpyrimidin-2(1*H*)-one **35**, the precursor of thymine, provided two products (N^1-alkylate **35a** and O^2-alkylate **35b**) in the ratio almost 1:1. The procedure is depicted in Figure 4.9. Compound **35a** was further hydrolyzed under acid conditions to provide thymine derivative **36**. It is worth mentioning that attempts to prepare thymine derivative of **35b** failed. No reaction was observed in the reaction with 80% CH_3COOH and Dowex 50 (H^+ form). Decomposition of the phosphonate was achieved under more drastic conditons (heating with 2 M HCl). The subsequent deprotection of tetraester **36** with bromotrimethylsilane in acetonitrile followed by hydrolysis yielded the corresponding free bisphosphonic acid **37**, which was isolated from the deionized product by ion exchange chromatography. Both isolated isomers **35a** and **35b** could again be distinguished by NMR spectroscopy. All signals of hydrogen and carbon atoms were assigned using 1D and 2D NMR experiments. The position of the substituent is clear from the chemical shift of the carbon atom bonded to the pyrimidine ring (65 ppm for the O^2 derivative and 48 ppm for the N^1 derivative) and is confirmed also by 2D-HMBC spectra.

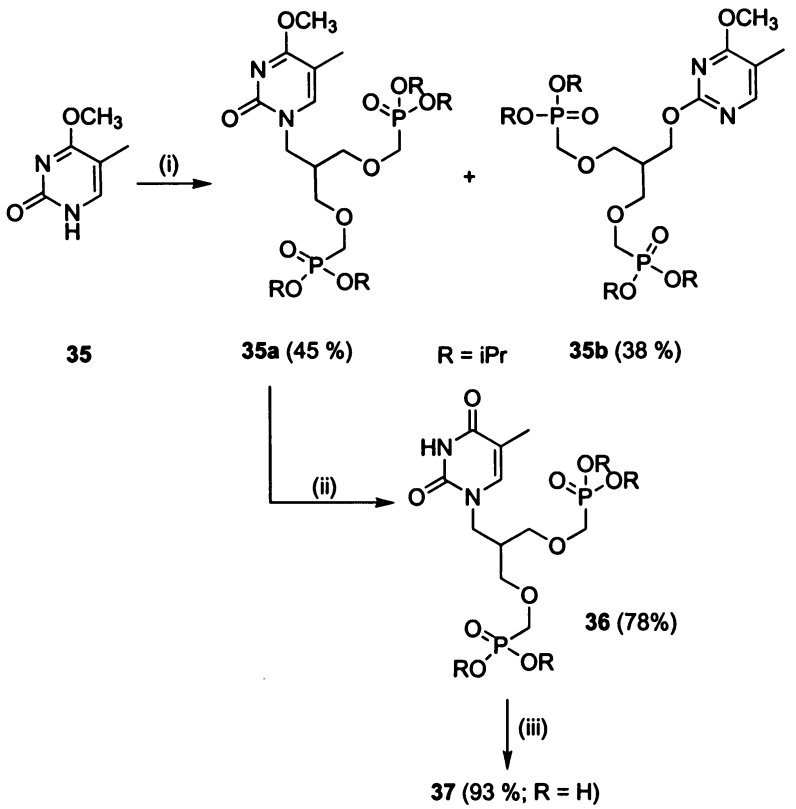


Figure 4.9: Alkylation of 4-methoxy-5-methylpyrimidin-2(1*H*)-one

4.3 Conclusions

A novel bisphosphonate building block, tetraisopropyl {2-[(mesyloxy)methyl]propane-1,3-diyl}bis(oxymethylene)bisphosphonate, was synthesized using transformation of diethyl 2,2-bis(hydroxymethyl)malonate as starting compound. The obtained alkylating agent was further used in alkylation of various nucleobases in the presence of Cs_2CO_3. While, the alkylation of 2-amino-6-chloropurine afforded a mixture of N^9- and N^7-substituted purine derivatives, the formation of N^9-substituted nucleobases were obtained in the reaction with adenine and 2-amino-6-(cyclopropyl)aminopurine. A mixture of N^1 and O^2-regioisomers were obtained in the alkylation of cytosine and 4-methoxy-5-methylpyrimidin-2(1*H*)-one. The alkylation of uracil afforded a mixture of N^1-mono and N^1,N^3-bisalkylates. The corresponding free bisphosphonic acids were obtained after the ester cleavage from N^9-substituted purine and N^1-substituted pyrimidine derivatives. All the target compounds were subjected to the screening of inhibitory activity murine leukemia L1210 cells, human

promyelocytic leukemia HL60 cells, murine L929 cells, human cervix carcinoma HeLa S3 cells and human T-lymphoblastoid CCRF-CEM cell line. They are also inactive both in the antiviral assays of DNA viruses, RNA viruses and retroviruses, specifically against hepatitis B and C viruses and against HIV, against herpesviruses (HSV-1 and HSV-2, VZV and HCMV) and RNA virus models of human pathogens. Nonetheless, the data are not considered to be conclusive, since some tests are still in progress. However, as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor, they might possess this activity. This hypothesis is currently being tested.

4.4 Experimental part

Unless otherwise stated, solvents were evaporated at 40 °C / 2 kPa and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. NMR spectra were measured on a Bruker Avance-500 instruments (500.0 MHz for ^1H and 125.7 MHz for ^{13}C). Chemical shifts are in ppm (δ-scale) and coupling constants (J) in Hz. Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using FAB or EI. UV spectra were taken on a Beckman DU-65 spectrophotometer in aqueous solution. Elemental analyses were carried out on a Perkin Elmer CHN Analyser 2400, Series II Sys. Chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic). Dimethylformamide, dichloromethane and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å). Tetrahydrofuran was distilled over sodium with benzophenone as indicator. Acetone was dried over anhydrous $CuSO_4$. Diethyl ether was distilled from $LiAlH_4$.

Diethyl 2,2-dimethyl-1,3-dioxane-5,5-dicarboxylate (12)
Synthetic strategy and analysis corresponds to ref.[82]

Ethyl 2,2-dimethyl-1,3-dioxane-5-carboxylate (13)
Synthetic strategy and analysis corresponds to ref.[83–85]

(2,2-Dimethyl-1,3-dioxan-5-yl)methanol (14)
$LiAlH_4$ (4.6 g, 120.9 mmol) and dry diethyl ether (250 ml) were introduced into a well-dried three-neck vessel with condenser. The reaction mixture was cooled to 0 °C. Subsequently, compound **13** (28.08 g, 149.2 mmol) was added dropwise in 150 ml of diethyl ether during 30 min in argon atmosphere. The reaction proceeded under gentle reflux. The mixture was stirred under these conditions for another 3 h and at room temperature overnight. The residue was cooled with ice bath and 4.7 ml of water was slowly added. After that, 4.7 ml of 15% NaOH and 14.1 ml of water were added. The solution was stirred until a white precipitate formed. The mixture was filtered through Celite. The solid was washed with 200 ml of

diethyl ether (twice). The filtrate was evaporated and used without further purification. FABMS: 147.1 (MH^+) (70). ^1H NMR (500 MHz, DMSO-d_6): 4.54 (t, 1H, $J_{OH,CH_2}=5.2$, OH); 3.82 (dd, 2H, $J_{2a,3}=4.4$, $J_{gem}=11.8$, H-2a); 3.61 (dd, 2H, $J_{2b,3}=7.2$, $J_{gem}=11.8$, H-2b); 3.49 (dd, 2H, $J_{4,3}=6.7$, $J_{4,OH}=5.2$, H-4); 1.69 (m, 1H, H-3); 1.30 and 1.29 (2 x s, 6H, 2 x CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 97.32 (C-1); 61.05 (C-2); 59.81 (C-4); 36.68 (C-3); 24.97 and 23.33 (2 x CH_3).

5-[(Benzyloxy)methyl]-2,2-dimethyl-1,3-dioxane (15)

Compound **14** (19 g, 130 mmol) was added to a suspension of NaH (7.28 g of 60% suspension in mineral oil prewashed with n-hexane; 182 mmol) in dry THF (350 ml) at 0 °C under argon atmosphere. The reaction mixture was then cooled to −10 °C and benzyl bromide (28.9 g, 169 mmol) in THF (200 ml) was added dropwise during 1 h. The mixture was stirred at −10 °C for 30 min and at room temperature overnight, under argon. When the reaction was complete (TLC), methanolic ammonia (30 ml) was added. After stirring the solution for 1 h, the solvent was evaporated. The residue in chloroform (500 ml) was washed with water (500 ml). The organic layer was dried with anhydrous $MgSO_4$, filtered and evaporated to yield crude **15**, which was used without further purification.

2-[(Benzyloxy)methyl]propane-1,3-diol (16)

A crude mixture of **15** (30.7 g, 130 mmol) in 80% methanol (300 ml) was refluxed with Dowex 50 X 8 (H^+ form) (10 g) for 4 h. The resin was filtered off, the solution neutralized with aqueous ammonia and the solvent was evaporated. The crude product was purified by silica gel column chromatography, using the chloroform – methanol gradient 0-6 %, to yield 20.4 g (80 %) of pure **16** as colorless oil. FABMS: 197.2 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 7.36-7.25 (m, 5H, $H_{arom.}$); 4.44 (s, 2H, OCH_2Ph); 4.40 (t, 2H, $J_{OH,3}=5.2$, OH); 3.44 (t, 4H, $J_{3,OH}\sim J_{3,2}=5.3$, H-3); 3.43 (d, 2H, $J_{1,2}=6.1$, H-1); 1.80 (h, 1H, $J_{2,3}\sim J_{2,1}=6.0$, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 139.03; 128.46 (2C); 127.57 (2C); 127.54; 72.39 (OCH_2Ph); 68.79 (d, $J_{C,P}=12.7$, C-1); 59.73 (C-3); 44.59 (C-2).

Tetraisopropyl[{2-[(benzyloxy)methyl]propane-1,3-diyl}bis(oxymethylene)]bisphosphonate (17)

A mixture of compound **16** (12.3 g, 62.7 mmol), paraformaldehyde (2.2 equiv.) and $CaCl_2$ (4 g) was saturated with gaseous HCl for 45 min at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, then allowed to reach room temperature, evaporated and dried. The residue was further used in the Arbuzov reacton with triisopropyl phosphite. The crude mixture was stirred at 100 °C and $P(OiPr)_3$ (30 ml, 2.1 equiv.) was slowly added and stirred for 1 h at 130 °C. The excess of phosphite and isopropyl chloride was distilled off (oil bath 80 °C) and the residue were purified by column chromatography on silica gel, using the chloroform – methanol gradient 0-3 %, to yield 25 g (85 %) of pure **17** as yellowish oil. FABMS: 553.3 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 7.36-7.25 (m, 5H, $H_{arom.}$); 4.74 (m, 4H, CHipr); 4.48 (s, 2H, OCH_2Ph); 3.69 (d, 4H, $J_{CH,P}=8.6$, OCH_2P); 3.62 (d, 4H, $J_{1,2}=6.0$, H-1); 3.53 (d, 2H, $J_{3,2}=6.0$, H-3); 2.26 (m, 1H, $J_{2,3}\sim J_{2,1}=6.0$, H-2); 1.32 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 138.37; 128.32 (2C); 127.52; 127.48 (2C); 73.15 (OCH_2Ph); 71.75 (d, $J_{C,P}=12.7$, C-1); 71.18 (m, CHipr); 66.79 (C-3); 66.04 (d, $J_{C,P}=168.0$, OCH_2P); 40.20 (C-2); 24.06 (m, CH_3).

Tetraisopropyl {[2-(hydroxymethyl)propane-1,3-diyl]bis(oxymethylene)}bisphosphonate (18)

Palladium on activated charcoal (10% Pd, 0.2 g) and conc. HCl (0.1 ml) were added to a solution of **17** (3.68 g, 6.66 mmol) in methanol (30 ml). The reaction mixture was hydrogenated at atmospheric pres-

sure and room temperature overnight. The catalyst was filtered off through a Celite pad, the filtrate was neutralized with Et_3N and evaporated. The crude product was purified by column chromatography on silica gel, using the chloroform – methanol gradient 0-6 %, to yield 2.95 g (96 %) of pure **18** as yellowish oil. FABMS: 463.4 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 4.74 (dh, 4H, $J_{CH,P}=7.6$, $J_{CH,CH_3}=6.2$, CHipr); 3.68 (d, 2H, $J_{3,2}=5.6$, H-3); 3.66 (d, 4H, $J_{CH,P}=8.6$, OCH_2P); 3.64 (m, 4H, H-1); 3.44 (bt, 1H, $J_{OH,3}=6.5$, OH); 2.13 (m, 1H, H-2); 1.34 and 1.33 (2 x d, 24H, $J_{CH_3,CH}=6.2$ and 6.2, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 72.02 (d, $J_{C,P}=9.8$, C-1); 71.11 (m, CHipr); 65.91 (d, $J_{C,P}=167.4$, OCH_2P); 61.29 (C-3); 41.73 (C-2); 24.02 (m, CH_3).

Tetraisopropyl {2-[(mesyloxy)methyl]propane-1,3-diyl}bis(oxymethylene)bisphosphonate (19)

A mixture of **18** (1.3 g, 2.8 mmol), and Et_3N (1.5 equiv.) in dry dichloromethane (30 ml) was stirred at 0 °C with a $CaCl_2$ protecting tube. Mesyl chloride (1.1 equiv.) was added. The mixture was stirred at 0 °C for 1 h and then kept overnight in refrigerator. The solution was diluted with ice water (300 ml) and the layers separated. The organic layer was dried with anhydrous $MgSO_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel, using the chloroform – methanol gradient 0-4 %, to yield 1.5 g (99 %) of pure **19** as yellowish oil. For $C_{19}H_{42}O_{11}P_2S$ (540.54) calcd: C, 42.22; H, 7.83; P, 11.46; S, 5.93. Found: C, 42.25; H, 7.87; P, 11.56; S, 6.05. FABMS: 541.3 (MH^+) (90). ^1H NMR (500 MHz, $CDCl_3$): 4.74 (dh, 4H, $J_{CH,P}=7.6$, $J_{CH,CH_3}=6.2$, CHipr); 4.32 (d, 2H, $J_{3,2}=5.6$, H-3); 3.71 (d, 4H, $J_{CH,P}=8.6$, OCH_2P); 3.64 (m, 4H, H-1); 3.04 (s, 3H, Ms-CH_3); 2.39 (h, 1H, $J_{2,3}\sim J_{2,1}=6.0$, H-2); 1.34 and 1.33 (2 x d, 24H, $J_{CH_3,CH}=6.2$ and 6.1, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 71.07 (d, $J_{C,P}=6.6$, CHipr); 70.35 (d, $J_{C,P}=11.8$, C-1); 67.59 (C-3); 66.14 (d, $J_{C,P}=168.5$, OCH_2P); 39.59 (C-2); 37.00 (Ms-CH_3); 24.04 (m, CH_3).

4.4.1 General procedure for alkylation of nucleobases

A mixture of an appropriate nucleobase (**20-22**, **31**, **33**, **35**; 1.0 equiv.) and Cs_2CO_3 (0.5 equiv.) in dry DMF was stirred at room temperature for 1 h under a $CaCl_2$ protecting tube. The reaction mixture was heated at 60 °C and bisphosphonate **19** (1.0 equiv.) was added. The mixture was then stirred at 100 °C for 24 h. The solvent was evaporated and the residue was co-evaporated with toluene. The residue dissolved in hot chloroform was filtered through a Celite pad, evaporated and purified on a silica gel column in chloroform – methanol

2-Amino-6-chloro-9-(2-{[bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)purine (20a)

Material: 2.8 mmol of **20**, 1.4 mmol Cs_2CO_3, 2.8 mmol of **19**, 50 ml of DMF. Column chromatography (silica gel, 1-4 % gradient of MeOH in $CHCl_3$) afforded the product **20a** as yellowish oil (yield 77 %). FABMS: 615.1 (MH^+) (90). ^1H NMR (500 MHz, $CDCl_3$): 7.90 (s, 1H, Pu-8); 5.46 (bs, 2H, NH_2); 4.78 (m, 4H, CHipr); 4.26 (d, 2H, $J_{3',2'}=7.5$, H-3'); 3.72 (m, 4H, OCH_2P); 3.50 (dd, 2H, $J_{gem}=9.1$ and $J_{1'a,2'}=6.0$, H-1'a); 3.48 (dd, 2H, $J_{gem}=9.0$ and $J_{1'b,2'}=5.6$, H-1'b); 2.52 (m, 1H, H-2'); 1.36, 1.35, 1.34 and 1.33 (4 x d, 4 x 6H, $J_{CH_3,CH}=6.2$, 6.2, 6.4 and 6.3, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.33

(Pu-2); 154.17 (Pu-4); 151.16 (Pu-6); 143.43 (Pu-8); 124.96 (Pu-5); 71.18 and 71.16 (2 x d, $J_{C,P}=6.6$ and 6.7, CHipr); 70.94 (d, $J_{C,P}=12.9$, C-1'); 66.11 (d, $J_{C,P}=169.3$, OCH_2P); 41.46 (C-3'); 39.84 (C-2'); 24.08 (m, CH_3).

2-Amino-6-chloro-7-(2-{[bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)purine (20b)
Material: 2.8 mmol of **20**, 1.4 mmol Cs_2CO_3, 2.8 mmol of **19**, 50 ml of DMF. Column chromatography (silica gel, 1-6 % gradient of MeOH in $CHCl_3$) afforded the product **20b** as yellowish oil (yield 10 %). FABMS: 615.1 (MH^+) (90). ^1H NMR (500 MHz, $CDCl_3$): 8.18 (s, 1H, Pu-8); 5.17 (bs, 2H, NH_2); 4.76 (m, 4H, CHipr); 4.46 (d, 2H, $J_{3',2'}=7.5$, H-3'); 3.69 (m, 4H, OCH_2P); 3.63 (dd, 2H, $J_{gem}=9.5$ and $J_{1'a,2'}=5.2$, H-1'a); 3.49 (dd, 2H, $J_{gem}=9.5$ and $J_{1'b,2'}=5.0$, H-1'b); 2.51 (m, 1H, H-2'); 1.36, 1.35, 1.34 and 1.33 (4 x d, 4 x 6H, $J_{CH_3,CH}=6.2$, 6.2, 6.4 and 6.3, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.38 (Pu-4); 159.33 (Pu-2); 150.03 (Pu-8); 143.29 (Pu-6); 116.17 (Pu-5); 71.15 (m, CHipr); 70.81 (d, $J_{C,P}=11.6$, C-1'); 66.11 (d, $J_{C,P}=168.8$, OCH_2P); 45.06 (C-3'); 41.06 (C-2'); 24.05 (m, CH_3).

9-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)adenine (21a)
Material: 0.74 mmol of **21**, 0.37 mmol Cs_2CO_3, 0.74 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1-8 % gradient of MeOH in $CHCl_3$) afforded the product **21a** as yellowish oil (yield 76 %). FABMS: 580.5 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 8.33 (s, 1H, Pu-8); 8.00 (s, 1H, Pu-2); 5.82 (bs, 2H, NH_2); 4.76 (m, 4H, CHipr); 4.32 (d, 2H, $J_{3',2'}=6.7$, H-3'); 3.71 (m, 4H, OCH_2P); 3.58 (dd, 2H, $J_{gem}=9.6$ and $J_{1'a,2'}=5.8$, H-1'a); 3.52 (dd, 2H, $J_{gem}=9.6$ and $J_{1'b,2'}=5.6$, H-1'b); 2.60 (m, 1H, H-2'); 1.34 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.37 (Pu-6); 152.91 (Pu-2); 150.33 (Pu-4); 141.87 (Pu-8); 119.53 (Pu-5); 71.10 (m, CHipr and C-1'); 66.08 (d, $J_{C,P}=168.5$, OCH_2P); 41.95 (C-3'); 39.90 (C-2'); 24.07 (m, CH_3).

6-(Cyclopropyl)amino-9-(2-{[bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)purine (22a)
Material: 0.74 mmol of **22**, 0.37 mmol Cs_2CO_3, 0.74 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1-5 % gradient of MeOH in $CHCl_3$) afforded the product **22a** as yellowish oil (yield 60 %). FABMS: 620.6 (MH^+) (70). ^1H NMR (500 MHz, $CDCl_3$): 8.45 (s, 1H, Pu-2); 7.91 (s, 1H, Pu-8); 5.99 (bs, 1H, NH); 4.76 (m, 4H, CHipr); 4.31 (d, 2H, $J_{3',2'}=6.7$, H-3'); 3.70 (d, 4H, $J_{CH,P}=8.6$, OCH_2P); 3.57 (dd, 2H, $J_{gem}=9.5$ and $J_{1'a,2'}=5.8$, H-1'a); 3.51 (dd, 2H, $J_{gem}=9.5$ and $J_{1'b,2'}=5.7$, H-1'b); 3.04 (m, 1H, $CH_{cycl.}$); 2.59 (m, 1H, H-2'); 1.35 (d, 12H, $J_{CH_3,CH}=6.1$, CH_3); 1.33 (d, 12H, $J_{CH_3,CH}=6.1$, CH_3); 0.93 (m, 2H, $CH_{2cycl.}$); 0.67 (m, 2H, $CH_{2cycl.}$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.72 (Pu-6); 153.16 (Pu-2); 149.22 (Pu-4); 141.21 (Pu-8); 119.81 (Pu-5); 71.10 (m, C-1' and CHipr); 66.07 (d, $J_{C,P}=168.4$, OCH_2P); 41.87 (C-3'); 39.94 (C-2'); 24.07 (m, CH_3).

1-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)cytosine (31a)
Material: 0.87 mmol of **31**, 0.43 mmol Cs_2CO_3, 0.87 mmol of **19**, 40 ml of DMF. Column chromatography (silica gel, 1-15 % gradient of MeOH in $CHCl_3$) afforded the product **31a** as yellowish oil (yield 49 %). FABMS: 556.4 (MH^+) (45). ^1H NMR (500 MHz, $CDCl_3$): 7.51 (d, 1H, $J_{6,5}=7.2$, Py-6); 5.75 (d, 1H, $J_{5,6}=7.2$, Py-5); 4.74 (m, 4H, CHipr); 3.81 (d, 2H, $J_{3',2'}=6.8$, H-3'); 3.68 (m, 4H, OCH_2P); 3.60 (dd, 2H, $J_{gem}=9.6$ and $J_{1'a,2'}=5.3$, H-1'a); 3.54 (dd, 2H, $J_{gem}=9.6$ and $J_{1'b,2'}=5.8$, H-1'b); 2.52 (m, 1H, H-2'); 1.33 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 165.87 (Py-4); 155.75 (Py-2); 147.26 (Py-6); 93.78 (Py-5); 71.32 (d, $J_{1',P}=12.9$, C-1'); 71.06 (m, CHipr); 65.93 (d, $J_{C,P}=169.0$, OCH_2P); 48.60 (C-3');

38.63 (C-2'); 24.07 (m, CH_3).

2-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyloxy)cytosine (31b)
Material: 0.87 mmol of **31**, 0.43 mmol Cs_2CO_3, 0.87 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1-7 % gradient of MeOH in $CHCl_3$) afforded the product **31b** as yellowish oil (yield 15 %). FABMS: 556.5 (MH^+) (45). ^1H NMR (500 MHz, $CDCl_3$): 7.96 (d, 1H, $J_{6,5}=5.5$, Py-6); 6.11 (d, 1H, $J_{5,6}=5.7$, Py-5); 5.64 (bs, 1H, NH_2); 4.74 (m, 4H, CHipr); 4.34 (d, 2H, $J_{3',2'}=6.8$, H-3'); 3.76-3.68 (m, 8H, H-1' and OCH_2P); 2.44 (m, 1H, H-2'); 1.32 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.83 and 164.78 (Py-4 and Py-2); 156.90 (Py-6); 99.19 (Py-5); 71.13 (d, $J_{1',P}=12.6$, C-1'); 70.80 (d, $J_{CH,P}=6.6$, CHipr); 65.79 (d, $J_{C,P}=168.2$, OCH_2P); 64.19 (C-3'); 39.18 (C-2'); 23.82 (d, $J_{C,P}=3.7$, CH_3); 23.72 (d, $J_{C,P}=4.5$, CH_3).

1-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)uracil (33a)
Material: 0.87 mmol of **33**, 0.43 mmol Cs_2CO_3, 0.87 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1-5 % gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **33a** and **33b**. Compound 33a was separated from 33b on thin layer chromatography in 10% MeOH/$CHCl_3$ as yellowish oil (54 %). FABMS: 557.5 (MH^+) (90). ^1H NMR (500 MHz, $CDCl_3$): 8.72 (bs, 1H, NH); 7.51 (d, 1H, $J_{6,5}=7.9$, Py-6); 5.68 (dd, 1H, $J_{5,6}=7.9$, $J_{5,3'}=2.3$, Py-5); 4.75 (m, 4H, CHipr); 3.82 (d, 2H, $J_{3',2'}=6.7$, H-3'); 3.69 (m, 4H, OCH_2P); 3.61 (dd, 2H, $J_{gem}=9.6$ and $J_{1'a,2'}=5.6$, H-1'a); 3.57 (dd, 2H, $J_{gem}=9.6$ and $J_{1'b,2'}=5.5$, H-1'b); 2.42 (m, 1H, H-2'); 1.34 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 163.55 (Py-4); 150.88 (Py-2); 146.17 (Py-6); 101.68 (Py-5); 71.26 (d, $J_{1',P}=12.2$, C-1'); 71.10 (m, CHipr); 66.04 (d, $J_{C,P}=169.1$, OCH_2P); 47.62 (C-3'); 38.98 (C-2'); 24.06 (m, CH_3).

1,3-{Bis(2,2'-{[bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)}uracil (33b)
Material: 0.87 mmol of **33**, 0.43 mmol Cs_2CO_3, 0.87 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1-5 % gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **33a** and **33b**. Compound 33b was separated from 33a on thin layer chromatography in 10% MeOH/$CHCl_3$ as yellowish oil (15 %). FABMS: 1001.9 (MH^+) (85). ^1H NMR (500 MHz, $CDCl_3$): 7.45 (d, 1H, $J_{6,5}=7.9$, Py-6); 5.68 (d, 1H, $J_{5,6}=7.9$, Py-5); 4.74 (m, 8H, CHipr); 4.00 (d, 2H, $J_{3'',2''}=6.9$, H-3"); 3.80 (d, 2H, $J_{3',2'}=6.8$, H-3'); 3.75-3.53 (m, 16H, H-1', H-1", OCH_2P, OCH_2P'); 2.39 (m, 2H, H-2' and H-2"); 1.35-1.31 (m, 48H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 163.32 (Py-4); 151.72 (Py-2); 144.06 (Py-6); 100.99 (Py-5); 72.38 (d, $J_{1'',P}=12.8$, C-1"); 71.29-70.85 (m, C-1' and CHipr); 66.08 and 66.04 (d, $J_{C,P}=167.8$ and 168.9, OCH_2P and OCH_2P'); 48.58 (C-3'); 40.77 (C-3"); 38.85 (C-2'); 38.51 (C-2"); 24.15-24.00 (m, CH_3).

1-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)-4-methoxythymine (35a)
Material: 1.85 mmol of **35**, 0.92 mmol Cs_2CO_3, 1.85 mmol of **19**, 25 ml of DMF. Column chromatography (silica gel, 1-3 % gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **35a** and **35b**. Compound 35a was separated from 35b on thin layer chromatography in 5% MeOH/$CHCl_3$ as yellowish oil (45 %). FABMS: 585.5 (MH^+) (70). ^1H NMR (500 MHz, $CDCl_3$): 7.51 (q, 1H, $J_{6,CH_3}=1.1$, Py-6); 4.74 (m, 4H, CHipr); 3.97 (s, 3H, OCH_3); 3.87 (d, $J_{3',2'}=6.9$, H-3'); 3.68 (m, 4H, OCH_2P); 3.60 (dd, 2H, $J_{gem}=9.5$ and $J_{1'a,2'}=5.2$, H-1'a); 3.52 (dd, 2H, $J_{gem}=9.5$ and $J_{1'b,2'}=6.0$, H-1'b); 2.53 (m, 1H, H-2'); 1.96 (d, 3H, $J_{CH_3,6}=1.1$, Py-CH_3); 1.35-1.31 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 170.79 (Py-4); 156.88 (Py-2); 145.64 (Py-6); 104.29 (Py-5); 71.24-70.96 (m, C-1'and CHipr); 65.97 (d, $J_{C,P}=169.1$, OCH_2P); 54.44 (OCH_3); 48.43 (C-3'); 38.61 (C-2'); 24.07 (m, CH_3); 11.80 (Py-CH_3).

2-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyloxy)4-methoxythymine (35b)
Material: 1.85 mmol of **35**, 0.92 mmol Cs_2CO_3, 1.85 mmol of **19**, 20 ml of DMF. Column chromatography (silica gel, 1-3 % gradient of MeOH in $CHCl_3$) afforded the product as a mixture of **35a** and **35b**. Compound 35b was separated from 35a on thin layer chromatography in 5% MeOH/$CHCl_3$ as yellowish oil (38 %). FABMS: 585.6 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 7.95 (s, 1H, Py-6); 4.73 (m, 4H, CHipr); 4.37 (d, 2H, $J_{3',2'} = 6.2$, H-3'); 3.98 (s, 3H, OCH_3); 3.74-3.70 (m, 8H, H-1' and OCH_2P); 2.49 (m, 1H, H-2'); 2.05 (d, 3H, $J_{CH_3,6} = 0.7$, Py-CH_3); 1.31 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 169.61 (Py-4); 163.60 (Py-2); 157.00 (Py-6); 111.10 (Py-5); 71.40 (d, $J_{1',P} = 12.7$, C-1'); 70.97 (d, $J_{CH,P} = 6.3$, CHipr); 66.10 (d, $J_{C,P} = 168.1$, OCH_2P); 65.14 (C-3'); 53.86 (OCH_3); 39.42 (C-2'); 24.04 (m, CH_3); 11.84 (Py-CH_3).

9-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)guanine (23)
A solution of **20a** (370 mg, 0.6 mmol) in 80% acetic acid (20 ml) was refluxed for 6 h. The solution was neutralized with Et_3N and the volatile were evaporated in vacuo. Column chromatography (silica gel, 1-15 % gradient of MeOH in $CHCl_3$) afforded compound **23** as a white amorphous powder (yield 63%). FABMS: 596.3 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 11.43 (bs, 1H, NH); 7.58 (s, 1H, Pu-8); 7.02 (bs, 2H, NH_2); 4.80 (m, 4H, CHipr); 4.09 (d, 2H, $J_{3',2'} = 7.6$, H-3'); 3.69 (m, 4H, OCH_2P); 3.56 (dd, 2H, $J_{gem} = 9.1$ and $J_{1'a,2'} = 6.0$, H-1'a); 3.47 (dd, 2H, $J_{gem} = 9.0$ and $J_{1'b,2'} = 4.4$, H-1'b); 2.49 (m, 1H, H-2'); 1.35 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 157.89 (Pu-6); 154.46 (Pu-2); 150.82 (Pu-4); 138.23 (Pu-8); 117.60 (Pu-5); 71.61 (m, CHipr and C-1'); 66.10 (d, $J_{C,P} = 170.7$, OCH_2P); 42.01 (C-3'); 39.42 (C-2'); 24.05 (m, CH_3).

2,6-Diamino-9-(2-{[bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)purine (24)
A solution of **20a** (570 mg, 0.93 mmol) in methanolic ammonia (50 ml) was heated (100 °C) in autoclave for 13 h. The solvent was then evaporated. Purification of the residue by column chromatography (silica gel, 1-12 % gradient of MeOH in $CHCl_3$) afforded compound **24** as yellowish foam (yield 65 %). FABMS: 595.3 (MH^+) (70). ^{1}H NMR (500 MHz, $CDCl_3$): 7.63 (s, 1H, Pu-8); 5.52 (bs, 2H, 6-NH_2); 4.91 (bs, 2H, 2-NH_2); 4.77 (m, 4H, CHipr); 4.16 (d, 2H, $J_{3',2'} = 6.5$, H-3'); 3.76 and 3.70 (2 x dd, 4H, $J_{gem} = 13.5$ and 13.5, $J_{CH,P} = 8.9$ and 8.8, OCH_2P); 3.54 (dd, 2H, $J_{gem} = 9.5$ and $J_{1'a,2'} = 6.1$, H-1'a); 3.49 (dd, 2H, $J_{gem} = 9.6$ and $J_{1'b,2'} = 5.4$, H-1'b); 2.53 (m, 1H, H-2'); 1.35 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.90 (Pu-2); 155.73 (Pu-6); 152.41 (Pu-4); 139.16 (Pu-8); 114.20 (Pu-5); 71.07 (m, CHipr and C-1'); 66.03 (d, $J_{C,P} = 168.8$, OCH_2P); 41.16 (C-3'); 39.77 (C-2'); 24.08 (m, CH_3).

2-Amino-6-(cyclopropyl)amino-9-(2-{[bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)purine (25)
A solution of **20a** (430 mg, 0.7 mmol) and cyclopropylamine (9.0 equiv.) in dioxane (20 ml) was refluxed for 2 days. The solvent and excess of amine were evaporated to dryness. Purification of compound by column chromatography (silica gel, 1-6 % gradient of MeOH in $CHCl_3$) afforded the product **25** as yellowish oil (yield 84 %). FABMS: 635.6 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 7.57 (s, 1H, Pu-8); 5.81 (bs, 1H, $NH_{cycl.}$); 4.93 (bs, 2H, NH_2); 4.77 (m, 4H, CHipr); 4.15 (d, 2H, $J_{3',2'} = 6.6$, H-3'); 3.75 and 3.70 (2 x dd, 4H, $J_{gem} = 13.5$ and 13.5, $J_{CH,P} = 9.0$ and 8.8, OCH_2P); 3.53 (dd, 2H, $J_{gem} = 9.6$ and $J_{1'a,2'} = 6.1$, H-1'a); 3.48 (dd, 2H, $J_{gem} = 9.6$ and $J_{1'b,2'} = 5.4$, H-1'b); 2.99 (bs, 1H, $CH_{cycl.}$); 2.52 (m, 1H, H-2'); 1.34 (m, 24H, CH_3); 0.85 (m, 2H, $CH_{2cycl.}$); 0.61 (m, 2H, $CH_{2cycl.}$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 160.07 (Pu-2); 156.17 (Pu-6); 151.36 (Pu-4); 138.38 (Pu-8); 114.38 (Pu-5); 71.07 (m, CHipr and C-1'); 66.01 (d,

$J_{C,P}$ = 168.9, OCH_2P); 41.08 (C-3'); 39.80 (C-2'); 24.07 (m, CH_3); 23.60 ($CH_{cycl.}$); 7.37 ($CH_{2cycl.}$).

1-(2-{[Bis(diisopropoxyphosphoryl)methoxy]methyl}ethyl)thymine (36)
A solution of **35a** (480 mg, 0.8 mmol) in 80% acetic acid (15 ml) was refluxed for 24 h. The solution was neutralized with Et_3N. Solvent and excess of acetic acid were evaporated. The purification of the residue by column chromatography (silica gel, 1-3 % gradient of MeOH in $CHCl_3$) afforded the product **36** as yellowish oil (yield 78 %). FABMS: 571.2 (MH^+) (60). ^1H NMR (500 MHz, $CDCl_3$): 8.59 (s, 1H, NH); 7.33 (d, 1H, J_{6,CH_3} = 1.2, Py-6); 4.75 (m, 4H, CHipr); 3.79 (d, 2H, $J_{3',2'}$ = 6.8, H-3'); 3.69 (m, 4H, OCH_2P); 3.60 (dd, 2H, J_{gem} = 9.5 and $J_{1'a,2'}$ = 5.5, H-1'a); 3.51 (dd, 2H, J_{gem} = 9.5 and $J_{1'b,2'}$ = 5.6, H-1'b); 1.95 (d, 3H, $J_{CH_3,6}$ = 1.1, Py-CH_3); 1.34 (m, 24H, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.16 (Py-4); 150.91 (Py-2); 141.89 (Py-6); 110.32 (Py-5); 71.23 (d, $J_{1',P}$ = 12.7, C-1'); 71.07 (d, J_{C-O-P} = 7.0, CHipr); 71.03 (d, J_{C-O-P} = 6.9, CHipr); 66.01 (d, $J_{C,P}$ = 169.2, OCH_2P); 47.19 (C-3'); 39.07 (C-2'); 24.05 (m, CH_3); 12.06 (Py-CH_3).

4.4.2 General procedure for preparation of free phosphonic acids

Dried esters (**21a**, **22a**, **23**, **24**, **25**, **31a**, **33a** and **36**), acetonitrile and $BrSiMe_3$ (excess) were stirred at room temperature overnight. After evaporation and co-distillation with acetonitrile, the residue was treated with water and aqueous ammonia. The mixture was evaporated to dryness, and the residue dissolved in water was applied onto a column of Dowex 50 X 8 (H^+ form).

1. Elution with water and evaporation in vacuo afforded free bisphosphonic acids **26-28**, **32**, **34**, and **36**.

2. Elution with dilute ammonia and evaporation afforded ammonium salts, which were applied onto Dowex 50 (Na^+ form). Elution with water and evaporation gave bisphosphonic acids **29** and **30** as tetrasodium salts.

9-(2-{[Bis(phosphono)methoxy]methyl}ethyl)adenine (26)
Material: 0.50 mmol of **21a**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 80 %), mp 248.9 °C (D). For $C_{11}H_{19}N_5O_8P_2$ (411.24) calcd: C, 32.13; H, 4.66; N, 17.03; P, 15.06. Found: C, 32.07; H, 4.76; N, 17.15; P, 15.13. FABMS: 412.1 (MH^+) (45). ^1H NMR (500 MHz, D_2O + NaOD): 8.22 and 8.21 (s, 2H, Pu-8 and Pu-2); 4.37 (d, 2H, $J_{3',2'}$ = 6.7, H-3'); 3.58 (m, 8H, H-1' and OCH_2P); 2.56 (m, 1H, H-2'). ^{13}C NMR (125.7 MHz, D_2O + NaOD): 156.10 (Pu-6); 152.93 (Pu-2); 149.79 (Pu-4); 144.04 (Pu-8); 119.03 (Pu-5); 71.50 (d, $J_{1',P}$ = 11.6, C-1'); 67.95 (d, $J_{C,P}$ = 156.2, OCH_2P); 43.15 (C-3'); 39.85 (C-2'). UV spectrum: (0.01 M HCl) λ_{max} = 258 nm (ϵ_{max} = 13768); (H_2O) λ_{max} = 259 nm (ϵ_{max} = 14352); (0.01 M NaOH) λ_{max} = 259 nm (ϵ_{max} = 13402).

6-(Cyclopropyl)amino-9-(2-{[bis(phosphono)methoxy]methyl}ethyl)purine (27)
Material: 0.39 mmol of **22a**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 83 %), mp 205.0 °C

(D). For $C_{14}H_{23}N_5O_8P_2$ (451.31) calcd: C, 37.26; H, 5.14; N, 15.52; P, 13.73. Found: C, 37.37; H, 5.25; N, 15.68; P, 13.82. FABMS: 452.3 (MH^+) (60). ^1H NMR (500 MHz, D_2O): 8.49 (s, 1H, Pu-2); 8.38 (s, 1H, Pu-8); 4.49 (d, 2H, $J_{3',2'} = 6.7$, H-3'); 3.69-3.60 (m, 8H, H-1' and OCH_2P); 2.91 (bs, 1H, $CH_{cycl.}$); 2.62 (m, 1H, H-2'); 1.11 (m, 2H, $CH_{2cycl.}$); 0.90 (m, 2H, $CH_{2cycl.}$). ^{13}C NMR (125.7 MHz, D_2O): 150.61 (Pu-6); 148.35 (Pu-4); 146.08 (Pu-8); 144.71 (Pu-2); 119.08 (Pu-5); 71.72 (d, $J_{1',P} = 11.8$, C-1'); 67.24 (d, $J_{C,P} = 158.2$, OCH_2P); 44.04 (C-3'); 39.89 (C-2'); 23.42 ($CH_{cycl.}$); 7.33 ($CH_{2cycl.}$). UV spectrum: (0.01 M HCl) $\lambda_{max} = 266$ nm ($\epsilon_{max} = 18646$); (H_2O) $\lambda_{max} = 266$ nm ($\epsilon_{max} = 18340$); (0.01 M NaOH) $\lambda_{max} = 268$ nm ($\epsilon_{max} = 17518$).

9-(2-{[Bis(phosphono)methoxy]methyl}ethyl)guanine (28)

Material: 0.37 mmol of **23**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 67 %), mp 206.9 °C (D). For $C_{11}H_{19}N_5O_9P_2$ (427.24) calcd: C, 30.92; H, 4.48; N, 16.39; P, 14.50. Found: C, 30.85; H, 4.58; N, 16.50; P, 14.56. FABMS: 428.3 (MH^+) (60). ^1H NMR (500 MHz, D_2O): 8.98 (s, 1H, Pu-8); 4.44 (d, 2H, $J_{3',2'} = 6.5$, H-3'); 3.72-3.61 (m, 8H, H-1' and OCH_2P); 2.64 (m, 1H, H-2'). ^{13}C NMR (125.7 MHz, D_2O): 156.02 and 155.97 (Pu-6 and Pu-2); 150.92 (Pu-4); 138.95 (Pu-8); 108.45 (Pu-5); 71.73 (d, $J_{1',P} = 12.4$, C-1'); 67.28 (d, $J_{C,P} = 158.1$, OCH_2P); 45.17 (C-3'); 39.12 (C-2'). UV spectrum: (0.01 M HCl) $\lambda_{max} = 252$ nm ($\epsilon_{max} = 10558$); (H_2O) $\lambda_{max} = 251$ nm ($\epsilon_{max} = 10890$); (0.01 M NaOH) $\lambda_{max} = 266$ nm ($\epsilon_{max} = 9320$).

Sodium 2,6-diamino-9-(2-{[bis(phosphono)methoxy]methyl}ethyl)purine (29)

Material: 0.60 mmol of **24**, 3.5 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 88 %), mp 327.1 °C (D). FABMS: 515.0 (MH^+) (30). HR MS for $C_{11}H_{16}N_6Na_4O_8P_2$: calc. 515.01546, found 515.01739. ^1H NMR (500 MHz, D_2O): 7.91 (s, 1H, Pu-8); 4.23 (d, 2H, $J_{3',2'} = 6.8$, H-3'); 3.95 (m, 8H, H-1' and OCH_2P); 2.51 (m, 1H, H-2'). ^{13}C NMR (125.7 MHz, D_2O): 160.35 (Pu-6); 156.61 (Pu-2); 151.79 (Pu-4); 141.86 (Pu-8); 113.49 (Pu-5); 71.51 (d, $J_{1',P} = 11.8$, C-1'); 68.56 (d, $J_{C,P} = 154.5$, OCH_2P); 42.62 (C-3'); 39.68 (C-2'). UV spectrum: (0.01 M HCl) $\lambda_{max} = 284$ nm ($\epsilon_{max} = 9092$); (H_2O) $\lambda_{max} = 278$ nm ($\epsilon_{max} = 10120$); (0.01 M NaOH) $\lambda_{max} = 278$ nm ($\epsilon_{max} = 9442$).

Sodium 2-amino-6-(cyclopropyl)amino-9-(2-{[bis(phosphono)methoxy]methyl}ethyl)purine (30)

Material: 0.54 mmol of **25**, 3.5 ml of TMSBr, 15 ml of CH_3CN. Yellow solid (yield 73 %), mp 338.8 °C (D). FABMS: 555.0 (MH^+) (30). HR MS for $C_{14}H_{20}N_6Na_4O_8P_2$: calc. 555.0460, found 555.0487. Mp = 338.8 °C (D). ^1H NMR (500 MHz, D_2O): 7.89 (s, 1H, Pu-8); 4.24 (d, 2H, $J_{3',2'} = 6.7$, H-3'); 3.54 (m, 8H, H-1' and OCH_2P); 2.86 (bs, 1H, $CH_{cycl.}$); 2.52 (m, 1H, H-2'); 0.88 (m, 2H, $CH_{2cycl.}$); 0.67 (m, 2H, $CH_{2cycl.}$). ^{13}C NMR (125.7 MHz, D_2O): 160.59 (Pu-2); 156.99 (Pu-6); 150.75 (Pu-4); 141.39 (Pu-8); 113.85 (Pu-5); 71.52 (d, $J_{1',P} = 9.6$, C-1'); 68.83 (d, $J_{C,P} = 153.7$, OCH_2P); 42.58 (C-3'); 39.69 (C-2'); 23.88 ($CH_{cycl.}$); 7.37 ($CH_{2cycl.}$). UV spectrum: (0.01 M HCl) $\lambda_{max} = 282$ nm ($\epsilon_{max} = 13712$); (H_2O) $\lambda_{max} = 282$ nm ($\epsilon_{max} = 13736$); (0.01 M NaOH) $\lambda_{max} = 290$ nm ($\epsilon_{max} = 12008$).

1-(2-{[Bis(phosphono)methoxy]methyl}ethyl)cytosine (32)

Material: 0.36 mmol of **31a**, 3.0 ml of TMSBr, 15 ml of CH_3CN. White solid (yield 57 %), mp 208.5 °C (D). For $C_{10}H_{19}N_3O_9P_2$ (387.22) calcd: C, 31.02; H, 4.95; N, 10.85; P, 16.00. Found: C, 31.05; H, 4.85; N, 10.75; P, 16.15. FABMS: 388.3 (MH^+) (30). ^1H NMR (500 MHz, D_2O): 7.93 (d, 1H, $J_{6,5} = 7.7$, Py-6); 6.17 (d, 1H, $J_{5,6} = 7.7$, Py-5); 4.00 (d, 2H, $J_{3',2'} = 6.9$, H-3'); 3.65 (m, 8H, H-1' and OCH_2P); 2.47 (m, 1H, H-2'). ^{13}C NMR (125.7 MHz, D_2O): 160.19 (Py-4); 151.21 (Py-6); 149.99 (Py-2); 94.92 (Py-5); 71.91 (d,

$J_{1',P} = 12.1$, C-1'); 67.27 (d, $J_{C,P} = 158.1$, OCH_2P); 50.03 (C-3'); 38.50 (C-2'). UV spectrum: (0.01 M HCl) $\lambda_{max} = 281$ nm ($\epsilon_{max} = 11478$); (H_2O) $\lambda_{max} = 279$ nm ($\epsilon_{max} = 10066$); (0.01 M NaOH) $\lambda_{max} = 272$ nm ($\epsilon_{max} = 7846$).

1-(2-{[Bis(phosphono)methoxy]methyl}ethyl)uracil (34)

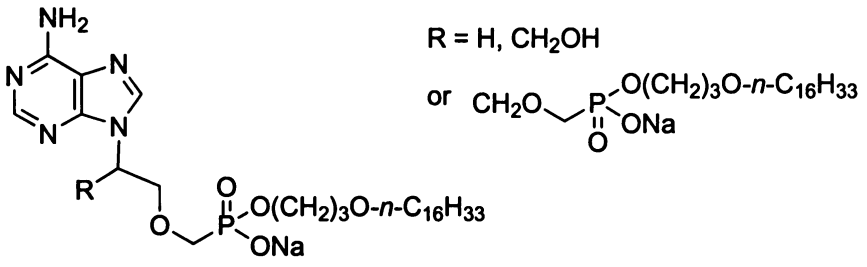
Material: 0.50 mmol of **33a**, 3.5 ml of TMSBr, 15 ml of CH_3CN. Hygroscopic yellow solid (yield 63 %). For $C_{10}H_{18}N_2O_{10}P_2 \cdot \frac{1}{2} H_2O$ (397.20) calcd: C, 30.24; H, 4.82; N, 7.05; P, 15.60. Found: C, 30.37; H, 4.95; N, 7.19; P, 15.73. FABMS: 389.3 (MH^+) (90). ^1H NMR (500 MHz, D_2O): 7.67 (d, 1H, $J_{6,5} = 7.9$, Py-6); 5.83 (d, 1H, $J_{5,6} = 7.8$, Py-5); 3.91 (d, 2H, $J_{3',2'} = 7.1$, H-3'); 3.75 (d, 4H, $J_{CH,P} = 8.7$, OCH_2P); 3.67 (d, 4H, $J_{1',2'} = 5.6$, H-1'); 2.45 (m, 1H, H-2'). ^{13}C NMR (125.7 MHz, D_2O): 167.59 (Py-4); 153.11 (Py-2); 148.68 (Py-6); 102.00 (Py-5); 71.89 (d, $J_{1',P} = 11.9$, C-1'); 66.67 (d, $J_{C,P} = 159.5$, OCH_2P); 48.82 (C-3'); 38.70 (C-2'). UV spectrum: (0.01 M HCl) $\lambda_{max} = 264$ nm ($\epsilon_{max} = 6682$); (H_2O) $\lambda_{max} = 265$ nm ($\epsilon_{max} = 8416$); (0.01 M NaOH) $\lambda_{max} = 264$ nm ($\epsilon_{max} = 8516$).

1-(2-{[Bis(phosphono)methoxy]methyl}ethyl)thymine (37)

Material: 0.66 mmol of **36**, 3.5 ml of TMSBr, 15 ml of CH_3CN. Hygroscopic white solid (yield 93 %). For $C_{11}H_{20}N_2O_{10}P_2 \cdot \frac{1}{2}$ (411.23) calcd: C, 32.13; H, 5.15; N, 6.81; P, 15.06. Found: C, 32.27; H, 5.27; N, 6.89; P, 15.22. FABMS: 403.3 (MH^+) (45). ^1H NMR (500 MHz, D_2O): 7.52 (q, 1H, $J_{6,CH_3} = 1.2$, Py-6); 3.87 (d, 2H, $J_{3',2'} = 7.2$, H-3'); 3.75 (d, 4H, $J_{CH,P} = 8.7$, OCH_2P); 3.66 (d, 4H, $J_{1',2'} = 5.6$, H-1'); 2.44 (m, 1H, H-2'); 1.89 (d, 3H, $J_{CH_3,6} = 1.2$, CH_3). ^{13}C NMR (125.7 MHz, D_2O): 167.82 (Py-4); 153.18 (Py-2); 144.46 (Py-6); 111.31 (Py-5); 71.91 (d, $J_{1',P} = 12.1$, C-1'); 66.63 (d, $J_{C,P} = 159.7$, OCH_2P); 48.55 (C-3'); 38.74 (C-2'); 12.00 (CH_3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 270$ nm ($\epsilon_{max} = 8660$); (H_2O) $\lambda_{max} = 270$ nm ($\epsilon_{max} = 8846$); (0.01 M NaOH) $\lambda_{max} = 269$ nm ($\epsilon_{max} = 6760$).

Chapter 5

Synthesis of lipophilic ANbPs and ANPs based on glycerol and PME



5.1 Introduction

Considerable effort in medicinal chemistry is directed towards the search for compounds with antiviral activity. We are continually investigating and developing acyclic nucleoside phosphonates (ANPs). These compounds deserve special attention owing to their significant biological activity.[23,86] However, the oral delivery of many acyclic nucleoside phosphonates, *e.g.*, PMEA or (*R*)-PMPA (Figure 5.1), is hindered by their poor resorption in the intestine caused by the negative charges in the ANP structure.[24,87–89] The introduction of a suitable masking moiety to the polar phosphonate group, thus decreasing the polarity of the molecule, and can solve this problem. For instance, the application of the dipivoxil group to adefovir (PMEA) or disoproxil group to tenofovir ((*R*)-PMPA) can serve as good examples (Figure 5.1).[25,89–92] Several other phosphonate-masking groups

have been applied to acyclic nucleoside phosphonates, *e.g.*, phosphonamidates, cycloSal esters, and lipophilic alkyl esters.[93–95] The latter, having 14 to 20 carbons in the moiety are most frequently selected.[95,96] They provide bioavailable prodrugs of ANPs with potency comparable to that of the corresponding non-derivatized drugs.[97–99]

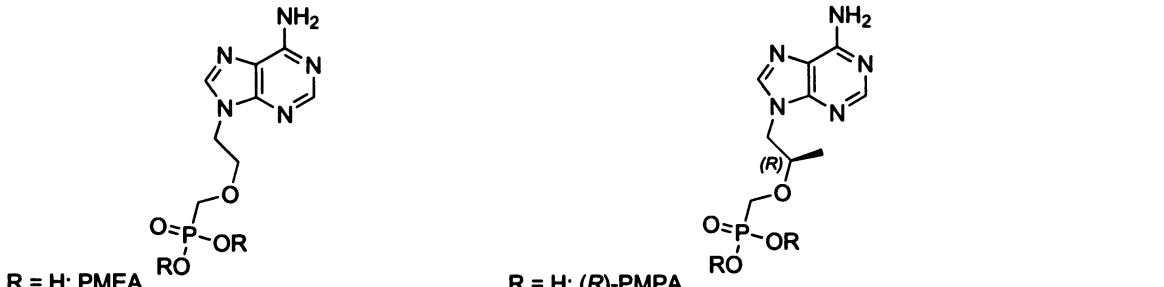


Figure 5.1: Adefovir and tenofovir

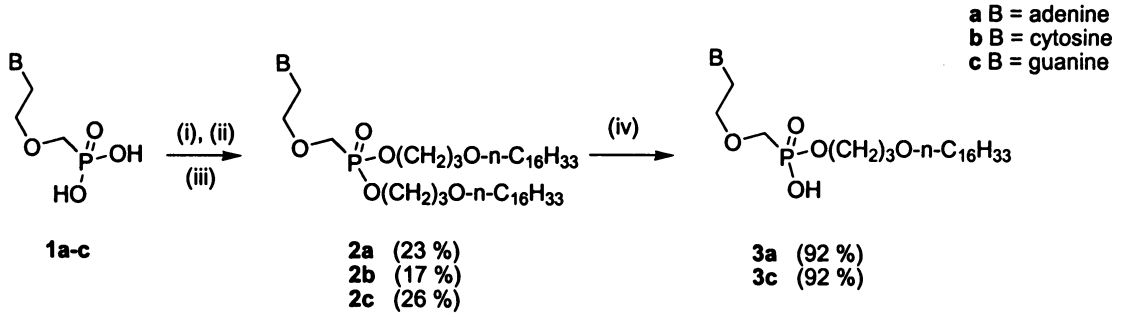
5.2 Results and discussion

The previous project describes the synthesis of a large variety of symmetrical 1,3-bis(phosphonoethoxy)propan-2-yl] derivatives of purines and pyrimidines. The present project discusses the synthetic pathways leading to decreasing the polarity of such prepared bisphosphonic acid derivatives. The hindrance to oral delivery of ANPs is due to the two negative charges. The permeability through the cell membrane, which is decisive for biological activity of bisphosphonates possessing four negative charges in the molecule, must be expected to be even lower. Therefore, it has been examined the effect of masking the 1,3-bisphosphonates. For this purpose it was used the hexadecyloxypropyl group described in the literature.[95–98,100] In principle, it is possible to prepare such lipophilic phosphonates by activating either the corresponding free phosphonic acids or building appropriate lipophilic phosphonate chain by the stepwise procedure.

5.2.1 Preparation of lipophilic phosphonate derivatives by free phosphonic acid activation

The conversion of free PME acids (PMEA, PMEC, PMEG) to bis[3-{(hexadecyloxy)propyl}-ethoxy]methylphosphonate by *N,N*-carbonyldiimidazole, *N,N*-dimethylformamide dineopentyl

or 1,3-propylene acetal was not successful. The free acids of the PME-structure derived from adenine, cytosine or guanine (**1a-c**), as depicted in Figure 5.2, were first converted by treatment with oxalyl chloride and DMF into the respective chlorides whose formation was monitored by HPLC. Lipophilic derivatives of PME chain **2a-c** were prepared by the subsequent reaction with 3-(hexadecyloxy)propanol in the presence of pyridine and triethylamine at 0 °C under argon atmosphere. In the reaction, the NH_2 groups of PMEA and PMEC are simultaneously transformed into the corresponding amidine (dimethylaminomethylene) derivatives. They are easily released under acidic (PMEC or PMEG) or basic conditions (PMEA). Finally, one of the lipophilic chains was removed exclusively, using the earlier described reaction – by treatment with an excess of LiN_3 in DMF at 100 °C to give the monoesters **3a,c** as the sole products.[101] This approach can be applied in those cases where the starting compound is easily available. However, a different synthetic strategy has to be used in sensitive systems or compounds that are accessible by multistep syntheses only.



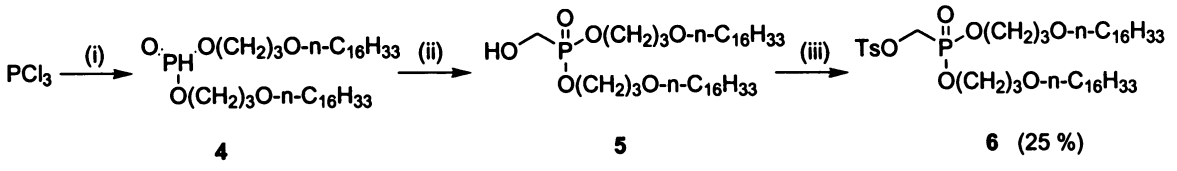
(i) Oxalyl chloride, CH_2Cl_2, DMF, reflux; (ii) 3-(hexadecyloxy)propanol, pyridine, Et_3N, CH_2Cl_2, 0 °C; (iii) methanolic ammonia, RT or 80% CH_3COOH, reflux; (iv) LiN_3, DMF, 100 °C

Figure 5.2: Preparation of lipophilic phosphonate derivatives by the free phosphonic acid activation

5.2.2 Stepwise construction of the lipophilic phosphonate

For the synthesis of the lipophilic 1,3-bis(phosphorylethoxy)propan-2-yl derivative was applied the stepwise building of propan-1,3-diol-2-yl derivative on the adenine moiety followed by etherification with the lipophilic phosphorylmethyl derivative with a good leaving group (*e.g.*, tosylate **6** or **9**). Compound **6** was prepared by treatment of 3-(hexadecyloxy)propanol with freshly distilled PCl_3 in pyridine/ether mixture at 0 °C followed by a standard method for hydroxymethylation and tosylation[102] (Figure 5.3). This compound can be directly used as an etherifying/alkylating agent for diol **12** or one

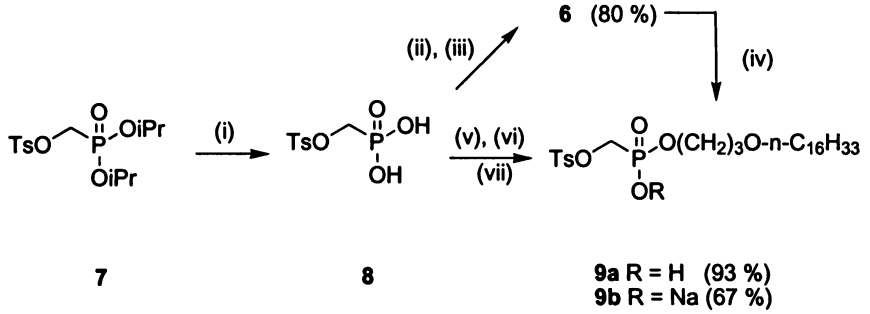
lipophilic chain of the intermediate can be easily removed on heating with LiN_3 in DMF to provide, finally, compound **9a** (see Figure 5.4).



(i) 3-(hexadecyloxy)propanol, pyridine, ether; (ii) $(CH_2O)_n$, Et_3N, 100 °C; (iii) TsCl, DMAP, Et_3N, CH_3CN

Figure 5.3: Synthesis of the lipophilic building block I

Although the compound **6** could be obtained by this procedure, its yield was low. Therefore, we decided to use a different approach. The attempts to convert triethyl phosphite and/or diphenyl phosphite by transesterification to their hexadecyloxypropyl esters failed. It has been, therefore, applied the standard method for deprotection of ester groups using the strategy reported in the literature[95,100] and depicted in Figure 5.4.



(i) TMSBr, RT, CH_3CN; for compound **9a**: (ii) oxalyl chloride, DMF, CH_2Cl_2, reflux; (iii) 3-(hexadecyloxy)propanol, pyridine, Et_3N, CH_2Cl_2, 0 °C; (iv) LiN_3, DMF, 100 °C; for compound **9b**: (v) oxalyl chloride, DMF, CH_2Cl_2, 0 °C; (vi) 3-(hexadecyloxy)propanol, pyridine, Et_2O, RT; (vii) $NaHCO_3$, 0 °C

Figure 5.4: Synthesis of the lipophilic building block II

The stepwise building of compound **13** and **14** started with the alkylation of 2-phenyl-1,3-dioxan-5-yl tosylate **10**. Only N^9 substituted adenine **11** was found in this reaction step, the formation of other possible (N^7) isomer was not observed. NMR analysis was used to identify the position of substitution of the purine moiety. All signals of hydrogen and carbon atoms were assigned usig 2D-^{1}H,^{13}C HSQC and 2D-^{1}H,^{13}C HMBC experiments. In the case of N^9-isomers, protons on carbon atom next to the purine nitrogen have strong crosspeaks in HMBC spectra with carbons C-4 and C-8 of the purine moiety.

Interestingly, no reaction occurred when the corresponding mesylate or the reaction under Mitsunobu conditions was used for the alkylation. After cleavage of the benzylidene-protecting group, compound **12** was obtained. It was treated with sodium 3-(hexadecyloxy)-propyl tosyloxymethylphosphonate **9b** in the presence of NaH, in DMF at 50 °C to give the final products **13** and **14** (Figure 5.5).

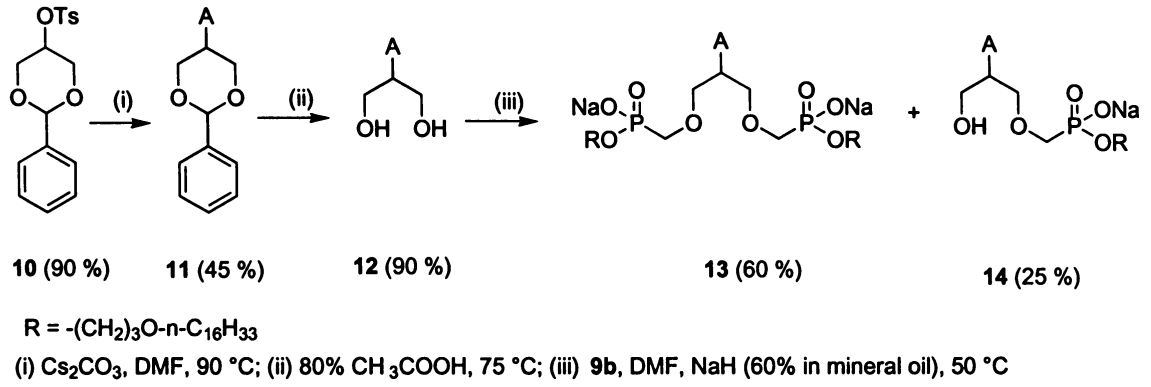


Figure 5.5: The stepwise buildup of the lipophilic bisphosphonate derivatives

Antiviral activity in vitro against DNA, RNA, and retroviruses was determined under the standard conditions.[103,104] Compounds **3a**, **13**, and **14** were examined for their inhibitory effect on the replication of varicella-zoster virus (VZV), human cytomegalovirus (HCMV), and herpes simplex virus (HSV-1 and HSV-2) in human embryonic lung (HEL) cells. Further, they were examined for their inhibitory effect on the replication of human immunodeficiency virus (HIV) in human T-lymphocyte (CEM) cells.

The parent compound, PMEA **1a**, is highly active against HIV-1 with the measured value of EC_{50} of 6.22 ± 0.73 μmol/l.[105] Against VZV and HIV-2, it exhibited inhibitory activity with the EC_{50}'s of 7.32 and 6.59, respectively. Although these results are very promising themselves, it is worth mentioning that the introduction of suitable masking moiety to the parent compound has a considerable effect on the antiviral activity. While the lipophilic compound **2a** exhibits a lower activity compared to the corresponding parent compound, the antiviral activity of its mono-lipophilic congener **3a** is increased severalfold in all tested assays (see Table 5.1).

Also the lipophilic analogues **13** and **14** proved to be active (see Table 5.1). While the free bisphosphonic acids showed only negligible activity,[1] their lipophilic congeners **13** and **14** exhibit significant inhibitory activity against herpesviruses, namely varicella-zoster virus and human cytomegalovirus. Also the compounds **13** and **14** showed anti-HSV

Table 5.1: Antiviral activity of studied compounds *in vitro*.

Type of virus	EC50[a] [μM] (14)	(13)	(3a)	PMEA
Retroviruses				
HIV-1	>32.91	>19.88	0.072	6.22 ± 0.73
HIV-2	>32.91	>19.88	0.14	6.59
CC_{50}[b] [μM]	54.63 ± 8.86	38.86 ± 5.66	3.56 ± 0.18	129.58 ± 5.12
Herpesviruses				
HSV-1	>32.91	>19.88	1.08 ± 0.54	>100
HSV-2	>32.91	>19.88	0.54 ± 0.36	73.21
HCMV				
AD-169 strain	>6.58	>19.88	0.13	>100
Davis strain	>6.58	10.93	<0.058	>100
VZV				
TK^+ OKA strain	>6.58	>3.98	<0.058	30.38
TK^- 07/1 strain	>6.58	3.98	<0.058	7.32
Picornaviruses				
Coxsackie virus B4	>6.58	3.98	>1.44	>100

[a] EC_{50} = 50 % effective concentration

[b] CC_{50} = 50 % cytostatic concentration

activity comparable or even better to that observed for the reference compound PMEA. Furthermore, the antiviral, other than anti-HIV, activities of compounds **13** and **14** are higher then in case of parent compound PMEA.

Suprisingly, all lipophilic compounds **3a**, **13**, and **14** showed also activity against the Coxsackie virus B4 (HeLa cell culture). In general, in this series of ANPs the activity against RNA viruses is very rare. It remains to be seen whether this finding could result in a new lead.

Furthermore, some of the prepared lipophilic compounds were subject to cytotoxicity measurements. In this case, PMEG **1c** was chosen as the parent and reference compound for the lipophilic analogues. PMEG is an extremely active cytostatic, exhibiting significant activity *in vivo* in rat and mouse carcinomas and sarcomas.[106–108] The cytostatic activities of compounds **3c** and PMEG were obtained by classic technique, estimating the cell count in a haematological analyzer. In parallel the cell viability was determined by XTT (Cell Proliferation Kit II, Roche) test. Compounds were tested for cytostatic effect on the cultures of murine leukemia L1210 cells, human cervix carcinoma HeLa S3 cells, human promyelocytic leukemia HL60 cells, and in human T-lymphoblastoid CCRF-CEM cell

Table 5.2: Cytostatic activity of studied compounds *in vitro*.

Compound	% of control, (IC_{50}, mmol.l^{-1}), [XTT]			
	L1210	**HL60**	**HeLa S3**	**CCRF-CEM**
(3c)	15 (0.18 ± 0.02)	(3.6 ± 0.2) [0.059 ± 0.004]	41 (2 ± 0.15)	38 (2 ± 0.12) [0.033 ± 0.004]
PMEG	(3.1 ± 0.17) N.D.	(4.5 ± 0.31) [3.68 ± 0.12]	N.D.	(2.66 ± 0.16) [2.44 ± 0.122]
(14)	79	92	104	55
(3a)	62	86	98	45

N.D. not determined

line. The presented data (Table 5.2, CCRF-CEM and HL-60 cells) show that the growth inhibitory effect of compound **3c** is two orders of magnitude more efficient than that of parent compound PMEG. The data on viability in case of compound **3c** show significantly low values of IC_{50} compared to the cell count IC_{50}. This difference is proportional to a high extent of apoptosis found in **3c** treated cells. Also, the PMEA analogues **3a** and **14** showed the cytostatic activity, however, it was necessary to prolonge the time to evoke the apoptosis.

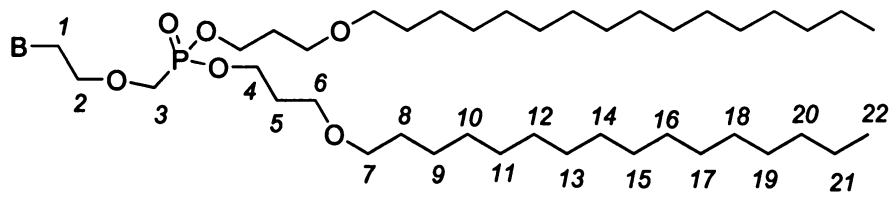
5.3 Conclusions

A new series of acyclic nucleoside phosphonates with lipophilic ester groups as a part of the phosphorylmethoxyethyl or 1,3-bis(phosphorylmethoxy)propan-2-yl side chain was prepared. The best results were obtained by activation of acids (PMEA, PMEC, PMEG) with oxalyl chloride and subsequent treatment with 3-(hexadecyloxy)propanol. In the case of the sequential building of 1,3-bis(phosphorylmethoxy)propan-2-yl building block on the nucleobase, Beadle's procedure was the strategy of choice. In conclusion, this study further confirmed that the introduction of suitable masking moiety to the polar phosphonate group helps with the transport of free phosphonic acids through the cell membrane and may provide bioavailable prodrugs of ANPs with potency comparable or even better to that of the corresponding non-derivatized drugs.

5.4 Experimental part

Unless otherwise stated, solvents were evaporated at 40 °C / 2 kPa and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. NMR spectra were measured on a Bruker Avance-500 instruments (500.0 MHz for ^1H and 125.7 MHz for ^{13}C). Chemical shifts are given in ppm (δ-scale); coupling constants (J) in Hz. Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using FAB or EI. UV spectra (λ in nm) were taken on a Beckman Coulter, DU-800 spectrophotometer. Elemental analyses were carried out on a Perkin–Elmer CHN Analyser 2400, Series II Sys (Perkin–Elmer, Norwolk, CT, U.S.A.). IR spectra were recorded on a FTIR spectrometer Bruker Equinox 55. Chemicals were purchased from Sigma–Aldrich (Prague, Czech Republic). Dimethylformamide, acetonitrile and dichloromethane were distilled from P_2O_5 and stored over molecular sieves (4 Å). Diethyl ether was distilled from $LiAlH_4$. Paraformaldehyde was dried over H_2SO_4.

Numbering of the chain for NMR analysis



B = adenine, guanine, cytosine

5.4.1 General procedure for the conversion of phosphonic acids to lipophilic phosphonates

Oxalyl chloride (6-7 equiv.) was slowly added to the slurry of free phosphonic acid (0.8 mmol, of **1a-c**; 1.5 mmol of **8**) and DMF (3 drops) in dry CH_2Cl_2 (25 ml). The resulting mixture was stirred under reflux with $CaCl_2$ protecting tube. The reaction was monitored by analysis of a small aliquot of the reaction mixture with anhydrous MeOH containing 1 drop of triethylamine. The resulting intermediate was analyzed by HPLC (mobile phase: Solvent A = water; Solvent B = MeOH/water = 50/50; Solvent C = MeOH; gradient: 100 %A (10 min); 0-100 %B (5 min); 0-100 %B (15 min); 1.0 ml/min.; inj. vol.= 10 ml; UV detection at 254 nm). After the completion, the reaction mixture was cooled to room

temperature and evaporated in vacuo. The residue in CH_2Cl_2 (15 ml) was treated slowly with pyridine (0.25 ml) at 0 °C and then added to a solution of 3-(hexadecyloxy)propanol (2 equiv.) and triethylamine (1.3 ml) in CH_2Cl_2 (15 ml) at –25 °C. The reaction mixture was stirred in argon atmosphere for 5 h and then at the room temperature overnight. The crude mixture was concentrated in vacuo, codistilled with toluene and worked up as described for compounds **2a-c**.

Bis(3-hexadecyloxypropyl) [2-(adenin-9-yl)ethoxy]methylphosphonate (2a)

The evaporated and dried crude product was dissolved in methanolic amonia and stirred at room temperature overnight. The resulting dark viscous residue was evaporated and purified by silica gel chromatography (0-10 % MeOH/$CHCl_3$) to yield 0.16 g (23 %) of **2a** as yellowish solid (mp 96-98 °C). For $C_{46}H_{88}N_5O_6P$ (838.19) calcd: C, 65.91; H, 10.58; N, 8.36; P, 3.70. Found: C, 65.93; H, 10.59; N, 8.29; P, 3.65. FABMS: 839.6 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 8.13 and 8.05 (2 x s, 2 x 1H, Pu-8 and Pu-2); 7.08 (bs, 2H, NH_2); 4.32 (t, 2H, $J_{1,2}=5.2$, H-1); 3.96 (m, 2 x 2H, H-4); 3.89 (t, 2H, $J_{2,1}=5.2$, H-2); 3.85 (d, 2H, $J_{3,P}=8.0$, H-3); 3.35 and 3.31 (4 x t, 2 x 4H, $J_{6,7}=6.5$ and 6.0, H-6 and H-7); 1.73 (2 x q, 2 x 2H, $J_{5,4}=6.3$, $J_{5,6}=6.3$, H-5); 1.47 (2 x m, 2 x 2H, H-8); 1.31-1.25 (bs, 52H, CH_2); 0.86 (2 x t, 2 x 3H, $J_{22,21}=7.2$, H-22). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.9 (Pu-6); 152.3 (Pu-2); 149.6 (Pu-4); 140.9 (Pu-8); 118.8 (Pu-5); 70.3 (d, $J_{2,P}=11.3$, C-2); 70.1 (C-7); 65.9 (C-6); 63.8 (d, $J_{3,P}=162.7$, C-3); 63.0 (d, $J_{4,P}=6.4$, C-4); 42.4 (C-1); 31.3 (2C); 30.4 (d, $J_{5,P}=5.4$, C-5); 29.0 (2C); 28.9 (2C); 28.8 (2C); 28.6 (2C); 25.6 (2C); 22.0 (2C); 21.2 (2C); 13.9 (2C, C-22). λ_{max}(KBr) 2955, 2919, 2851, 1640, 1600, 1579, 1468, 1417, 1378, 1327, 799, 646, 1250, 1034, 1010, 995, 1126, 1058, 722 cm^{-1}.

Bis(3-hexadecyloxypropyl) [2-(cytosin-1-yl)ethoxy]methylphosphonate (2b)

The evaporated mixture was dissolved in 80% CH_3COOH (20 ml) and dioxane (10 ml) and refluxed for 5 h. The crude residue was evaporated and purified by silica gel column chromatography (0-8 % MeOH/$CHCl_3$) to yield 0.11 g (17 %) of **2b** as yellowish solid (mp 98-100 °C). For $C_{45}H_{88}N_3O_7P$ (814.17) calcd: C, 66.38; H, 10.89; N, 5.16; P, 3.80. Found: C, 66.35; H, 10.82; N, 5.05; P, 3.72. FABMS: 815.0 (MH^+) (60). ^1H NMR (500 MHz, $CDCl_3$): 7.41 (d, 1H, $J_{Py-6,Py-5}=7.0$, Py-6); 5.72 (d, 1H, $J_{Py-5,Py-6}=6.3$, Py-5); 4.15 (2 x q, 2 x 2H, $J_{4,5}=6.8$, $J_{4,P}=6.8$, H-4); 3.97 (bs, 2H, H-1); 3.82 (bs, 2H, H-2); 3.77 (d, 2H, $J_{3,P}=8.4$, H-3); 3.47 (2 x t, 2 x 2H, $J_{6,5}=6.1$, H-6); 3.39 (2 x t, 2 x 2H, $J_{7,8}=6.7$, H-7); 1.91 (2 x p, 2 x 2H, $J_{5,4}=6.2$, $J_{5,6}=6.2$, H-5); 1.54 (2 x m, 2 x 2H, H-8); 1.32-1.25 (bs, 52H, CH_2); 0.88 (2 x t, 2 x 3H, $J_{22,21}=6.9$, H-22). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.8 (Py-4); 155.4 (Py-2); 146.9 (Py-6); 93.3 (Py-5); 71.2 (d, $J_{2,P}=12.0$, C-2); 71.2 (C-7); 66.5 (C-6); 65.1 (d, $J_{3,P}=167.2$, C-3); 63.8 (d, $J_{4,P}=6.5$, C-4); 49.7 (C-1); 31.9 (2C); 30.9 (d, $J_{5,P}=5.9$, C-5); 29.7 (2C); 29.7 (2C) 29.7 (2C); 29.5 (2C); 29.3 (2C); 26.2 (2C); 22.7 (2C); 14.1 (2C, C-22). λ_{max} (KBr) 2957, 2919, 2851, 1654, 1611, 1526, 1491, 1469, 1388, 1380, 1272, 1246, 1122, 1057, 1021, 791, 721 cm^{-1}.

Bis(3-hexadecyloxypropyl) [2-(guanin-9-yl)ethoxy]methylphosphonate (2c)

The residue after evaporation was dissolved in 80% CH_3COOH (20 ml) and refluxed for 5 h. The crude residue was evaporated and purified by silica gel column chromatography (0-12 % MeOH/$CHCl_3$) to yield 0.18 g (26 %) of **2c** as white solid (mp 100-102 °C). For $C_{46}H_{88}N_5O_7P$ (854.19) calcd: C, 64.68; H, 10.38; N, 8.20; P, 3.63. Found: C, 64.62; H, 10.35; N, 8.19; P, 3.68. FABMS: 855.5 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 11.92 (bs, 1H, NH); 7.66 (s, 1H, Pu-8); 6.34 (bs, 2H, NH_2); 4.22 (t, 2 H, $J_{1,2}=6.7$, H-1);

4.15 (2 x m, 2 x 2H, $J_{4,5} \sim J_{4,P} = 6.5$, H-4); 3.92 (t, 2H, $J_{2,1} = 5.3$, H-2); 3.82 (d, 2H, $J_{3,P} = 8.2$, H-3); 3.48 (2 x t, 2 x 2H, $J_{6,5} = 6.2$, H-6); 3.39 (2 x t, 2 x 2H, $J_{7,8} = 6.8$, H-7); 1.92 (2 x p, 2 x 2H, $J_{5,4} \sim J_{5,6} = 6.2$, H-5); 1.58 (2 x m, 2 x 2H, H-8); 1.32-1.25 (m, 52H, CH_2); 0.88 (2 x t, 2 x 3H, $J_{22,21} = 7.1$, H-22). ^{13}C NMR (125.7 MHz, $CDCl_3$): 158.9 (Pu-6); 153.7 (Pu-2); 151.6 (Pu-4); 138.2 (Pu-8); 116.9 (Pu-5); 71.4 (d, $J_{2,P} = 10.9$, C-2); 71.2 (C-7); 66.5 (C-6); 65.1 (d, $J_{3,P} = 166.5$, C-3); 64.0 (d, $J_{4,P} = 6.4$,C-4); 43.1 (C-1); 31.9 (2C, C-8); 30.9 (d, $J_{5,P} = 5.9$, C-5); 29.7 (2C); 29.7 (2C); 29.5 (2C); 29.4 (2C); 26.2 (2C); 22.7 (2C); 21.2 (2C); 14.1 (C-22). λ_{max} (KBr) 2923, 2851,1689, 1654, 1625, 1540, 1481, 1476, 1445, 1398, 1384, 1365,1260, 1236, 1121, 1072, 1037, 781, 721 cm^{-1}.

5.4.2 General procedure for the selective removal of one ester group from bis[3-(hexadecyloxy)propyl]phosphonates

LiN_3 (1.1 mmol) was added to a stirred solution of compounds **2a** or **2c** (0.16 mmol) or **6** (1.5 mmol) in dry DMF (15 ml). The reaction mixture was stirred with $CaCl_2$ protecting tube at 100 °C for 12 h. The solvent was evaporated in vacuo and the crude residue was purified by silica gel column chromatography (solvent H1 – EtOAc:EtOH:aceton:H_2O = 4:1:1:1; solvent H3 - EtOAc:EtOH:aceton:H_2O = 6:1:1:0.5).

3-Hexadecyloxypropyl hydrogen [2-(adenin-9-yl)ethoxy]methylphosphonate (3a)
Silica gel column chromatography in system H3 (600 ml) followed by H1 (400 ml) gave compound **3a** (81 mg, 92 %) as yellowish solid (mp 78-79 °C). For $C_{27}H_{50}N_5O_5P$ (555.69) calcd: C, 58.36; H, 9.07; N, 12.60; P, 5.57. Found: C, 58.48; H, 8.92; N, 12.45; P, 5.49. FABMS: 556.53 (MH^+) (70). ^1H NMR (500 MHz, $CDCl_3$): 8.21 and 8.19 (2 x s, 2 x 1H, Pu-8 and Pu-2); 4.36 (t, 2H, $J_{1,2} = 4.4$, H-1); 3.84 (q, 2H, $J_{4,5} = 6.5$, H-4); 3.80 (bs, 2H, H-2); 3.61 (d, 2 H, $J_{3,P} = 9.2$, H-3); 3.32 (t, 2H, $J_{6,5} = 6.2$, H-6); 3.26 (t, 2H, $J_{7,8} = 6.9$, H-7); 1.71 (p, 2H, $J_{5,4} \sim J_{5,6} = 6.4$, H-5); 1.46 (m, 2H, H-8); 1.32-1.21 (m, 26H, CH_2); 0.88 (t, 3H, $J_{22,21} = 7.0$, H-22). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.2 (Pu-6); 151.4 (Pu-2); 149.3 (Pu-4); 142.6 (Pu-8); 118.0 (Pu-5); 71.1 (C-7); 70.9 (d, $J_{2,P} = 12.5$, C-2); 66.9 (C-6); 66.3 (d, $J_{3,P} = 161.9$, C-3); 62.4 (d, $J_{4,P} = 5.6$, C-4); 43.4 (C-1); 31.9 (C-8); 30.9 (d, $J_{5,P} = 6.4$, C-5); 29.8; 29.7; 29.7; 29.6; 29.4; 26.1; 22.6; 14.1 (2C, C-22); λ_{max} (KBr) 3427, 3199, 2923, 2853, 1647, 1600, 1578, 1490, 1468, 1419, 1377, 1328, 1238, 1211, 1113, 1077, 995, 799, 720, 649 cm^{-1}.

3-Hexadecyloxypropyl hydrogen [2-(guanin-9-yl)ethoxy]methylphosphonate (3c)
Silica gel column chromatography in system H3 (400 ml) followed by H1 (600 ml) gave compound **3c** (84 mg, 92 %) as white amorphous solid. For $C_{27}H_{50}N_5O_6P$ (571.69) calcd: C, 56.72; H, 8.82; N, 12.25; P, 5.42. Found: C, 56.68; H, 8.69; N, 12.36; P, 5.43. FABMS: 572.40 (MH^+) (80). NMR spectra of the free acid form of the monoester are unavailable due to poor solubility of compound **3c** in common deuterated solvents and their mixtures, and due to their broad signals in ^1H NMR spectra. λ_{max} (KBr) 2922, 2852, 2123, 1669, 1649, 1625, 1572, 1541, 1485, 1468, 1412, 1370, 1224, 1187, 1125, 1072, 1030, 781, 720, 704 cm^{-1}.

[(3-Hexadecyloxypropoxy)(hydroxy)phosphoryl]methyl tosylate (9a)

The reaction mixture was obtained according to the above described procedure. Evaporated and purified on silica gel column chromatography (elution solvent 15-20 % MeOH/$CHCl_3$) to give 0.76 g (93 %) of compound **9a** as white foam. ^{1}H NMR (500 MHz, $CDCl_3$): 7.76 (d, 2H, $J_{2,3}=7.8$, Ts-2); 7.30 (d, 2H $J_{3,2}=7.8$, Ts-3); 4.04 and 3.88 (bs, 2 x 2H, OCH_2P and H-1); 3.37 and 3.32 (bs and t, 2 x 2H, H-3 and H-4); 2.38 (s, 3 H, Ts-CH_3); 1.72 (bs, 2H, H-2); 1.50 (bs, 2H, H-5); 1.33-1.20 (bs, 26H, CH_2); 0.88 (t, 3H, $J_{19,18}=7.0$, H-19). ^{13}C NMR (125.7 MHz, $CDCl_3$): 144.8 (Ts-4); 132.4 (Ts-1); 129.9 and 128.2 (Ts-2 and Ts-3); 71.1 (C-4); 67.2 (C-3); 64.3 and 63.4 (bs, OCH_2P and C-1); 31.9, 30.8, 29.8, 29.7, 29.4, 26.2, 22.7, 21.6 (Ts-CH_3); 14.1 (C-19). λ_{max} (KBr) 2918, 2815, 1598, 1487, 1468, 1400, 1374, 1308, 1295, 1262, 1191, 1179, 1122, 1097, 1061,1033, 1021, 1010, 819, 770, 721, 702, 664, 579, 554 cm^{-1}.

[Bis(3-hexadecyloxypropoxy)phosphoryl]methyl tosylate (6)

Method A: 3-(Hexadecyloxy)propanol (5.8 mmol, 2.5 equiv.) was added to a mixture of freshly distilled PCl_3 (2.3 mmol) and pyridine (3 equiv.) in dry diethylether (20 ml) for 15 minutes, keeping the temperature below 10 °C. The mixture was then stirred at RT for 3 h, the solid was filtered off, the solution evaporated and codistilled with toluene. The crude **4** in toluene (30 ml) was treated with Et_3N (0.4 ml). Paraformaldehyde (12.0 equiv.) was added to this mixture and refluxed with the $CaCl_2$ protecting tube for 3 h. The mixture was cooled to RT, evaporated and codistilled with CH_2Cl_2. The solution of crude **5** in CH_2Cl_2 (40 ml) was treated with DMAP (0.1 g), Et_3N (0.4 ml) and TsCl (0.5 g); stirred at 0 °C for 2 h and then at RT overnight. The reaction mixture was washed with ice-cold water and purified by silica gel column chromatography (HE:EA = 2:1, then 3:2) to give 0.45 g (25 %) of **6** as white foam.

Method B: (Diisopropoxyphosphoryl)methyl tosylate **7** (0.5 g, co-distilled with acetonitrile) in acetonitrile (20 ml) and $BrSiMe_3$ (2.5 ml) were stirred at RT overnight. After evaporation and co-distillation with acetonitrile, the residue was codistilled with water (three times), toluene and finally with CH_2Cl_2. The crude mixture of **8** was evaporated to dryness, and the residue dissolved in CH_2Cl_2. Treatment with oxalyl chloride and further work up followed the general procedure (see above). The crude residue was purified on silica gel column chromatography (HE:EA = 2:1, then 3:2) to give 0.95 g (80 %) of **6** as white foam. FABMS: 832.1 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 7.76 (d, 2H, $J_{2,3}=7.8$, Ts-2); 7.30 (d, 2H, $J_{3,2}=7.8$, Ts-3); 4.18 (d, 2H, $J_{P,CH}=9.9$, OCH_2P); 3.88 (2 x m, 2 x 2H, H-1); 3.42 (2 x t, 2 x 2H, $J=$ 6.2, H-3); 3.38 (2 x t, 2 x 2H, $J=6.7$, H-4); 2.40 (s, 3H, Ts-CH_3); 1.80 (2 x t, 2 x 2H, $J=6.5$, H-2); 1.50 (2 x m, 2 x 2H, H-5); 1.33-1.25 (bs, 52H, CH_2); 0.88 (2 x t, 2 x 3H, $J_{19,18}=7.1$, H-19). ^{13}C NMR (125.7 MHz, $CDCl_3$): 145.5 (Ts-4); 131.7 (Ts-1); 130.0 and 128.2 (Ts-2 and Ts-3); 71.1 (2C, C-4); 67.2 (2C, C-3); 64.3 and 63.5 (bs, 3C, OCH_2P and C-1); 31.9 (2C); 29.7 (2C); 29.7 (6C); 29.6 (2C); 29.4 (2C); 26.2 (2C); 22.7 (2C); 21.7 (Ts-CH_3); 14.1 (2C, C-19).

2-Phenyl-1,3-dioxan-5-yl tosylate (10)

Tosyl chloride (1.2 equiv.) was added to a stirred solution of 2-phenyl-1,3-dioxan-5-ol (5.47 g, 30.35 mmol), DMAP (0.1 equiv.), and Et_3N (1.2 equiv.) in dry CH_2Cl_2 (80 ml) at 0 °C with a $CaCl_2$ protecting tube. The mixture was stirred at 0 °C for 3 h and at room temperature overnight. The reaction mixture was quenched with ice-cold water (20 ml) and vigorously stirred for 0.5 h. The layers were separated and organic layer was washed with ice water and evaporated. The crystallization from ethanol yielded 9.12 g (90 %) of white solid (mp 127-129 °C). For $C_{17}H_{18}O_5S$ (334.39) calcd: C, 61.06; H, 5.43; S, 9.59. Found: C, 60.99; H, 5.38; S, 9.71. FABMS: 335.1 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 7.89 and 7.52 (2

x d, 4H, $H_{arom.}$); 7.36 (m, 5H, $H_{arom.}$); 5.55 (s, 1H, OCHO); 4.50 (tt, 1H, $J = 5.3, 5.3, 10.1, 10.1$, OCH); 4.07 (dd, 2H, $J = 11.3, 5.3$, OCH_2); 3.81 (dd, 2H, $J = 11.3, 10.3$, OCH_2); 2.44 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6) 145.8, 137.2, 132.5, 130.6 (2C); 129.2, 128.3 (2C); 127.9 (2C); 126.3 (2C); 100.3 (OCHO); 68.8 (CH); 67.5 (CH_2); 21.3 (CH_3). λ_{max} (KBr) 3065, 3034, 1595, 1499, 1493, 1453, 1392, 1355, 1317, 1309, 1296, 1283, 1247, 1220, 1192, 1175, 1143, 1122, 1082, 1041, 1029, 1020, 991, 985, 952, 870, 848, 811, 746, 742, 705, 696, 658, 618, 568, 555, 475, 420 cm^{-1}.

9-(2-Phenyl-1,3-dioxan-5-yl)adenine (11)

A suspension of adenine (17 mmol) in dry DMF (50 ml) was treated with Cs_2CO_3 (0.5 equiv.) at room temperature under a $CaCl_2$ protecting tube for 0.5 h. The reaction mixture was then heated at 60 °C and **10** (17 mmol, 1.0 equiv.) was added. The mixture was stirred at 100 °C for 24 h. The solvent was taken down and the residue was co-evaporated with toluene. The residue in chloroform was filtered through a Celite pad, evaporated and purified on a silica gel column using chloroform–methanol gradient (3-4 %). Crystalization from ethanol yielded 1.2 g (45 %) as white amorphous powder. FABMS: 298.6 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 8.26 and 8.17 (2 x s, 2H, H-2 and H-8); 7.53-7.38 (m, 5H, $H_{arom.}$); 7.30 (bs, 2H, NH_2); 5.74 (s, 1H, OCHO); 4.86 (m, 1H, N-CH); 4.52 (dd, 2H, $J_{gem} = 10.9$, $J_{CH_2,CH} = 10.9$, CH_2); 4.39 (dd, 2H, $J_{gem} = 10.9$, $J_{CH_2,CH} = 5.0$, CH_2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 156.3 (C-6); 152.7 (C-2); 149.6 (C-4); 139.8 (C-8); 137.9, 129.2, 128.4, 126.4, 119.0 (C-5); 100.7 (OCHO); 68.1 (CH_2); 47.7 (N-CH).

9-(1,3-Dihydroxyprop-2-yl)adenine[102] (12)

A solution of **11** (0.3 g, 1.0 mmol) in 80 % CH_3COOH (20 ml) was heated at 75 °C for 7 h. The solvent was evaporated. The potentially formed N^6-acetyl group was removed by the methanolysis of the crude product. After neutralization with HCl, methanol was evaporated and the residue was purified by silica gel column chromatography, using chloroform–methanol (elution with 30% MeOH/$CHCl_3$) yielded 0.18 g (90 %) as white solid (mp 192-194 °C; lit. 193-195 °C). For $C_8H_{11}N_5O_2$ (209.21) calcd: C, 45.93; H, 5.30; N, 33.48. Found: C, 45.83; H, 5.25; N, 33.40. FABMS: 210.1 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 8.11 and 8.10 (2 x s, 2H, H-2 and H-8); 7.17 (bs, 2H, NH_2); 5.04 (t, 2H, OH); 4.51 (m, 1H, N-CH); 3.83 (2 x m, 2 x 2H, CH_2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 156.1 (C-6); 152.1 (C-2); 149.9 (C-4); 140.5 (C-8); 119.0 (C-5); 60.1 (CH_2); 59.2 (N-CH). λ_{max} (KBr) 3424, 3350, 3240, 1637, 1613, 1570, 1480, 1335, 1212, 1095, 1087, 1069, 794, 652 cm^{-1}.

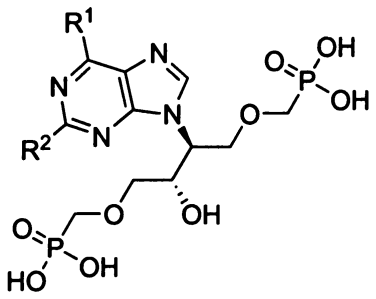
Sodium bis(3-hexadecyloxypropyl) [2-(adenin-9-yl)propane-1,3-diyl]bis(oxy)bis(methylene)]-bisphosphonate (13) and sodium 3-hexadecyloxypropyl [2-(adenin-9-yl)-3-hydroxypropoxy]-methylphosphonate (14)

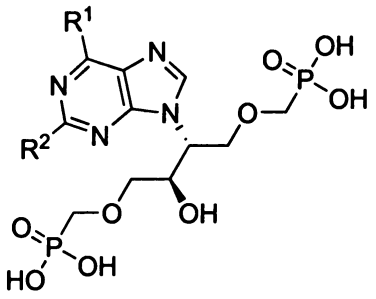
Sodium hydride (2.5 equiv., 60% in mineral oil) was added to a stirred solution of compound **12** (0.14 g, 0.7 mmol) in dry DMF (20 ml) at 0 °C. After 15 min compound **9b**[95] (0.8 g, 1.4 mmol) was added in one portion at room temperature, and the reaction mixture was stirred at 50 °C for 24 h. The mixture was then evaporated and purified by column chromatography, compound **13** was eluted with 30% MeOH/$CHCl_3$ (60 % yield, white solid, mp 232-234 °C) followed by compound **14** with 50% MeOH/$CHCl_3$ (25 % yield, yellowish solid, mp 91-92 °C). Compound **13**: UV spectrum: ($CHCl_3$) $\lambda_{max} = 259$ nm ($\epsilon_{max} = 10362$). FAB HRMS found: 1006.6118; calculated for $C_{48}H_{91}N_5Na_2O_{10}P$: 1006.6115. FABMS: 1006.9 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 8.46 and 8.22 (2 x bs, 2 x 1H, Pu-8 and Pu-2); 4.97 (bs, 1H, N-CH resp. H-1); 4.02 (m, 8H, H-2 and H-4); 3.76 (bs, 4H, H-3); 3.41 (bs, 4H, H-6); 3.32 (t, 4 H, $J_{7,8} = 6.9$, H-7); 1.80

(bs, 4H, H-5); 1.49 (m, 4H, H-8); 1.32-1.20 (m, 52H, CH_2); 0.88 (t, 6H, $J_{CH_3,CH_2} = 7.1$, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): 154.8 (Pu-6); 150.7 (Pu-2); 148.9 (Pu-4); 141.9 (Pu-8); 117.2 (Pu-5); 71.1 (C-7); 70.6 (C-2); 66.9 (C-6); 66.3 (d, $J_{3,P} = 159.9$, C-3); 62.4 (d, $J_{4,P} = 6.4$, C-4); 54.1 (C-1); 31.9 (d, $J_{5,P} = 5.6$, C-5); 30.9 (2C); 30.8 (2C); 29.7 (2C); 29.6 (2C); 29.6 (2C); 29.5 (2C); 29.3 (2C); 26.1 (2C); 22.6 (2C); 14.0 (2C, CH_3). λ_{max} (KBr) 2923, 2853, 1634, 1468, 1416, 1379, 1330, 1236, 1216, 1203, 1193, 1128, 1051, 1041, 1014, 799, 720 cm^{-1}. Compound **14**: UV spectrum: (0.01 M HCl) $\lambda_{max} = 259$ nm ($\epsilon_{max} = 5242$); (H_2O) $\lambda_{max} = 258$ nm ($\epsilon_{max} = 10254$); (0.01 M NaOH) $\lambda_{max} = 259$ nm ($\epsilon_{max} = 10944$). FAB HRMS found: 608.3557; calculated for $C_{28}H_{51}N_5NaO_6P$: 608.3553. FABMS: 608.5 (MH^+) (60). ^1H NMR (500 MHz, $CDCl_3$ + CD_3COOD): 8.30 and 8.17 (2 x bs, 2 x 1H, Pu-8 and Pu-2); 4.81 (m, 1H, N-CH resp. H-1); 4.05 (m, 3H, CH_2OH and H-2a); 3.94 (dd, 1H, $J_{gem} = 9.9$, $J_{2,1} = 4.5$, H-2b); 3.86 (m, 2H, H-4); 3.67 (d, 2H, $J_{3,P} = 8.6$, H-3); 3.36 (t, 2 H, $J_{6,5} = 6.4$, H-6); 3.29 (t, 2H, $J_{7,8} = 7.0$, H-7); 1.74 (p, 2 H, $J_{5,4} \sim J_{5,6} = 6.3$, H-5); 1.47 (m, 2H, H-8); 1.31-1.21 (m, 26H, CH_2); 0.88 (t, 3H, $J_{CH_3,CH_2} = 7.0$, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$ + CD_3COOD): 155.5 (Pu-6); 151.4 (Pu-2); 149.0 (Pu-4); 141.8 (Pu-8); 118.0 (Pu-5); 71.6 (C-2); 71.1 (C-7); 66.8 (C-6); 66.4 (d, $J_{3,P} = 160.0$, C-3); 62.5 (d, $J_{4,P} = 6.4$ Hz, C-4); 61.1 (CH_2OH); 56.5 (C-1); 31.9 (d, $J_{5,P} = 5.7$, C-5); 30.8, 29.7, 29.6, 29.6, 29.5, 29.3, 26.1, 22.6, 14.1 (CH3). λ_{max} (KBr) 3428, 3267, 3210, 2924, 2853, 1643, 1602, 1577, 1482, 1469, 1417, 1370, 1332, 1245, 1209, 1115, 1073, 994, 799, 720, 651 cm^{-1}.

Chapter 6

Synthesis of L-threitol and D-mannitol based ANbPs





6.1 Introduction

Most of the antiviral compounds that are currently used in the treatment of herpes simplex virus (HSV), human immunodeficiency virus (HIV), hepatitits B virus (HBV), varicella zoster virus (VZV), and cytomegalovirus (CMV) infections can be described as acyclic nucleoside or nucleotide analogues.[86,109–111] The activity of this class of chemical substances stays behind ongoing intensive research aimed at cancer therapy and viral diseases treatment.[112,113] It is worth mentioning that the absolute configuration of studied compounds often play an important role and significantly influences their biological potency.[114]

Among the acyclic nucleoside analogues bearing phosphonomethyl ether group, 9-(2-phosphonomethoxyethyl)adenine (PMEA, adefovir) was approved as an antiviral agent[115] active both against DNA viruses and retroviruses, including HIV.[116–119] The discovery of PMEA

was the beginning of search for other similar biologically potential compounds. A study of the structure activity relationships (SAR) in the series of newly synthesized phosphonomethoxyalkyl purine and pyrimidine derivatives revealed that several nucleobases substituted by an 3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) or an 2-phosphonomethoxypropyl (PMP) moiety, (*R*)-PMPA or (*S*)-HPMPC, respectively; (see Figure 6.1), show a broad spectrum of potent antiviral activity.[29,31,32,35–37]

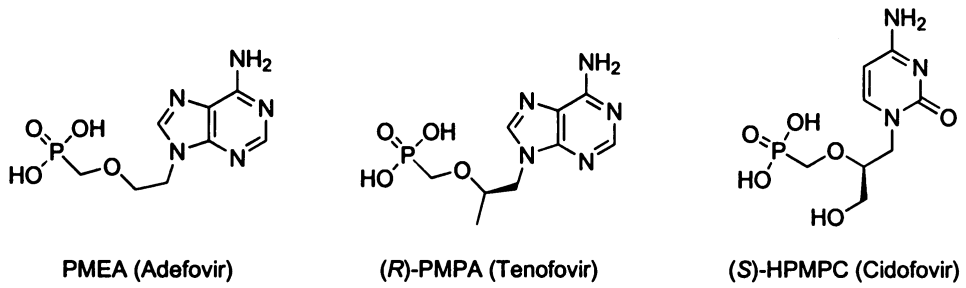


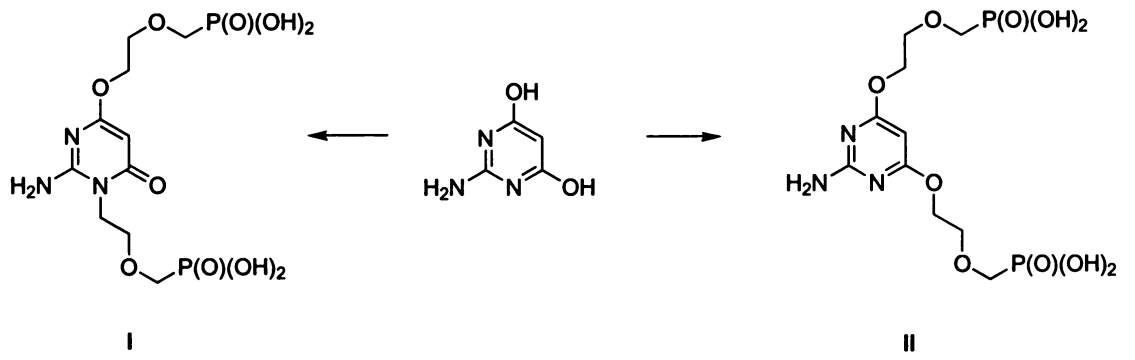
Figure 6.1: Adefovir, Tenofovir and Cidofovir

Recently, attention was turned to the synthesis of a new type of ANPs originating from 2-substituted 4-amino-6-hydroxypyrimidines.[44] In these investigations, a significant activity of 6-[2-(phosphonomethoxy)ethoxy]pyrimidine derivatives, compounds derived from 2,4-diaminopyrimidine and 2-amino-4-hydroxypyrimidine,[45] and their C5-substituted congeners[46,47] was discovered.

Among the products isolated in these studies, bisphosphonates **I** and **II** were also identified (Figure 6.2).[44] Despite the fact that these compounds constitute a new class of possible antiviral agents, they have not yet received much attention. The aim of this work was to explore the potential biological activity of such substances, which could be also considered as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor.[1,2,69] Additional goal was to study the effect of introduction of chiral centers to synthesized compounds, since the chirality seems to play an important role in EI-complex formation in certain enzymes.

6.2 Results and discussion

As an outcome of our research, we propose the following synthetic pathway leading to novel type of chiral four-carbon acyclic nucleoside bisphosphonates, derivatives of L-threitol



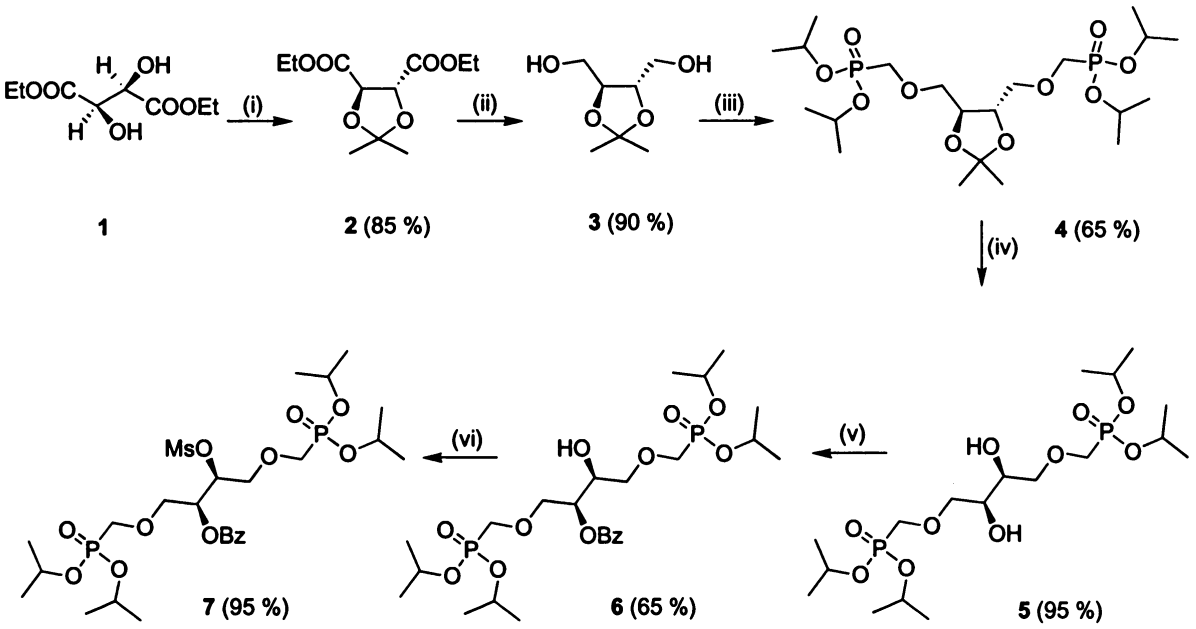
I II

Figure 6.2: Fist ANbPs isolated in our group

and D-mannitol, respectively. The strategy of the work was to prepare an appropriate chiral bisphosphonate building block and use it as a common alkylation reagent for various nucleobases. Synthesis of this building block made use of our earlier experience on synthesis of *threo-* and *erythro*-butyl analogues of acyclic nucleosides.[120] It differed for (2*S*,3*S*) and (2*R*,3*R*) bisphosphonate enantiomers. While (2*S*,3*S*) bisphosphonate was prepared from (+)-diethyl L-tartrate **1** (Figure 6.3), D-mannitol was used as starting compound for the opposite enantiomer (2*R*,3*R*) (Figure 6.4).

The starting (4*S*,5*S*)-(2,2-dimethyl-1,3-dioxolane-4,5-diyl)dimethanol **3**[120] was prepared from commercially available (+)-diethyl L-tartrate **1** as described in Figure 6.3. The thus-obtained diol intermediate **3** was alkylated with (diisopropoxyphosphoryl)methyl tosylate[75] and isopropylidene protecting group was subsequently removed with Dowex 50 X 8 (H^+ form) to give compound **5**. The resulting chiral bisphosphonate agent **7** was prepared by mono benzoylation of **5** and subsequent mesylation of **6**. No racemization was observed during this multistep reaction (NMR analysis, optical rotation), so we assume the configuration of final chiral four-carbon bisphosphonate as (2*S*,3*S*).

Multistep synthesis of (2*R*,3*R*)-bisphosphonate (Figure 6.4) started by protection of primary hydroxyls of compound **8** (D-mannitol) with benzoyl groups. Cis-oriented hydroxyl groups were then protected by isopropylidene group. This reaction step was followed by debenzoylation. Cleavage of **11**[121] with $NaIO_4$ in the presence of $Ba(OAc)_2 \cdot 2H_2O$ and $NaBH_4$ reduction gave 3,4-di-*O*-isopropylidene protected 1,4-diol **12**.[121] Alkylating bisphosphonate agent **16** was prepared in following four steps: attachment of phosphonate moiety to **12** formed compound **13**, which was transformed to **14** with Dowex 50 X 8 (H^+ form). Its monobenzoylation provided compound **15**, which gave the final alkylating agent **16** by mesylation. No racemization was observed during this multistep reaction ac-

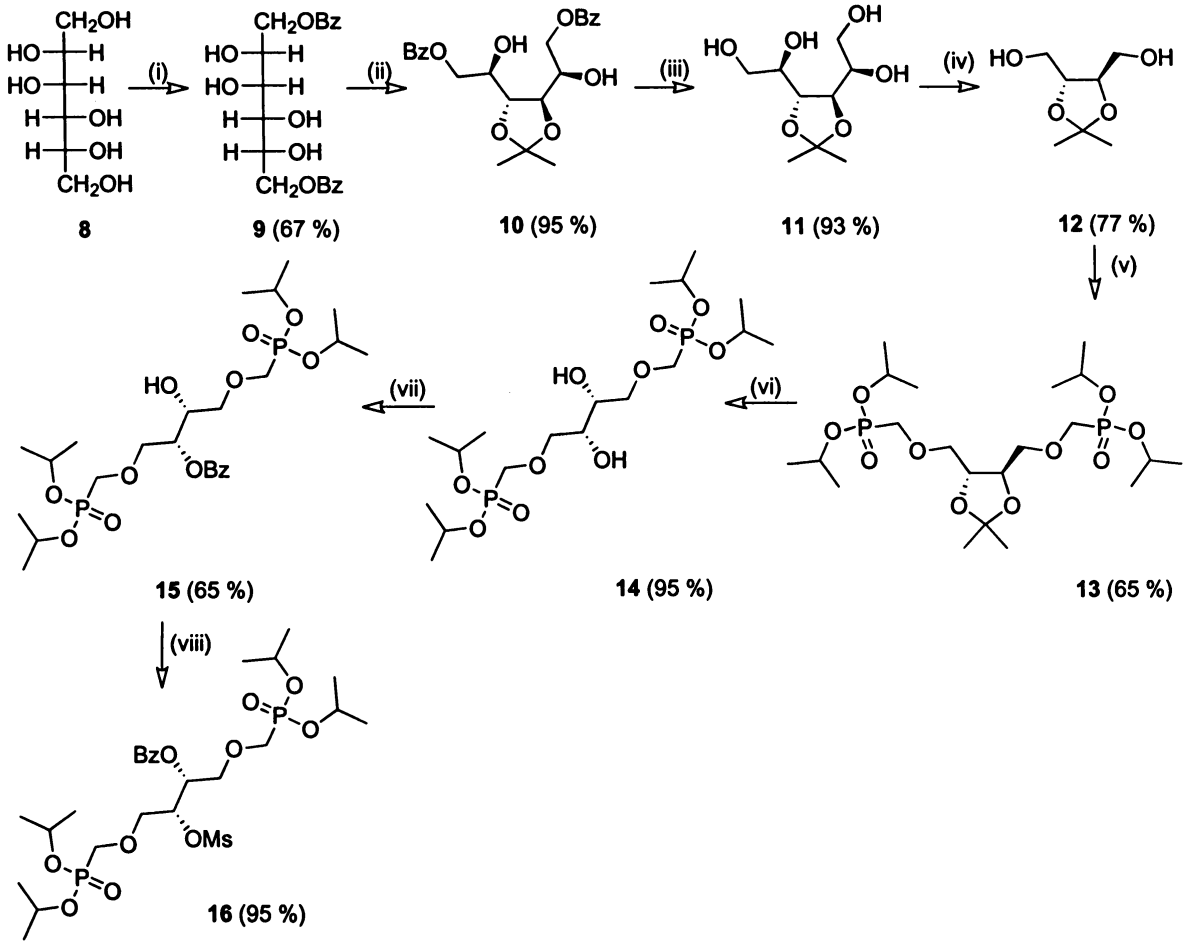


(i) $HC(OEt)_3$, 4.5 M HCl/DMF, acetone, RT; (ii) $LiAlH_4$, ether, RT; (iii) NaH, DMF, $TsOCH_2OP(O)(OiPr)_2$, 0 °C, then RT; (iv) Dowex 50 X 8 (H^+ form), 80 % isopropyl alcohol; (v) BzCl, pyridine, 0 °C; (vi) MsCl, pyridine, 0 °C.

Figure 6.3: The synthesis of (2*S*,3*S*)-bisphosphonate building block

cording to NMR analysis, and optical rotation; therefore, we assign the configuration of final chiral four-carbon bisphosphonate as (2*R*,3*R*).

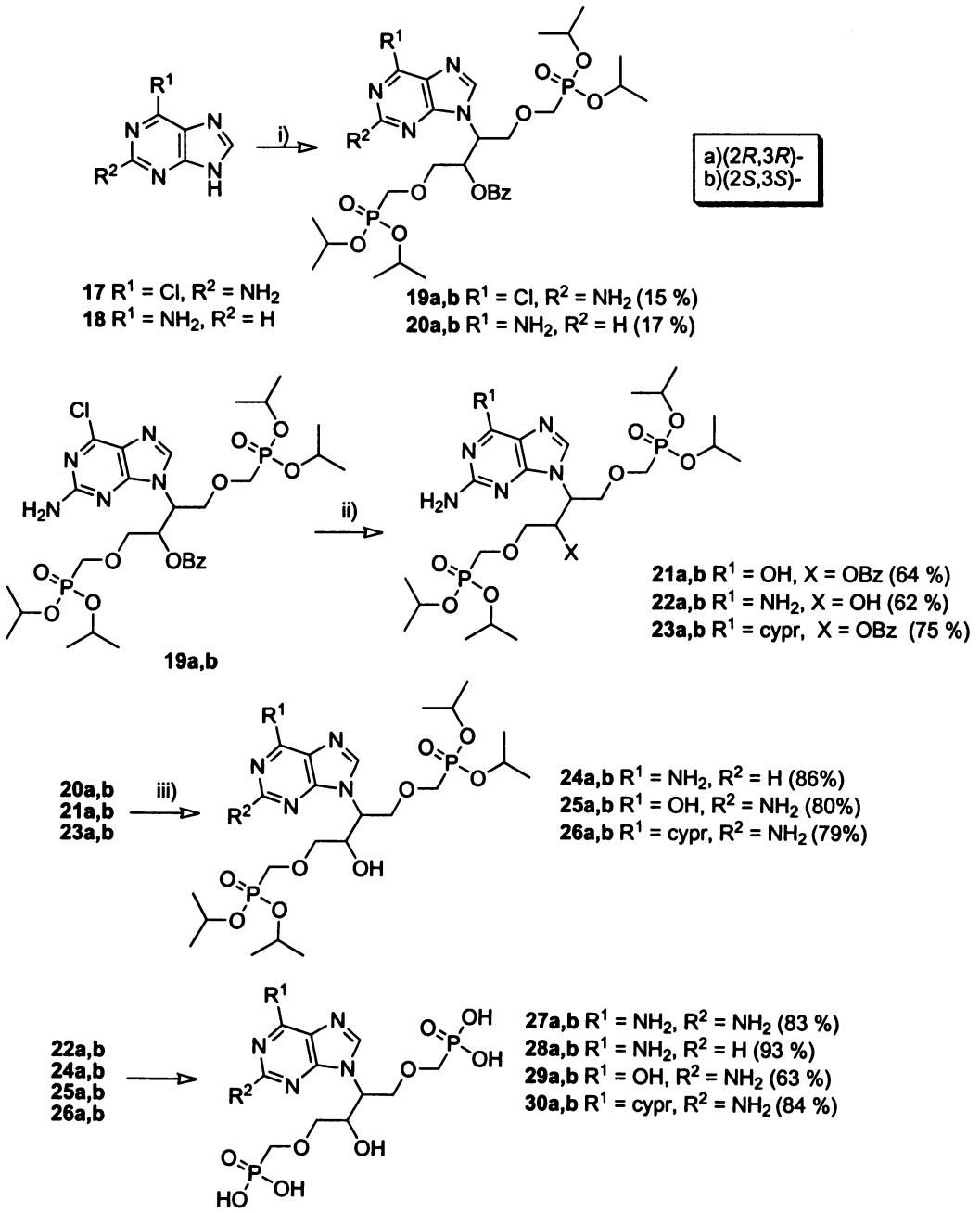
Alkylation of 2-amino-6-chloropurine (**17**) and adenine (**18**) with both chiral four-carbon bisphosphonates **7** and **16** provided N^9-substituted derivatives as the only product, however, the mechanism of alkylation and also the final configuration on stereocenter C2 could not be unambiguously determined (Figure 6.5). We used benzoyl-protected moiety on stereocenter C3, which is generally used as a participating group for glycosylations in the chemistry of carbohydrates.[122,123] The formation of single product indicated the possibility of either S_N2 or S_N1 mechanism. It is known that participation of the benzoyl group during glycosylation reaction (S_N1) on the rigid carbohydrate moiety affords the formation of cyclic intermediate **IV**, which allows nucleophile (*e.g.*, R^1OH) to attack the anomeric carbon only from the opposite side and thus create highly stereoselective trans-product **V** (Figure 6.6). In spite of the fact that free rotation of bisphosphonate chains **7** and **16** probably occurred in our case (Figure 6.5), the participation of benzoyl group could not be excluded.



(i) BzCl, pyridine, 0 °C; (ii) $HCOEt_3$, 4.5 M HCl/DMF, acetone, RT; (iii) MeONa/MeOH, MeOH, RT; (iv) $NaIO_4$, $Ba(OAc)_2$ * $2H_2O$, then $NaBH_4$, H_2O, acetone; (v) $TsOCH_2P(O)(OiPr)_2$, NaH, DMF, 0 °C; (vi) Dowex 50 X 8 (H^+ form), 80% isopropyl alcohol; (vii) BzCl, pyridine, 0 °C; (viii) MsCl, pyridine, 0 °C.

Figure 6.4: The synthesis of (2*R*,3*R*)-bisphosphonate building block

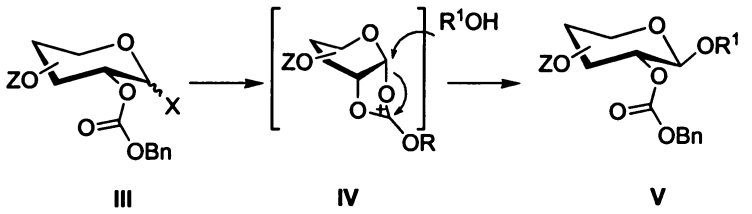
Mechanism of the alkylation reaction was clarified after the replacement of participating benzoyl group with non-participating *tert*-butyldimethylsilyl group (Figure 6.7). For this purpose compound **33** was prepared and used as alkylating agent. Alkylation of adenine under the same conditions as used for bisphosphonate **7** (DMSO, Cs_2CO_3, 110 °C) provided N^9-substituted derivative **34**. Since only one product was isolated the mechanism was still not certain. Therefore, the protecting silyl group was removed with 1M TBAF in THF. The structure of thus-obtained compound **35** was compared with **24a**. NMR study of both measured derivatives proved that they are identical. Supplementary optical rotation analysis also confirmed that **35** indeed is compound **24a**.



i) **7** or **16**, Cs_2CO_3, DMF or DMSO, 110 °C; ii) for compounds **21a,b**: 80% CH_3COOH, reflux; for compounds **22a,b**: methanolic ammonia, 100 °C; for compounds **23a,b**: cyclopropylamine, dioxane, reflux; iii) 1M CH_3ONa/CH_3OH, RT; iv) TMSBr, CH_3CN, RT.

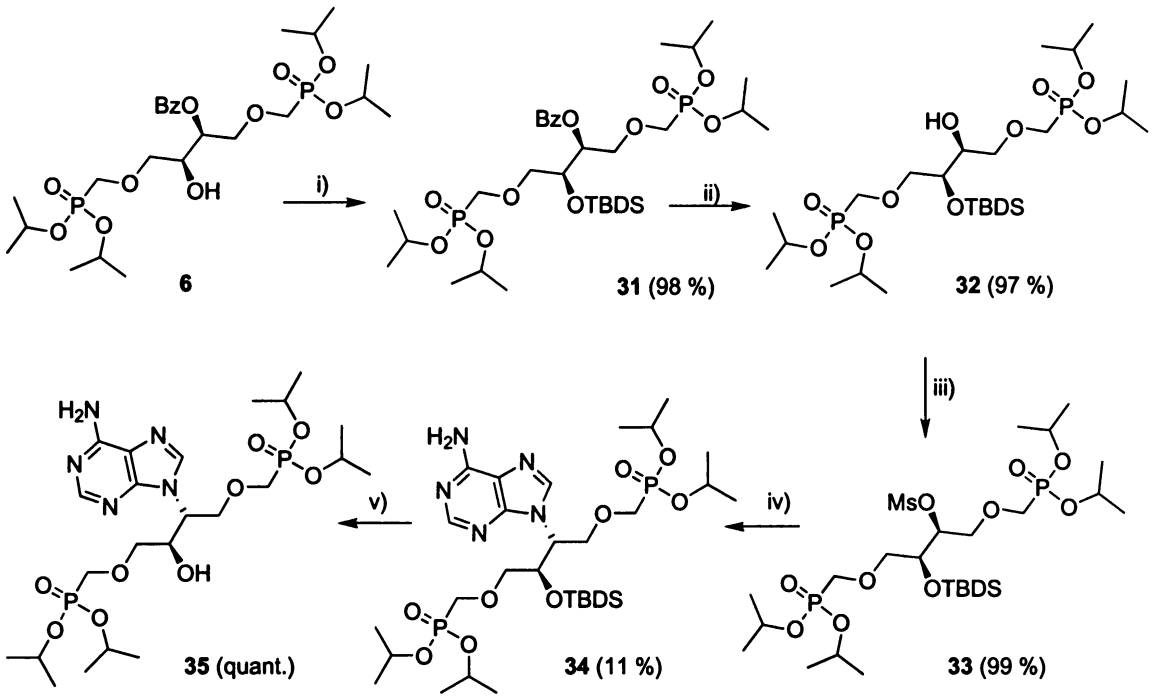
Figure 6.5: Alkylation reaction with (2*R*,3*R*) or (2*S*,3*S*) bisphosphonate

NMR spectroscopy, optical rotation and exclusion the participation of silyl group, therefore, revealed that the alkylation of compound **7**, **16** and **33** (Figure 6.5, 6.7) proceeded by the



Z = the system of protecting groups on saccharides skeleton
X = leaving group

Figure 6.6: Glycosylation reaction using participating benzoyl group



(i) TBDSCl, imidazole, DMF, RT; (ii) 1M MeONa/MeOH, RT; (iii) MsCl, pyridine, 0 °C; iv) adenine **18**, Cs_2CO_3, DMSO, 110 °C; v) 1M TBAF/THF, RT.

Figure 6.7: Alkylation reaction with (2*R*,3*R*) or (2*S*,3*S*) bisphosphonate

S_N2 mechanism; *i.e.*, nucleophile (nucleobase) attacks the C2 of bisphosphonate **7**, **16** and **33** from the opposite side than the mesyl group leaves and it causes the conversion of configuration on stereocenter C2. As an outcome of this study, it can be said that the alkylation of (2*S*,3*S*)-bisphosphonate **7** gave (2*R*,3*R*)-alkylated derivatives **19a** and **20a**, while opposite enantiomers **19b** and **20b** were formed during the alkylation with

(2*R*,3*R*)-bisphoshonate building block **16** (Figure 6.5). As found by NMR analysis the C3 stereocenter was not changed during the alkylation (no formation of diastereomer congener was observed).

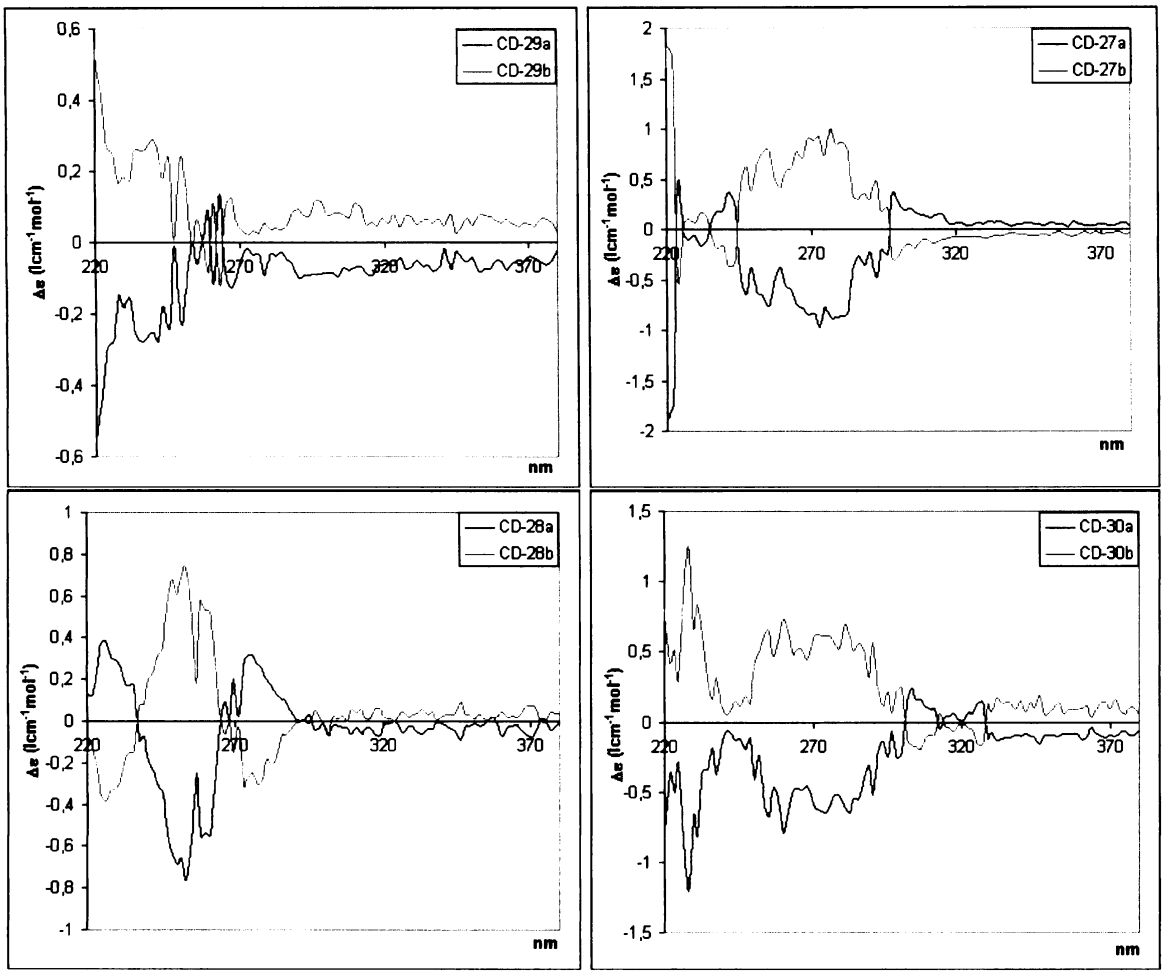


Figure 6.8: CD spectra of enantiomeric free bisphosphonic acids

Transformation of the C-Cl bond of purines **19a,b** under standard conditions[1] afforded the bisphosphonates **21a,b-23a,b** (Figure 6.5). Methanolysis catalyzed by sodium methoxide provided compounds **24a,b-26a,b**. Cleavage of diisopropyl esters with bromotrimethylsilane in acetonitrile, followed by hydrolysis, gave their chiral (2*R*,3*R*)-bisphosphonic acids **27a-30a** and (2*S*,3*S*)-bisphosphonic acids **28b** and **29b**, which were isolated from the deionised product by ion exchange chromatography. Purification of compounds **27b** and **30b** was slightly different. The crude products were applied onto Dowex 50 X 8 (H^+ form) and washed with water, followed by dilute aqueous ammonia. The thus-obtained ammo-

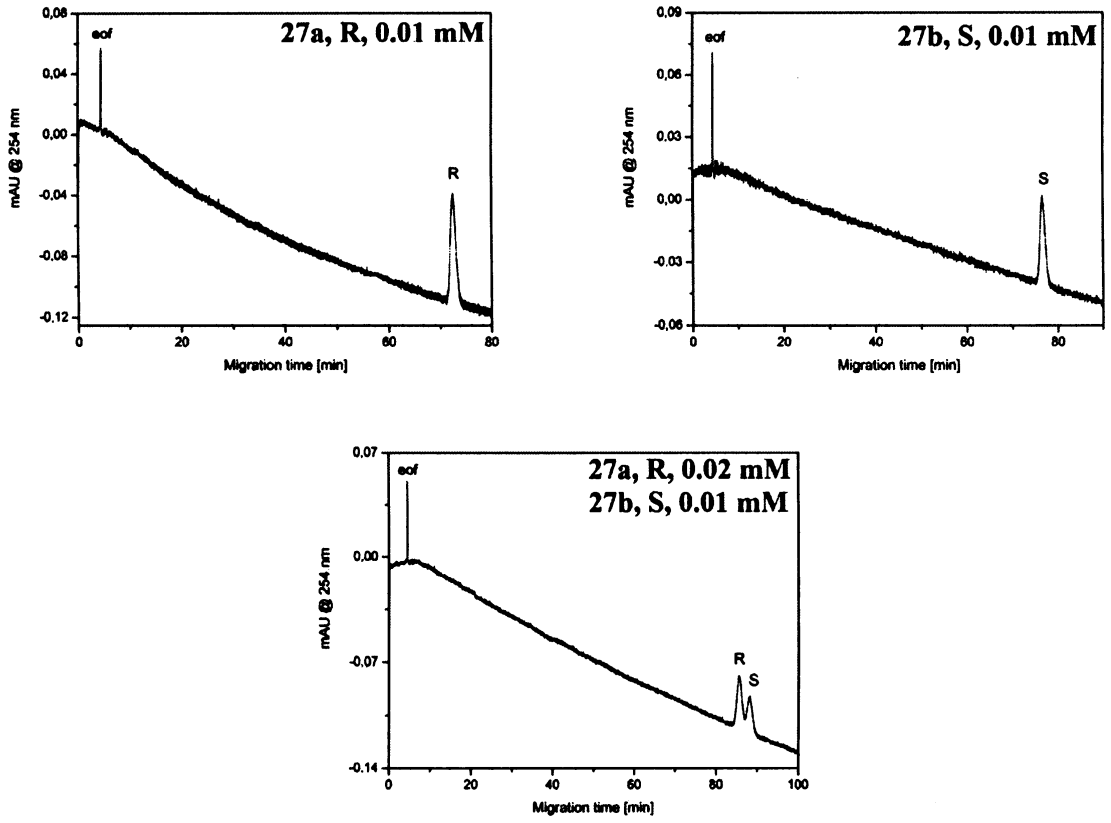


Figure 6.9: Confirmation of the enantiomeric purity of free bisphosphonic acids **27a,b** by CZE.

nium salts were transformed into sodium salts of bisphosphonic acids by Dowex 50 X 8 (Na^+ form) ion exchange chromatography. Free bisphosphonic acids of original sodium salts of compounds **27b** and **30b** were obtained after the treatment with HCl in water. The optical purity of final free bisphosphonic acids was among others witnessed by CD spectra (see Figure 6.8) or capillary zone electrophoresis (CZE, Figures 6.9 and 6.10).[124]

6.3 Conclusions

Chiral four-carbon bisphosphonate alkylating agents were prepared from optically active (+)-diethyl L-tartrate and D-mannitol. It was used then for the synthesis of N^9-alkylated nucleobases, 2-amino-6-chloropurine and adenine, respectively. As verified in the attempts with non-participating silyl group, the alkylation proceeds by S_N2 mechanism, which causes the inversion of configuration on the stereocenter C2. No racemization on stereocenter C3

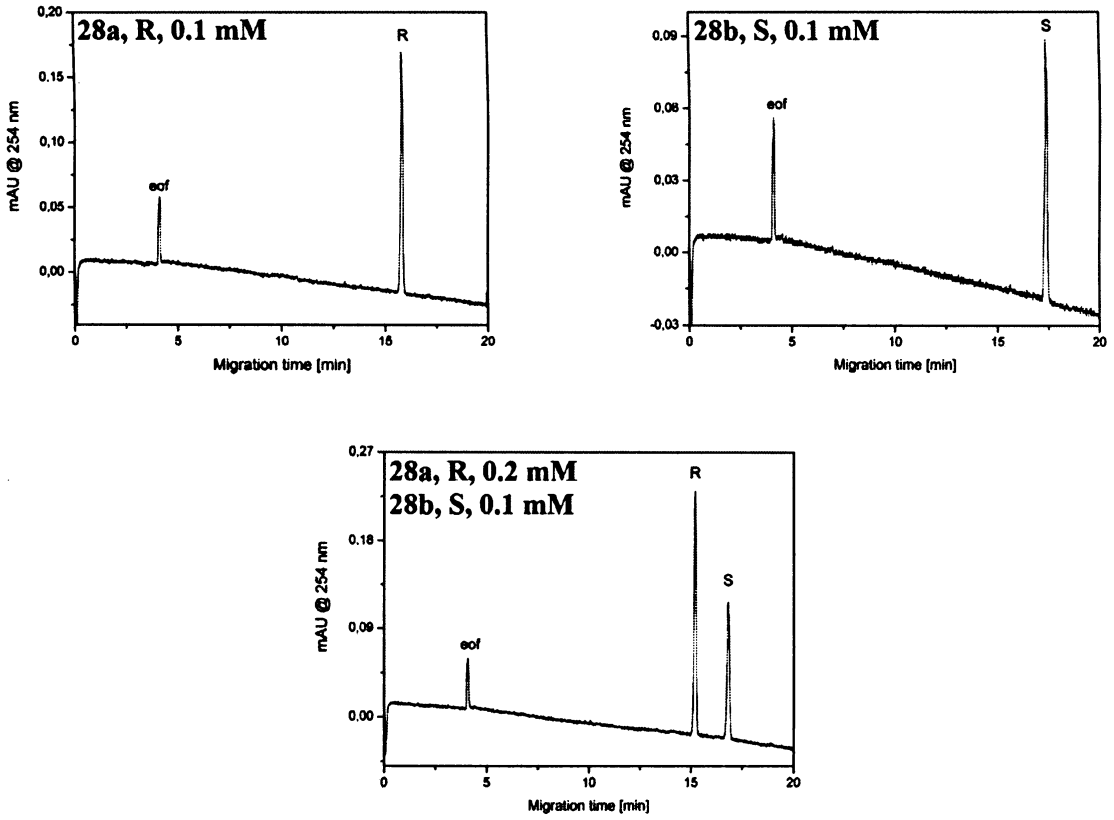


Figure 6.10: Confirmation of the enantiomeric purity of free bisphosphonic acids **28a,b** by CZE.

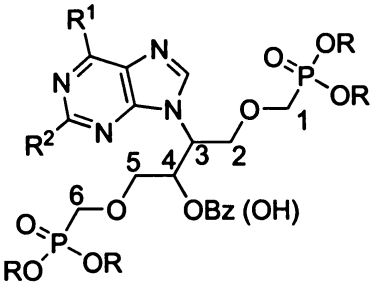
took place during the reactions. Therefore, we predict configuration of final bisphosphonic acids to be (2*R*,3*R*) when starting from (2*S*,3*S*) bisphosphonate or (2*S*,3*S*) when starting from (2*R*,3*R*) bisphosphonate. Optical purity was confirmed with NMR, CD, or CZE analysis. CZE analyses of compounds **27a,b** and **28a,b** provided single peaks both in the non-chiral BGE and chiral BGE 2 (see Figures 6.9 and 6.10). No enantioseparation was achieved in the case of enantiomeric pairs **29a,b** and **30a,b**, despite the fact that several chiral BGEs were tested for their separation. It is probably due to the fact that the used chiral selector, beta-cyclodextrin, was not able to distinguish among these, because the strength of interactions in both enantiomer-cyclodextrin complexes was the same. When the interaction differs among the enantiomers, it is possible to separate them by means of electrophoresis, as was observed for enantiomeric pairs **27a,b** and **28a,b**. The CD spectra and optical rotation indicate that in all cases the respective enantiomeric pairs were obtained. For all compounds the spectra correspond also quantitatively.

All synthesized compounds are currently undergoing screening for their potential antiviral and cytostatic activity. Initial cytostatic screening shows very promising activity for the (2*S*,3*S*) guanine derivative **29b**. The (2*R*,3*R*) derivative **29a**, on the other hand, does not exhibit any activity (for detail results see Chapter 8). Further testing is currently in progress.

6.4 Experimental part

Unless otherwise stated, solvents were evaporated at 40 °C / 2 kPa, and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus and are uncorrected. NMR spectra were measured on a Bruker Avance-500 instrument (500.0 MHz for ^{1}H and 125.7 MHz for ^{13}C). Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV) or using EI (electron energy 70 eV). UV spectra (λ in nm) were taken on a Beckman Coulter, DU 800 spectrophotometer. Elemental analyses were carried out on a Perkin–Elmer 2400 Series II CHNS/O Analyser. Optical rotation was measured on Autopol IV (Rudolph Research Analytical). CD spectra were recorded on *J*asco *J*-810 spectrometer in silicon cells with thickness 0.5-1.0 nm. Aqueous solution with approximate concentration of 0.002 mol/l were used. The results were averaged over three scans (point spacing 0.5 nm, time constant 2 sec) due to unfavorable CD signal/absorption ratio. Capillary zone electrophoresis (CZE) analyses were performed in a commercial P/ACE MDQ apparatus (Beckman Coulter, Fullerton, CA, USA), equipped with an internally non-coated fused silica capillary with outer polyimide coating (Polymicro Technilogies, Phoenix, AR, USA). The analyses were performed both in non-chiral and chiral background electrolytes (BGEs). Chemicals were purchased from Sigma–Aldrich (Prague, Czech Republic). Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å). Acetone was dried over anhydrous $CuSO_4$. Diethylether was distilled from $LiAlH_4$. Pyridine was dried over KOH and distilled with $KMnO_4$. Methanol was distilled over magnesium pellets.

Numbering for NMR analysis



(4*R*,5*R*)-Diethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (2)

A mixture of (+)-diethyl L-tartrate (309 g; 1.5 mol), dry acetone (450 ml), ethyl orthoformate (332 ml; 1.3 equiv.) and 4.5 M HCl in DMF (9 ml) was set aside at room temperature under a $CaCl_2$ cap for 3 days. After the neutralization with Et_3N the mixture was evaporated. The residue diluted with diethylether (900 ml), washed with water (2 x 300 ml), dried over $MgSO_4$, filtrated and evaporated. Distillation in vacuo afforded 310 g (85 %) of pure **2** as yellowish oil, Bp. 84-86 °C/0.1 torr. $[\alpha]_D^{20} = +41.2$ (c 1, MeOH). FABMS: 247.2 (MH^+) (55). ^{1}H NMR (500 MHz, DMSO-d_6): 4.81 (s, 2H, OCH); 4.18 (q, 4H, $J_{CH_2,CH_3} = 7.1$, OCH_2); 1.38 (s, 6H, CH_3); 1.21 (t, 6H, $J_{CH_3,CH_2} = 7.1$, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 169.60 (2C, CO); 113.04 (C-iPr); 76.74 (2C, OCH); 61.46 (2C, OCH_2); 26.48 and 14.08 (2C and 2C, iPr-CH_3 and CH_3).

(4*S*,5*S*)-(2,2-Dimethyl-1,3-dioxolane-4,5-diyl)dimethanol (3)

Compound **2** (269 g, 1.1 mol) in dry diethylether (300 ml) was added dropwise to a stirred and ice-cooled solution of Red-Al (564 ml, 65 wt. % solution in toluene) in absolute diethylether (500 ml). The temperature being held below 35 °C. The mixture was then stirred at room temperature for 3 hours. The excess of hydride was decomposed with ethyl acetate, ethanol, water, and 4 M NaOH. The solids were filtered off and the solution was evaporated. The residue was diluted with hot water and neutralized with HCl. The solids were again filtered through the Celite pad and the filtrate washed twice with diethylether. Water layer was evaporated and co-evaporated with ethanol. The residue was diluted with ethyl acetate and the solids filtered through the Celite pad. Organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was distilled, yielding 108 g (90 %) of **3**. Bp. 91-93 °C/0.1 torr. $[\alpha]_D^{20} = +10.8$ (c 0.5, MeOH). FABMS: 163.0 (MH^+) (50). ^{1}H NMR (500 MHz, DMSO-d_6): 4.65 (bs, 2H, OH); 3.74 (m, 2H, OCH); 3.51 (dd, 2H, $J = 4.1$ and 11.6, OCH_2); 3.47 (dd, 2H, $J = 5.2$ and 11.6, OCH_2); 1.30 (s, 6H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 108.20 (C-iPr); 78.99 (2C, OCH); 62.14 (2C, OCH_2); 27.31 (2C, CH_3).

(4*S*,5*S*)-[4,5-Bis(diisopropoxyphosphoryl)methoxymethyl]-2,2-dimethyl-1,3-dioxolane (4)

A solution of **3** (20 g, 124 mmol) in dry DMF (200 ml) was added dropwise to a stirred suspension of NaH (12.4 g of 60% suspension in mineral oil, prewashed with n-hexane, 310 mmol) in dry DMF (300 ml) at 0 °C under a $CaCl_2$ protecting tube. (Diisopropoxyphosphoryl)methyl tosylate (91 g, 260 mmol) was added dropwise and the mixture was stirred at room temperature overnight. The reaction mixture was neutralized with 4.5 M HCl in DMF and solvent evaporated. The residue was co-evaporated with toluene, dissolved in ethyl acetate (300 ml) and washed with water (3 x 300 ml). The organic layer was dried with anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography,

using chloroform – methanol gradient 0-2 %, to yield 42 g (65 %) of pure **4** as yellowish oil. $[\alpha]_D^{20} = +39.4$ (c 0.43, MeOH); FABMS: 519.0 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 4.60 (m, 4H, CH-iPr); 3.91 (m, 2H, OCH); 3.78 (dd, 4H, $J_{P,CH} = 8.3$, PCH_2); 3.67 (dd, 2H, $J = 3.1$ and 10.7, OCH_2); 3.62 (dd, 2H, $J = 5.1$ and 10.7, OCH_2); 1.31 (s, 6H, CH_3); 1.25 and 1.24 (4 x d, 24H, $J_{CH_3,CH} = 6.2$, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 108.99 (C-iPr); 76.50 (2C, OCH); 72.67 (d, 2C, $J_{P,C} = 11.7$, OCH_2); 70.31 (d, 4C, $J_{P,C} = 6.4$, CH-iPr); 65.48 (d, 2C, $J_{P,C} = 164.5$, PCH_2); 27.01 (2C, CH_3); 23.99 (d, 4C, $J_{P,C} = 3.9$, CH_3); 23.88 (d, 4C, $J_{P,C} = 4.4$, CH_3).

(2*S*,3*S*)-[1,4-Bis(diisopropoxyphosphoryl)methoxy]butane-2,3-diol (5)

A solution of **4** (42 g, 81 mmol) in 80% isopropyl alcohol (300 ml) was refluxed with Dowex 50 X 8 (H^+ form) (5 g) for 12 h. The resin was filtered off, the solution neutralized with aqueous ammonia and the solvent was evaporated. The crude product was purified by silica gel column chromatography, using chloroform – methanol gradient 0-5 %, to yield 37.5 g (95 %) of pure **5** as colorless oil. $[\alpha]_D^{20} = +2.2$ (c 0.35, MeOH); FABMS: 479.0 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 4.62 (bs, 2H, OH); 4.60 (m, 4H, CH-iPr); 3.74 and 3.71 (dd, 4H, $J_{P,CH} = 8.2$, $J_{gem} = 13.9$, PCH_2); 3.57 (m, 4H, OCH_2); 3.43 (m, 2H, OCH); 1.24 and 1.23 (2 x d, 24H, $J_{CH_3,CH} = 6.2$ and 6.3, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 74.16 (d, 2C, $J_{P,C} = 11.2$, OCH_2); 70.28 and 70.27 (d, 4C, $J_{P,C} = 6.4$, CH-iPr); 69.57 (2C, OCH); 65.38 (d, 2C, $J_{P,C} = 164.5$, PCH_2); 24.03 (d, 4C, $J_{P,C} = 3.9$, CH_3); 23.92 (d, 4C, $J_{P,C} = 4.4$, CH_3).

(2*S*,3*S*)-3-Hydroxy-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl benzoate (6)

Benzoyl chloride (8.7 g, 62 mmol) was added dropwise to a stirred solution of **5** (37 g, 77 mmol) in dry pyridine (300 ml) at 0 °C with a $CaCl_2$ protecting tube. The mixture was stirred for 2 h at 0 °C and then at room temperature overnight. The solvent was evaporated and the residue was purified by column chromatography on silica gel, using chloroform – methanol gradient 0-5 %, to yield 29.2 g (65 %) of pure **6** as yellowish oil. $[\alpha]_D^{20} = +5.0$ (c 0.1, MeOH); FABMS: 583.1 (MH^+) (100). ^1H NMR (500 MHz, DMSO-d_6): 8.05 (d, 2H, $H_{arom.}$); 7.66 (t, 1H, $H_{arom.}$); 7.53 (t, 2H, $H_{arom.}$); 5.26 (bs, 1H, OH); 5.22 (m, 1H, OCH); 4.58 and 4.52 (m, 4H, CH-iPr); 3.88 (m, 1H, OCH); 3.81 (d, 2H, $J = 5.0$, OCH_2); 3.58 (dd, 1H, $J = 4.8$ and 10.2, OCH_2); 3.53 (dd, 1H, $J = 6.2$ and 10.2, OCH_2); 3.77 (dd, 1H, $J_{P,CH} = 8.2$, $J_{gem} = 13.9$, P-CH_2); 3.75 (d, 2H, $J_{P,CH} = 7.8$, P-CH_2); 3.73 (dd, 1H, $J_{P,CH} = 8.2$, $J_{gem} = 13.9$, P-CH_2); 1.23-1.13 (8 x d, 24H, $J_{CH_3,CH} = 6.2$, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): 165.40 (CO); 133.49; 129.99; 129.58 (2C), 128.77 (2C); 73.80 (d, $J_{P,C} = 10.2$, OCH_2); 73.17 (O-CH); 71.30 (d, $J_{P,C} = 12.2$, OCH_2); 70.34 and 70.30 (2 x d, $J_{P,C} = 5.9$, CH-iPr); 65.48 and 65.19 (2 x d, $J_{P,C} = 164.1$, P-CH_2); 23.99 (d, 4C, $J_{P,C} = 3.9$, CH_3); 23.80 (d, 4C, $J_{P,C} = 4.4$, CH_3).

(2*S*,3*S*)-3-(Benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl mesylate (7)

Mesyl chloride (7.8 g, 68 mmol) was added dropwise to a stirred solution of **6** (28.5 g, 49 mmol) in dry pyridine (200 ml) at 0 °C with a $CaCl_2$ protecting tube. The mixture was stirred for 2 h at 0 °C and then at room temperature overnight. The solvent was evaporated and the residue was purified by column chromatography on silica gel, using chloroform – methanol gradient 0-3 %, to yield 29.8 g (95 %) of pure **7** as yellowish oil. $[\alpha]_D^{20} = +27.5$ (c 0.2, MeOH); For $C_{26}H_{46}O_{13}P_2S$ (660.65) calcd: C, 47.27; H, 7.02; P, 9.38; S, 4.85. Found: C, 47.23; H, 7.14; P, 9.55; S 4.92. FABMS: 661.20 (MH^+) (40). ^1H NMR (500 MHz, $CDCl_3$): 8.08 (m, 2H, $H_{arom.}$); 7.59 (m, 1H, $H_{arom.}$); 7.46 (m, 2H, $H_{arom.}$); 5.48 (q, 1H, $J_{CH_{Bz},CH_{Ms}} \sim J_{CH_{Bz},CH_a} \sim J_{CH_{Bz},CH_b} = 4.8$, CH-Bz); 5.16 (m, 1H, CH-Ms); 4.72 (m, 4H, CH-iPr); 4.00-3.87 (m, 4H, OCH_2); 3.88-3.68 (m, 4H, P-CH_2); 1.34-1.27 (m, 24H, CH_3); ^{13}C NMR (125.7 MHz,

$CDCl_3$): 166.45 (CO); 135.54; 129.95 (2C); 129.15; 128.50 (2C); 79.05 (C-Ms); 72.09 (d, $J_{P,C} = 11.9$, OCH_2); 71.33-71.13 (m, CH-iPr); 70.78 (d, $J_{P,C} = 10.6$, OCH_2); 70.77 (C-Bz); 66.31 (d, $J_{P,C} = 167.4$ and 168.6, P-CH_2); 38.82 (Ms-CH_3); 24.06-23.93 (m, CH_3).

(2*S*,3*S*)-1,4-[Bis(diisopropoxyphosphoryl)methoxy]-3-(*tert*-butyldimethylsilyloxy)butan-2-yl benzoate (31)

A solution of *tert*-butyldimethylsilyl chloride (5.2 g, 34.4 mmol) in dry DMF (50 ml) was slowly added to a solution of **6** (5.0 g, 8.6 mmol) and imidazole (4.1 g, 60.0 mmol) in dry DMF (150 ml). The reaction mixture was stirred overnight at room temperature. It was then concentrated under reduced pressure, dissolved in chloroform, and washed with 0.1 M aq. HCl (twice). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Column chromatography (silica gel, chloroform–methanol gradient 1–5 %) afforded the product **31** as yellowish oil (5.87 g, yield 98 %). $[\alpha]_D^{20} = +5.0$ (c 0.25, MeOH); FABMS: 697.7 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 8.04 (m, 1H, Bz-2); 7.56 (m, 1H, Bz-4); 7.44 (m, 2H, Bz-3); 5.34 (m, 1H, H-3); 4.70 (m, 4H, CH-iPr); 4.13 (m, 1H, H-4); 3.89 (m, 2H, H-2); 3.84-3.69 (m, 4H, H-1 and H-6); 3.65 (m, 2H, H-5); 1.34-1.23 (m, 24H, CH_3-iPr); 0.88 (s, 9H, C-$(CH_3)_3$); 0.11 and 0.09 (2 x s, 6H, CH_3-Si). ^{13}C NMR (125.7 MHz, $CDCl_3$): 165.69 (CO); 133.06 (Bz-4); 130.02 (Bz-1); 129.74 (Bz-2); 128.32 (Bz-3); 74.61 (d, $J_{5,P} = 10.6$, C-5); 73.43 (C-3); 71.25 (d, $J_{2,P} = 11.8$, C-2); 71.03 (m, 4 x CH-iPr); 70.33 (C-4); 66.49 and 66.04 (2 x d, $J_{C,P} = 167.2$ and 167.6, C-1 and C-6); 25.74 (C-$(CH_3)_3$); 24.00 (m, 8 x CH_3-iPr); 17.98 (C-$(CH_3)_3$); -4.48 (CH_3-Si).

(2*S*,3*S*)-1,4-[Bis(diisopropoxyphosphoryl)methoxy]-3-(*tert*-butyldimethylsilyloxy)-butane--2-ol (32)

Synthesis viz General procedure for debenzoylation. Column chromatography (silica gel, chloroform–methanol gradient 1–5 %) afforded the product **32** as yellowish oil (4.4 g, yield 97 %). $[\alpha]_D^{20} = +8.5$ (c 0.50, MeOH); FABMS: 593.7 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 4.75 (m, 4H, CH-iPr); 3.90 (m, 1H, H-4); 3.84 (m, 1H, H-3); 3.75 (m, 4H, H-1 and H-6); 3.67 (dd, 1H, $J_{gem} = 9.5$, $J_{5,4} = 6.0$, H-5); 3.64 (dd, 1H, $J_{gem} = 9.9$, $J_{2,3} = 5.3$, H-2); 3.59 (dd, 1H, $J_{gem} = 9.9$, $J_{2',3} = 6.9$, H-2'); 3.57 (dd, 1H, $J_{gem} = 9.5$, $J_{5',4} = 5.9$, H-5'); 1.34 (m, 24H, CH_3-iPr); 0.89 (s, 9H, C-$(CH_3)_3$); 0.10 and 0.09 (2 x s, 6H, CH_3-Si). ^{13}C NMR (125.7 MHz, $CDCl_3$): 74.39 and 74.37(2 x d, $J_{C,P} = 10.6$ and 10.4, C-5 and C-2); 71.02 (m, 4 x CH-iPr); 70.60 (C-4); 70.24 (C-3); 66.23 and 66.22 (2 x d, $J_{C,P} = 167.4$ and 167.7, C-1 and C-6); 25.81 (C-$(CH_3)_3$); 24.00 (m, 8 x CH_3-iPr); 18.05 (C-$(CH_3)_3$); -4.38 and -5.03 (CH_3-Si).

(2*S*,3*S*)-1,4-[Bis(diisopropoxyphosphoryl)methoxy]-3-(*tert*-butyldimethylsilyloxy)butan-2-yl mesylate (33)

Viz mesylation of compound **6**. Column chromatography (silica gel, chloroform–methanol gradient 0-2 %) afforded the product **33** as yellowish oil (4.7 g, yield 99 %). $[\alpha]_D^{20} = +25.0$ (c 0.43, MeOH); FABMS: 671.7 (MH^+) (90). For $C_{25}H_{56}O_{12}P_2SSi$ (670.8) calcd: C, 44.76; H, 8.41; P, 9.23; S, 4.78; Si, 4.19. Found: C, 44.68; H, 8.39; P, 9.19; S, 4.86. ^{1}H NMR (500 MHz, $CDCl_3$): 4.74 (m, 5H, CH-iPr and H-3); 4.03 (m, 1H, H-4); 3.84 (m, 2H, H-2); 3.79 and 3.71 (2 x m, 4H, H-1 and H-6); 3.65 (m, 1H, H-5); 3.14 (s, 3H, Ms-CH_3); 1.33 (m, 24H, CH_3-iPr); 0.89 (s, 9H, C-$(CH_3)_3$); 0.11 (s, 6H, CH_3-Si). ^{13}C NMR (125.7 MHz, $CDCl_3$): 81.42 (C-3); 73.27 and 71.91 (2 x d, $J_{C,P} = 10.8$ and 13.3, C-5 and C-2); 71.06 (m, 4 x CH-iPr); 70.77 (C-4); 66.36 and 66.22 (2 x d, $J_{C,P} = 167.5$ and 169.3, C-1 and C-6); 38.54 (Ms-CH_3); 25.73 (C-$(CH_3)_3$); 24.04 (m, 8 x CH_3-iPr); 17.98 (C-$(CH_3)_3$); -4.60 and -5.08 (CH_3-Si).

1,6-Di-*O*-benzoyl-D-mannitol (9)

Benzoyl chloride (308.6 g, 2.2 mol) was added dropwise to a stirred solution of D-mannitol (200 g, 1.1 mol) in dry pyridine (850 ml) at 0 °C. The mixture was stirred with a $CaCl_2$ protecting tube at 0 °C for 2 h and then at room temperature overnight. The solvent was evaporated and co-evaporated with toluene. The residue was slowly poured into vigorously stirred water. The white precipitate was filtered and dried on the air. The crystallization from ethanol yielded 288 g (67 %) of **9** as a white powder. Mp 179-180 °C (lit. 182 °C). $[\alpha]_D^{20} = +15.4$ (c 0.5, acetone). For $C_{20}H_{22}O_8$ (390.38) calcd: C, 61.53; H, 5.68. Found: C, 61.63; H, 5.67. FABMS: 391.12 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 7.95 (d, 4H, $H_{arom.}$); 7.56 (t, 2H, $H_{arom.}$); 7.40 (t, 4H, $H_{arom.}$); 4.47 and 4.45 (2 x dd, 2H, $J = 1.4$ and 11.4, $H_{b_{1,6}}$); 4.30 and 4.27 (2 x dd, 2H, $J = 5.8$ and 11.4, $H_{a_{1,6}}$); 3.82 and 3.80 (2 x m, 2H, $H_{2,5}$); 3.75 and 3.73 (2 x d, 2H, $J = 9.4$, $H_{3,4}$); ^{13}C NMR (125.7 MHz, DMSO-d_6): 165.92 (CO); 132.89, 130.10, 129.15, 128.75, 70.56 (2C, $CH_{3,4}$); 68.54 (2C, CH_2); 67.15 ($CH_{2,5}$).

1,6-Dibenzoyl-3,4-di-*O*-isopropylidene-D-mannitol (10)

A mixture of **9** (287 g; 735 mmol), dry acetone (600 ml), ethyl orthoformate (159 ml; 1.3 equiv.) and 4.5 M HCl in DMF (5 ml) was set aside for 7 days at room temperature under a $CaCl_2$ protecting tube. After the neutralization with Et_3N the mixture was evaporated, the residue diluted with ethyl acetate (900 ml) and washed with water (2 x 300 ml), dried over $MgSO_4$, filtrated and evaporated. Crystallization from ethyl acetate/petrolether afforded 242 g (95 %) of **10** as a white powder. Mp 95-96 °C. $[\alpha]_D^{20} = +32.3$ (c 1, MeOH). For $C_{23}H_{26}O_8$ (430.45) calcd: C, 64.18; H, 6.09. Found: C, 63.92; H, 6.05. FABMS: 431.2 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 8.02 (d, 4H, $H_{arom.}$); 7.65 (t, 2H, $H_{arom.}$); 7.52 (t, 4H, $H_{arom.}$); 5.59 (d, 1H, $J_{OH,CH} = 5.4$, OH); 4.47 and 4.45 (2 x dd, 2H, $J = 3.2$ and 11.4, $H_{b_{1,6}}$); 4.30 and 4.26 (2 x dd, 2H, $J = 6.7$ and 11.4, $H_{a_{1,6}}$); 4.10 and 4.05 (2 x d, 2H, $J = 9.4$, $H_{3,4}$); 3.94 and 3.92 (2 x m, 2H, $H_{2,5}$); 1.34 (s, 3H, CH_3); ^{13}C NMR (125.7 MHz, DMSO-d_6): 165.97 (CO); 133.49, 130.01, 129.48 (2C); 128.87 (2C); 109.44 (C-iPr); 79.54 and 69.82 (2C,CH-OH); 66.59 (OCH_2); 27.55 (CH_3).

3,4-Di-*O*-isopropylidene-D-mannitol (11)

Catalytic amount of 1 M sodium methoxide in methanol (10 ml) was added to solution of **10** (241 g; 560 mmol) in dry methanol (500 ml) and stirred at room temperature for 5 h. After the neutralization with Dowex 50 X 8 (H^+ form) the mixture was filtrated, and dowex was washed with hot methanol. The solution was evaporated and the residue was diluted with water and washed with diethylether. The water layer was evaporated and co-evaporated with ethanol to give 115.6 g (93 %) of **11** as white powder. Mp 86-87 °C (lit. 85-88 °C) °C. $[\alpha]_D^{20} = +31.2$ (c 0.8, MeOH); For $C_9H_{18}O_6$ (222.24) calcd: C, 48.64; H, 8.16. Found: C, 48.58; H, 8.27. FABMS: 223.12 (MH^+) (100). ^{1}H NMR (500 MHz, DMSO-d_6): 5.08 (d, 2H, $J_{OH,CH} = 4.6$, OH); 4.46 (t, 2H, $J_{OH,CH_2} = 5.7$, OH); 3.85 and 3.47 (2 x dd, 2 x 2H, $OCH_{3,4}$); 3.54 (ddd, 2H, $J = 3.1$ and 5.6 and 11.2, OCH_2); 3.35 (dt, 2H, $J = 6.0$ and 6.0 and 11.2, $OCH_{2,5}$); 1.28 (s, 6H, CH_3); ^{13}C NMR (125.7 MHz, DMSO-d_6): 108.48 (C-iPr); 79.25 and 73.07 (4C, OCH); 63.18 (2C, OCH_2); 27.44 (CH_3).

(4*R*,5*R*)-(2,2-Dimethyl-1,3-dioxolane-4,5-diyl)dimethanol (12)

$NaIO_4$ (225 g) was disolved in hot water (830 ml) and then cooled to 40 °C. Acetone (1000 ml) was added and the reaction mixture was cooled to 15 °C. 3,4-Di-*O*-isopropylidene-D-mannitol **11** (115 g, 517 mmol) was added and the reaction mixture was stirred at 10-15 °C for 2 h. The suspension was filtered off and washed with acetone. Acetone was evaporated from the water – acetone solution and $Ba(OAc)_2 \cdot 2H_2O$

(11 g disolved in minimum water) was added. The precipitate was filtered through the Celite pad and the residue was cooled to 5 °C. $NaBH_4$ (24 g) was added portionwise keeping the temperature under 20 °C. The reaction mixture was stirred at 15 °C for 2 h and at room temperature overnight. The solution was neutralized with acetic acid, evaporated to 150 ml of final volume and continually extracted with chloroform for 15 h to yield 65 g (77 %) of **12** as colorless oil. The analysis data correspond with the compound **3**. $[\alpha]_D^{20}$ =-11.2 (c 0.52, MeOH).

(4*R*,5*R*)-[4,5-Bis(diisopropoxyphosphoryl)methoxymethyl]-2,2-dimethyl-1,3-dioxolane (13)
The synthetic procedure and analysis data correspond with the compound **4**. $[\alpha]_D^{20}$ =-38.9 (c 0.50, MeOH).

(2*R*,3*R*)-[1,4-Bis(diisopropoxyphosphoryl)methoxy]butane-2,3-diol (14)
The synthetic procedure and analysis data correspond with the compound **5**. $[\alpha]_D^{20}$ =-2.4 (c 0.25, MeOH).

(2*R*,3*R*)-3-Hydroxy-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl benzoate (15)
The synthetic procedure and analysis data correspond with the compound **6**. $[\alpha]_D^{20}$ =-5.2 (c 0.30, MeOH).

(2*R*,3*R*)-3-(Benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl mesylate (16)
The synthetic procedure and analysis data correspond with the compound **7**. $[\alpha]_D^{20}$ =-27.8 (c 0.45, MeOH).

6.4.1 General procedure for alkylation of nucleobases

A solution of an appropriate nucleobase (**17, 18**, 2 equiv.) in dry DMF or DMSO (50 ml) was treated with Cs_2CO_3 (1 equiv.) for 1 h at room temperature under $CaCl_2$ protecting tube. The reaction mixture was then heated at 60 °C and bisphosphonate **7, 16** or **33** (0.5 equiv.) was added portionwise. The mixture was stirred at 110 °C for 48 h. The solvent was evaporated and the residue was co-evaporated with toluene (twice). The residue in 10% MeOH in chloroform was filtered through a Celite pad, evaporated and purified on a silica gel column in chloroform–methanol.

(2*R*,3*R*)-2-Amino-9-{3-(benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}--6-chloropurine (19a)
Column chromatography (silica gel, chloroform–methanol gradient 1–3 %) afforded the product **19a** as yellowish oil (2 g, yield 15 %). $[\alpha]_D^{20}$ =+21.8 (c 0.3, MeOH); FABMS: 735.21 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 8.05-8.02 (m, 3H, Pu-8 and Bz-2); 7.64-7.59 (m, 1H, Bz-4); 7.49-7.45 (m, 2H, Bz-3); 5.75 (dt 1H, $J_{4,5} \sim J_{4,5'}$ =3.7, $J_{4,3}$ =8.2, H-4); 5.30 (bs, 2H, NH_2); 5.23 (dt, 1H, $J_{3,4} \sim J_{3,2}$ =7.7, $J_{3,2'}$ =3.4; H-3); 4.78-4.57 (m, 4H, CH-iPr); 4.40 (dd, 1H, J_{gem} =10.2, $J_{2,3}$ =7.4, H-2); 4.02 (dd, 1H, J_{gem} =10.2, $J_{2',3}$ =3.4, H-2'); 3.84 (dd, 1H, J_{gem} =11.1, $J_{5,4}$ =3.6, H-5); 3.70-3.59 (m, 4H, H-1 and H-6); 3.41 (dd, 1H, J_{gem} =11.1, $J_{5',4}$ =3.8, H-5'); 1.30-1.17 (m, 24 H, CH_3-iPr). ^{13}C NMR (125.7 MHz, $CDCl_3$): 165.19 (CO); 159.10 (Pu-2); 153.90 (Pu-4); 151.40 (Pu-6); 142.63 (Pu-8); 133.63 (Bz-4); 129.81 (Bz-2); 129.25 (Bz-1); 128.59 (Bz-3); 125.02 (Pu-5); 71.42-71.18 (m, 4 x CH-iPr); 70.60 (C-4); 70.59 (d, $J_{5,P}$ =12.2, C-5); 70.26 (d, $J_{2,P}$ =9.8, C-2); 66.38 and 66.24 (2 x d, $J_{P,C}$ =169.0 and 167.5, C-1 and C-6); 54.68 (C-3); 24.03-23.90 (m, 8 x CH_3-iPr).

(2*S*,3*S*)-2-Amino-9-{3-(benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}--6-chloropurine (19b)

The synthetic procedure and analysis data correspond with the compound **19a**. $[\alpha]_D^{20}$ =-21.2 (c 0.4, MeOH).

(2*R*,3*R*)-9-{3-(Benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}adenine (20a)

Column chromatography (silica gel, chloroform–methanol gradient 1–6 %) afforded the product **20a** as yellowish oil (0.42 g, yield 17 %). $[\alpha]_D^{20}$ = +18.7 (c 0.32, MeOH); FABMS: 700.45 (MH^+) (90). ^{1}H NMR (500 MHz, DMSO-d_6): 8.17 (s, 1H, Pu-8); 8.09 (s, 1H, Pu-2); 7.97 (m, 2H, Bz-2); 7.69 (m, 1H, Bz-4); 7.54 (m, 2H, Bz-3); 7.25 (bs, 2H, NH_2); 5.77 (dt 1H, $J_{4,5} \sim J_{4,5'} = 3.3$, $J_{4,3} = 6.4$, H-4); 5.14 (dt, 1H, $J_{3,4} \sim J_{3,2} = 7.3$, $J_{3,2'} = 3.3$; H-3); 4.55 (m, 4H, CH-iPr); 4.37 (dd, 1H, $J_{gem} = 10.2$, $J_{2,3} = 6.8$, H-2); 4.06 (dd, 1H, $J_{gem} = 10.5$, $J_{2',3} = 4.0$, H-2'); 3.83 (dd, 1H, $J_{gem} = 11.0$, $J_{5,4} = 4.0$, H-5); 3.72 (dd, 1H, $J_{gem} = 11.0$, $J_{5',4} = 4.1$, H-5'); 3.70 and 3.67 (2 x d, 2 x 2H, $J_{H,P} = 8.7$ and 8.2, H-1 and H-6); 1.15-1.04 (m, 24H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 164.89 (CO); 156.17 (Pu-6); 152.53 (Pu-2); 149.77 (Pu-4); 140.25 (Pu-8); 133.76 (Bz-4); 129.60 (Bz-2); 129.29 (Bz-1); 128.80 (Bz-3); 118.91 (Pu-5); 71.01 (C-4); 70.72 (d, $J_{5,P} = 10.7$, C-5); 70.37-70.27 (m, 4 x CH-iPr); 69.46 (d, $J_{2,P} = 11.7$, C-2); 65.25 and 64.99 (2 x d, $J_{P,C} = 164.1$ and 163.6, C-1 and C-6); 54.38 (C-3); 23.90-23.58 (m, 8 x CH_3-iPr).

(2*S*,3*S*)-9-{3-(Benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}adenine (20b)

The synthetic procedure and analysis data correspond with the compound **20a**. $[\alpha]_D^{20}$ =-18.8 (c 0.4, MeOH).

(2*R*,3*R*)-9-{1,4-[Bis(diisopropoxyphosphoryl)methoxy]butan-2-yl-3-(*tert*-butyldimethylsilyl-oxy)}adenine (34)

Column chromatography (silica gel, chloroform–methanol gradient 2–3 %) afforded the product **34** as yellowish oil (0.31 g, yield 11 %). $[\alpha]_D^{20}$ = +18.2 (c 0.59, MeOH); FABMS: 700.2 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 8.31 (s, 1H, Pu-2); 8.04 (s, 1H, Pu-8); 5.70 (bs, 2H, NH_2); 4.87 (ddd 1H, $J_{3,2'} = 3.7$, $J_{3,2} \sim J_{3,4} = 6.8$ and 8.2, H-3); 4.72-4.60 and 4.61-4.54 (2 x m, 4H, CH-iPr); 4.38 (m, 2H, H-2 and H-4); 3.97 (dd, 1H, $J_{gem} = 10.1$, $J_{2',3} = 3.7$, H-2'); 3.69-3.54 (m, 4H, H-1 and H-6); 3.47 (dd, 1H, $J_{gem} = 10.3$, $J_{5,4} = 4.6$, H-5); 3.28 (dd, 1H, $J_{gem} = 10.3$, $J_{5',4} = =$ 4.8, H-5'); 1.29-1.12 (m, 24H, CH_3-iPr); 0.90 (s, 9H, C-$(CH_3)_3$); 0.07 and –0.04 (2 x s, 6H, CH_3-Si). $_{13}$C NMR (125.7 MHz, $CDCl_3$): 155.26 (Pu-6); 152.64 (Pu-2); 150.05 (Pu-4); 141.35 (Pu-8); 119.39 (Pu-5); 74.34 (d, $J_{5,P} = 9.7$, C-5); 71.00 (m, 4 x CH-iPr); 70.25 (C-4); 70.00 (d, $J_{2,P} = 11.5$, C-2); 66.31 and 65.98 (2 x d, $J_{C,P} = 167.1$ and 168.1, C-1 and C-6); 56.91 (C-3); 25.77 (C-$(CH_3)_3$); 23.90 (m, 8 x CH_3-iPr); -4.36 and –5.28 (CH_3-Si).

(2*R*,3*R*)-9-{3-(Benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}guanine (21a)

A solution of **19a** (0.6 g, 0.8 mmol) in 80% acetic acid (25 ml) was refluxed for 6 h. The solution was neutralized with Et_3N and the volatile were evaporated in vacuo. Column chromatography (silica gel, 1-12 % gradient of MeOH in $CHCl_3$) afforded compound **21a** as yellowish oil (0.37 g, yield 64 %). $[\alpha]_D^{20}$ = +20.3 (c 0.3, MeOH); FABMS: 716.32 (MH^+) (50). ^{1}H NMR (500 MHz, $CDCl_3$): 11.78 (bs, 1H, NH); 8.10-8.01 (m, 2H, Bz-2); 7.78 (s, 1H, Pu-8); 7.64-7.56 (m, 1H, Bz-4); 7.50-7.43 (m, 2H, Bz-3); 6.54

(bs, 2H, NH_2); 5.72 (dt 1H, $J_{4,5} \sim J_{4,5'} = 3.8$, $J_{4,3} = 8.2$, H-4); 5.09 (dt, 1H, $J_{3,4} \sim J_{3,2} = 7.3$, $J_{3,2'} = 3.3$; H-3); 4.77-4.59 (m, 4H, CH-iPr); 4.33 (dd, 1H, $J_{gem} = 10.2$, $J_{2,3} = 6.8$, H-2); 4.02 (dd, 1H, $J_{gem} = 10.2$, $J_{2',3} = 3.3$, H-2); 3.84 (dd, 1H, $J_{gem} = 11.0$, $J_{5,4} = 4.0$, H-5); 3.53 (dd, 1H, $J_{gem} = 11.0$, $J_{5',4} = 4.1$, H-5'); 3.71 and 3.69 (2 x d, 2 x 2H, $J_{H,P} = 8.7$ and 8.2, H-1 and H-6); 1.29-1.19 (m, 24H, 8 x CH_3-iPr). ^{13}C NMR (125.7 MHz, $CDCl_3$): 165.18 (CO); 158.91 (Pu-6); 153.74 (Pu-2); 151.70 (Pu-4); 137.34 (Pu-8); 133.56 (Bz-4); 129.82 (Bz-2); 129.35 (Bz-1); 128.57 (Bz-3); 116.92 (Pu-5); 71.55-71.27 (m, 4 x CH-iPr); 70.88 (d, $J_{2,P} = 9.7$, C-2); 70.85 (C-4); 70.78 (d, $J_{5,P} = 12.6$, C-5); 66.38 and 66.24 (2 x d, $J_{P,C} = 169.4$ and 167.5, C-1 and C-6); 53.94 (C-3); 23.87, 23.88-24.05 (m, 8 x CH_3-iPr).

(2*S*,3*S*)-9-{3-(Benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}guanine (21b)

The synthetic procedure and analysis data correspond with the compound **19a**. $[\alpha]_D^{20} = -20.2$ (c 0.35, MeOH).

(2*R*,3*R*)-2,6-Diamino-9-{3-(hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl} purine (22a)

A solution of **19a** (0.6 g, 0.8 mmol) in methanolic ammonia (50 ml) was heated (100 °C) in autoclave for 13 h. The solvent was then evaporated. Purification of the residue by column chromatography (silica gel, 1-11 % gradient of MeOH in $CHCl_3$) afforded compound **22a** as yellowish foam (0.30 g, yield 62 %). $[\alpha]_D^{20} = +17.3$ (c 0.23, MeOH + 2 drops of $CHCl_3$). FABMS: 611.58 (MH^+) (90). ^1H NMR (500 MHz, $CDCl_3$): 7.63 (s, 1H, Pu-8); 6.04 (bs, 1H, OH); 5.62 (bs,2H, 6-NH_2); 4.81 (bs, 2H, 2-NH_2); 4.80-4.62 (m, 4H, CH-iPr); 4.59 (dt, 1H, $J_{3,4} \sim J_{3,2} = 7.5$, $J_{3,2'} = 3.4$, H-3); 4.32 (dt 1H, $J_{4,5} \sim J_{4,5'} = 3.7$, $J_{4,3} = 8.2$, H-4); 4.13 (dd, 1H, $J_{gem} = 10.3$, $J_{2,3} = 7.7$, H-2); 3.99 (dd, 1H, $J_{gem} = 10.2$, $J_{2',3} = 3.3$, H-2'); 3.83 and 3.77 (2 x d, 2 x 2H, $J_{H,P} = 8.7$ and 8.2, H-1 and H-6); 3.72 (dd, 1H, $J_{gem} = 11.0$, $J_{5,4} = 4.0$, H-5); 3.65 (dd, 1H, $J_{gem} = 11.0$, $J_{5',4} = 4.1$, H-5'); 1.34-1.24 (m, 24H, CH_3-iPr). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.08 (Pu-2); 156.00 (Pu-6); 151.08 (Pu-4); 139.04 (Pu-8); 114.38 (Pu-5); 74.16 (d, $J_{5,P} = 10.0$, C-5); 71.24-71.09 (m, 4 x CH-iPr); 70.83 (d, $J_{2,P} = 10.5$, C-2); 70.20 (C-4); 66.41 and 66.05 (2 x d, $J_{P,C} = 165.0$ and 163.6, C-1 and C-6); 57.68 (C-3); 24.08-23.87 (m, 8 x CH_3-iPr).

(2*S*,3*S*)-2,6-Diamino-9-{3-(hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl} purine (22b)

The synthetic procedure and analysis data correspond with the compound **22a**. $[\alpha]_D^{20} = -17.5$ (c 0.20, $CHCl_3$).

(2*R*,3*R*)-2-Amino-9-{3-(benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}--6-(cyclopropyl)aminopurine (23a)

A solution of **19a** (0.5 g, 0.68 mmol) in dry dioxane (20 ml) was refluxed with cyclopropylamine (0.38 ml, 5.44 mmol) for 13 h. The solvent was then evaporated. Purification of the residue by column chromatography (silica gel, 1-4 % gradient of MeOH in $CHCl_3$) afforded compound **23a** as yellowish oil (0.38 g, yield 75 %). $[\alpha]_D^{20} = +24.5$ (c 0.25, $CHCl_3$); FABMS: 755.54 (MH^+) (90). ^1H NMR (500 MHz, $CDCl_3$): 8.06 (s, 1H, Bz-2); 7.69 (s, 1H, Pu-8); 7.60 (m, 1H, Bz-4); 7.47 (m, 2H, Bz-3); 5.80 (dt 1H, $J_{4,5} \sim J_{4,5'} = 3.8$, $J_{4,3} = 8.3$, H-4); 5.09 (ddd, 1H, $J_{3,4} = 8.4$, $J_{3,2'} = 7.4$, $J_{3,2} = 3.4$; H-3); 4.83 (bs, 2H, NH_2); 4.74-4.55 (m, 4H, CH-iPr); 4.35 (dd, 1H, $J_{gem} = 10.1$, $J_{2,3} = 7.4$, H-2); 3.97 (dd, 1H, $J_{gem} = 10.1$, $J_{2',3} = 3.4$, H-2'); 3.80 (dd, 1H, $J_{gem} = 11.0$, $J_{5,4} = 3.3$, H-5); 3.70 and 3.65 (2 x d, 2 x 2H, $J_{H,P} = 8.7$ and 8.2, H-1 and H-6); 3.42

(dd, 1H, J_{gem} = 11.0, $J_{5',4}$ = 4.2, H-5'); 2.98 (bs, 1H, $CH_{cycloprop.}$); 1.29-1.16 (m, 24H, CH_3-iPr); 0.90-0.82 (m, 2H, CH_2); 0.64-0.59 (m, 2H, CH_2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 165.26 (CO); 159.91 (Pu-2); 156.19 (Pu-6); 151.05 (Pu-4); 137.62 (Pu-8); 133.49 (Bz-4); 129.81 (Bz-2); 129.39 (Bz-1); 128.51 (Bz-3); 114.25 (Pu-5); 71.26-71.07 (m, 4 x CH-iPr); 70.80 (d, $J_{5,P}$ = 12.3, C-5); 70.77 (d, $J_{2,P}$ = 11.3, C-2); 70.75 (C-4); 66.21 and 66.15 (2 x d, $J_{P,C}$ = 168.1 and 167.1, C-1 and C-6); 54.07 (C-3'); 24.02-23.77 (m, 8 x CH_3, $CH_{cycloprop.}$); 7.32 and 6.47 (2 x CH_2).

(2*S*,3*S*)-2-Amino-9-{3-(benzoyloxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}--6-(cyclopropyl)aminopurine (23b)
The synthetic procedure and analysis data correspond with the compound **23a**. $[\alpha]_D^{20}$ = -24.6 (c 0.20, $CHCl_3$).

6.4.2 General procedure for debenzoylation

A catalytic amount of 1 M sodium methoxide in methanol was added to a well-dried compounds (**20a,b**; **21a,b**; **23a,b**; 0.5-0.6 mmol) in dry methanol and stirred at RT under $CaCl_2$ protecting tube until the full conversion of starting compound. The mixture was neutralized with HCl or CH_3COOH, evaporated and purified on a silica gel column in chloroform–methanol.

(2*R*,3*R*)-9-{3-(Hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}adenine (24a)
Column chromatography (silica gel, chloroform–methanol gradient 1–8 %) afforded the product **24a** as yellowish oil (0.31 g, yield 86 %). $[\alpha]_D^{20}$ = +12.4 (c 0.20, MeOH); FABMS: 596.35 (MH^+) (80). ^1H NMR (500 MHz, DMSO-d_6): 8.12 (s, 1H, Pu-8); 8.10 (s, 1H, Pu-2); 7.20 (bs, 2H, NH_2); 5.63 (d, 1H, $J_{OH,4}$ = 5.8, OH); 4.72 (ddd, 1H, $J_{3,2'}$ = 3.8, $J_{3,4}$ = 6.6, $J_{3,2}$ = 9.2, H-3); 4.58 and 4.38 (2 x m, 4H, CH-iPr); 4.25 (t, 1H, $J_{2,3} = J_{gem}$ = 9.8, H-2); 4.13 (m, 1H, H-4); 4.01 (dd, 1H, J_{gem} = 10.5, $J_{2',3}$ = 3.7, H-2'); 3.80-3.64 (2 x d, 2 x 2H, $J_{H,P}$ = 8.8 and 8.4, H-1 and H-6); 3.45 (dd, 1H, J_{gem} = 11.2, $J_{5,4}$ = 4.0, H-5); 3.36 (dd, 1H, J_{gem} = 11.2, $J_{5',4}$ = 4.1, H-5'); 1.23-1.02 (m, 24H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 156.17 (Pu-6); 152.32 (Pu-2); 149.68 (Pu-4); 140.38 (Pu-8); 118.91 (Pu-5); 74.21 (d, $J_{5,P}$ = 10.0, C-5); 70.34 (m, 4 x CH-iPr); 70.22 (d, $J_{2,P}$ = 12.2, C-2); 68.86 (C-4); 65.44 (d, $J_{6,P}$ = 163.5, C-6); 64.94 (d, $J_{1,P}$ = 163.6, C-1); 55.93 (C-3); 24.02-23.67 (m, 8 x CH_3-iPr).

(2*S*,3*S*)-9-{3-(Hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}adenine (24b)
The synthetic procedure and analysis data correspond with the compound **24a**. $[\alpha]_D^{20}$ = -12.6 (c 0.20, MeOH).

(2*R*,3*R*)-9-{3-(Hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}guanine (25a)
Column chromatography (silica gel, chloroform–methanol gradient 1–8 %) afforded the product **25a** as yellowish oil (0.26 g, yield 80 %). $[\alpha]_D^{20}$ = +18.2 (c 0.2, $CHCl_3$); FABMS: 612.23 (MH^+) (50). ^1H NMR (500 MHz, DMSO-d_6): 10.73 (bs, 1H, NH); 7.68 (s, 1H, Pu-8); 6.59 (bs, 2H, NH_2); 5.60 (d, 1H, $J_{OH,4}$ = 5.8, OH); 4.62-4.52 (m, 4H, CH-iPr); 4.40 (dt, 1H, $J_{3,4} \sim J_{3,2}$ = 7.5, $J_{3,2'}$ = 3.4; H-3); 4.12 (dd, 1H, J_{gem} = 10.4, $J_{2,3}$ = 6.6, H-2); 4.03 (dt 1H, $J_{4,5} \sim J_{4,5'}$ = 3.7, $J_{4,3}$ = 8.2, H-4); 3.94 (dd, 1H, J_{gem} = 10.5, $J_{2',3}$ = 3.6,

H-2'); 3.77 and 3.61 (2 x d, 2 x 2H, $J_{H,P}$ = 8.7 and 8.2, H-1 and H-6); 3.45 (dd, 1H, J_{gem} = 11.0, $J_{5,4}$ = 4.0, H-5); 3.40 (dd, 1H, J_{gem} = 11.0, $J_{5',4}$ = 4.1, H-5'); 1.23-1.10 (m, 24H, CH_3-iPr). ^{13}C NMR (125.7 MHz, DMSO-d_6): 156.83 (Pu-6); 153.63 (Pu-2); 151.16 (Pu-4); 136.72 (Pu-8); 116.54 (Pu-5); 74.13 (d, $J_{5,P}$ = 12.6, C-5); 70.50-70.20 (m, 4 x CH-iPr, C-2); 68.86 (C-4); 65.48 and 65.01 (2 x d, $J_{P,C}$ = 165.0 and 163.6, C-1 and C-6); 55.52 (C-3); 24.00-23.60 m, 8 x CH_3-iPr).

(2*S*,3*S*)-9-{3-(Hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}guanine (25b)
The synthetic procedure and analysis data correspond with the compound **25a**. $[\alpha]_D^{20}$ = -18.9 (c 0.20, $CHCl_3$).

(2*R*,3*R*)-2-Amino-6-(cyclopropyl)amino-9-{3-(hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}purine (26a)
Column chromatography (silica gel, chloroform–methanol gradient 1–5 %) afforded the product **26a** as yellowish oil (0.26 g, yield 79 %). $[\alpha]_D^{20}$ = +19.9 (c 0.22, $CHCl_3$). FABMS: 651.45 (MH^+) (80). ^{1}H NMR (500 MHz, $CDCl_3$): 7.63 (s, 1H, Pu-8); 6.07 (bs, 1H, OH); 5.67 (bs, 2H, NH_2); 4.80-4.62 (m, 4H, CH-iPr); 4.60 (ddd, 1H, $J_{3,4} \sim J_{3,2}$ = 7.5, $J_{3,2'}$ = 3.4, H-3); 4.26 (dt 1H, $J_{4,5} \sim J_{4,5'}$ = 3.7, $J_{4,3}$ = 8.2, H-4); 4.15 (dd, 1H, J_{gem} = 10.1, $J_{2,3}$ = 7.5, H-2); 4.01 (dd, 1H, J_{gem} = 10.2, $J_{2',3}$ = 3.3, H-2'); 3.83 and 3.77 (2 x d, 2 x 2H, $J_{H,P}$ = 8.7 and 8.2, H-1 and H-6); 3.72 (dd, 1H, J_{gem} = 11.0, $J_{5,4}$ = 4.0, H-5); 3.65 (dd, 1H, J_{gem} = 11.0, $J_{5',4}$ = 4.1, H-5'); 2.95 (bs, 1H, $CH_{cycloprop.}$); 1.34-1.24 (m, 24H, CH_3-iPr); 0.85 (m, 2H, CH_2); 0.60 (m, 2H, CH_2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.72 (Pu-2); 156.35 (Pu-6); 151.18 (Pu-4); 139.24 (Pu-8); 114.48 (Pu-5); 74.14 (d, $J_{5,P}$ = 10.0, C-5); 71.11 (m, 4 x CH-iPr); 70.81 (d, $J_{2,P}$ = 10.5, C-2); 70.20 (C-4); 66.41 (d, $J_{6,P}$ = 163.5, C-6); 66.05 (d, $J_{1,P}$ = 163.6, C-1); 57.68 (C-3); 24.05-23.87 (m, 8 x CH_3, $CH_{cycloprop.}$); 7.31 and 6.49 (2 x CH_2).

(2*S*,3*S*)-2-Amino-6-(cyclopropyl)amino-9-{3-(hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}purine (26b)
The synthetic procedure and analysis data correspond with the compound **26a**. $[\alpha]_D^{20}$ = -20.1 (c 0.20, $CHCl_3$).

(2*R*,3*R*)-9-{3-(Hydroxy)-1,4-[bis(diisopropoxyphosphoryl)methoxy]butan-2-yl}adenine (35)
A oven-dried bottomed flask under argon atmosphere was charged with **34** (110 mg, 0.15 mmol) and 1 M solution of TBAF in THF (0.85 ml, 0.85 mmol). The reaction was aged at RT for 2 h and concentrated. Purification on thin layer chromatography with UV silica gel sorbent (eluent 10 % MeOH in $CHCl_3$) afforded pure **35** as yellowish oil (90 mg, quant. yield). $[\alpha]_D^{20}$ = +12.7 (c 0.71, MeOH). For $C_{23}H_{43}N_5O_9P_2$ (595.56) calcd: C, 46.38; H, 7.28; N, 11.76; P, 10.40. Found: C, 46.43; H, 7.35; N, 11.72; P, 10.50. Other analysis data (FABMS and NMR) correspond with the compound **24a**.

6.4.3 General procedure for preparation of free phosphonic acids

Dried esters (**22a,b**; **24a,b**; **25a,b**; **26a,b**; 0.4-0.5 mmol), acetonitrile (15 ml) and $BrSiMe_3$ (2-3 ml) were stirred at room temperature overnight. After evaporation and co-distillation with acetonitrile, the residue was treated with water and aqueous ammonia. The mixture

was evaporated to dryness, and the residue dissolved in water was applied onto a column of Dowex 50 X 8 (H^+ form).

1. Elution with water and evaporation in vacuo afforded free phosphonic acids **27a**; **28a,b**; **29a,b**; **30a**.

2. Elution with dilute ammonia and evaporation afforded ammonium salts, which were applied onto Dowex 50 (Na^+ form). Elution with water and evaporation gave phosphonic acids **27b** and **30b** as tetrasodium salts. Precipitation of free bisphosphonic acids were obtained after the treatment of sodium salts with HCl in water.

(2*R*,3*R*)-2,6-Diamino-9-{3-(hydroxy)-1,4-[bis(phosphonomethoxy)]butan-2-yl}purine (27a)
White solid (0.18 g, yield 83 %), mp 142.1 °C (D). $[\alpha]_D^{20} = +19.2$ (c 0.34, H_2O). For $C_{11}H_{20}N_6O_9P_2$ (442.26) calcd: C, 29.87; H, 4.56; N, 19.00; P, 14.01. Found: C, 29.95; H, 4.62; N, 19.12; P, 13.95. FABMS: 443.3 (MH^+) (40). ^{1}H NMR (500 MHz, D_2O): 8.20 (s, 1H, Pu-8); 4.82 (dt, 1H, $J_{3,4} \sim J_{3,2} = 7.4$, $J_{3,2'} = 3.2$, H-3); 4.32 (ddd, 1H, $J_{4,3} = 7.2$, $J_{4,5} = 5.2$, $J_{4,5'} = 3.8$, H-4); 4.30 (dd, 1H, $J_{gem} = 11.0$, $J_{2,3} = 7.7$, H-2); 4.09 (dd, 1H, $J_{gem} = 11.0$, $J_{2',3} = 3.3$, H-2'); 3.80 (dd, 1H, $J_{gem} = 13.3$, $J_{1,P} = 8.8$, H-1); 3.73 (dd, 1H, $J_{gem} = 13.3$, $J_{1',P} = 9.0$, H-1'); 3.65-3.61 (m, 3H, H-6,6', H-5); 3.44 (dd, 1H, $J_{gem} = 10.8$, $J_{5',4} = 5.2$, H-5'). ^{13}C NMR (125.7 MHz, D_2O): 153.03 (Pu-2); 151.02 (Pu-6); 150.94 (Pu-4); 142.27 (Pu-8); 111.17 (Pu-5); 73.74 (d, $J_{5,P} = 12.3$, C-5); 70.53 (d, $J_{2,P} = 12.7$, C-2); 69.22 (C-4); 67.52 and 67.47 (2 x d, $J_{P,C} = 157.8$ and 157.5, C-1 and C-6); 56.85 (C-3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 250$ nm ($\epsilon_{max} = 7804$); (H_2O) $\lambda_{max} = 251$ nm ($\epsilon_{max} = 7286$); (0.01 M NaOH) $\lambda_{max} = 254$ nm ($\epsilon_{max} = 6562$).

(2*S*,3*S*)-2,6-Diamino-9-{3-(hydroxy)-1,4-[bis(phosphonomethoxy)]butan-2-yl}purine (27b)
The synthetic procedure (followed by method 2), NMR and UV data correspond with the compound **27a**. White solid (0.23 g, yield 88 %). $[\alpha]_D^{20} = -19.0$ (c 0.20, H_2O).

(2*R*,3*R*)-9-{3-(Hydroxy)-1,4-[bis(phosphonomethoxy)]butan-2-yl}adenine (28a)
White solid (0.21 g, yield 93 %), mp 147.2 °C D. $[\alpha]_D^{20} = +12.2$ (c 0.26, H_2O). For $C_{11}H_{19}N_5O_9P_2$ (427.24) calcd: C, 30.92; H, 4.48; N, 16.39; P, 14.50. Found: C, 30.98; H, 4.56; N, 16.35; P, 14.61. FABMS: 428.3 (MH^+) (40). ^{1}H NMR (500 MHz, D_2O): 8.56 (s, 1H, Pu-8); 8.41 (s, 1H, Pu-2); 5.04 (dt, 1H, $J_{3,4} \sim J_{3,2} = 7.5$, $J_{3,2'} = 3.3$, H-3); 4.44 (ddd, 1H, $J_{4,3} = 7.6$, $J_{4,5} = 5.2$, $J_{4,5'} = 3.7$, H-4); 4.34 (dd, 1H, $J_{gem} = 11.1$, $J_{2,3} = 7.4$, H-2); 4.10 (dd, 1H, $J_{gem} = 11.0$, $J_{2',3} = 3.4$, H-2'); 3.73 (dd, 1H, $J_{gem} = 13.4$, $J_{1,P} = 8.8$, H-1); 3.69 (dd, 1H, $J_{gem} = 13.4$, $J_{1',P} = 8.8$, H-1'); 3.63 (m, 1H, H-5); 3.58 (m, 2H, 6 and 6'); 3.45 (dd, 1H, $J_{gem} = 10.8$, $J_{5',4} = 5.2$, H-5'). ^{13}C NMR (125.7 MHz, D_2O): 150.54 (Pu-6); 149.27 (Pu-4); 145.07 (Pu-8); 144.94 (Pu-2); 118.55 (Pu-5); 73.86 (d, $J_{5,P} = 11.7$, C-5); 70.76 (d, $J_{2,P} = 12.2$, C-2); 69.26 (C-4); 67.50 and 67.38 (2 x d, $J_{P,C} = 158.2$ and 157.7, C-1 and C-6); 57.36 (C-3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 257$ nm ($\epsilon_{max} = 12992$); (H_2O) $\lambda_{max} = 258$ nm ($\epsilon_{max} = 13274$); (0.01 M NaOH) $\lambda_{max} = 259$ nm ($\epsilon_{max} = 13122$).

(2*S*,3*S*)-9-{3-(Hydroxy)-1,4-[bis(phosphonomethoxy)]butan-2-yl}adenine (28b)
The synthetic procedure and analysis data correspond with the compound **28a**. $[\alpha]_D^{20} = -11.9$ (c 0.2, H_2O).

(2*R*,3*R*)-9-{3-(Hydroxy)-1,4-[bis(phosphonomethoxy]butan-2-yl}guanine (29a)

White solid (0.12 g, yield 63 %), mp 163.3 °C D. $[\alpha]_D^{20} = +10.9$ (c 0.23, H_2O). For $C_{11}H_{19}N_5O_{10}P_2$ (443.24) calcd: C, 29.81; H, 4.32; N, 15.80; P, 13.98. Found: C, 29.92; H, 4.36; N, 15.85; P, 13.87. FABMS: 444.2 (MH^+) (60). ^{1}H NMR (500 MHz, D_2O): 9.08 (bs, 1H, Pu-8); 5.00 (dt, 1H, $J_{3,4} \sim J_{3,2} = 6.9$, $J_{3,2'} = 3.3$, H-3); 4.38 (dt, 1H, $J_{4,3} = 7.1$, $J_{4,5} \sim J_{4,5'} = 4.6$, H-4); 4.29 (dd, 1H, $J_{gem} = 11.0$, $J_{2,3} = 6.6$, H-2); 4.04 (dd, 1H, $J_{gem} = 11.0$, $J_{2',3} = 3.3$, H-2'); 3.74 (m, 2H, H-1); 3.70 (dd, 1H, $J_{gem} = 10.7$, $J_{5,4} = 4.2$, H-5); 3.65 (d, 2H, $J = 6.6$, H-6); 3.58 (dd, 1H, $J_{gem} = 10.7$, $J_{5',4} = 5.0$, H-5'). ^{13}C NMR (125.7 MHz, D_2O): 155.98 and 155.96 (Pu-6 and Pu-2); 150.86 (Pu-4); 138.34 (Pu-8); 108.26 (Pu-5); 73.77 (d, $J_{5,P} = 12.1$, C-5); 69.98 (d, $J_{2,P} = 12.5$, C-2); 68.80 (C-4); 67.43 and 67.39 (2 x d, $J_{P,C} = 158.3$ and 158.0, C-1 and C-6); 57.76 (C-3). UV spectrum: (0.01 M HCl) $\lambda_{max} = 258$ nm ($\epsilon_{max} = 10960$); (H_2O) $\lambda_{max} = 258$ nm ($\epsilon_{max} = 10968$); (0.01 M NaOH) $\lambda_{max} = 282$ nm ($\epsilon_{max} = 10796$).

(2*S*,3*S*)-9-{3-(Hydroxy)-1,4-[bis(phosphonomethoxy]butan-2-yl}guanine (29b)

The synthetic procedure and analysis data correspond with the compound **29a**. $[\alpha]_D^{20} = -10.5$ (c 0.16, H_2O).

(2*R*,3*R*)-2-Amino-6-(cyclopropyl)amino-9-{3-(hydroxy)-1,4-[bis(phophosphono)methoxy]butan-2-yl}purine (30a)

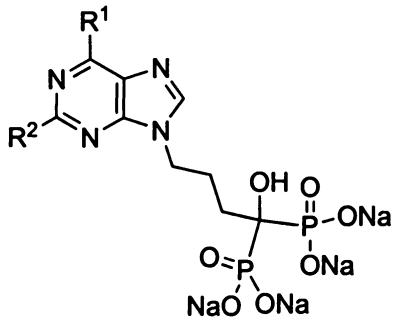
White solid (0.16 g, yield 84 %), mp 164.3 °C D. $[\alpha]_D^{20} = +12.9$ (c 0.25, H_2O). For $C_{14}H_{24}N_6O_9P_2$ (482.32) calcd: C, 34.86; H, 5.02; N, 17.42; P, 12.84. Found: C, 34.96; H, 4.96; N, 17.35; P, 12.71. FABMS: 483.1 (MH^+) (65). ^{1}H NMR (500 MHz, D_2O): 8.16 (bs, 1H, Pu-8); 4.80 (dt, 1H, $J_{3,4} \sim J_{3,2} = 7.2$, $J_{3,2'} = 3.1$, H-3); 4.34 (ddd, 1H, $J_{4,3} = 7.8$, $J_{4,5} = 3.7$, $J_{4,5'} = 5.1$, H-4); 4.26 (dd, 1H, $J_{gem} = 10.9$, $J_{2,3} = 7.2$, H-2); 4.05 (dd, 1H, $J_{gem} = 10.9$, $J_{2',3} = 3.2$, H-2'); 3.76-3.57 (m, 5H, H-1,1', H-5, H-6,6'); 3.41 (dd, 1H, $J_{gem} = 10.8$, $J_{5',4} = 5.1$, H-5'); 2.95 (bs, 1H, $CH_{cycloprop.}$); 1.04 (m, 2H, CH_2); 0.84 (m, 2H, CH_2). ^{13}C NMR (125.7 MHz, D_2O): 153.26 and 151.43 (Pu-2 and Pu-6); 149.62 (Pu-4); 141.95 (Pu-8); 111.71 (Pu-5); 73.74 (d, $J_{5,P} = 12.1$, C-5); 70.72 (d, $J_{2,P} = 12.2$, C-2); 69.20 (C-4); 67.66 and 67.58 (2 x d, $J_{P,C} = 157.7$ and 157.6, C-6 and C-1); 56.72 (C-3); 23.44 (bs, $CH_{cycloprop.}$); 7.47 (bs, 2C, 2 x CH_2). UV spectrum: (0.01 M HCl) $\lambda_{max} = 253$ nm and 292 nm ($\epsilon_{max} = 9216$ and 11408); (H_2O) $\lambda_{max} = 253$ nm and 290 nm ($\epsilon_{max} = 9156$ and 11394); (0.01 M NaOH) $\lambda_{max} = 258$ nm and 282 nm ($\epsilon_{max} = 7590$ and 12546).

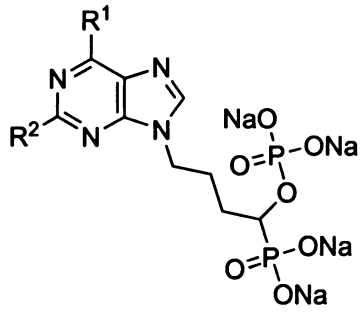
(2*S*,3*S*)-2-Amino-6-(cyclopropyl)amino-9-{3-(hydroxy)-1,4-[bis(phophosphono)methoxy]butan-2-yl}purine (30b)

The synthetic procedure (followed by method 2), NMR and UV data correspond with the compound **30a**. White solid (0.20 g, yield 88 %). $[\alpha]_D^{20} = -13.1$ (c 0.16, H_2O).

Chapter 7

Synthesis of bisphosphonate and phosphate-phosphonate acyclic nucleosides based on alendronate





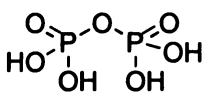
7.1 Introduction

Over the past two decades, several methylene bisphosphonates (BPs) were established for the treatment of various diseases which are associated with excessive bone resorption,[125] including Paget's disease, myeloma and bone metastases, hypercalcemia (raised blood calcium) of malignancy, and osteoporosis.[126] Their therapeutic use, however, is hindered by rather poor oral bioavailability.[127,128]

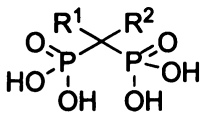
Bisphosphonates are synthetic stable analogues of inorganic pyrophosphate, characterized by a basic phosphate-carbon-phosphate core. The P-C-P moiety is responsible for the

strong affinity of the BPs to hydroxyapatite. This ability of BPs is enhanced with R^1 substituent being hydroxyl group.[129,130]

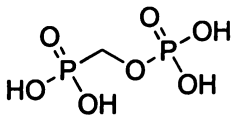
Phosphate-phosphonates (PPs) are structurally similar to both pyrophosphate and bisphosphonates (being a combination of both). It can be, therefore, expected, that they could function similarly, *i.e.*, to control bone mineralization. In blood plasma or upon entering the cell membrane, the phosphate group is known to be hydrolyzed by phosphatase[20] (the sole reason for using enzymatically stable phosphonate group instead of phosphate), providing P-C moiety and hydroxyl group at position equivalent to R^1 in BP structure. The affinity to hydroxyapatite might be lowered by the fact that only one phosphorus-containing group is left. However, these compounds are still worth investigating, since the exact mechanism of the BP function is not completely clear. We, therefore, synthesized a series of PPs structurally equivalent to prepared BPs (see below).



inorganic pyrophosphate



general structure of BP



phosphate-phosphonate

Bisphosphonates can be classified into two classes according to their chemical structure and mechanism of action. Non-nitrogen-containing BPs (such as etidronate and clodronate), and the more potent nitrogen-containing BPs (such as alendronate, ibandronate, zoledronate, pamidronate and risedronate). See Figure 7.1 for the structures of BPs used in clinical practice.

Bisphosphonates, especially those containing a tertiary nitrogen or nitrogen atom as a part of the heterocyclic ring, are the most potent at inhibiting bone resorption.[131] Although all BPs have similar physicochemical properties, their antiresorbing activities differ. The goal in the development of new BPs is, therefore, to find compounds with higher antiresorptive activity, giving broader safety with respect to normal bone mineralization.

Quite a few BPs carrying simple heteroaromatic moieties have been prepared and tested for their osteoporosis activity.[132,133] However, purine containing BPs have not been studied with respect to osteoporosis treatment. Following our previous studies on similar compounds[3,4] we wanted to contribute to this area of research. We synthesized new BP (alendronate like structure) and phosphate-phosponate containing imidazole ring (zoledronate) as a part of the purine moiety. The aim of this work was also to explore potential biological activity (not limited to osteoporosis) of such substances.

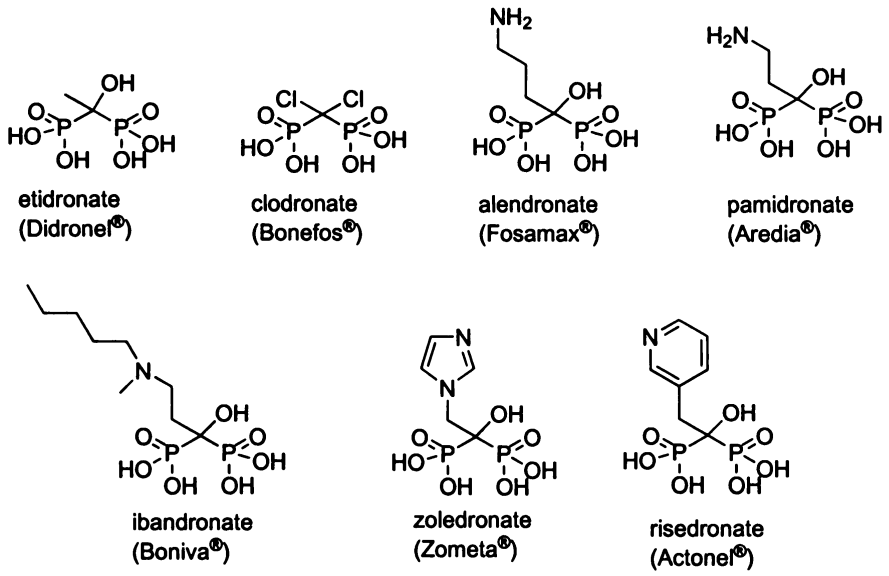


Figure 7.1: Bisphosphonates used in clinical medicine

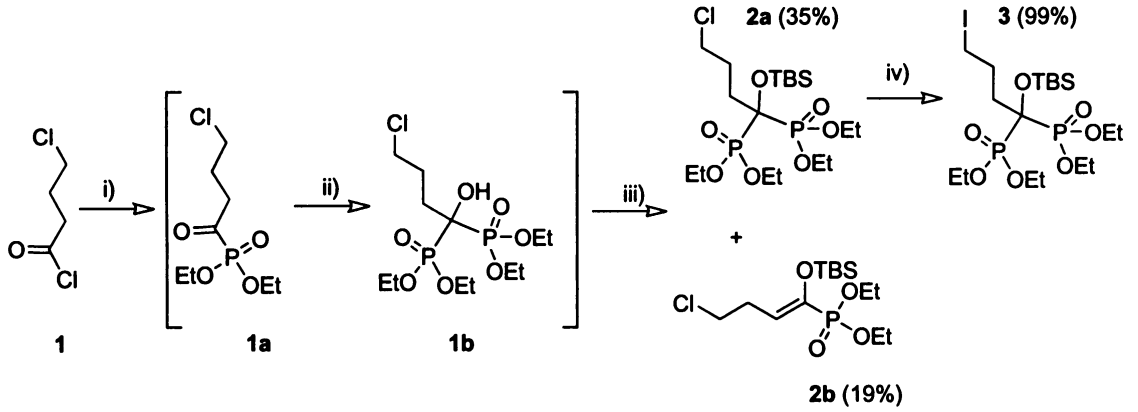
7.2 Results and discussion

The synthetic strategy was firstly directed to the preparation of appropriate alendronate bisphosphonate building block, which was further used as an alkylating agent for various nucleobases.

First, 4-bromobutyryl chloride was used as starting compound and was treated with triisopropyl phosphite, diisopropyl phosphite, DMAP, and *tert*-butyldimethylsilyl chloride in dichloromethane according to the literature[134] but the desired product could not be isolated. Another reaction path - substitution of 4-bromo to 4-chloro derivative and subsequent reaction with above mentioned reagents under the same conditions also did not lead to the product. Original recipe suggested using 4-chloro derivative. In the alkylation reactions, however, much better yields are observed for bromo-substituted agents, which we tried to use in our case. Furthermore, isopropyl groups on the phosphonate moiety were used due to their higher stability (when compared to ethyl) in alkylation reactions and nucleobase transformations (see below - Transformation of the C-Cl bond).

In both cases we observed conversion in the Michaelis-Arbuzov reaction (first step), however, no product was formed after the addition of diisopropyl phosphite to α-ketophosphonate (TLC). This situation probably happened due to high reactivity of bromide and the possible attack of P-nucleophile. Another effect could come from the size of the isopropyl

ester groups. They are much bigger than ethyl ester groups reported in the original article,[134] possibly causing steric hindrance. Due to inability to isolate the desired product, we reverted back to using the ethyl ester groups in the subsequent reactions.



i) $P(OEt)_3$, 0 °C; ii) $HP(O)(OEt)_2$, DMAP, CH_2Cl_2, RT; iii) TBSCl, RT; iv) NaI, acetone, RT.

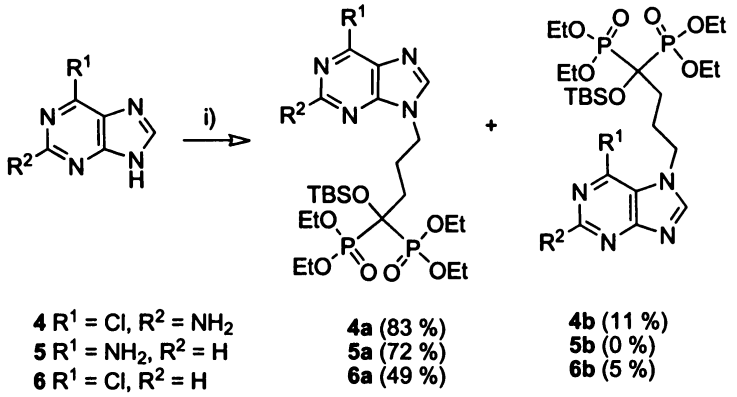
Figure 7.2: Synthesis of methylene bisphosphonate building block

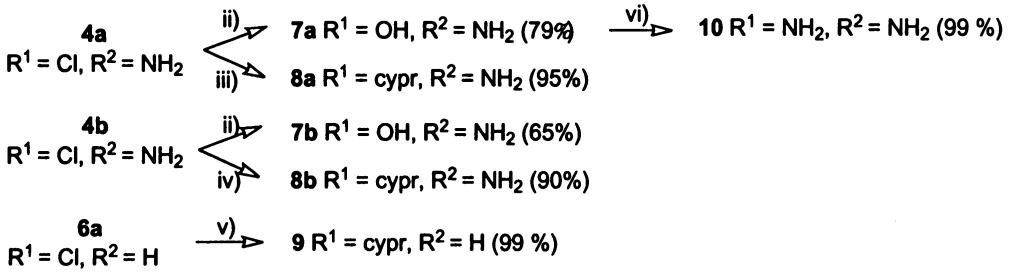
The alendronate BP **2a** was synthesized using Michaelis-Arbuzov[135] and Pudovik[136] reactions performed in a one-pot sequence (Figure 7.2). It was, however, necessary to modify reaction conditions to obtain yields comparable to values reported previously.[134] The formation of side-product, (*E*)-diethyl 1-(*tert*-butyldimethylsilyloxy)-4-chlorobut-1-enylphosphonate (**2b**), can be used to explain the lower yield of **2a**. The configuration of side product **2b** was determined by NMR and acording to the literature.[137]

Subsequent alkylation reaction of 4-chloro BP **2a** with various nucleobases failed under the standard conditions (Cs_2CO_3, DMF, 90 °C). Higher temperature and the presence of not particularly stable diethyl ester phosphonate groups caused the fact that only N^9-ethyl substituted nucleobases were isolated. Alkylation at lower temperature (70 °C) in the presence of catalytic amount of sodium iodide provided desired poduct in modest yield (22 %). Using of 4-iodo derivative **3**, prepared from its 4-chloro congener **2a**, in alkylation reaction was the strategy of choice (Figure 6.5). Introduction of more reactive leaving group enabled us to decrease the reaction temperature (40 °C) and thus overtake the formation of side N^9-ethyl substituted derivative.

The alkylation reaction of 2-amino-6-chloropurine **4** and 6-chloropurine **6** easily proceeded with **3** at both N^9 and N^7 positions (Figure 7.3). These isomers were separated by silica gel column chromatography. The alkylation of adenine **5** gave exclusively the N^9-isomer. In all the discussed cases, NMR analysis was used to identify the position of substitution on

the purine moiety. All signals of hydrogen and carbon atoms were assigned usig 2D-$^{1}H,^{13}C$ HSQC and 2D-$^{1}H,^{13}C$ HMBC experiments. In the case of N^9-isomers, protons on carbon atom next to the purine nitrogen have strong crosspeaks in HMBC spectra with carbons C-4 and C-8 of the purine moiety. In N^7-isomers these protons correlate with C-5 and C-8 atoms.



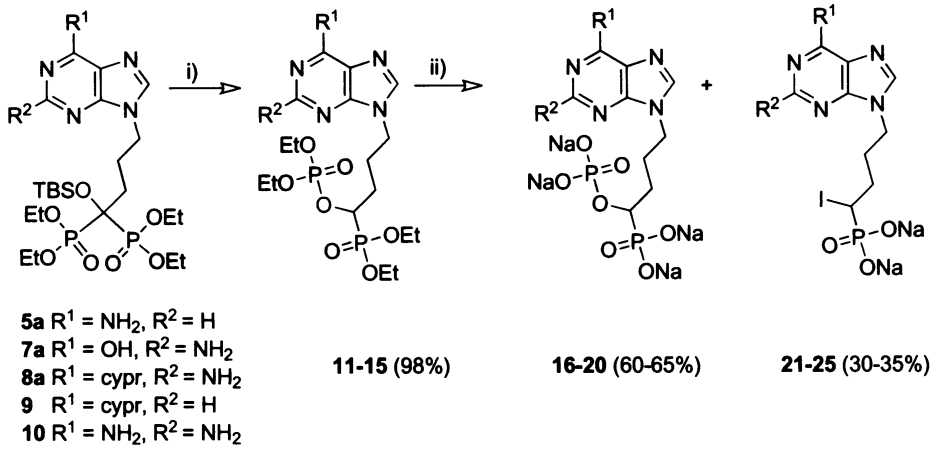


i) **3**, Cs_2CO_3, DMF, 40 °C; ii) 80 % CH_3COOH, reflux; iii) cyclopropylamine, 40 °C; iv) cyclopropylamine, CH_3CN, 60 °C; v) cyclopropylamine, RT; vi) $(CF3CO)_2O$, pyridine, 0 °C, Ar, then gaseous NH_3, 0 °C -> RT

Figure 7.3: Alkylation of nucleobases with alendronate-like bisphosphonate

Transformation of the C-Cl bond of purines **4a,b** and **6a** under standard conditions[1,2] afforded the bisphosphonates **7a,b**, **8a,b** and **9** (Figure 7.3). Due to the presence of not very stable ethyl ester groups of phosphonates, the typical conversion of 2-amino-6-chloro purine **4a** to its 2,6-diamino derivative **10** by amonolysis had to be replaced by a reaction under mild conditions. Therefore, 2,6-diamino derivative **10** was prepared from guanine BP **7a** in reaction with trifluoroacetanhydride in pyridine, followed by the treatment with gaseous ammonia.

An interesting property of α-hydroxyphosphonates and bisphosphonates, is the ability of the P-C-O arrangement to isomerize to C-O-P.[138,139] This reaction must be taken into



5a $R^1 = NH_2$, $R^2 = H$
7a $R^1 = OH$, $R^2 = NH_2$
8a R^1 = cypr, $R^2 = NH_2$
9 R^1 = cypr, $R^2 = H$
10 $R^1 = NH_2$, $R^2 = NH_2$

11-15 (98%) **16-20** (60-65%) **21-25** (30-35%)

i) 1M TBAF in THF, RT; ii) TMSI, CH_3CN, RT.

Figure 7.4: Rearrangement of P-C(OH)-P to P-C-O-P

account when all phosphonic hydroxyl groups are substituted, and at pH greater than 8 and/or at temperature above 60 °C. This rearrangement was indeed observed in our attempt to cleave TBS group of **5a**, **7a**, **8a**, **9** and **10** (Figure 7.4). NMR study indicated that P-C-P (bisphosphonate) bridge of **5a**, **7a**, **8a**, **9** and **10** rearranges to P-C-O-P core of **11-15**. The formation of phosphate-phosphonate moiety was confirmed also using hydrolysis catalyzed by alkaline phosphatase. The progress of the reaction and creation of the degradation product was monitored by HPLC. (For the result see Figure 7.5).

Dealkylation of both diethyl ester groups of **11-15** with iodotrimethylsilane, followed by hydrolysis, gave a mixture of two products **16-20** and **21-25** in ratio 2:1 in favour of phosphate-phosphonate **16-20** (Figure 7.4). The presence of the iodo derivative can be explained as substitution of less stable phosphate group by iodide coming from iodotrimethylsilane. The sodium salts of compounds **16-25** were obtained after their purification by ion exchange chromatography Dowex 50 X 8 (H^+ form) or HPLC and ion exchange chromatography Dowex 50 X 8 (Na^+ form).

To avoid the rearrangement discussed above, another synthetic strategy had to be used. It is known that isomerisation does not occur if less than four hydroxyl groups of phosphonate are substituted. Therefore, the dealkylation of ester groups of bisphosphonate **5a**, **7a,b**, **8a,b**, **9**, and **10**, followed by cleavage of TBS was our new strategy of choice (Figure 7.6). This reaction sequence indeed prevented the formation of phosphate-phosphonate derivatives and allowed us to obtain free 1-hydroxy-1,1-bisphosphonic acids or their amonium

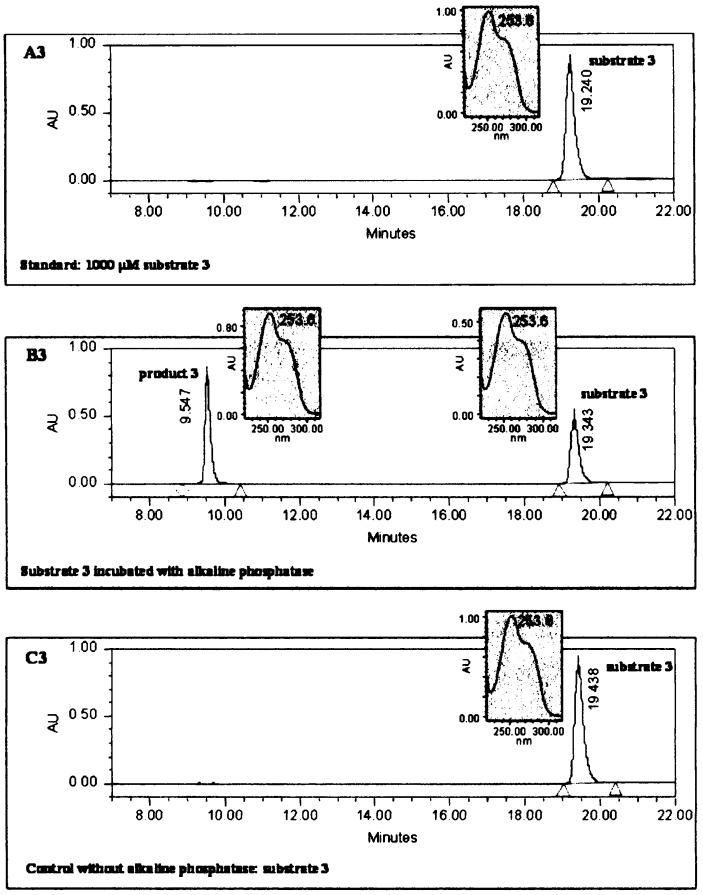


Figure 7.5: Enzymatic hydrolysis of phosphate bond of compound **17**

salts after the purification by ion exchange chromatography or HPLC. Isolated derivatives were further converted to the respective sodium salts **26-32** by Dowex 50 X 8 (Na^+ form) ion exchange chromatography.

7.3 Conclusions

New series of bisphosphonate acyclic nucleosides based on alendronate was synthesized. Also new series of phosphate-phosphonate acyclic nucleosides was prepared as an outcome of rearrangement occuring during the cleavage of TBS group. The aim of this work was to evaluate biological, not only osteporosis-related activity of these compounds, and with the aim to find compounds with higher antiresorptive activity. Purine moiety was introduced as a new part of the alendronate-like structure.

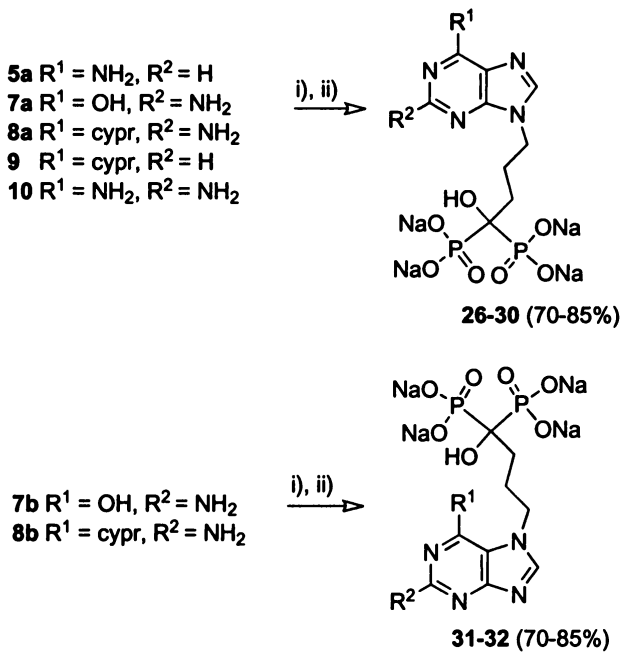


Figure 7.6: The synthesis of 1-hydroxy-1,1-bisphosphonates

Finally, new series of α-iodophosphonate acyclic nucleosides was obtained after the treatment of phosphate-phosphonate moiety with iodotrimethylsilane in acetonitrile. These compounds could be very interesting for antiviral screening.

All substances are currently undergoing testing for anti-osteoporotic, antiviral, and cytostatic activity.

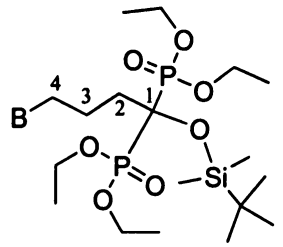
7.4 Experimental part

Unless otherwise stated, solvents were evaporated at 40 °C / 2 kPa, and compounds were dried over P_2O_5 at 2 kPa. NMR spectra were measured on a Bruker Avance-500 instrument (500.0 MHz for ^{1}H and 125.7 MHz for ^{13}C). Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. Mass spectra were measured on a ZAB-EQ (Micromass, Manchester, U.K.) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV) or using EI (electron energy 70 eV). UV spectra (λ in nm) were taken on a Beckman Coulter, DU 800 spectrophotometer. Elemental analyses were carried out on

a Perkin–Elmer 2400 Series II CHNS/O Analyser. Preparative HPLC was performed on Waters 2487 Delta 600 (Terra RP18 column, 10 μm, 19 x 150 mm). Dimethylformamide, acetonitrile and dichloromethane were distilled from P_2O_5 and stored over molecular sieves (4 Å). Pyridine was dried over KOH and distilled with $KMnO_4$. Acetone was dried over anhydrous $CuSO_4$. Alkaline phosphatase from Escherichia coli, bovine serum albumin, tri-*n*-octylamine, tetrabutylammonium hydrogen sulfate were purchased from Sigma-Aldrich (Prague, Czech Republic), 1,1,2,-trichlorotrifluoroethane from Merck (Darmstadt, Germany), glycine from Lachema (Czechoslovakia), TCA, potassium dihydrogen-phosphate, potassium hydrogen-phosphate, $MgCl_2 \cdot 6H_2O$ from Serva (Heidelberg, Grmany), Supelcosil LC-18T column (4 mm x 15 cm, 3 μm) from Supelco (St. Louis, USA), acetonitrile and $ZnCl_2$ from Fluka (St. Louis, USA).

HPLC analysis samples were analyzed using Waters Alliance System 2795 (2996 PDA Detector, PDA Software Millenium[32]). The analytical separation was performed on Supelcosil LC-18T column (4 mm x 15 cm, 3 μm) at the flow rate of 0.75 ml.min^{-1}. Mobile phase consisted of acetonitrile in 50 mM potassium phosphate buffer (pH 5.1) and 3 mM tetrabutylammonium hydrogen sulfate. A non-linear gradient of acetonitrile in phosphate buffer was used for the separation of substrates and products. Substrates were identified on the basis of their retention times and comparison of UV spectra with library of external standards.

Numbering for NMR analysis



B = nucleobase

Tetraethyl 1-(*tert*-butyldimethylsilyloxy)-4-chlorobutane-1,1-diyldiphosphonate (2a)
Oven-dried flask was charged with 4-chlorobutyryl chloride (25 g, 0.18 mmol) under argon atmosphere. Triethyl phosphite (26.5 g, 0.16 mmol) was added dropwise at 0 °C. The mixture was allowed to reach room temperature and stirred for additional 20 minutes. Anhydrous dichloromethane (500 ml), diethyl phosphite (22.4 g, 0.16 mmol) and DMAP (22 g, 0.18 mmol) were sequentially added. The reaction mixture was stirred at room temperature for 1 h. *Tert*-butyldimethylsilyl chloride (27 g, 0.18 mmol) was added

in the reaction and stirred at room temperature for another 18 hours. The reaction mixture was then washed with 0.1 M aqueous HCl (twice). The organic layer was evaporated and the residue was purified by silica gel comlumn chromatography, using hexane-ethylacetate 1:2 as elution solvent, to yield 31.2 g (35 %) of pure **2a** as yellowish oil. FABMS: 496.1 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 4.22 (m, 8H, P-O-CH_2); 3.55 (t, 2H, $J_{4,3}=6.0$, H-4); 2.19 (m, 4H, H-2 and H-3); 1.35 (t, 12H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 0.91 (s, 9H, C-$(CH_3)_3$); 0.21 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 77.68 (t, $J_{1,P}=157.0$, C-1); 62.85 (m, P-O-CH_2); 45.54 (C-4); 33.49 (C-2); 27.12 (t, $J_{3,P}=5.3$, C-3); 25.79 (C-$(CH_3)_3$); 18.99 (C-$(CH_3)_3$); 16.44 (m, P-O-CH_2CH_3); -2.58 (Si$(CH_3)_2$).

Diethyl 1-(*tert*-butyldimethylsilyloxy)-4-chlorobut-1-enylphosphonate (2b)
The synthetic procedure corresponds to the compound **2a**. The organic layer was evaporated and the residue was purified by silica gel column chromatography, using hexane-ethylacetate 2:1 as elution solvent, to yield 12.2 g (19 %) of pure **2b** as yellowish oil. FABMS: 357.2 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 5.86 (dt, 1H, $J_{2,3}=7.1$, $J_{2,P}=10.3$, H-2); 4.10 (m, 4H, P-O-CH_2); 3.54 (t, 2H, $J_{4,3}=7.0$, H-4); 2.65 (dq, 2H, $J_{3,4}\sim J_{3,2}=7.0$, $J_{3,P}=3.0$, H-3); 1.34 (dt, 6H, $J_{CH_3,CH_2}=7.1$, $J_{CH_3,P}=0.5$, P-O-CH_2CH_3); 0.97 (s, 9H, C-$(CH_3)_3$); 0.20 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 143.28 (d, $J_{1,P}=217.9$, C-1); 123.39 (d, $J_{2,P}=35.4$, C-2); 62.02 (d, $J_{CH_2,P}=5.6$, P-O-CH_2); 42.89 (d, $J_{4,P}=1.9$, C-4); 28.86 (d, $J_{3,P}=13.4$, C-3); 25.75 (C-$(CH_3)_3$); 18.53 (C-$(CH_3)_3$); 16.24 (m, $J_{CH_3,P}=6.5$, P-O-CH_2CH_3); -4.33 (Si$(CH_3)_2$).

Tetraethyl 1-(*tert*-butyldimethylsilyloxy)-4-iodobutane-1,1-diyldiphosphonate (3)
Sodium iodide (4 equiv.) was added to a stirred mixture of **2a** (30 g, 60 mmol) in dry acetone (500 ml) at room temperature. The reaction mixture was aged at room temperature for 24 h. The precepitate was filtered and the residue was purified by silica gel column chromatography (1-2 % gradient of MeOH in $CHCl_3$), to yield 35.0 g (99 %) of pure **3** as yellowish oil. FABMS: 587.3 (MH^+) (85). ^{1}H NMR (500 MHz, $CDCl_3$): 4.21 (m, 8H, P-O-CH_2); 3.19 (m, 2H, H-4); 2.16 (m, 4H, H-2 and H-3); 1.36 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 1.35 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 0.91 (s, 9H, C-$(CH_3)_3$); 0.21 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 77.48 (t, $J_{1,P}=156.8$, C-1); 62.95 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.4$, P-O-$CH_2$); 62.80 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.3$, P-O-$CH_2$); 37.11 (C-2); 27.66 (t, $J_{3,P}=5.2$, C-3); 25.78 (C-$(CH_3)_3$); 18.97 (C-$(CH_3)_3$); 16.47 (m, P-O-CH_2CH_3); 7.37 (C-4); -2,52 (Si$(CH_3)_2$).

7.4.1 General procedure for alkylation of nucleobases

A solution of an appropriate nucleobase (**4**, **5**, **6**, 1.0 equiv.) in dry DMF was treated with Cs_2CO_3 (0.5 equiv.) under a $CaCl_2$ protecting tube at 60 °C for 1 h. The reaction mixture was then heated at 40 °C and bisphosphonate **3** (1.0 equiv.) was added. The mixture was stirred at 40 °C for 24 h. The mixture was neutralized with 4.5 M HCl/DMF, solvent was evaporated and the residue was co-evaporated with toluene (twice). The residue dissolved in 10% MeOH in chloroform was filtered through a Celite pad, evaporated and purified on a silica gel column in chloroform–methanol gradient.

Tetraethyl 4-(2-amino-6-chloro-9*H*-purin-9-yl)-1-(*tert*-butyldimethylsilyloxy)butane-1,1-diyldiphosphonate (4a)

Material: 5.2 mmol of **4**, 2.6 mmol Cs_2CO_3, 5.2 mmol of **3**, 60 ml of DMF.

Purification on column chromatography (silica gel, 1-5 % gradient of MeOH in $CHCl_3$) afforded the product **4a** as yellowish foam (yield 83 %). FABMS: 629.2 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 7.78 (s, 1H, Pu-8); 5.16 (bs, 2H, NH_2); 4.17 (m, 8H, P-O-CH_2); 4.09 (t, 2H, $J_{4,3}=6.6$, H-4); 2.26 (m, 2H, H-3); 2.04 (m, 2H, H-2); 1.31 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 1.29 (t, 6H, $J_{CH_3,CH_2}=7.0$, P-O-CH_2CH_3); 0.86 (s, 9H, C-$(CH_3)_3$); 0.10 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 158.98 (Pu-2); 153.96 (Pu-4); 151.24 (Pu-6); 142.19 (Pu-8); 125.22 (Pu-5); 77.35 (t, $J_{1,P}=157.0$, C-1); 63.01 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.4$, P-O-$CH_2$); 62.84 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.3$, P-O-$CH_2$); 43.84 (C-4); 33.02 (C-2); 25.67 (C-$(CH_3)_3$); 24.61 (t, $J_{3,P}=4.8$, C-3); 18.89 (C-$(CH_3)_3$); 16.40 (m, P-O-CH_2CH_3); -2.71 (Si$(CH_3)_2$).

Tetraethyl 4-(2-amino-6-chloro-7*H*-purin-7-yl)-1-(*tert*-butyldimethylsilyloxy)butane-1,1-diyldiphosphonate (4b)

Material: 5.2 mmol of **4**, 2.6 mmol Cs_2CO_3, 5.2 mmol of **3**, 60 ml of DMF.

Purification on column chromatography (silica gel, 1-7 % gradient of MeOH in $CHCl_3$) afforded the product **4b** as yellowish foam (yield 11 %). FABMS: 629.1 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 7.96 (s, 1H, Pu-8); 5.08 (bs, 2H, NH_2); 4.33 (t, 2H, $J_{4,3}=6.9$, H-4); 4.18 (m, 8H, P-O-CH_2); 2.30 (m, 2H, H-3); 2.02 (m, 2H, H-2); 1.31 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 1.30 (t, 6H, $J_{CH_3,CH_2}=7.0$, P-O-CH_2CH_3); 0.85 (s, 9H, C-$(CH_3)_3$); 0.12 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 164.35 (Pu-4); 159.25 (Pu-2); 148.34 (Pu-8); 143.41 (Pu-6); 116.40 (Pu-5); 77.24 (t, $J_{1,P}=156.5$, C-1); 63.08 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.4$, P-O-$CH_2$); 62.92 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.3$, P-O-$CH_2$); 47.29 (C-4); 32.77 (C-2); 25.86 (t, $J_{3,P}=5.1$, C-3); 25.67 (C-$(CH_3)_3$); 18.92 (C-$(CH_3)_3$); 16.44 (m, P-O-CH_2CH_3); -2.68 (Si$(CH_3)_2$).

Tetraethyl 4-(6-amino-9*H*-purin-9-yl)-1-(*tert*-butyldimethylsilyloxy)butane-1,1-diyldiphosphonate (5a)

Material: 2.6 mmol of **5**, 1.3 mmol Cs_2CO_3, 2.6 mmol of **3**, 30 ml of DMF.

Purification on column chromatography (silica gel, 1-7 % gradient of MeOH in $CHCl_3$) afforded the product **5a** as white solid (yield 72 %). FABMS: 594.5 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 8.34 (s, 1H, Pu-8); 8.02 (s, 1H, Pu-2); 5.83 (bs, 2H, NH_2); 4.22 (t, 2H, $J_{4,3}=6.8$, H-4); 4.16 (m, 8H, P-O-CH_2); 2.30 (m, 2H, H-3); 2.04 (m, 2H, H-2); 1.30 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 1.29 (t, 6H, $J_{CH_3,CH_2}=7.0$, P-O-CH_2CH_3); 0.85 (s, 9H, C-$(CH_3)_3$); 0.09 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.36 (Pu-6); 152.83 (Pu-2); 150.25 (Pu-4); 140.34 (Pu-8); 119.41 (Pu-5); 77.42 (t, $J_{1,P}=156.9$, C-1); 63.00 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.4$, P-O-$CH_2$); 62.83 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.3$, P-O-$CH_2$); 43.85 (C-4); 33.02 (C-2); 25.68 (C-$(CH_3)_3$); 24.91 (t, $J_{3,P}=4.5$, C-3); 18.88 (C-$(CH_3)_3$); 16.39 (m, P-O-CH_2CH_3); -2.71 (Si$(CH_3)_2$).

Tetraethyl 1-(*tert*-butyldimethylsilyloxy)-4-(6-chloro-9*H*-purin-9-yl)butane-1,1-diyldiphosphonate (6a)

Material: 2.6 mmol of **6**, 1.3 mmol Cs_2CO_3, 2.6 mmol of **3**, 30 ml of DMF.
Purification on column chromatography (silica gel, 1-4 % gradient of MeOH in $CHCl_3$) afforded the product **6a** as yellowish oil (yield 49 %). FABMS: 614.1 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 8.74 (s, 1H, Pu-8); 8.16 (s, 1H, Pu-2); 4.32 (t, 2H, $J_{4,3}=6.9$, H-4); 4.16 (m, 8H, P-O-CH_2); 2.34 (m, 2H, H-3); 2.05 (m, 2H, H-2); 1.31 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 1.29 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 0.84 (s, 9H, C-$(CH_3)_3$); 0.09 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 152.01 (Pu-4); 151.90 (Pu-2); 151.02 (Pu-6); 145.04 (Pu-8); 131.51 (Pu-5); 77.00 (t, $J_{1,P}=156.9$, C-1); 63.08 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.4$, P-O-$CH_2$); 62.88 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.3$, P-O-$CH_2$); 44.50 (C-4); 32.98 (C-2); 25.65 (C-$(CH_3)_3$); 24.75 (t, $J_{3,P}=4.6$, C-3); 18.89 (C-$(CH_3)_3$); 16.41 (m, P-O-CH_2CH_3); -2.74 (Si$(CH_3)_2$).

Tetraethyl 1-(*tert*-butyldimethylsilyloxy)-4-(6-chloro-7*H*-purin-7-yl)butane-1,1-diyldiphosphonate (6b)

Material: 2.6 mmol of **6**, 1.3 mmol Cs_2CO_3, 2.6 mmol of **3**, 30 ml of DMF.
Purification on column chromatography (silica gel, 1-6 % gradient of MeOH in $CHCl_3$) afforded the product **6b** as yellowish oil (yield 5 %). FABMS: 614.1 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 8.82 (s, 1H, Pu-2); 8.29 (s, 1H, Pu-8); 4.47 (t, 2H, $J_{4,3}=6.9$, H-4); 4.10 (m, 8H, P-O-CH_2); 2.30 (m, 2H, H-3); 1.99 (m, 2H, H-2); 1.25 (t, 6H, $J_{CH_3,CH_2}=7.0$, P-O-CH_2CH_3); 1.24 (t, 6H, $J_{CH_3,CH_2}=7.2$, P-O-CH_2CH_3); 0.79 (s, 9H, C-$(CH_3)_3$); 0.05 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 161.70 (Pu-4); 152.22 (Pu-2); 149.09 (Pu-8); 142.91 (Pu-6); 122.28 (Pu-5); 76.98 (t, $J_{1,P}=157.0$, C-1); 63.09 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.4$, P-O-$CH_2$); 62.92 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.3$, P-O-$CH_2$); 47.32 (C-4); 32.50 (C-2); 25.96 (t, $J_{3,P}=4.6$, C-3); 25.48 (C-$(CH_3)_3$); 18.74 (C-$(CH_3)_3$); 16.27 (m, P-O-CH_2CH_3); -2.83 (Si$(CH_3)_2$).

Tetraethyl 4-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-1-(*tert*-butyldimethylsilyloxy)butane-1,1--diyldiphosphonate (7a)

A solution of **4a** (600 mg, 0.95 mmol) in 80% acetic acid (20 ml) was refluxed for 3 h. Reaction mixture was neutralized with Et_3N and the volatile were evaporated in vacuo. Column chromatography (silica gel, 1-14 % gradient of MeOH in $CHCl_3$) afforded compound **7a** as a white solid (yield 79 %). FABMS: 610.5 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 11.93 (bs, 1H, NH); 7.60 (s, 1H, Pu-8); 6.46 (bs, 2H, NH_2); 4.18 (m, 8H, P-O-CH_2); 4.01 (t, 2H, $J_{4,3}=6.8$, H-4); 2.23 (m, 2H, H-3); 2.08 (m, 2H, H-2); 1.32 (t, 6H, $J_{CH_3,CH_2}=7.1$, P-O-CH_2CH_3); 1.30 (t, 6H, $J_{CH_3,CH_2}=7.0$, P-O-CH_2CH_3); 0.86 (s, 9H, C-$(CH_3)_3$); 0.13 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 158.85 (Pu-6); 153.72 (Pu-2); 151.70 (Pu-4); 137.40 (Pu-8); 117.07 (Pu-5); 77.52 (t, $J_{1,P}=157.3$, C-1); 63.14 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.4$, P-O-$CH_2$); 63.02 (t, $J_{C-O-P}=J_{C-O-P-C-P}=3.2$, P-O-$CH_2$); 43.66 (C-4); 33.03 (C-2); 25.72 (C-$(CH_3)_3$); 24.86 (t, $J_{3,P}=6.0$, C-3); 18.91 (C-$(CH_3)_3$); 16.44 (m, P-O-CH_2CH_3); -2.59 (Si$(CH_3)_2$).

Tetraethyl 4-(2-amino-6-oxo-1*H*-purin-7(6*H*)-yl)-1-(*tert*-butyldimethylsilyloxy)butane-1,1--diyldiphosphonate (7b)

A solution of **4b** (450 mg, 0.71 mmol) in 80% acetic acid (20 ml) was refluxed for 3 h. Reaction mixture was neutralized with Et_3N and the volatile were evaporated in vacuo. Column chromatography (silica gel, 1-16 % gradient of MeOH in $CHCl_3$) afforded compound **7b** as a white solid (yield 65 %). FABMS: 610.4 (MH^+) (90). ^{1}H NMR (500 MHz, DMSO-d_6): 10.79 (bs, 1H, NH); 7.91 (s, 1H, Pu-8); 6.06 (bs, 2H, NH_2); 4.20 (t, 2H, $J_{4,3}=6.0$, H-4); 4.01 (m, 8H, P-O-CH_2); 2.05 (m, 2H, H-3); 1.79 (m, 2H, H-2); 1.19 (t, 6H,

J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 1.18 (t, 6H, J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 0.80 (s, 9H, C-$(CH_3)_3$); 0.03 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, DMSO-d_6): 160.18 (Pu-4); 154.77 (Pu-6); 152.83 (Pu-2); 143.36 (Pu-8); 108.37 (Pu-5); 77.39 (t, $J_{1,P}$ = 156.1, C-1); 62.42 (m, P-O-CH_2); 46.11 (C-4); 32.26 (C-2); 25.81 (C-$(CH_3)_3$); 25.69 (t, $J_{3,P}$ = 4.2, C-3); 18.78 (C-$(CH_3)_3$); 16.37 (m, P-O-CH_2CH_3); -2.65 (Si$(CH_3)_2$).

Tetraethyl 4-(2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl)-1-(*tert*-butyldimethylsilyloxy)butane-1,1-diyldiphosphonate (8a)

Compound **4a** (500 mg, 0.8 mmol) was dissolved in cyclopropylamine (2 ml) and stirred at 40 °C for 4 h. The solution was concentrated. Purification on column chromatography (silica gel, 0-6 % gradient of MeOH in $CHCl_3$) afforded the product **8a** as yellowish oil (yield 95 %). FABMS: 649.7 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 7.47 (s, 1H, Pu-8); 5.71 (bs, 1H, NH); 4.74 (bs, 2H, NH_2); 4.16 (m, 8H, P-O-CH_2); 4.02 (t, 2H, $J_{4,3}$ = 6.8, H-4); 3.00 (m, 1H, $CH_{cycloprop.}$); 2.22 (m, 2H, H-3); 2.04 (m, 2H, H-2); 1.31 (t, 6H, J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 1.29 (t, 6H, J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 0.86 (s, 9H, C$(CH_3)_3$); 0.85 (m, 2H, $CH_{2cycloprop.}$); 0.61 (m, 2H, $CH_{2cycloprop.}$); 0.11 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.91 (Pu-2); 156.21 (Pu-6); 151.34 (Pu-4); 137.20 (Pu-8); 114.60 (Pu-5); 77.56 (t, $J_{1,P}$ = 157.5, C-1); 62.96 and 62.81 (2 x t, $J_{C-O-P} \sim J_{C-O-P-C-P}$ = 3.4 and 3.3, P-O-CH_2); 43.32 (C-4); 33.04 (C-2); 25.72 (C$(CH_3)_2$); 24.80 (t, $J_{3,P}$ = 4.9, C-3); 23.64 ($CH_{cycloprop.}$); 18.91 (C$(CH_3)_2$); 16.42 (m, P-O-CH_2CH_3); 7.43 ($CH_{2cycloprop.}$), -2.69 (Si$(CH_3)_2$).

Tetraethyl 4-(2-amino-6-(cyclopropylamino)-7*H*-purin-7-yl)-1-(*tert*-butyldimethylsilyloxy)butane-1,1-diyldiphosphonate (8b)

Cyclopropylamine (1.2 ml) was added to a solution of **4b** (470 mg, 0.75 mmol) in acetonitrile (15 ml) and stirred at 60 °C for 24 h. The solution was concentrated. Purification on column chromatography (silica gel, 0-16 % gradient of MeOH in $CHCl_3$) afforded the product **8b** as yellowish foam (yield 90 %). FABMS: 649.6 (MH^+) (90). ^{1}H NMR (500 MHz, $CDCl_3$): 7.64 (s, 1H, Pu-8); 6.14 (bs, 1H, NH); 5.06 (bs, 2H, NH_2); 4.19 (m, 10H, H-4 and P-O-CH_2); 2.96 (m, 1H, $CH_{cycloprop.}$); 2.17 (m, 2H, H-3); 2.07 (m, 2H, H-2); 1.33 (t, 6H, J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 1.32 (t, 6H, J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 0.87 (s, 9H, C$(CH_3)_3$); 0.81 (m, 2H, $CH_{2cycloprop.}$); 0.74 (m, 2H, $CH_{2cycloprop.}$); 0.13 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 160.81 (Pu-4); 159.29 (Pu-2); 152.31 (Pu-6); 143.36 (Pu-8); 106.44 (Pu-5); 77.01 (t, $J_{1,P}$ = 157.2, C-1); 63.15 and 63.05 (2 x t, $J_{C-O-P} \sim J_{C-O-P-C-P}$ = 3.5 and 3.3, P-O-CH_2); 47.86 (C-4); 32.30 (C-2); 26.29 (t, $J_{3,P}$ = 4.6, C-3); 25.72 (C$(CH_3)_2$); 24.29 ($CH_{cycloprop.}$); 18.92 (C$(CH_3)_2$); 16.43 (m, P-O-CH_2CH_3); 7.05 ($CH_{2cycloprop.}$), -2.54 (Si$(CH_3)_2$).

Tetraethyl 1-(*tert*-butyldimethylsilyloxy)-4-(6-(cyclopropylamino)-9*H*-purin-9-yl)butane-1,1-diyldiphosphonate (9)

Compound **6a** (360 mg, 0.59 mmol) was dissolved in cyclopropylamine (0.5 ml) and stirred at room temperature for 2 h. The solution was concentrated. Purification on column chromatography (silica gel, 0-5 % gradient of MeOH in $CHCl_3$) afforded the product **9** as yellowish oil (yield 99 %). FABMS: 634.5 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 8.47 (s, 1H, Pu-2); 7.76 (s, 1H, Pu-8); 5.90 (bs, 1H, NH); 4.21 (t, 2H, $J_{4,3}$ = 6.8, H-4); 4.16 (m, 8H, P-O-CH_2); 3.05 (bs, 1H, $CH_{cycloprop.}$); 2.28 (m, 2H, H-3); 2.03 (m, 2H, H-2); 1.30 (t, 6H, J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 1.28 (t, 6H, J_{CH_3,CH_2} = 7.1, P-O-CH_2CH_3); 0.94 (m, 2H, $CH_{2cycloprop.}$); 0.85 (s, 9H, C-$(CH_3)_3$); 0.66 (m, 2H, $CH_{2cycloprop.}$); 0.09 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.73 (Pu-6); 153.13 (Pu-2); 149.46 (Pu-4); 139.72 (Pu-8); 119.82 (Pu-5); 77.46 (t, $J_{1,P}$ = 156.9, C-1); 62.97 (t, $J_{C-O-P} = J_{C-O-P-C-P}$ = 3.3, P-O-CH_2);

62.81 (t, $J_{C-O-P} = J_{C-O-P-C-P} = 3.3$, P-O-$CH_2$); 43.76 (C-4); 33.04 (C-2); 25.69 (C-$(CH_3)_3$); 24.94 (t, $J_{3,P} = 4.6$, C-3); 23.69 ($CH_{cycloprop.}$); 18.90 (C-$(CH_3)_3$); 16.43 (m, P-O-CH_2CH_3); 7.41 ($CH_{2cycloprop.}$); -2.71 (Si$(CH_3)_2$).

Tetraethyl 1-(*tert*-butyldimethylsilyloxy)-4-(2,6-diamino-9*H*-purin-9-yl)butane-1,1-diyldiphosphonate (10)

To a well-dried **7a** (0.56 g, 0.92 mmol, codistilled with pyridine) in dry pyridine (20 ml) was added dropwise trifluoroacetanhydride (1 ml, 7.3 mmol) under argon atmosphere at 0 °C. The reaction mixture was aged at 0 °C for 1 h and subsequently treated with gaseous ammonia for 10 minutes keeping the temperature below 5 °C. The reaction mixture was stirred at room temperature additional 2 h. The solvent was evaporated, co-evaporated with toluene and the resiue was purified on column chromatography (silica gel, 0-8 % gradient of MeOH in $CHCl_3$). The residue was dissolved in 70% aqueous ethanol and applied on Dowex 1 (Cl^- form). The elution with 70% aqueous ethanol afforded the product **10** as white solid (yield 99 %). FABMS: 609.6 (MH^+) (100). ^{1}H NMR (500 MHz, $CDCl_3$): 7.54 (s, 1H, Pu-8); 5.55 (bs, 2H, NH_2); 4.80 (bs, 2H, NH_2); 4.15 (m, 8H, P-O-CH_2); 4.04 (t, 2H, $J_{4,3} = 6.8$, H-4); 2.23 (m, 2H, H-3); 2.04 (m, 2H, H-2); 1.31 (t, 6H, $J_{CH_3,CH_2} = 7.1$, P-O-CH_2CH_3); 1.29 (t, 6H, $J_{CH_3,CH_2} = 7.1$, P-O-CH_2CH_3); 0.86 (s, 9H, C-$(CH_3)_3$); 0.11 (s, 6H, Si$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.19 (Pu-2); 155.29 (Pu-6); 152.24 (Pu-4); 138.15 (Pu-8); 114.11 (Pu-5); 77.48 (t, $J_{1,P} = 157.1$, C-1); 63.01 (t, $J_{C-O-P} = J_{C-O-P-C-P} = 3.3$, P-O-$CH_2$); 62.84 (t, $J_{C-O-P} = J_{C-O-P-C-P} = 3.3$, P-O-$CH_2$); 43.46 (C-4); 33.00 (C-2); 25.70 (C-$(CH_3)_3$); 24.79 (t, $J_{3,P} = 4.3$, C-3); 18.91 (C-$(CH_3)_3$); 16.41 (m, P-O-CH_2CH_3); -2.69 (Si$(CH_3)_2$).

7.4.2 General procedure for rearrangement of bisphoshonate to phosphate-phosphonate

1 M solution of TBAF in THF (6.0 mmol, 6 ml) was added to well dried compounds **5a**, **7a**, **8a**, **9** and **10** (1.0 mmol), respectively, under inert (argon) atmosphere. The reaction was aged at room temperature for 2 h and evaporated.

4-(6-Amino-9*H*-purin-9yl)-1-(diethoxyphosphoryl)butyl diethyl phosphate (11)

Purification on column chromatography (silica gel, 1-6 % gradient of MeOH in $CHCl_3$) afforded compound **11** as a yellowish oil (yield 98 %). FABMS: 480.1 (MH^+) (60). ^{1}H NMR (500 MHz, $CDCl_3$): 8.33 (bs, 1H, Pu-2); 7.87 (s, 1H, Pu-8); 6.02 (bs, 2H, NH_2); 4.69 (m, 1H, H-1); 4.27 (t, 2H, $J_{4,3} = 4.6$, H-4); 4.21-4.07 (m, 8H, P-O-CH_2); 2.23 and 2.11 (2 x m, 2H, H-3); 1.94 (m, 2H, H-2); 1.35-1.28 (m, 12H, P-O-CH_2CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.41 (Pu-6); 152.73 (Pu-2); 149.85 (Pu-4); 140.26 (Pu-8); 119.39 (Pu-5); 71.78 (dd, $J_{C,P} = 171.5$, $J_{C-O-P} = 7.2$, C-1); 64.08 and 62.87 (2 x m, P-O-CH_2); 42.87 (C-4); 27.81 (C-2); 25.63 (t, $J_{3,P} = 10.3$, C-3); 16.26 and 15.86 (2 x m, P-O-CH_2CH_3). 31P NMR (500 MHz, $CDCl_3$, $H_3PO_4 = 0$): 19.81 (d, $J_{P,P} = 22.9$, C-P); -0.29 (d, $J_{P,P} = 22.9$, O-P).

4-(2-Amino-6-oxo-1*H*-purin-9(6*H*)-yl)-1-(diethoxyphosphoryl)butyl diethyl phosphate (12)

Purification on column chromatography (silica gel, 1-15 % gradient of MeOH in $CHCl_3$) afforded compound **12** as a white amorphous powder (yield 98 %). FABMS: 496.2 (MH^+) (80). ^{1}H NMR (500 MHz, $CDCl_3$):

12.11 (bs, 1H, NH); 7.64 (s, 1H, Pu-8); 6.55 (bs, 2H, NH_2); 4.77 (m, 1H, H-1); 4.23-4.10 (m, 8H, P-O-CH_2); 4.07 (t, 2H, $J_{4,3}=7.0$, H-4); 2.14 and 2.07 (2 x m, 2H, H-3); 1.96 (m, 2H, H-2); 1.36-1.29 (m, 12H, P-O-CH_2CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 158.94 (Pu-6); 153.84 (Pu-2); 151.62 (Pu-4); 137.40 (Pu-8); 116.96 (Pu-5); 72.13 (dd, $J_{C,P}=171.0$, $J_{C-O-P}=7.7$, C-1); 64.37 and 63.15 (2 x m, P-O-CH_2); 42.69 (C-4); 27.99 (C-2); 25.83 (d, $J_{3,P}=10.4$, C-3); 16.44 and 16.05 (2 x m, P-O-CH_2CH_3).

4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-1-(diethoxyphosphoryl)butyl diethyl phosphate (13)

Purification on column chromatography (silica gel, 1-5 % gradient of MeOH in $CHCl_3$) afforded compound **13** as a yellowish oil (yield 98 %). FABMS: 535.4 (MH^+) (80). ^1H NMR (500 MHz, $CDCl_3$): 7.48 (s, 1H, Pu-8); 5.74 (bs, 1H, NH); 4.81 (bs, 2H, NH_2); 4.72 (m, 1H, H-1); 4.20-4.05 (m, 10H, H-4, P-O-CH_2); 2.99 (m, 1H, $CH_{cycloprop.}$); 2.15 and 2.05 (2 x m, 2H, H-3); 1.92 (m, 2H, H-2); 1.35-1.27 (m, 12H, P-O-CH_2CH_3); 0.84 (m, 2H, $CH_{2cycloprop.}$); 0.60 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.95 (Pu-2); 156.18 (Pu-6); 151.23 (Pu-4); 137.25 (Pu-8); 114.61 (Pu-5); 71.81 (dd, $J_{C,P}=171.2$, $J_{C-O-P}=7.2$, C-1); 64.16 and 62.97 (2 x m, P-O-CH_2); 42.41 (C-4); 27.98 (C-2); 25.70 (d, $J_{3,P}=10.43$, C-3); 23.65 ($CH_{cycloprop.}$); 16.40 and 16.01 (2 x m, P-O-CH_2CH_3); 7.38 ($CH_{2cycloprop.}$).

4-[6-(Cyclopropylamino)-9*H*-purin-9-yl]-1-(diethoxyphosphoryl)butyl diethyl phosphate (14)

Purification on column chromatography (silica gel, 1-5 % gradient of MeOH in $CHCl_3$) afforded compound **14** as a yellowish oil (yield 98 %). FABMS: 520.3 (MH^+) (70). ^1H NMR (500 MHz, $CDCl_3$): 8.46 (s, 1H, Pu-2); 7.79 (s, 1H, Pu-8); 5.99 (bs, 1H, NH); 4.68 (m, 1H, H-1); 4.26 (t, 2H, $J_{4,3}=7.3$, H-4); 4.21-4.06 (m, 8H, P-O-CH_2); 3.05 (bs, 1H, $CH_{cycloprop.}$); 2.23 and 2.11 (2 x m, 2H, H-3); 1.94 (m, 2H, H-2); 1.35-1.28 (m, 12H, P-O-CH_2CH_3); 0.93 (m, 2H, $CH_{2cycloprop.}$); 0.67 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 155.75 (Pu-6); 153.13 (Pu-2); 149.11 (Pu-4); 139.86 (Pu-8); 119.88 (Pu-5); 71.97 (dd, $J_{C,P}=171.6$, $J_{C-O-P}=7.2$, C-1); 64.23 and 63.01 (2 x m, P-O-CH_2); 43.02 (C-4); 27.99 (C-2); 25.81 (d, $J_{3,P}=10.3$, C-3); 23.65 ($CH_{cycloprop.}$); 16.41 and 16.01 (2 x m, P-O-CH_2CH_3); 7.35 ($CH_{2cycloprop.}$).

4-(2,6-Diamino-9*H*-purin-9-yl)-1-(diethoxyphosphoryl)butyl diethyl phosphate (15)

Purification on column chromatography (silica gel, 1-10 % gradient of MeOH in $CHCl_3$) afforded compound **15** as white amorphous powder (yield 98 %). FABMS: 495.0 (MH^+) (70). ^1H NMR (500 MHz, $CDCl_3$): 7.63 (s, 1H, Pu-8); 5.50 (bs, 2H, 6-NH_2); 4.80 (bs, 2H, 2-NH_2); 4.73 (m, 1H, H-1); 4.20-4.06 (m, 10H, H-4, P-O-CH_2); 2.15 and 2.05 (2 x m, 2H, H-3); 1.91 (m, 2H, H-2); 1.34-1.27 (m, 12H, P-O-CH_2CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): 159.75 (Pu-2); 155.67 (Pu-6); 152.09 (Pu-4); 137.94 (Pu-8); 114.27 (Pu-5); 71.97 (dd, $J_{C,P}=171.1$, $J_{C-O-P}=7.1$, C-1); 64.15 and 62.96 (2 x m, P-O-CH_2); 42.43 (C-4); 27.91 (C-2); 25.58 (d, $J_{3,P}=10.4$, C-3); 16.36 and 15.96 (2 x m, P-O-CH_2CH_3).

7.4.3 General procedure for transformation of bisphosphonate and phosphate-phosphonate esters to sodium salts of the phosphonic acids

Dried esters (**5a**, **7a,b**, **8a,b**, **9**, **10**, and **11-15**, 1 mmol), acetonitrile (15 ml) and iododtrimethylsilane (1 ml) were stirred at room temperature under argon atmosphere

overnight. After evaporation and co-distillation with acetonitrile, the residue was treated with aqueous ammonia.

1. The mixture was dissolved in water and washed with dichloromethane three times. The water layer was evaporated to dryness. The residue was dissolved in water and applied onto a column of Dowex 50 X 8 (H^+ form).

   (a) Elution with water and subsequent evaporation gave phosphonic acids which were further applied onto Dowex 50 X 8 (Na^+ form). Elution with water and evaporation gave phosphonic acids **16**, **17** and **19** as tetrasodium salts.

   (b) Elution with dilute ammonia and evaporation afforded ammonium salts, which were applied onto Dowex 50 X 8 (Na^+ form). Elution with water and evaporation gave compounds **21**, **22** and **24** as disodium salts.

2. The mixture was dissolved in water and washed with dichloromethane three times. The water layer was evaporated to dryness. The residue was dissolved in 0.1 M TEAB and purified by HPLC.

   (a) Elution with 0.1 M TEAB and evaporation gave phosphonic acids which were further applied onto Dowex 50 X 8 (Na^+ form). Elution with water and evaporation gave phosphonic acids **18** and **20** as tetrasodium salts.

   (b) Elution with water-methanol gradient and evaporation gave phosphonic acids which were further applied onto Dowex 50 X 8 (Na^+ form). Elution with water and evaporation gave phosphonic acids **23** and **25** as disodium salts.

3. The residue was evaporated and dried over P_2O_5. Well dried compounds were further treated wih 1 M solution of TBAF in THF (see Page 116) under inert atmosphere of argon. The reaction was aged at room temperature for 2 h, neutralized with 1 M aqueous HCl, evaporated, dissolved in water and washed with dichloromethane three times. The water layer was evaporated to dryness. The residue was dissolved in water and applied onto a column of Dowex 50 X 8 (H^+ form).

   (a) Elution with water and evaporation gave phosphonic acids which were further applied onto Dowex 50 (Na^+ form). Elution with water and evaporation gave phosphonic acids **26**, **27**, **29**, and **31** as tetrasodium salts.

(b) Elution with dilute ammonia and evaporation afforded ammonium salts, which were applied onto Dowex 50 (Na^+ form). Elution with water and evaporation gave compounds **28**, **30**, and **32** as tetrasodium salts.

Sodium 4-(6-amino-9*H*-purin-9-yl)-1-phosphonatobutyl phosphate (16)
Purification according to method 1a afforded compound **16** as a white amorphous powder (yield 63 %). For $C_9H_{11}N_5Na_4O_7P_2 \cdot H_2O$ (473.13). Calcd: C, 22.85; H, 2.77; N, 14.80; Na, 19.44; P, 13.09. Found: C, 22.78; H, 2.74; N, 14.94; P, 13.15. FABMS: 456.1 (MH^+) (40). ^{1}H NMR (500 MHz, D_2O): 8.19 (s, 1H, Pu-8); 8.15 (s, 1H, Pu-2); 4.26 (t, 2H, $J_{4,3} = 7.2$, H-4); 4.04 (m, 1H, H-1); 2.16 and 2.00 (m, 2H, H-3); 1.78 and 1.71 (m, 2H, H-2). ^{13}C NMR (125.7 MHz, D_2O): 155.92 (Pu-6); 152.67 (Pu-2); 149.37 (Pu-4); 143.28 (Pu-8); 118.98 (Pu-5); 73.21 (dd, $J_{C,P} = 151.2$, $J_{C-O-P} = 6.4$, C-1); 44.63 (C-4); 28.76 (C-2); 26.90 (d, $J_{3,P} = 11.2$, C-3). UV spectrum: (H_2O) $\lambda_{max} = 259$-260 nm ($\epsilon_{max} = 14743$); (0.01 M HCl) $\lambda_{max} = 258$ nm ($\epsilon_{max} = 14415$); (0.01 M NaOH) $\lambda_{max} = 259$-260 nm ($\epsilon_{max} = 14392$).

Sodium 4-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-1-phosphonatobutyl phosphate (17)
Purification according to method 1a afforded compound **17** as a white amorphous powder (yield 62 %). For $C_9H_{11}N_5Na_4O_8P_2 \cdot H_2O$ (489.13). Calcd: C, 22.10; H, 2.68; N, 14.32; Na, 18.80; P, 12.66. Found: C, 22.18; H, 2.64; N, 14.28; P, 12.70. FABMS: 472.1 (MH^+) (30). ^{1}H NMR (500 MHz, D_2O): 7.86 (s, 1H, Pu-8); 4.11 (t, 2H, $J_{4,3} = 7.2$, H-4); 4.08 (m, 1H, H-1); 2.10 and 1.92 (m, 2H, H-3); 1.74 (m, 2H, H-2). ^{13}C NMR (125.7 MHz, D_2O): 159.61 (Pu-6); 154.22 (Pu-2); 152.07 (Pu-4); 140.98 (Pu-8); 116.59 (Pu-5); 73.16 (dd, $J_{C,P} = 151.7$, $J_{C-O-P} = 6.4$, C-1); 44.20 (C-4); 28.41 (C-2); 26.74 (d, $J_{3,P} = 11.8$, C-3). UV spectrum: (H_2O) $\lambda_{max} = 250$ nm ($\epsilon_{max} = 17354$); (0.01 M HCl) $\lambda_{max} = 276$ nm ($\epsilon_{max} = 16844$); (0.01 M NaOH) $\lambda_{max} = 267$ nm ($\epsilon_{max} = 19216$).

Sodium 4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9yl]-1-phosphonatobutyl phosphate (18)
Purification according to method 2a afforded compound **18** as a white amorphous powder (yield 64 %). For $C_{12}H_{16}N_6Na_4O_7P_2 \cdot H_2O$ (528.21). Calcd: C, 27.29; H, 3.43; N, 15.91; Na, 17.41; P, 11.73. Found: C, 27.28; H, 3.44; N, 15.88; P, 11.70. FABMS: 511.2 (MH^+) (40). ^{1}H NMR (500 MHz, D_2O): 7.85 (s, 1H, Pu-8); 4.08 (t, 2H, $J_{4,3} = 7.4$, H-4); 4.03 (m, 1H, H-1); 2.83 (bs, 1H, $CH_{cycloprop.}$); 2.11 and 1.99 (m, 2H, H-3); 1.83 and 1.75 (m, 2H, H-2); 0.86 (m, 2H, $CH_{2cycloprop.}$); 0.65 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O): 160.60 (Pu-2); 156.88 (Pu-6); 150.70 (Pu-4); 140.53 (Pu-8); 113.88 (Pu-5); 73.47 (dd, $J_{C,P} = 154.6$, $J_{C-O-P} = 6.9$, C-1); 44.41 (C-4); 29.70 (C-2); 26.88 (d, $J_{3,P} = 8.8$, C-3); 23.79 ($CH_{cycloprop.}$); 7.34 ($CH_{2cycloprop.}$). UV spectrum: (H_2O) $\lambda_{max} = 257$-258 and 281-282 nm ($\epsilon_{max} = 14194$ and 17257); (0.01 M HCl) $\lambda_{max} = 252$-253 and 291 nm ($\epsilon_{max} = 15250$ and 16888); (0.01 M NaOH) $\lambda_{max} = 257$ and 282 nm ($\epsilon_{max} = 13865$ and 17016).

Sodium 4-[6-(Cyclopropylamino)-9*H*-purin-9-yl]-1-phosphonatobutyl phosphate (19)
Purification according to method 1a afforded compound **19** as a white amorphous powder (yield 61%). For $C_{12}H_{15}N_5Na_4O_7P_2 \cdot H_2O$ (513.2). Calcd: C, 28.08; H, 3.34; N, 13.65; Na, 17.92; P, 12.07. Found: C, 28.18; H, 3.34; N, 13.78; P, 12.68. FABMS: 496.2 (MH^+) (60). ^{1}H NMR (500 MHz, D_2O): 8.26 (s, 1H, Pu-2); 8.18 (s, 1H, Pu-8); 4.29 (t, 2H, $J_{4,3} = 7.1$, H-4); 4.20 (m, 1H, H-1); 2.85 (bs, 1H, $CH_{cycloprop.}$); 2.15 and 2.02 (m, 2H, H-3); 1.80 and 1.75 (m, 2H, H-2); 0.95 (m, 2H, $CH_{2cycloprop.}$); 0.73 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O): 154.25 (Pu-6); 150.06 (Pu-2); 148.20 (Pu-4); 143.48 (Pu-8); 119.15 (Pu-5);

73.22 (dd, $J_{C,P}=156.9$, $J_{C-O-P}=7.0$, C-1); 44.49 (C-4); 28.48 (C-2); 26.42 (d, $J_{3,P}=11.0$, C-3); 23.79 ($CH_{cycloprop.}$); 7.22 ($CH_{2cycloprop.}$). UV spectrum: (H_2O) $\lambda_{max}=268$ nm ($\epsilon_{max}=19523$); (0.01 M HCl) $\lambda_{max}=266$ nm ($\epsilon_{max}=20507$); (0.01 M NaOH) $\lambda_{max}=268$-269 nm ($\epsilon_{max}=18972$).

Sodium 4-(2,6-diamino-9*H*-purin-9yl)-1-phosphonatobutyl phosphate (20)
Purification according to method 2a afforded compound **20** as a white amorphous powder (yield 65 %). For $C_9H_{12}N_6Na_4O_7P_2 \cdot H_2O$ (488.15). Calcd: C, 22.14; H, 2.89; N, 17.22; Na, 18.84; P, 12.69. Found: C, 22.18; H, 2.84; N, 17.28; P, 12.70. FABMS: 471.2 (MH^+) (50). ^{1}H NMR (500 MHz, D_2O): 7.86 (s, 1H, Pu-8); 4.09 (t, 2H, $J_{4,3}=7.2$, H-4); 4.07 (m, 1H, H-1); 2.09 and 1.91 (m, 2H, H-3); 1.74 (m, 2H, H-2). ^{13}C NMR (125.7 MHz, D_2O): 159.30 (Pu-2); 155.75 (Pu-6); 151.03 (Pu-4); 141.02 (Pu-8); 113.18 (Pu-5); 73.04 (dd, $J_{C,P}=151.4$, $J_{C-O-P}=6.0$, C-1); 44.03 (C-4); 28.29 (C-2); 26.69 (d, $J_{3,P}=12.4$, C-3). UV spectrum: (H_2O) $\lambda_{max}=278$ nm ($\epsilon_{max}=15773$); (0.01 M HCl) $\lambda_{max}=285$-286 nm ($\epsilon_{max}=14879$); (0.01 M NaOH) $\lambda_{max}=278$ nm ($\epsilon_{max}=15122$).

Sodium 4-(6-amino-9*H*-purin-9-yl)-1-iodobutylphosphonate (21)
Purification according to method 1b (see Page 118) afforded compound **21** as a white amorphous powder (yield 30 %). For $C_9H_{11}IN_5Na_2O_3P$ (441.07). Calcd: C, 24.51; H, 2.51; I, 28.77; N, 15.88; Na, 10.42; P, 7.02. Found: C, 24.52; H, 2.54; I, 28.80; N, 15.84; P, 10.50. FABMS: 442.1 (MH^+) (40). NMR (500 MHz, D_2O): 8.19 (s, 1H, Pu-8); 8.15 (s, 1H, Pu-2); 4.05 (m, 2H, H-4); 3.69 (ddd, 1H, $J=11.3$, 9.3, 2.7, H-1); 2.16 (m, 1H, H-3a); 1.95-1.73 (m, 3H, H-2, H-3b). ^{13}C NMR (125.7 MHz, D_2O): 155.92 (Pu-6); 152.67 (Pu-2); 151.43 (Pu-4); 142.76 (Pu-8); 118.56 (Pu-5); 43.43 (C-4); 32.56 (C-2); 31.45 (d, $J_{3,P}=12.0$, C-3); 30.97 (d, $J_{1,P}=132.0$, C-1). UV spectrum: (H_2O) $\lambda_{max}=259$-260 nm ($\epsilon_{max}=13752$); (0.01 M HCl) $\lambda_{max}=258$ nm ($\epsilon_{max}=13471$); (0.01 M NaOH) $\lambda_{max}=259$-260 nm ($\epsilon_{max}=14552$).

Sodium 4-(2-Amino-6-oxo-1*H*-purin-9(6*H*)-yl)-1-iodobutylphosphonate (22)
Purification according to method 1b afforded compound **22** as a white amorphous powder (yield 33 %). For $C_9H_{11}IN_5Na_2O_4P$ (457.07). Calcd: C, 23.65; H, 2.43; I, 27.76; N, 15.32; Na, 10.06; P, 6.78. Found: C, 23.68; H, 2.44; I, 27.80; N, 15.28; P, 6.80. FABMS: 458.2 (MH^+) (70). ^{1}H NMR (500 MHz, D_2O): 7.79 (s, 1H, Pu-8); 4.06 (m, 2H, H-4); 3.71 (ddd, 1H, $J=11.2$, 9.4, 2.6, H-1); 2.15 (m, 1H, H-3a); 1.98-1.76 (m, 3H, H-2, H-3b). ^{13}C NMR (125.7 MHz, D_2O): 163.19 (Pu-6); 157.11 (Pu-2); 151.96 (Pu-4); 140.15 (Pu-8); 117.21 (Pu-5); 43.53 (C-4); 32.58 (C-2); 31.63 (d, $J_{3,P}=12.0$, C-3); 30.96 (d, $J_{1,P}=132.5$, C-1). UV spectrum: (H_2O) $\lambda_{max}=250$ nm ($\epsilon_{max}=14784$); (0.01 M HCl) $\lambda_{max}=275$-276 nm ($\epsilon_{max}=13934$); (0.01 M NaOH) $\lambda_{max}=266$ nm ($\epsilon_{max}=15473$).

Sodium 4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-1-iodobutylphosphonate (23)
Purification according to method 2b afforded compound **23** as a white amorphous powder (yield 32 %). For $C_{12}H_{16}IN_6Na_2O_3P$ (496.15). Calcd: C, 29.05; H, 3.25; I, 25.58; N, 16.94; Na, 9.27; P, 6.24. Found: C, 29.08; H, 3.24; I, 25.60; N, 16.98; P, 6.30. FABMS: 497.2 (MH^+) (50). ^{1}H NMR (500 MHz, D_2O): 7.77 (s, 1H, Pu-8); 4.05 (m, 2H, H-4); 3.68 (ddd, 1H, $J=11.3$, 9.3, 2.7, H-1); 2.81 (bs, 1H, $CH_{cycloprop.}$); 2.14 (m, 1H, H-3a); 1.98-1.74 (m, 3H, H-2, H-3b); 0.85 (m, 2H, $CH_{cycloprop.}$); 0.64 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O): 160.53 (Pu-2); 156.77 (Pu-6); 150.50 (Pu-4); 140.17 (Pu-8); 113.86 (Pu-5); 43.40 (C-4); 32.59 (C-2); 31.53 (d, $J_{3,P}=12.5$, C-3); 31.01 (d, $J_{1,P}=132.2$, C-1); 23.93 ($CH_{cycloprop.}$); 7.35 ($CH_{2cycloprop.}$). UV spectrum: (H_2O) $\lambda_{max}=258$ and 282 nm ($\epsilon_{max}=14483$ an 17302); (0.01 M HCl) $\lambda_{max}=293$ nm ($\epsilon_{max}=17045$); (0.01 M NaOH) $\lambda_{max}=257$-258 and 282 nm ($\epsilon_{max}=14794$ and 17707).

Sodium 4-[6-(cyclopropylamino)-9*H*-purin-9-yl]-1-iodobutylphosphonate (24)
Purification according to method 1b afforded compound **24** as a white amorphous powder (yield 34 %). For $C_{12}H_{15}IN_5Na_2O_3P$ (481.14). Calcd: C, 29.96; H, 3.14; I, 26.38; N, 14.56; Na, 9.56; P, 6.44. Found: C, 29.98; H, 3.14; I, 26.30; N, 14.58; P, 6.40. FABMS: 482.3 (MH^+) (70). ^{1}H NMR (500 MHz, D_2O): 8.16 (s, 1H, Pu-2); 8.05 (s, 1H, Pu-8); 4.20 (m, 2H, H-4); 3.69 (ddd, 1H, $J = 11.6$, 9.5, 2.5, H-1); 2.80 (bs, 1H, $CH_{cycloprop.}$); 2.18 (m, 1H, H-3a); 1.93 (m, 2H, H-2a, H-3b); 1.78 (m, 1H, H-2b); 0.90 (m, 2H, CHcycloprop.); 0.66 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O): 155.89 (Pu-6); 152.54 (Pu-2); 148.23 (Pu-4); 142.47 (Pu-8); 119.24 (Pu-5); 49.35 (C-4); 32.58 (C-2); 31.65 (d, $J_{3,P} = 12.0$, C-3); 30.92 (d, $J_{1,P} = 132.4$, C-1); 23.84 ($CH_{cycloprop.}$); 7.20 ($CH_{2cycloprop.}$). ^{31}P NMR (500 MHz, $CDCl_3$, $H_3PO_4 = 0$): 14.69. UV spectrum: (H_2O) $\lambda_{max} =$ 268-269 nm ($\epsilon_{max} = 15524$); (0.01 M HCl) $\lambda_{max} = 266$ nm ($\epsilon_{max} = 17104$); (0.01 M NaOH) $\lambda_{max} = 268$ nm ($\epsilon_{max} = 16076$).

Sodium 4-(2,6-diamino-9*H*-purin-9-yl)-1-iodobutylphosphonate (25)
Purification according to method 2b afforded compound **25** as a white amorphous powder (yield 35 %). For $C_9H_{12}IN_6Na_2O_3P$ (456.09). Calcd: C, 23.70; H, 2.65; I, 27.82; N, 18.43; Na, 10.08; P, 6.79. Found: C, 23.68; H, 2.64; I, 27.80; N, 18.48; P, 6.80. FABMS: 457.1 (MH^+) (50). ^{1}H NMR (500 MHz, D_2O): 7.82 (s, 1H, Pu-8); 4.05 (m, 2H, H-4); 3.69 (ddd, 1H, $J = 11.3$, 9.3, 2.7, H-1); 2.14 (m, 1H, H-3a); 1.97-1.73 (m, 3H, H-2, H-3b). ^{13}C NMR (125.7 MHz, D_2O): 160.39 (Pu-2); 156.54 (Pu-6); 151.43 (Pu-4); 140.76 (Pu-8); 113.56 (Pu-5); 43.43 (C-4); 32.56 (C-2); 31.45 (d, $J_{3,P} = 12.0$, C-3); 30.97 (d, $J_{1,P} = 132.0$, C-1). UV spectrum: (H_2O) $\lambda_{max} =$ 251-253 and 278 nm ($\epsilon_{max} =$ 11347 and 11706); (0.01 M HCl) $\lambda_{max} =$ 250-251 and 286-288 nm ($\epsilon_{max} =$ 11756 an 11775); (0.01 M NaOH) $\lambda_{max} =$ 253 and 277-278 nm ($\epsilon_{max} =$ 11393 and 11753).

Sodium 4-(6-amino-9*H*-purin-9-yl)-1-hydroxybutane-1,1-diyldiphosphonate (26)
Purification according to method 3a (see Page 118) afforded compound **26** as a white amorphous powder (yield 77 %). For $C_9H_{11}N_5Na_4O_7P_2$ (455.12). Calcd: C, 23.75; H, 2.44; N, 15.39; Na, 20.21; P, 13.61. Found: C, 23.70; H, 2.40; N, 15.45; P, 13.55. FABMS: 456.2 (MH^+) (60). ^{1}H NMR (500 MHz, D_2O): 8.19 (s, 1H, Pu-8); 8.15 (s, 1H, Pu-2); 4.18 (t, 2H, $J_{4,3} = 7.3$, H-4); 2.19 (bs, 2H, H-3); 1.94 (bs, 2H, H-2). ^{13}C NMR (125.7 MHz, D_2O): 155.89 (Pu-6); 152.65 (Pu-2); 149.40 (Pu-4); 143.33 (Pu-8); 118.93 (Pu-5); 76.60 (t, $J_{1,P} = 134.1$, C-1); 45.68 (C-4); 33.65 (C-2); 25.73 (C-3). UV spectrum: (H_2O) $\lambda_{max} = 259$ nm ($\epsilon_{max} = 15804$); (0.01 M HCl) $\lambda_{max} = 258$ nm ($\epsilon_{max} = 15417$); (0.01 M NaOH) $\lambda_{max} = 260$ nm ($\epsilon_{max} = 15556$).

Sodium 4-(2-amino-6-oxo-1*H*-purin-9(6*H*)-yl)-1-hydroxybutane-1,1-diyldiphosphonate (27)
Purification according to method 3a afforded compound **27** as a white amorphous powder (yield 79 %). For $C_9H_{11}N_5Na_4O_8P_2$ (471.12). Calcd: C, 22.94; H, 2.35; N, 14.87; Na, 19.52; P, 13.15. Found: C, 22.89; H, 2.40; N, 14.77; P, 13.20. FABMS: 472.1 (MH^+) (50). ^{1}H NMR (500 MHz, D_2O): 7.79 (s, 1H, Pu-8); 4.00 (t, 2H, $J_{4,3} = 7.6$, H-4); 2.12 (m, 2H, H-3); 1.93 (m, 2H, H-2). ^{13}C NMR (125.7 MHz, D_2O): 168.89 and 161.69 (Pu-6 and Pu-2); 151.94 (Pu-4); 139.43 (Pu-8); 118.11 (Pu-5); 76.65 (t, $J_{1,P} = 134.2$, C-1); 45.13 (C-4); 33.70 (C-2); 25.84 (t, $J_{3,P} = 5.0$, C-3). UV spectrum: (H_2O) $\lambda_{max} =$ 249-250 nm ($\epsilon_{max} = 14562$); (0.01 M HCl) $\lambda_{max} =$ 275-276 nm ($\epsilon_{max} = 13792$); (0.01 M NaOH) $\lambda_{max} =$ 266-267 nm ($\epsilon_{max} = 15230$).

Sodium 4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-1-hydroxybutane-1,1-diyldiphosphonate (28)

Purification according to method 3b afforded compound **28** as a white amorphous powder (yield 75 %). For $C_{12}H_{16}N_6Na_4O_7P_2$ (510.20). Calcd: C, 28.25; H, 3.16; N, 16.47; Na, 18.02; P, 12.14. Found: C, 28.35; H, 3.26; N, 16.40; P, 12.04. FABMS: 511.1 (MH^+) (40). ^{1}H NMR (500 MHz, D_2O): 7.86 (s, 1H, Pu-8); 4.03 (t, 2H, $J_{4,3}=7.6$, H-4); 2.83 (m, 1H, $CH_{cycloprop.}$); 2.13 (m, 2H, H-3); 1.92 (m, 2H, H-2); 0.86 (m, 2H, $CH_{2cycloprop.}$); 0.65 (m, 2H, CH_2cycloprop). ^{13}C NMR (125.7 MHz, D_2O): 160.62 (Pu-2); 156.92 (Pu-6); 150.75 (Pu-4); 140.62 (Pu-8); 113.89 (Pu-5); 76.63 (t, $J_{1,P}=133.9$, C-1); 45.16 (C-4); 33.62 (C-2); 25.76 (C-3); 23.83 ($CH_{cycloprop.}$); 7.34 ($CH_{2cycloprop.}$). UV spectrum: (H_2O) $\lambda_{max}=257$ and 282 nm ($\epsilon_{max}=10319$ and 15173); (0.01 M HCl) $\lambda_{max}=253$ and 292 nm ($\epsilon_{max}=10525$ and 14127); (0.01 M NaOH) $\lambda_{max}=258$ and 282 nm ($\epsilon_{max}=8307$ and 14562).

Sodium 4-[6-(cyclopropylamino)-9*H*-purin-9-yl]-1-hydroxybutane-1,1-diyldiphosphonate (29)
Purification according to method 3a afforded compound **29** as a white amorphous powder (yield 79 %). For $C_{12}H_{15}N_5Na_4O_7P_2$ (495.18). Calcd: C, 29.11; H, 3.05; N, 14.14; Na, 18.57; P, 12.51. Found: C, 29.09; H, 3.11; N, 14.24; P, 12.60. FABMS: 496.2 (MH^+) (70). ^{1}H NMR (500 MHz, D_2O): 8.20 (s, 1H, Pu-2); 8.14 (s, 1H, Pu-8); 4.17 (t, 2H, $J_{4,3}=7.4$, H-4); 2.83 (bs, 1H, $CH_{cycloprop.}$); 2.17 (m, 2H, H-3); 1.92 (m, 2H, H-2); 0.89 (m, 2H, $CH_{2cycloprop.}$); 0.67 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O): 155.99 (Pu-6); 152.55 (Pu-2); 148.45 (Pu-4); 142.80 (Pu-8); 119.29 (Pu-5); 76.54 (t, $J_{1,P}=134.8$, C-1); 45.60 (C-4); 33.62 (C-2); 25.78 (C-3); 23.79 ($CH_{cycloprop.}$); 7.21 ($CH_{2cycloprop.}$). UV spectrum: (H_2O) $\lambda_{max}=268$ nm ($\epsilon_{max}=11274$); (0.01 M HCl) $\lambda_{max}=266$ nm ($\epsilon_{max}=11656$); (0.01 M NaOH) $\lambda_{max}=268$ nm ($\epsilon_{max}=11338$).

Sodium 4-(2,6-diamino-9*H*-purin-9-yl)-1-hydroxybutane-1,1-diyldiphosphonate (30)
Purification according to method 3b afforded compound **30** as a white amorphous powder (yield 80 %). For $C_9H_{12}N_6Na_4O_7P_2$ (470.13). Calcd: C, 22.99; H, 2.57; N, 17.88; Na, 19.56; P, 13.18. Found: C, 23.08; H, 2.63; N, 17.95; P, 13.25. FABMS: 471.2 (MH^+) (60). ^{1}H NMR (500 MHz, D_2O): 7.89 (s, 1H, Pu-8); 4.05 (t, 2H, $J_{4,3}=7.2$, H-4); 2.12 (bs, 2H, H-3); 1.93 (m, 2H, H-2). ^{13}C NMR (125.7 MHz, D_2O): 160.46 (Pu-2); 156.61 (Pu-6); 151.56 (Pu-4); 141.04 (Pu-8); 113.60 (Pu-5); 76.59 (t, $J_{1,P}=134.1$, C-1); 44.90 (C-4); 32.13 (C-2); 25.94 (C-3). UV spectrum: UV spectrum: (H_2O) $\lambda_{max}=252$-253 and 278 nm ($\epsilon_{max}=13391$ and 14370); (0.01 M HCl) $\lambda_{max}=251$ and 286-287 nm ($\epsilon_{max}=13840$ and 14091); (0.01 M NaOH) $\lambda_{max}=253$ and 278 nm ($\epsilon_{max}=13413$ and 14380).

Sodium 4-(2-amino-6-oxo-1*H*-purin-7(6*H*)-yl)-1-hydroxybutane-1,1-diyldiphosphonate (31)
Purification according to method 3a afforded compound **31** as a white amorphous powder (yield 77 %). For $C_9H_{11}N_5Na_4O_8P_2$ (471.12). Calcd: C, 22.94; H, 2.35; N, 14.87; Na, 19.52; P, 13.15. Found: C, 22.87; H, 2.42; N, 14.85; P, 13.22. FABMS: 472.1 (MH^+) (50). ^{1}H NMR (500 MHz, D_2O): 7.93 (s, 1H, Pu-8); 4.27 (t, 2H, $J_{4,3}=7.4$, H-4); 2.13 (m, 2H, H-3); 1.90 (m, 2H, H-2). ^{13}C NMR (125.7 MHz, D_2O): 166.02 and 161.69 (Pu-6 and Pu-2); 160.09 (Pu-4); 143.12 (Pu-8); 110.76 (Pu-5); 76.59 (t, $J_{1,P}=134.0$, C-1); 48.44 (C-4); 33.44 (C-2); 26.87 (t, $J_{3,P}=5.6$, C-3). UV spectrum: (H_2O) $\lambda_{max}=285$-286 nm ($\epsilon_{max}=11483$); (0.01 M HCl) $\lambda_{max}=246$-247 and 284 nm ($\epsilon_{max}=11316$ and 13237); (0.01 M NaOH) $\lambda_{max}=290$ nm ($\epsilon_{max}=10111$).

Sodium 4-[2-amino-6-(cyclopropylamino)-7*H*-purin-7-yl]-1-hydroxybutane-1,1-diyldiphosphonate (32)
Purification according to method 3b afforded compound **32** as a white amorphous powder (yield 79 %).

For $C_{12}H_{16}N_6Na_4O_7P_2$ (510.20). Calcd: C, 28.25; H, 3.16; N, 16.47; Na, 18.02; P, 12.14. Found: C, 28.28; H, 3.20; N, 16.55; P, 12.18. FABMS: 511.2 (MH^+) (70). ^{1}H NMR (500 MHz, D_2O): 7.94 (s, 1H, Pu-8); 4.26 (t, 2H, $J_{4,3}=7.6$, H-4); 2.75 (m, 1H, $CH_{cycloprop.}$); 2.12 (m, 2H, H-3); 1.95 (m, 2H, H-2); 0.88 (m, 2H, $CH_{2cycloprop.}$); 0.75 (m, 2H, $CH_{2cycloprop.}$). ^{13}C NMR (125.7 MHz, D_2O): 158.75 (Pu-2); 157.14 (Pu-4); 153.78 (Pu-6); 144.80 (Pu-8); 106.63 (Pu-5); 74.10 (t, $J_{1,P}=129.0$, C-1); 48.45 (C-4); 31.14 (C-2); 26.22 (C-3); 24.23 ($CH_{cycloprop.}$); 7.68 ($CH_{2cycloprop.}$). UV spectrum: (H_2O) $\lambda_{max}=279$-280 nm ($\epsilon_{max}=15143$); (0.01 M HCl) $\lambda_{max}=270$ nm ($\epsilon_{max}=14645$); (0.01 M NaOH) $\lambda_{max}=277$ nm ($\epsilon_{max}=14975$).

7.4.4 Dephosphorylation of phosphate-phosphonates with alkaline phosphatase

1-Phosphate-1-phosphonates **16**, **17** and **20** were dephosphorylated using alkaline phosphatase. The reaction mixture (100 μl) contained 100 mM glycine buffer (pH 10.4), 1 mM $ZnCl_2$, 1 mM $MgCl_2$, 100 $\mu g \cdot ml^{-1}$ bovine serum albumin, 6 $U \cdot ml^{-1}$ alkaline phosphatase and 1000 μM solution of tested compound. All reactions were carried out at 37 °C for 120 min and stopped by addition of 50% TCA. After 10 min of incubation on ice, the samples were centrifuged and TCA was removed from the supernatant by extraction with tri-*n*-octylamine/1,1,2,-trichlorotrifluoroethane mixture (1:4; v/v). Aqueous phase was then separated by centrifugation and an aliquot was used for HPLC analysis.

Chapter 8

Cytotoxic pre-screening of prepared ANbPs in liposomes *in vitro*

8.1 Introduction

Liposomes are nano-sized artificial vesicles of spherical shape that can be produced from natural phospholipids and cholesterol. Phospholipids combined with water immediately form bi-layered spheres because of their amphiphilic character (see Figure 8.1). Liposomes are extensively studied because of their ability to encapsulate drugs. Molecules which are soluble in water can be encapsulated within the interior of the liposome filled in water. Less recognized but very important property of liposomes is that molecules which are not water soluble can be entrapped in the hydrophobic part of the phospholipid bilayer. Therefore, liposomes can serve as carriers for all types of molecules including both water-soluble and water-insoluble compounds.

8.2 Methods of pre-screening

This pre-screening was performed in the laboratory of Immunopharmacology at Veterinary Research Institute (VRI) Brno. Cytotoxic effects of prepared bisphosphonates were tested on B16F10 mouse melanoma cell lines (cell line was purchased from Banca Cellule e Colture in GMP-IST Genova). The cytotoxicity was evaluated in these pre-screening experiments by examination of cell morphology using Hoffman modulation contrast and inverted mi-

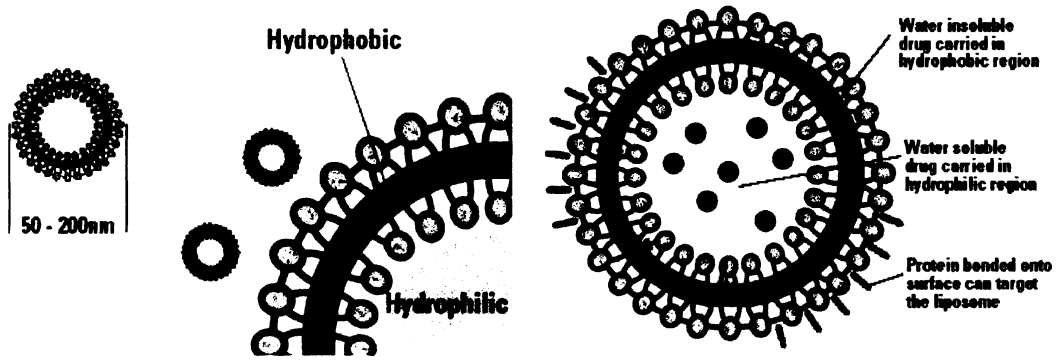


Figure 8.1: Structure of liposome

croscope T200 (Nikon, Japan). The cells were seeded on 96-well flat-bottom microplates at the density 2.5-3.0×10^4 per ml, 100 μl per well, and were allowed to grow for 16 to 24 hours in culture medium (RPMI supplemented with 10% foetal calf serum). The hydrophilic drugs (Chapter 3, 4, 6) dissolved in PBS (total volume of 20 ml) were added to wells and the cytotoxic effect was evaluated after 24, 48 and 72 hours of exposure in the concentration range 30, 60, 120 and 240 μM. The hydrophobic drugs (Chapter 5) were dissolved in DMSO, diluted with tissue culture medium or PBS and added to wells. This procedure was not found to be appropriate due to uncontrolled precipitation of drugs after dilution. Therefore, the data in Table 8.1 for free hydrophobic drug are classified by the symbol "+" to express that cytotoxic effect was observed for all applied concentrations of tested drugs. It could be explained by equilibrium between the free drug and its precipitate. Entrapment of hydrophobic drug into liposomes was found to be elegant solution of the solubility problem and accurate dosing.

Preparation of frozen and thawed multilamellar vesicles FTMLV

The lipid mixture, dissolved in chloroform was deposited onto the wall of a round-bottom flask (250 ml) by removal of the solvent in a rotary evaporator (40 °C, 4 h). The dried lipid film was then hydrated with the aqueous phase (PBS pH 7.2 previously filtered through a 0.22 μm filter) under continuous mixing on a mechanical reciprocal shaker for 2 h. The frozen-thawed MLV system[140] was obtained by freezing the MLVs in liquid nitrogen and thawing them in a 30 °C water bath, repeating the cycle five times. Liposomes were extruded through polycarbonate filters (pore size 0.2 μm).[141]

Table 8.1: Cytotoxic effect of BP analogues against B16F10 cell line

Tested compound	Cytotoxic activity	
	Free drug	Liposomal drug
SV327 (hydrophilic)	-	+ (IC_{50} < 30 μM) 24 h exposure
SV235 FCC:8+9	+	+ (IC_{50} < 60 μM) 48 h exposure
SV235	+	+ (IC_{50} < 30 μM) 48 h exposure
SV244	+	+ (IC_{50} < 60 μM) 48 h exposure
SV197	+	+ (IC_{50} < 60 μM) 48 h exposure

SV327 (compound **29b**, Chapter 6); SV235 FCC:8+9 (compound **14**, Chapter 5); SV235 (compound **13**, Chapter 5); SV244 (compound **3a**, Chapter 5); SV197 (compound **3c**, Chapter 5)

8.3 Results

Tested hydrophilic ANbPs (compounds from Chapter 3, 4, 6) were not active *in vitro* against the B16F10 mouse melanoma in the concentration range 30–240 μM despite the long exposure time used (up to 72 h). Only one compound SV327 (compound **29b**, Chapter 6) exhibited cytotoxic activity when encapsulated in liposomes, which improved penetration through cell membrane (Table 8.1). Enhanced penetration induced by liposomes brought relatively rapid cytotoxic effect (24 h exposure). Figure 8.2 shows the morphology of control cells and cells treated with the highest concentration of inactive BP analogue encapsulated into liposomes. The picture illustrates no additional cytotoxicity induced by liposomes. Treatment with liposomal SV327 induced both cytotoxic and cytostatic effect.

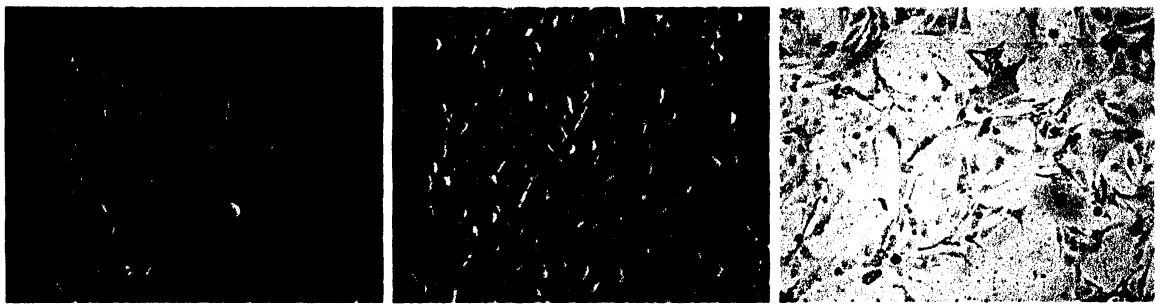


Figure 8.2: Hoffman modulation contrast microphotographs of B16F10 cancer cells treated with inactive BP liposomal analogue (200 μM) and active SV327 (60 μM). Control sample - untreated cells (left), cells treated with inactive BP liposomal analogue (middle), active liposomal SV327 (right)

Hydrophobic drugs (Chapter 5) seem to be more effective against melanoma cancer cells and their encapsulation into liposomes is one of the possible ways of modern targetable

application. Figure 8.3 shows cytotoxic effect of lipophilic drugs encapsulated in liposomes. The cytotoxic effect of lipophilic analogues is comparable to that of PMEG, which is drug with known anticancer activity.[106–108] Dead cells and cellular debris predominate, viable or dividing cells are rarely found in the optical field.

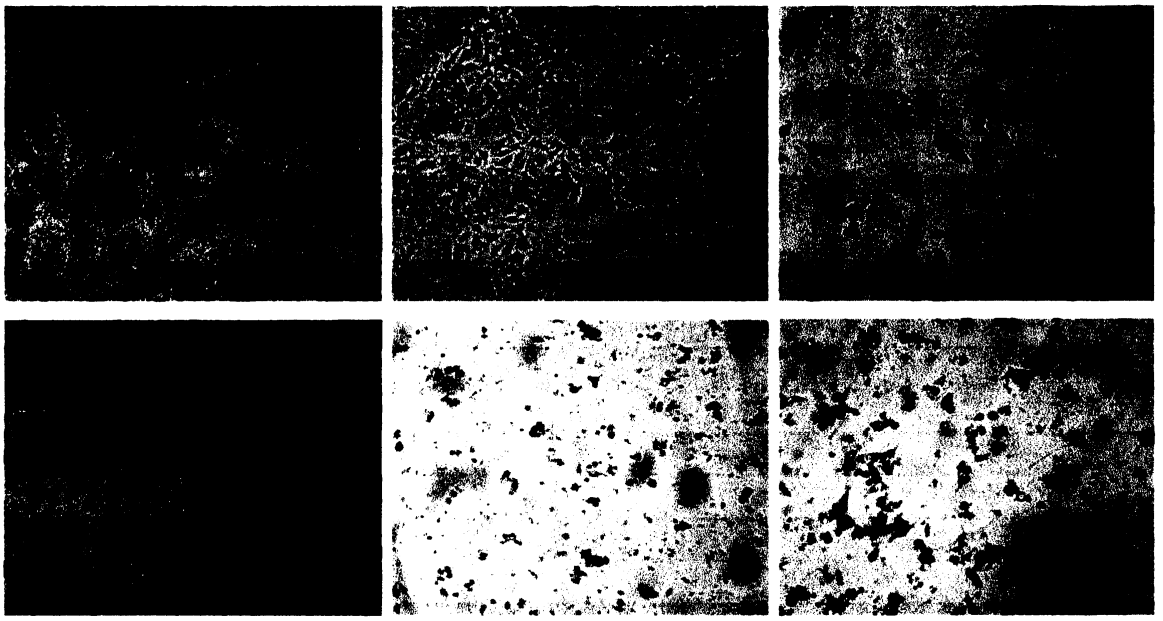


Figure 8.3: Hoffman modulation contrast microphotographs of B16F10 cancer cells treated with active liposomal hydrophobic analogues (60 μM, 48 h exposure). Liposomal PMEG was used as positive control. Control sample - untreated cells (top left); liposomal SV197 (compound **3c**, Chapter 5, top middle); liposomal SV235 FCC:8+9 (compound **14**, Chapter 5, top right); liposomal SV235 (compound **13**, Chapter 5, bottom left); liposomal SV244 (compound **3a**, Chapter 5, bottom middle); liposomal PMEG (bottom right).

8.4 Conclusions

Melanomas are generally resistant to chemotherapy and represents useful model for testing efficacy of new anticancer drugs. Hydrophilic BP analogues were not effective *in vitro* against this B16F10 melanoma cells and enhancement of their efficiency after entrapment into liposomes was observed only in one case (SV327). Promising activity was found within lipophilic derivatives (compounds from Chapter 5) which exerted cytotoxic effect comparable to liposomal PMEG which was used as positive control. Both lipophilic and hydrophilic derivatives are suitable for microencapsulation into various carriers such as

liposomes, which represents very efficient and successful nanocarriers for development of targetable anticancer drugs. Future research will be focused on testing of BP drugs on the broader panel cancer cell lines and development of their targetable liposomal formulations.

Chapter 9

Summary

Despite the advances in human knowledge, we still encounter new and more dangerous infectious or oncogenic diseases, which can not be treated using presently available drugs. Another problem lies in the ability of microorganisms and tumors to acquire resistency to pharmacological treatment. The development of new and more potent biologically active substances is, therefore, a crucial task.

This fact also lies behind this thesis. This work is extending the synthesis of acyclic nucleoside phosphonates, from which many are already used in clinical medicine(Hepsera, Viread, Vistide). We concentrated on so called acyclic nucleoside bisphosphonates (ANbPs), a group of relatively sparsely explored compounds. The main aim of this dissertation was to prepare new ANbPs and to assess their biological activity.

During the course of this work we prepared several different series of bisphosphonates. Tests for biological activity were carried out for many of them. In some cases, the evaluation of the activity is still in progress. The main synthetic strategy used was alkylation of nucleobases with a bisphosphonate building block prepared in advance. Further processing of the product by means of cleavage of ester protecting groups lead to free bisphosphonic acids or their sodium salts. These compounds were then subjected to biological screening.

First we synthesised symmetric ANbPs based on glycerol which could be also considered as analogues of nucleotide bisphosphate antagonists of the $P2Y_1$ receptor (Chapters 3 and 4).[1,2] These substances were found to be inactive against DNA, RNA, and herpesviruses. They do not possess any cytostatic activity and so far we have not found any evidence of their action as possible antagonists of the $P2Y_1$ receptor.

Due to their very high polarity, the penetration of ANPs and ANbPs into the cell is severely hindered. We therefore tried to prepare their lipophilic derivatives to make their membrane transport easier (Chapter 5). The lipophilic chains were attached to already known compounds containing PME chain (adenine, guanine, cytosine), and to glycerol based ANbPs (adenine). Antiviral and cytostatic screening revealed significant increase in the activity of the lipophilic derivatives, when compared to free acids.[3] As a surprise it was also found, that lipophilic drivatives attached to adenine are active against Coxsackie virus B4 (RNA virus). In general, in this series of ANPs the activity against RNA viruses is very rare.

In the following project, described in detail in Chapter 6, we studied ANbPs with chiral chain. We prepared two basic enantiomeric series of free phosphonic acids. The mechanism of the alkylation reaction was verified and the absolute configuration of the products was assigned. As in previous cases, one of the main goals was to assess the potential biological activity of these compounds. The additional goal was to study the effect of introduction of chiral centers to synthesized compounds, since the chirality seems to play an important role in EI-complex formation in certain enzymes. Neither antiviral nor cytostatic activity was found. This fact was not particularly surprising since the presence of four negative charges due to phosphonate groups indicated possible problems in membrane transport. To overcome this hindrance, we used liposomes, which are generally used for the transport of highly polar substances inside the cells. Initial cytostatic screening shows very promising activity for the *(S,S)* guanine derivative SV327 (compound **29b**, Chapter 6. The *(R,R)* derivative **29a**, on the other hand, does not exhibit any activity. Further testing is currently in progress.[4]

The last (but not least) part of our investigation of ANbPs concerned the synthesis of geminal (methylene) bisphosphonates (Chapter 7). Substances from this group (*e.g.*, alendronate, ibandronate, zoledronate) have received much attention due to their use in the treatment of osteoporosis and other bone diseases. It was our aim to study biological activity (not limited to osteoporosis) of similar compounds. We, therefore, prepared series of new ANbPs with alendronate side chain. We also synthesized acyclic nucleoside phosphate-phosphonates, emerging from the molecular rearrangement of the original bisphosphonate. We were also able to isolate α-iodophosphonates, as byproducts of the final removal of protecting ester groups from phosphate-phosphonate derivatives. Testing of these substances as potential agents for osteoporosis treatment, as well as antiviral and cytostatic tests are being carried out.

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