Charles University in Prague

Faculty of Science

Department of Organic and Nuclear Chemistry

Preparation of novel types of acyclic nucleoside phosphonates for study of their interaction with enzymes of metabolism of nucleic acids

Ph.D. Thesis

Prague 2014 Ing. Martin Maxmilian Kaiser

Univerzita Karlova v Praze

Přírodovědecká fakulta

Katedra organické a jaderné chemie

Příprava nových typů acyklických nukleosid-fosfonátů pro studium jejich interakce s enzymy metabolismu nukleových kyselin

Dizertační práce

Praha 2014 Ing. Martin Maxmilian Kaiser

Dedicated to the memory of Prof. Antonín Holý

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. I consent to publication of this thesis under Act No. 111/1998, Coll., the Higher Education Act, as amended by subsequent regulations. I have been informed of all duties and obligations applicable under Act No. 121/2000, Coll., the Copyright Act, as amended by subsequent regulations.

Neither the thesis nor any of its parts have been used previously for obtaining any academic degree.

Martin Maxmilian Kaiser

Abstrakt

Tato dizertační práce vznikla jako součást podrobného výzkumu v oblasti acyklických nukleosidfosfonátů ve skupině Chemie nukleových kyselin (prof. A. Holý) a později ve skupině Cílených analogů komponent nukleových kyselin (Dr. Z. Janeba) na Ústavu organické chemie a biochemie, Akademie věd České republiky. Byla vyvinuta metodika přípravy tří nových typů acyklických nukleosidfosfonátů (ANF), v prvé řadě karboxyfosfonoethoxymethyl (CPME), dále pak karboxyfosfono-ethoxyethyl (CPEE) a hydroxyfosfonoethoxypropyl (HPEP) derivátů s cílem studovat jejich biologické vlastnosti.

CPME deriváty byly navrženy na základě strukturní podobnosti s PMEA (9-[2-(fosfonomethoxy)ethyl]adenin, Adefovir) a (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-(fosfonomethoxy)propyl)adenine], tedy látkami s mimořádně vysokým protivirovým účinkem. Klíčovým krokem v jejich syntéze byla oxidace primární hydroxylové skupiny prekurzorů v řadě HPMP pomocí systému TEMPO/chloritan sodný/chlornan sodný. Jako velmi slibný analog s potenciální protivirovou (HIV) aktivitou se na základě počítačového modelování jevila sloučenina (*S*)-CPMEA [(*S*)-3-(adenin-9-yl)-2-(fosfonomethoxy)propanová kyselina)], 2‘-karboxy analog PMEA. I když se nakonec tato polární látka ukázala jako biologicky neaktivní, její dvě proléčiva vykazovala aktivitu proti viru hepatitidy C v submikromolárním měřítku. Připravená proléčiva (*S*)-CPMEA vykazovala rovněž slabou inhibici adenylátcyklázového toxinu bakterie *Bordetella pertussis*.

Další strategie syntézy CPME, CPEE a HPEP derivátů byla založena na tzv. synthonovém přístupu. V tomto případě bylo nutné připravit z výchozího tritylglycidolu meziprodukt obsahující fosfonátovou a hydroxymethyl/karboxylovou funkci a připojit jej následně pomocí Mitsunobuovy reakce k příslušné nukleobázi. Tento přístup byl využit zejména při syntéze ANF obsahujících 6-oxopurinové báze, cílených jako inhibitory plasmodiových fosforibosyltransferáz. Byla připravena a otestována série hypoxanthinových a guaninových derivátů, z nichž CPME byly zcela neaktivní, nicméně CPEE a HPEP deriváty vykazovaly účinnou inhibici lidského a plasmodiových enzymů. Výsledky tohoto studia ovšem naznačují, že pro vylepšení aktivity a/nebo selektivity mezi lidským a parazitickým enzymem bude zapotřebí dále modifikovat fosfonátový řetězec.

Dále byly připraveny HPEP monomery nesoucí adenin, thymin, cytosin a guanin pro syntézu modelových oligonukleotidů (nonamerů) sloužících ke studiu teplotní stability duplexů. Tato problematika je v současnosti předmětem dalšího studia.

Abstract

This Ph.D. thesis is a part of detailed SAR studies among acyclic nucleoside phosphonates carried out in the group of the Nucleic acid chemistry (Prof. A. Holý) and later in the group of the Targeted analogues of nucleic acid components (Dr. Z. Janeba) at the IOCB AS CR, v.v.i. Three novel series of acyclic nucleoside phosphonates, namely carboxyphosphonomethoxyethyl (CPME), carboxyphosphonoethoxyethyl (CPEE) and hydroxyphosphonoethoxypropyl (HPEP) derivatives were prepared in order to reveal their biological properties.

The CPME compounds were designed as structural analogues of PMEA (9-[2-(phosphonomethoxy)ethyl]adenine, Adefovir) and (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-(phosphonomethoxypropyl)adenine], well-known compounds with prominent antiviral effects. The key step in their synthesis was oxidation of primary hydroxyl group in HPMP precursors using TEMPO/sodium chlorite/sodium hypochlorite system. The initial docking studies indicated that (*S*)-CPMEA [(*S*)-3-(adenin-9-yl)-2-(phosphonomethoxy)propanoic acid)], 2‘-carboxy analogue of PMEA, could be a candidate with promising anti-HIV activity. Although this compound did not show desired biological activity, its two prodrugs exhibited submicromolar anti-HCV activity. Prepared prodrugs of (*S*)-CPMEA were also shown to be weak inhibitors of adenylate cyclase toxin of bacteria *Bordetella pertussis*.

Another strategy of synthesis of CPME, CPEE and HPEP derivatives was based on the synthon approach. Starting from tritylglycidol, it was necessary to prepare convenient intermediates containing phosphonate and hydroxymethyl/carboxylic moieties and attach them subsequently to the corresponding nucleobase *via* Mitsunobu reaction. This approach was used particularly for the synthesis of 6-oxopurine ANPs designed as inhibitors of plasmodial phosphoribosyltransferases. Series of hypoxanthine and guanine based compounds were prepared and tested. While CPME derivatives revealed no activity, CPEE and HPEP derivatives exhibited potent inhibitory effects on both human and plasmodial enzymes. However, the final results suggested that further modifications of the phosphonate linker may be necessary to achieve higher activity and/or better selectivity between the human and parasitic enzymes.

Furthermore, a novel series of HPEP monomers bearing adenine, cytosine, guanine and thymine was prepared for the subsequent synthesis of model oligonucleotides (nonamers). The nonamers were used for the study of thermal stability of their duplexes. This topic is currently subject of further study.

Acknowledgement

Firstly and foremost, I would like to thank to my former supervisor Prof. Antonín Holý who brought me along to the fascinating field of medicinal chemistry. I am especially very grateful to the head of our group and my supervisor, Dr. Zlatko Janeba for his valuable advices, expert guidance and for giving me the opportunity to work in his group. I would like to express my gratitude to Dr. Dana Hocková for her guidance in the malaria project. Many thanks belong to all the staff from Dr. Janeba group and particularly all members of my laboratory, Dr. Ondřej Baszczyňski, Dr. Petr Šimon, Bc. Lucie Čechová and Dr. Petr Jansa for creating friendly and creative working environment. I would like to express my thanks also to members of groups of Dr. Radim Nencka, Dr. Marcela Krečmerová and Dr. Ivan Rosenberg for establishing highly cooperative environment and for many precious advices.

My thanks are also due to members of the IOCB NMR team, especially Dr. Martin Dračínský and Dr. Lenka Poštová Slavětínská for measurement and interpretation of the NMR spectra, to Dr. Josef Cvačka (Mass Spectrometry, Head) and to Dr. Stanislava Matějková (Analytical Department, Head) for providing technical support and analyses of the prepared compounds.

I would like to acknowledge the group of Dr. Helena Mertlíková-Kaiserová (IOCB) and our external collaborators - Gilead Sciences (Foster City, CA, USA); Prof. Erik De Clercq, Prof. Jan Balzarini and Prof. Graciela Andrei (KU Leuven, Belgium); Dr. Dianne T. Keough and Prof. Luke W. Guddat (The University of Queensland, Brisbane, Australia) - for biological screening.

Special thanks belong to my family and my partner Tomáš Pernický, Ambassador Extraordinary and Plenipotentiary of the Czech Republic to Georgia, whose support, patience and understanding were limitless.

Table of contents

List of abbreviations

A	adenine
ACT	artemisinin-based combination therapy
AHPA	3-(adenine-9-yl)-2-hydroxypropanoic acid
Aldrithiol-2	2,2'-dithiopyridine
AMP	adenosine monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANP	acyclic nucleoside phosphonate
ATP	adenosine triphosphate
AZT	azidothymidine
Boc	*tert*-butoxycarbonyl
C	cytosine
cAMP	cyclic adenosine monophosphate
Cbz	benzyloxycarbonyl
CDI	1,1'-carbonyldiimidazole
CPEE	[2-carboxy-2-(phosphonoethoxy)ethyl]
CPME	[2-carboxy-2-(phosphonomethoxy)ethyl]
DABCO	1,4-diazabicyclo[2.2.2]octane
DAP	2,6-diaminopurine
DAPy	2,4-diaminopyrimidine
DCC	*N,N'*-dicyclohexylcarbodiimide
DEVP	diethyl vinylphosphonate
DHBV	Duck Hepatitis B virus
DHPA	9-(2,3-dihydroxypropyl)adenine
DIAD	diisopropyl azodicarboxylate
DMF	*N,N*-dimethylformamide
DMAP	4-(dimethylamino)pyridine
DMTrCl	4,4'-dimethoxytrityl chloride
DNA	deoxyribonucleic acid
EBV	Epstein-Baar virus
EDAC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

FDA	Food and Drug Administration, USA
G	guanine
GMP	guanosine monophosphate
GTP	guanosine triphosphate
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCMV	human cytomegalovirus
HDP	hexadecyloxypropyl
HGXPRT	hypoxanthine-guanine-xanthine phosphoribosyltranferase
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HIV	Human immunodeficiency virus
HPEP	9-[3-hydroxy-2-(phosphonoethoxy)propyl]
HPMP	9-[3-hydroxy-2-(phosphonomethoxy)propyl]
HPMPA	9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine
HPMPDAP	9-[3-hydroxy-2-(phosphonomethoxy)propyl]diaminopurine
HPLC	high performance liquid chromatography
HSV	Herpes simplex virus
Hx	hypoxanthine
H1	ethylacetate : acetone : ethanol : water = 15 : 3 : 4 : 3
IMP	inosine monophosphate
IOCB	Institute of Organic Chemistry and Biochemistry, Prague
MeCN	acetonitrile
MOM	methoxymethyl
m.p.	melting point
MSV	Murine sarcoma virus
MW	microwave (irradiation)
NECA	*N*-ethyl-1'-deoxy-1'-(6-amino-*9H*-purin-9-yl)-β-ribofuranuronamide
NMR	nuclear magnetic resonance
N(t)RTI	nucleoside/nucleotide reverse transcriptase inhibitor
PEE	phosphonoethoxyethyl
Pf	*Plasmodium falciparum*
PhSH	thiophenol
PMEA	9-[2-(phosphonomethoxy)ethyl]adenine, adefovir
PMEG	9-[2-(phosphonomethoxy)ethyl]guanine

PMPA	(*R*)-9-[2-(phosphonomethoxy)propyl]adenine, tenofovir
PRT	phosphoribosyltransferase
PTC	phase transfer catalysis
Pv	*Plasmodium vivax*
Py	pyridine
RNA	ribonucleic acid
RSV	respiratory syncytial virus
RT	reverse transcriptase
r.t.	room temperature
SAH	*S*-adenosyl-L-homocystein hydrolase
SAR	structure activity relationship
T	thymine
t-BuOH	*tert*-butyl alcohol
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TDF	tenofovir disoproxil fumarate
TEAB	tetraethylammonium bicarbonate
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxyl
THF	tetrahydrofurane
THP	tetrahydropyranyl
TMSCl	trimethylsilyl chloride
TMSBr	trimethylsilyl bromide
ÚOCHB	Ústav Organické Chemie a Biochemie AV ČR, v.v.i, Praha
VZV	Varicella zoster virus
WHO	World Health Organization

Note: abbreviations could be combined together

1. Introduction

1.1. Nucleic acid components as potent therapeutics

1.1.1. Nucleoside analogs

Purine and pyrimidine nucleotides are basic keystones of nucleic acids. These essential nucleic acids components play an important role not only in replication of hereditary information but they participate in many important regulatory processes in living systems. Their chemical modification leads to analogues of nucleic acid components that are able to influence basic life processes in cell or life cycle of cell parazites. This fact is commonly exploited for design of potential antibiotics, antivirotics, cytostatics and antiprotozoan agents.

The first generation of analogues of nucleic acid components is based on the principle of maximum resemblance to the natural metabolite which implies that active centre of the corresponding enzyme (with which the compound interacts) is not conformationally altered. This first generation includes important cures for leukemia such as cytarabine **1**[1] (araC, FDA approved in 1969, Fig. 1) which is still used in the therapy of acute myeloid leukemia, and vidarabine **2**[2] (araA, FDA approved in 1978, Fig. 1) which is utilized to treat myeloid leukemia as well.

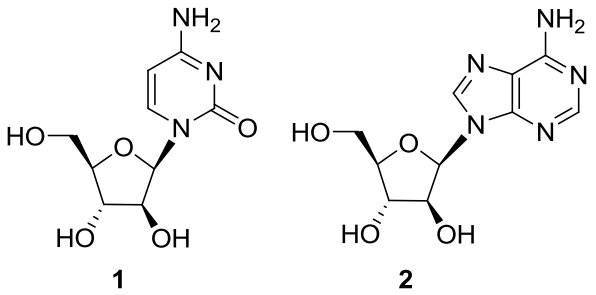


Figure 1. Nucleoside analogues containing arabinose as the sugar part.

Modified nucleobases were among the first of the nucleic acid components used in clinical arena. Pyrimidine as well as purine analogues have been utilized in oncology for more than sixty years. 5-Fluorouracil (5-FU, Fig. 2) **3**[3] represents the most appreciable example of modified pyrimidine, which is mainly used for the treatment of

colorectal carcinomas. Capecitabine **4**[4] (Fig. 2), a less toxic prodrug of 5-FU, is nowadays widely used in the treatment of colorectal and breast cancer. The noticeable examples of altered nucleobases with antimetabolic properties used in therapeutic practices are thioanalogues of purine - thiopurine **5**[5] (Fig. 2), thioguanine **6**[5] (used for the treatment of acute leukemias, chronic myelogenous leukemia, inflammatory bowel disease, Fig. 2) and azathioprine **7**[6] (Fig. 2) used in transplantology and for treatment of autoimmune diseases due to its immunosuppressive properties.

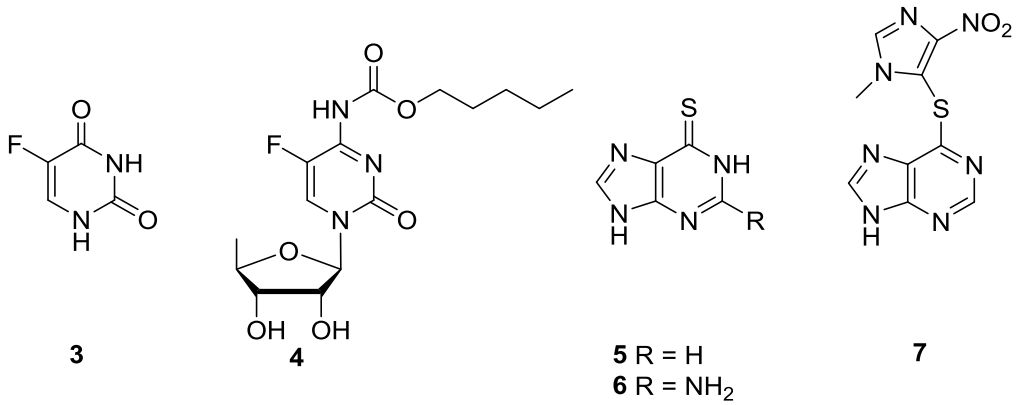


Figure 2. Modified nucleobases used in clinical practice.

Replacement of the methyl group of thymidine led to the novel class of modified nucleosides with significant antiviral activity. The oldest of them, 5-iodo-2'-deoxyuridine **8**[7] (idoxuridine, IUDR, Fig. 3), is used topically to treat herpes simplex keratitis. Structurally related to idoxuridine is 5-trifluoromethyl-2'-deoxyuridine **9**[8] (trifluridine, TFT, Fig. 3), used topically as eyedrops or ophthalmic cream for treatment HSV keratitis. Others include edoxudine **10**[9] (Fig. 3) with extraordinary activity against HSV and brivudine (BVDU) **11**[8b,10] (Fig. 3) utilized as antivirotic against HSV (type 1), VZV and some other herpesviruses.

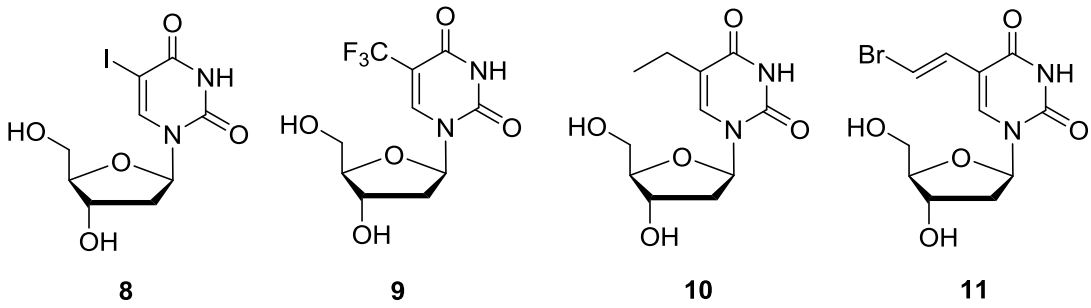


Figure 3. C-5 modified 2'-deoxyuridines as antiviral agents.

Zidovudine **12**[11] (AZT, ZDV, first reported in 1964, FDA approved in 1987, Fig. 4) belongs among antimetabolites of the first generation, as well. This compound was the first breakthrough in HIV/AIDS therapy due to its ability to significantly reduce replication of the virus leading to clinical and immunological improvements.[12] Other antiretroviral nucleosides were also introduced as an answer to the uprising epidemic of HIV in the 80s[13] – namely stavudine **13**[14] (d4T, first reported in 1966, FDA approved in 1994, Fig. 4), zalcitabine **14**[15] (ddC, first reported in 1967, FDA approved in 1992, Fig. 4) and didanosine **15**[16] (ddI, first reported in 1964, FDA approved in 1991, Fig. 4).

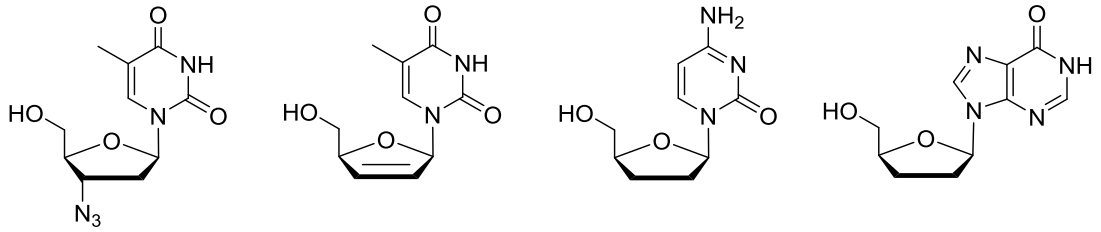


Figure 4. Antiretroviral nucleosides used in clinical practice.

Effect of these nucleosides is based on their incorporation into the growing chain of nucleic acid followed by chain termination. As chain terminators act also sugar modified nucleosides containing L-thioxolane ring – particularly lamivudine **16**[17] (3TC, first reported in 1988, FDA approved in 1995 as a combination drug with zidovudine, Fig. 5) and emtricitabine **17**[18] (FTC, FDA approved in 2003, Fig. 5) used for treatment of HIV and HBV infections.

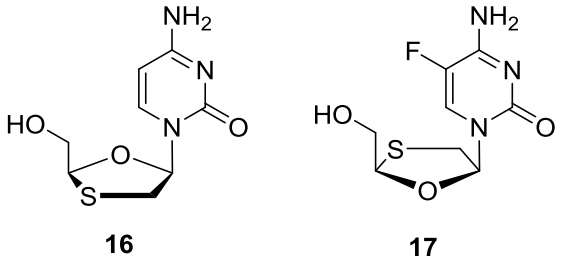
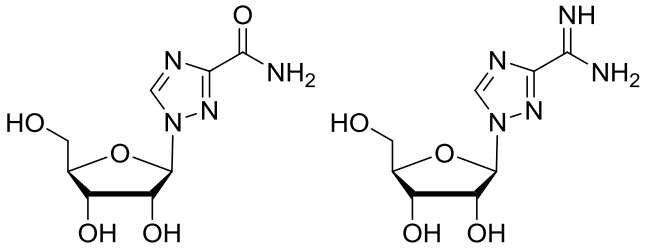


Figure 5. Examples of nucleoside reverse transcriptase inhibitors.

Applicability of modified nucleosides as antivirals is by far not limited to therapy for HSV and HIV infections. Viruses such as respiratory syncytital virus (RSV) or hepatitis C virus (HCV) are commonly treated with ribavirin **18**[19] (FDA approved in 1980 as an anti-RSV drug and in 1998 as anti-HCV drug to be used in combination

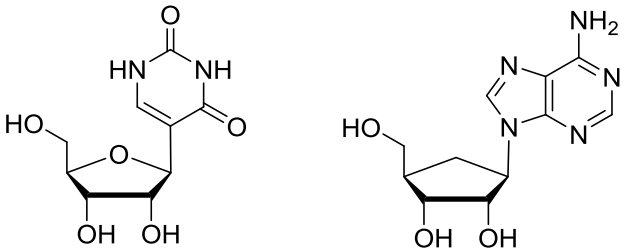
with interferon[20], Fig. 6). Taribavirin **19**[21] (Viramidine, Fig. 6) is a prodrug of ribavirin currently in Phase III human trials for treatment of chronic hepatitis C.



18 **19**

Figure 6. Ribavirin and its prodrug taribavirin.

Unfortunately, antimetabolites of the first generation are usually catabolically unstable. One of the possible solutions to overcome this problem consists in replacement of the hemiacetal bond between sugar and heterocycle. In this manner more enzymatically stable bond is formed. Examples of this phenomenon are modified nucleosides of natural origin – namely pseudouridine **20**[22] (C-nucleoside occurring in various types of RNA, Fig. 7) and aristeromycin **21**[23] (carbocyclic nucleoside antibiotic, Fig. 7).



20 **21**

Figure 7. Examples of C-nucleoside and carbocyclic nucleoside.

1.1.2. Acyclic analogues of nucleosides

The second generation of nucleoside analogues does not rely on the maximum structural resemblance to the natural metabolite. Its concept consists in the ability of the analogue to form numerous conformations so that some of them could lead to a stable complex with an enzyme. The requirement is adaptability both of the antimetabolite and of the active centre of the enzyme so that formed ES-complex would be thermodynamically optimal.

The new wave of interest in the development of novel nucleoside antimetabolites was initiated by the discovery of acyclovir **22**[24] in 1974 (FDA approved in 1982, Fig. 8) as the anti-HSV agent. Its derivative ganciclovir **23**[25] (FDA approved in 1994, Fig. 8) is used for treatment of human cytomegalovirus (HCMV) infections and its carba-analogue penciclovir **24**[26] (Fig. 8) exhibits potent activity against HSV-1 and HSV-2, varicella-zoster virus (VZV) and Epstein-Baar virus (EBV). Penciclovir is also used in the form of its prodrug with improved oral bioavailability called famciclovir **25**[27] (FDA approved in 2007, Fig. 8).

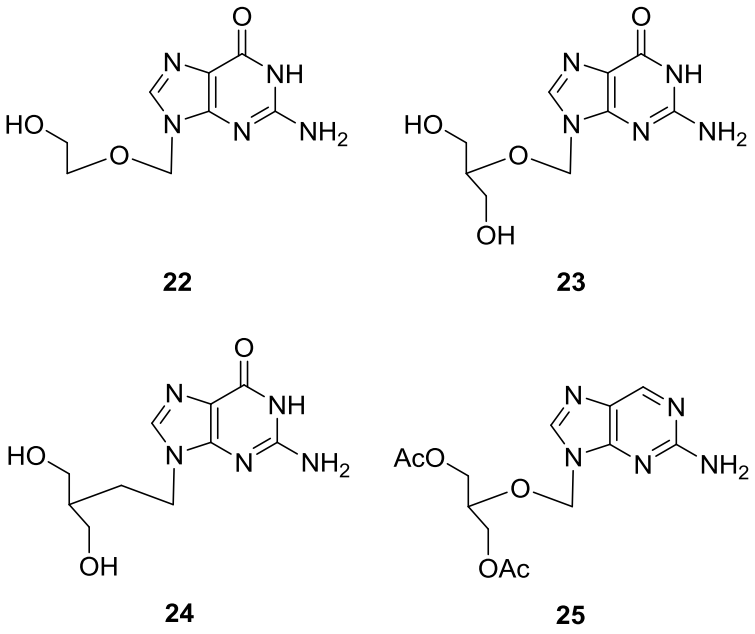
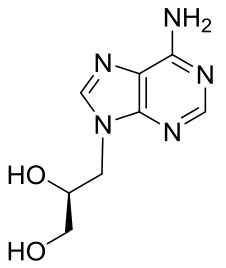


Figure 8. Acyclovir and related antiviral drugs used in clinical practice.

In 1978, four years after the discovery of acyclovir, De Clercq, Holý et al. reported (*S*)-DHPA **26**[28] [(*S*)-9-(2,3-dihydroxypropyl)adenine, Fig. 9] as an acyclic nucleoside with broad-spectrum antiviral activity against a number of RNA and DNA viruses. In comparison with acyclovir, the "gold standard" used for treatment of HSV-1 and HSV-2 infections, (*S*)-DHPA had only a limited appearance on the market in former Czechoslovakia (as Duvira gel) for treatment of herpes labialis (cold sores). (*S*)-DHPA, and several structurally related aliphatic nucleoside analogues, owe their antiviral activity due to an ability to inhibit *S*-adenosyl-L-homocysteine (SAH) hydrolase[29] (discussed in part 1.2.1.1.).



26

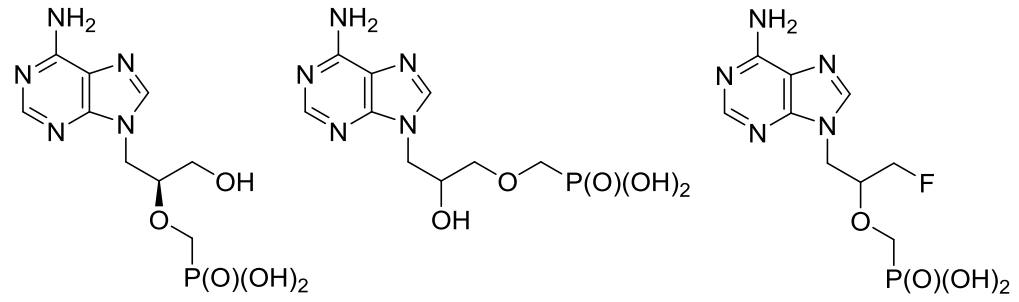
Figure 9. Structure of (*S*)-DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine]

1.1.3. Acyclic nucleoside phosphonates (ANPs)

This type of compounds was originally developed by A. Holý and I. Rosenberg (IOCB, Czech Republic) in cooperation with the team of E. De Clercq (KU Leuven, Belgium). Unlike natural nucleotides, ANPs contain a phosphonomethoxy group which is isopolar and bioisosteric with the phosphate group of nucleoside monophosphates but differs in its higher enzymatic stability. Another important feature is their ability to usually undergo enzymatic phosphorylation which converts them into the corresponding phosphonate diphosphates, analogues of the natural nucleoside triphosphates. Nucleoside phosphonate diphosphates then act as substrates and/or inhibitors for various enzymes.

Their concept was born in 1986 with the identification of the ANP prototype, (*S*)-HPMPA **27**[30] [(*S*)-9-(3-hydroxy-2-(phosphonomethoxy)propyl)adenine, Fig. 10], possessing broad-spectrum activity against DNA viruses including herpes-, adeno-, pox-, papova- and iridoviruses, as well as against retroviruses. HPMPA itself could be conceived as a kind of a construct resulting from the replacement of the carboxylate group of phosphonoacetic acid [PAA, the predecessor of the antiviral agent phosphonoformic acid (foscarnet, Foscavir)] by the acyclic nucleoside analogue DHPA (part 1.1.2.). Its 3'-*O*-phosphonomethyl regioisomer (*iso*-HPMPA) **28**[31] (Fig. 10) exhibits no antiviral activity.[30]

Replacement of the 3'-hydroxyl group of HPMPA by fluorine results in FPMPA **29**[32] [9-(3-fluoro-2-(phosphonomethoxy)propyl)adenine, Fig. 10]. The (*S*)-isomer has been further evaluated for its efficacy against FIV in cats.[33] FPMPA proved to be less antivirally effective but also less toxic than PMEA (see below).



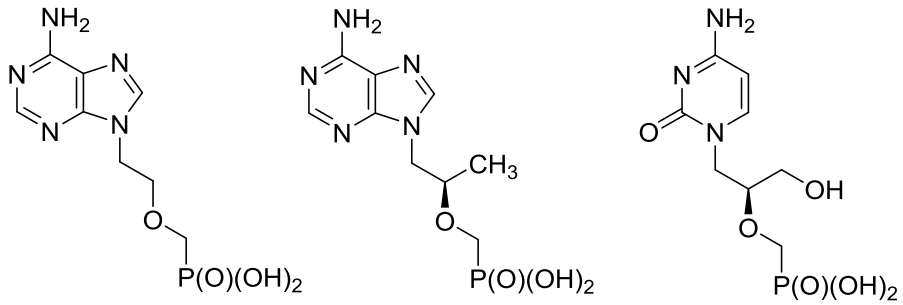
27 **28** **29**

Figure 10. (*S*)-HPMPA and related analogues.

The fact that three ANPs are currently on the market as active components of commonly used antivirals implies their essential influence on human health. First of them, PMEA **30**[34] [9-(2-(phosphonomethoxy)ethyl)adenine, adefovir, Fig. 11], was reported simultaneously with HPMPA in 1986 as an antiviral agent.[30] This compound in its bis(pivaloyloxymethyl) prodrug form [bis(POM)PMEA] was approved by FDA in 2002 for the treatment of chronic hepatitis B under the trade name of Hepsera.

The anti-HIV properties of (*R*)-PMPA **31**[34] ((*R*)-9-[2-(phosphonomethoxy) propyl]adenine, tenofovir, Fig. 11) were firstly described in 1993.[35] Like adefovir, tenofovir is not sufficiently bioavailable by the oral route. Thus, an oral prodrug has been developed, specifically tenofovir disoproxil [bis(isopropyloxycarbonyl-oxymethyl) ester of PMPA, or bis(POC)PMPA], which has been formulated as its salt, tenofovir disoproxil fumarate (TDF) or Viread. Viread was approved in 2001 for the treatment of HIV infections and in 2008 for the treatment of HBV. Viread is highly active against viral reverse transcriptase and is currently used in combinations with other virostatics (Truvada, Atripla, Complera/Eviplera, and Stribild).

The antiviral potential of (*S*)-HPMPC **32**[34] ((*S*)-9-[3-hydroxy-(2-phosphono-methoxy)propyl]cytosine, cidofovir, Fig. 11) was first reported by De Clercq et al.[36] Cidofovir exhibits an antiviral activity against many DNA viruses and was formally licensed in 1996 for clinical use, under the trade name of Vistide, for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients.



30 **31** **32**

Figure 11. Three ANPs approved worldwide for clinical use.

Many other ANPs were found to reveal important antiviral properties, e.g. derivatives of 2,6-diaminopurine (HPMPDAP, PMEDAP, PMPDAP)[34c] and "open ring" analogues of 2,4-diaminopyrimidine (HPMPO-DAPy, PMEO-DAPy, PMPO-DAPy).[34c,37] Like nucleotides,[38] due to their poor bioavailability, all ANPs have to be administered in the form of their prodrugs (or by injection in the case of cidofovir).[39]

1.2. Medicinal chemistry of nucleoside and nucleotide analogues containing a carboxylic group

1.2.1. Alkylated purine and pyrimidine derivatives

1.2.1.1. Derivatives of AHPA and related compounds

The carboxylic group plays an important role in medicinal chemistry in general. An oxidation of hydroxymethylene to carboxylic function has a strong impact on the physical, chemical and biological properties of bioactive compounds. Nucleoside analogues bearing carboxylic group are also important intermediates in synthesis of various biologically active compounds.

(*S*)-3-(Adenine-9-yl)-2-hydroxypropanoic acid **33**[40] [(*S*)-AHPA, Fig. 12] belongs among important nucleoside analogues structurally related to (*S*)-DHPA (part 1.1.2, Fig. 9). (*S*)-AHPA and its alkyl esters exhibit broad-spectrum antiviral activity (including vesicular stomatitis, vaccinia, reo, parainfluenza and measles viruses). These compounds are apparently targeted at *S*-adenosyl-L-homocystein hydrolase (SAH),[41] a key enzyme for the regulation of capping process (methylation of 5'-end-guanine of viral mRNA).[42]

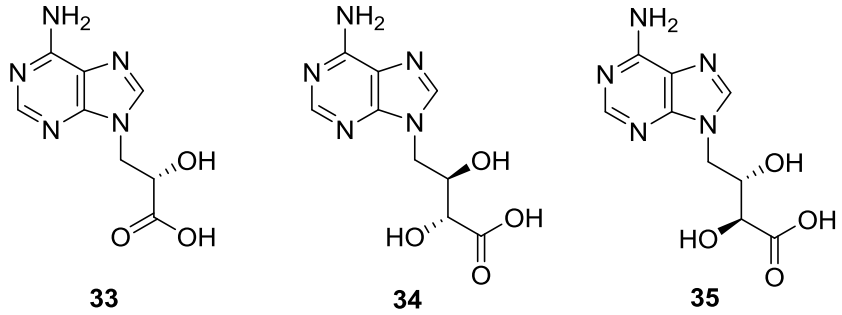


Figure 12. (*S*)-AHPA and related analogues.

As shown in Fig. 13, SAH hydrolase inhibitors block the cleavage of *S*-adenosylhomocysteine into two components, homocysteine (Hcy) and adenosine (Ado), which itself can be further metabolized into AMP, adenine and inosine. As a consequence of the SAH hydrolase inhibition, SAH accumulates and leads to an inhibition of the SAM-dependent methylation reactions, including those that are required for the maturation (i.e., 5'-capping) of viral mRNAs. Thus, maturation of viral mRNAs is suppressed, and so is the production of progeny virus particles.[29]

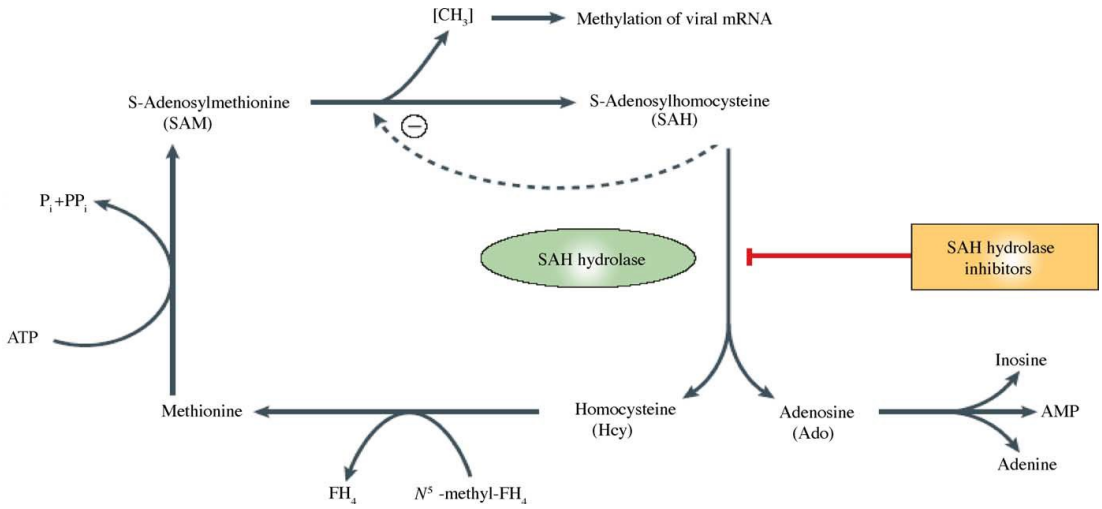
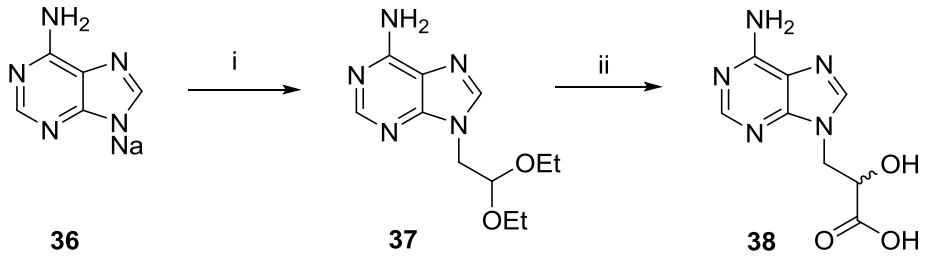


Figure 13. Mechanism of action of adenosine based inhibitors of SAH hydrolase.[29]

(*S*)- and (*R*)-AHPA were originally prepared by two-step oxidation of 5-(adenine-9-yl)-5-deoxyaldofuranoses; first step is oxidation of 1,2-diol moiety with sodium periodate, followed by conversion of the intermediate aldehyde to the desired carboxylic acid using sodium periodate in the presence of ruthenium catalyst.[43] Due to the high biological importance of the compound **33** and its derivatives it was desirable to develop a new synthetic approach (Scheme 1). The first step includes condensation of bromoacetaldehyde diethylacetal with sodium salt of adenine **36** (or adenine in the presence of potassium carbonate) in DMF at elevated temperature to yield acetal **37**. The free aldehyde, obtained by treatment of **37** with diluted aqueous acids, is treated with an excess of alkali metal cyanide to give the corresponding cyanohydrin derivative, which is finally hydrolysed to racemic AHPA **38**.[44]



36 **37** **38**

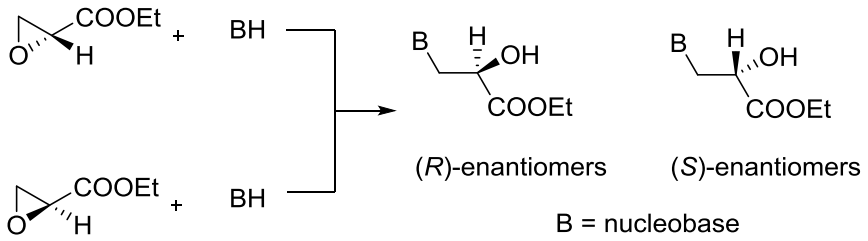
Scheme 1. Synthesis of racemic AHPA **38**. Reaction conditions: i) $BrCH_2CH(OEt)_2$, DMF, elevated temperature; ii) 1. HCl; 2. KCN; 3. HCl.

Eritadenine may be considered a homologous compound to AHPA. Natural D-eritadenine **34** [(*2R,3R*)-4-(adenin-9-yl)-2,3-dihydroxybutanoic acid, Fig. 12] is also an exceptionally potent inhibitor of SAH hydrolase.[45] This compound was

isolated from the edible Japanese mushroom *Lentinus edodes shiitake*[46] and exhibits a strong hypocholesterolemic effect.[47] Stereospecific synthesis of both *erythro*-derivatives **34** and **35** (Fig. 12) starts from 5-(adenin-9-yl)-5-deoxy-D-ribofuranose, -L or -D-arabinofuranose. Oxidation of these compounds in alkaline medium gives, among other products, compounds **34** or **35**. The yield of this reaction is low and isolation of the desired product is difficult. More efficient alternative method for preparation of *erythro*-isomers **34** and **35** is based on the condensation of sodium salt of adenine with 2,3-*O*-substituted erythronolactone. This synthetic approach requires a multiple-step preparation of the starting material, but purification of the products is much easier.[45] In contrast to the neutral open chain analogues which are reversible inhibitors of SAH hydrolase, eritadenines (as well as AHPA and its esters) inactivate the enzyme irreversibly.[42]

Although D-eritadenine is a much more potent inhibitor of SAH hydrolase than (*S*)-DHPA, it is not as active as an antiviral agent.[42] An explanation of this fact could be in its difficult penetration into cells caused by high polarity of the carboxyl moiety. This problem can be overcome by esterification of the carboxyl group and, truly, the methyl ester of D-eritadenine revealed more potent antiviral activity compared to the parent compound.[40b] With regard to the complicated large-scale synthesis of esters of D-eritadenine, it appeared much simpler to prepare alkyl esters of racemic 3-adenin-9-yl-2-hydroxypropanoic acid. From the racemic AHPA,[44] a series of esters were prepared by an acid-catalyzed esterification with excess of the corresponding alcohol or esterification mediated by *N,N'*-dicyclohexylcarbodiimide activation of the acid.[40a] Most alkyl esters exhibited either similar or slightly more potent antiviral activity compared to that of (*RS*)- or (*S*)-DHPA.[40a] These compounds are also poor inhibitors of SAH hydrolase.[40b]

As mentioned above, the previous synthesis of pure (*R*)- and (*S*)-enantiomers of AHPA was complicated by difficult purification and low yields of the products. Krečmerová et al.[48] developed simple and effective synthesis of pure enantiomers of AHPA starting from ethyl (*R*)- or (*S*)-oxiranecarboxylate. These chiral three-carbon atom synthons are commercially available. Nucleophilic opening of the oxirane ring with various nucleobases under basic conditions gave corresponding α-hydroxy esters (Scheme 2).



Scheme 2. General synthesis of enantiomeric AHPA esters and their analogues.

Surprisingly, during the reaction of ethyl (*R*)- or (*S*)-oxiranecarboxylate with some nucleobasis (e.g. adenine) a partial racemization was observed. The extent of racemization depends on the character of the heterocyclic base. In contrast to adenine, analogous reaction with 6-chloropurine afforded optically pure products. As all attempts with adenine to prepare the optically pure product failed, N^6-benzoyladenine was used for the reaction with ethyl (*R*)- or (*S*)-oxiranecarboxylate to give the desired product without racemization.[48]

Synthesis of the N^6-derivatives of racemic AHPA was described by Doláková et al.[49] These compounds were prepared under the conditions used for the original preparation of AHPA.[44] SAR study of these compounds contributed to clarifation of the mechanism of AHPA antiviral action.[50]

Replacement of the 2'-hydroxy group by amino function resulted in the willardiine analogues. Willardiine **39** [(*S*)-2-amino-3-(2,4-dioxo-3,4-dihydro-pyrimidin-1(*2H*)-yl)propanoic acid, Fig. 14] is a naturally occurring non-protein amino acid found in seeds of *Acacia* and *Mimosa*[51] and act as an AMPA receptor agonist while its 5-iodo derivative **40** (Fig. 14) is a selective kainate receptor agonist.[52]

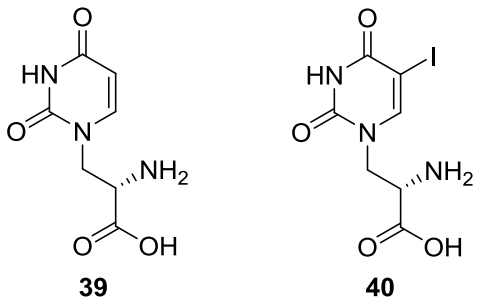


Figure 14. Willardiine **39** and its 5-iodo analogue **40**.

Dolman et al.[52] reported synthesis of N^3-substituted willardiine analogues bearing carboxylic moiety in a side chain and some of them had been shown to be potent kainate and/or AMPA receptor antagonists (Fig. 15).

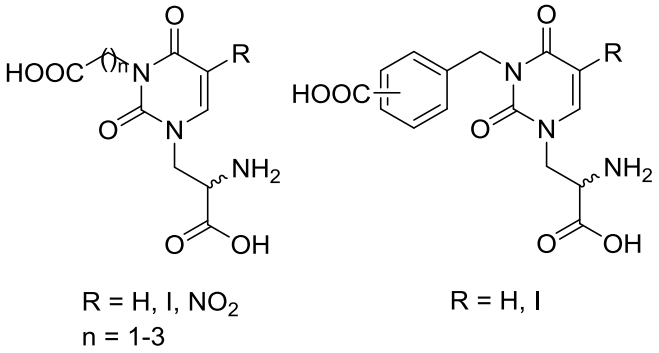
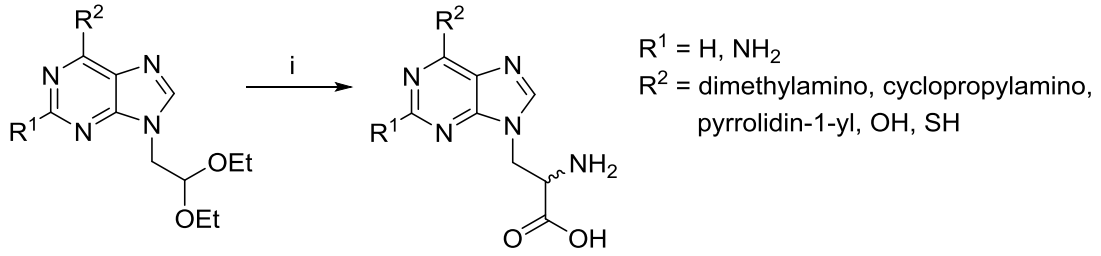


Figure 15. N^3-Substituted willardiine analogues.

Some of the willardiine analogues bearing purine nucleobases were described by Nollet et al.[53] Their synthesis is based on either Michael addition of the purine derivative to the α-chloroacrylate, followed by hydrolysis and amination or alkylation of the nucleobase with bromoacetaldehyde diethyl acetal, followed by hydrolysis to the free aldehyde and conversion into the 2-aminopropanoic acid by Strecker synthesis.[50] The second approach was used by Doláková et al.[49,50] for preparation of (*RS*)-2-amino-3-(purin-9-yl)propanoic acid derivatives bearing purines substituted at positions 2 and 6 (Scheme 3).



Scheme 3. Synthesis of willardiine analogues with purine bases. Reaction conditions: i) 1. HCl; 2. KCN, NH_4OH, NH_4Cl; 3. HCl.

These compounds were tested for their immunomodulatory and immunostimulatory potency. Some of them revealed immunobiological activity but none of them showed any interesting antiviral activity.[50]

1.2.2. Nucleosides bearing carboxylic group at the nucleobase

1.2.2.1. Nucleosides and nucleotides derived from orotidine

Orotidine **41** (Fig. 16), or 6-carboxyuridine, is a nucleoside bearing orotic acid as a nucleobase and its derivatives play an important role in medicinal chemistry. Orotidine was originally isolated from mutants of the fungus *Neurospora crassa*.[54] In the form of its 5'-phosphate (orotidylic acid), it occurs as an intermediate in the synthesis of pyrimidine nucleotides: by the action of orotidylate decarboxylase, orotidine 5'-phosphate is converted to uridine 5'-phosphate.[55] An orotidine regioisomer, 5-carboxyuridine **42** (Fig. 16) was described as a by-product of the oxidation of 5-hydroxymethyluridine.[56] An efficient synthesis of 5-ethoxycarbonyluridine **43** (Fig. 16) based on building of nucleobase from malonic ethyl ester chloride was developed by A. Holý.[55] Its phosphorylation gave the corresponding 5'-phosphate which was converted to the 5-carboxyuridine-5'-phosphate. These nucleotides represent good substrates for nonspecific phosphomonoesterases. 5-Ethoxycarbonyluridine-5'-phosphate is a good substrate for snake venom (*Crotalus terrificus terrificus*) 5'-nucleotidase, pancreatic ribonuclease and ribonuclease T 2.[55]

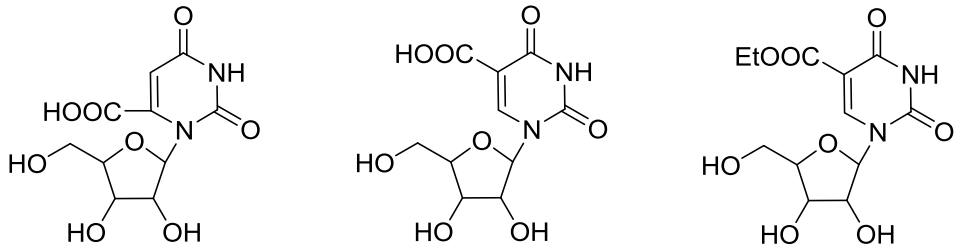


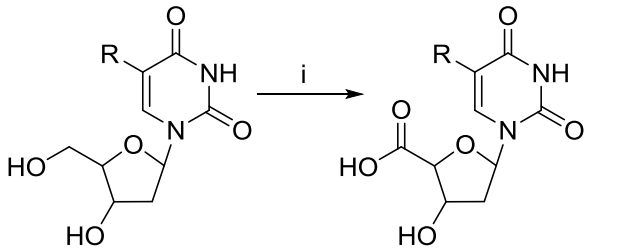
Figure 16. Orotidine **41** and its analogues.

1.2.3. Nucleoside-4'-carboxylic acids

Oxidation of the 5'-hydroxymethylene moiety of nucleosides to the 4'-carboxyl group is the key step in the preparation of a number of biologically active compounds.[57] There are several methods describing general preparation of such nucleoside-4'-carboxylic acids using various oxidizing agents.

1.2.3.1. Oxidation of the 5'-hydroxymethylene group using molecular oxygen and platinum catalyst

This method was applied for the oxidation of 5'-hydroxymethylene of the unprotected nucleosides[56,58], but it afforded relatively low yields in the case of 2',3'-isopropylidene-protected nucleosides.[59] Nevertheless, this reaction was used for the preparation of many important intermediates in synthesis of number of biologically active compounds.[60-63] Thomas et al.[64] applied this method for the synthesis of ribofuronic acid derivatives **46** and **47** from 2'-deoxyuridine **44** and 5-fluoro-2'-deoxyuridine **45**, respectively (Scheme 4).



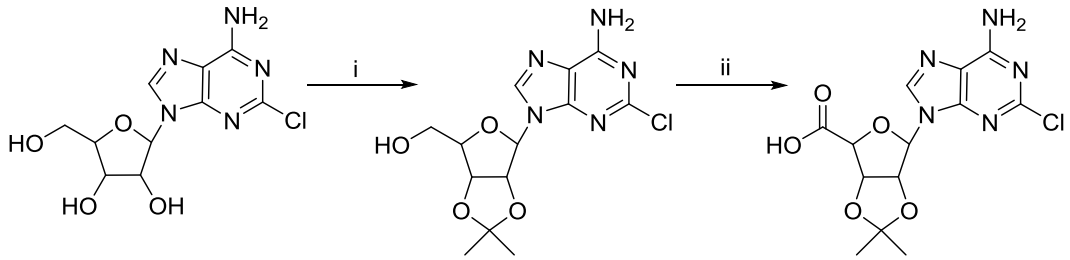
44 R = H
45 R = F

46 R = H (yield 48%)
47 R = F (yield 66%)

Scheme 4. Reaction conditions: i) freshly reduced PtO_2, O_2, aqueous $NaHCO_3$, 70 °C, 25-33 h.

1.2.3.2. Oxidation of the 5'-hydroxymethylene group using potassium permanganate

This methodology usually requires strongly alkaline reaction conditions, thus, limiting its use only for purine-containing nucleosides.[65] Transformation of the nucleosides to the corresponding to 2',3'-isopropylidene derivatives is also necessary before the oxidative step. Typical procedure using potassium permanganate was described by Hutchison et al.[65b] The starting nucleoside **48** was protected by treatment with 2,2-dimethoxypropane to give the protected intermediate **49** which was subsequently oxidized to uronic acid derivative **50** (Scheme 5).



48 49 50

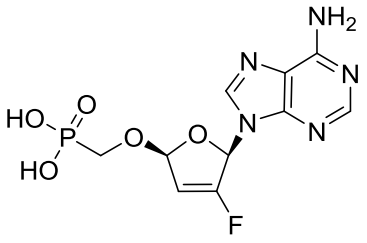
Scheme 5. Reaction conditions: i) 2,2-dimethoxypropane, acetone, camphorsulfonic acid , 16 h, yield 58%; ii) $KMnO_4$, KOH, water, 3 days, yield 74%.

An alternative oxidation using $KMnO_4$/AcOH has several advantages in the preparation of adenosine-5'-ribouronic acids over the alkaline permanganate method, namely lower excess of $KMnO_4$ and shorter reaction time. However, both methods with potassium permanganate degrade N^6-substituents.[66]

1.2.3.3. Oxidation of the 5'-hydroxymethylene group using chromium (VI) oxide

An approach employing CrO_3/AcOH was successfully used for synthesis of N^6-substituted adenosine-5'-uronic acids, where oxidation with $KMnO_4$/KOH is not applicable due to N^6-substituent oxidative cleavage.[66] These compounds are analogs of NECA (*N*-ethyl-1'-deoxy-1'-(6-amino-*9H*-purin-9-yl)-β-ribofuranuronamide), the prototypic A2 adenosine receptor agonist which is exceptionally potent coronary vasodilatator.[66]

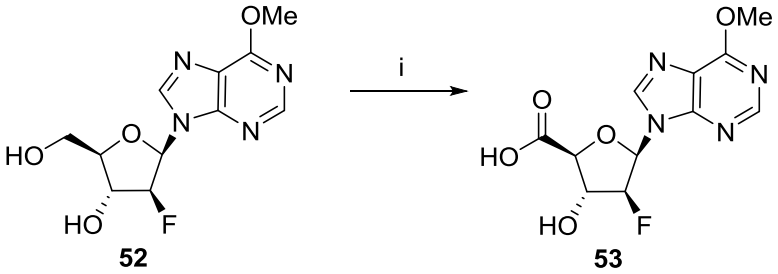
Oxidation with Jones' reagent (solution of chromium (VI) oxide in dilute sulfuric acid and acetone) was used by Mackman et al.[67] for the preparation of a key intermediate in the synthesis of GS-9148 **51** (Fig. 17) and its prodrugs.



51

Figure 17. Structure of GS-9148.

Nucleoside phosphonate **51** [5-(6-aminopurin-9-yl)-4-fluoro-2,5-dihydrofuran-2-yloxymethyl]phosphonic acid, is a HIV-1 reverse trancriptase (RT) inhibitor with an exceptional resistance profile toward N(t)RTI resistance mutations.[67] The key oxidation step in the synthesis of GS-9148 is depicted in Scheme 6.

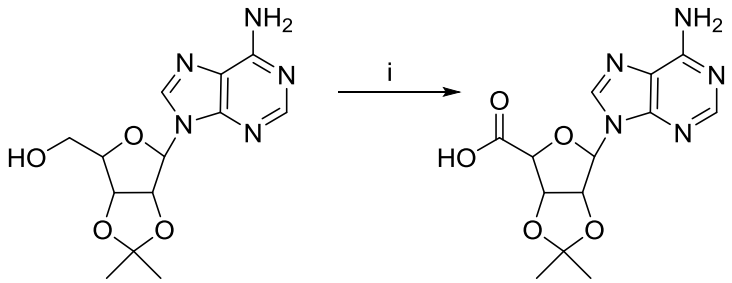


52 **53**

Scheme 6. Reaction conditions: i) Jones' reagent, aceton, Celite545, 0 °C - r.t., 14 h, yield 67%.

1.2.3.4. Oxidation of the 5'-hydroxymethylene group using ruthenium tetraoxide

Ruthenium tetroxide was introduced by Djerassi and Engle as an oxidant in organic synthesis in 1953.[68] This agent was successfully used for oxidative transformations of many organic compounds.[69] It's usually generated *in situ* from ruthenium trichloride employing sodium periodate as a co-oxidant under Sharpless conditions.[70] This non-alkaline method was used to obtain the 4'-carboxylic acids of 2',3'-isopropylidene-protected purine nucleosides in almost quantitative yields.[71] The typical procedure is shown in Scheme 7.

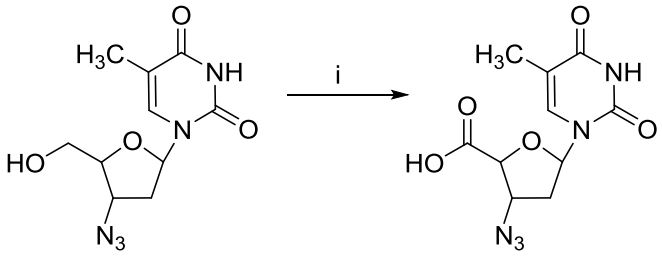


54 **55**

Scheme 7. Reaction conditions: i) $NaIO_4$, $RuCl_3.3H_2O$ in $CH_3CN/CCl_4/H_2O$ (1:1:1.5), r.t., quantitative yield.

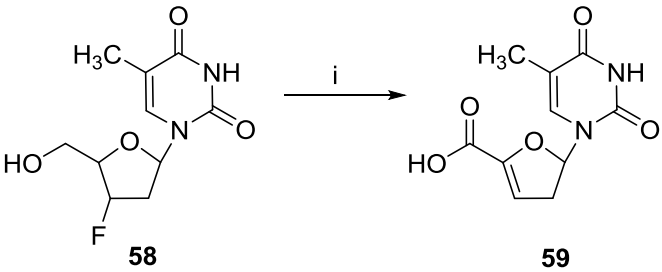
Unfortunately, this methodology has only limited application among purines and cannot be applied to pyrimidine nucleosides, as the reaction conditions cause

decomposition of the nucleoside base.[71] Extension of ruthenium trichloride-mediated oxidation to 2',3'-protected nucleosides bearing pyrimidine nucleobases requires use of both alkaline conditions and potassium persulfate.[72] This method usually affords 4'-carboxylic acid derivatives in good yields. The oxidation of AZT **56** (1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione) to the corresponding uronic acid **57** is a representative example of this procedure (Scheme 8).[72]



Scheme 8. Reaction conditions: i) $K_2S_2O_8$, $RuCl_3.3H_2O$, KOH (1 M in water), r.t., 3 h, yield 80%.

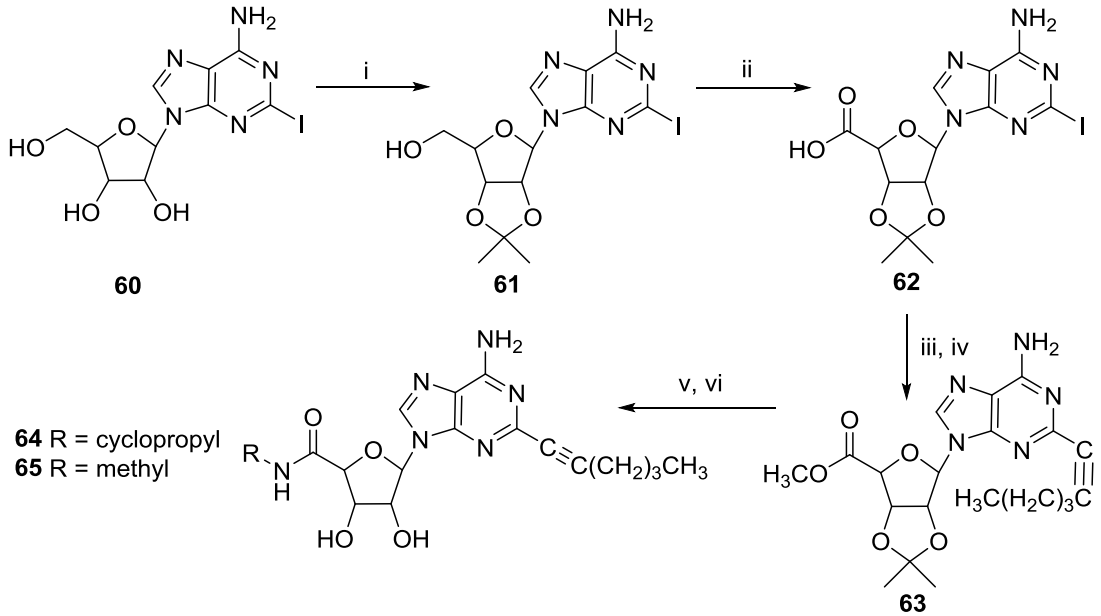
Oxidation using $RuCl_3$ and potassium persulfate under alkaline conditions was also used by Shakya et al.[73] for the synthesis of a new class of antiviral agents, namely dideoxy pyrimidine nucleosides bearing the 4'-carboxyl group. These nucleosides were shown to be inhibitors of HBV and HCV replication. Among these compounds, 3',4'-didehydrothymidine derivative **59** (Scheme 9) was the most potent analogue against DHBV, HBV and HCV.[73] In this special case, the oxidation of the 5'-hydroxymethylene group of compound **58** was accompanied by β-elimination of fluorine at the 3'-position (Scheme 9).



Scheme 9. Reaction conditions: i) $K_2S_2O_8$, $RuCl_3.3H_2O$, 1 M KOH (aq), r.t., 8 h, yield 46%.

Both methods employing ruthenium tetroxide were used by Debnath et al.[74] for oxidation of 5'-hydroxymethylene group of protected adenosine ($RuCl_3$, $NaIO_4$) and thymidine ($RuCl_3$, $K_2S_2O_8$, KOH) (deoxy)nucleosides. The prepared carboxyl derivatives revealed interesting inhibitory activity on ribonuclease A (RNase A).

As mentioned above, purine nucleoside-4'-carboxylic acids have been used as key intermediates in the preparation of esters, thioesters, amides and other derivatives possessing significant pharmacological activities. For example, esters and amides of adenosine-4'-carboxylic acids are effective coronary[75] and renal vasodilatators.[76] 2-(1-Hexyn-1-yl)adenosine-5'-*N*-cyclopropyluronamide **64** (Scheme 10) with potent activity on the A_2 receptor and 2-(1-hexyn-1-yl)adenosine-5'-*N*-methyluronamide **65** (Scheme 10) as the most selective agonist for the A_2 receptor belong to this class of compounds. Selective A_2 receptor agonists possess a potential for the treatment of cardiovascular diseases with minimized toxic effects.[77] Both adenosine-5'-uronamides **64** and **65** were synthetized by Homma et al.[77] The key synthetic step was the oxidation of the 5'-hydroxymethylene group with ruthenium tetroxide, prepared from RuO_2 and $NaIO_4$ in a mixture of CH_3CN, $CHCl_3$ and H_2O (1:1:2). The reaction afforded the desired carboxylic acid intermediate **62** in a good yield (Scheme 10). The iodo group at the C-2 position was not found to be oxidized during the procedure, unlike the oxidation using $KMnO_4$ in aqueous alkaline solution giving a complex reaction mixture due to the oxidation of the 2-iodo group.[77]



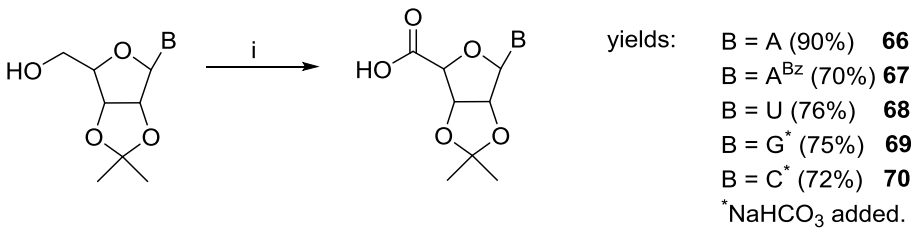
Scheme 10. Reaction conditions: i) acetone, 70% $HClO_4$; ii) RuO_2, $NaIO_4$ in CH_3CN,

$CHCl_3$ and H_2O (1:1:2), r.t., yield 68%; iii) $SOCl_2$ in MeOH, iv) hex-1-yn, $(Ph_3P)_2PdCl_2$, CuI, Et_3N in DMF; v) RNH_2 in MeOH; vi) 80% aqueous CF_3COOH.

1.2.3.5. Oxidation of the 5'-hydroxymethylene group using TEMPO/BAIB system

Nitroxyl radical catalyzed oxidations of alcohols play a significant role in organic synthesis.[78] De Mico et al.[79] described the oxidation of alcohols to aldehydes and ketons using catalytic amounts of (2,2,6,6-tetramethylpiperidin-1yl)oxyl (TEMPO) and stoichiometric amounts of an organic oxidant, [bis(acetoxy)iodo]benzene (BAIB). The main advantage of this method is its mildness and efficiency. The active oxidant is an *N*-oxoammonium salt generated from TEMPO. BAIB is essential for TEMPO regeneration *via* oxidation of the corresponding hydroxylamine group of TEMPO. The reaction generates iodobenzene and acetic acid as byproducts and unlike most other TEMPO-mediated oxidations it doesn't form inorganic salt contaminants.[79] TEMPO-mediated oxidations are compatible with double and triple bonds, esters, ethers, acetals, epoxides, amides, halides, and azides. Finally, protecting groups such as TBDMS, THP, MOM, Boc, Cbz, benzyl, benzoyl, and acetyl are also stable under the reaction conditions.[78a]

N-oxoammonium salts convert aliphatic alcohols to their respective carboxylic acids in the presence of high concentrations of water.[78a] Epp et al.[80] described application of the TEMPO/BAIB system in the mixture of acetonitrile and water (1:1) for preparation of the nucleoside-4'-carboxylic acids in high yields (Scheme 11).

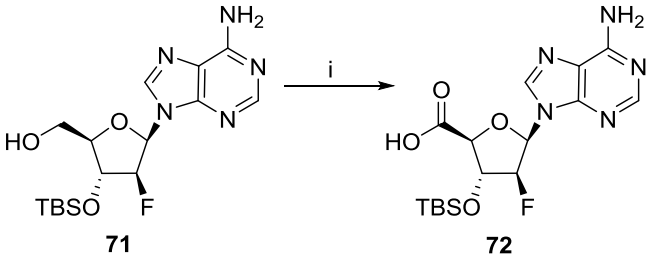


Scheme 11. Reaction conditions: i) TEMPO, BAIB, CH_3CN/H_2O (1:1), r.t., 3 h.

This methodology was extended, with slight procedural modification, to 2',3'-isopropylidene derivatives of cytidine and guanosine. Oxidation of these compounds under the previously described conditions gave poor yields of the desired products.

Increased yields were achieved by adding a second equivalent of sodium bicarbonate which might be necessary to neutralize one equivalent of the acetic acid generated as a reaction by-product.[80] The authors also exploited the fact that *N*-oxoammonium salts are known to be more stable at 0 °C than at room temperature.[81]

TEMPO/BAIB system was also employed in another synthetic approach towards GS-9148 **51** (Fig. 17), a nucleoside phosphonate RT inhibitor with good activity against wild-type HIV (EC_{50} = 12 μM).[82] A crucial oxidative step of the synthesis is shown in Scheme 12.



Scheme 12. Reaction conditions: i) TEMPO, BAIB, CH_2Cl_2, H_2O, r.t., yield 80%.

Analogous approach was used by Mackman et al.[83] for the preparation of several thymidine phosphonomethoxy nucleoside analogues. The phosphonates ddTP **73** and d4TP **74** (Fig. 18) are potent inhibitors of HIV reverse transcriptase, although weaker than the corresponding parent nucleosides ddT and d4T, respectively. The oxidative step of the synthesis of compounds **73** and **74** is shown in Scheme 13.

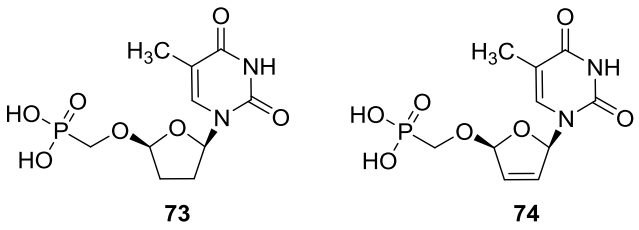
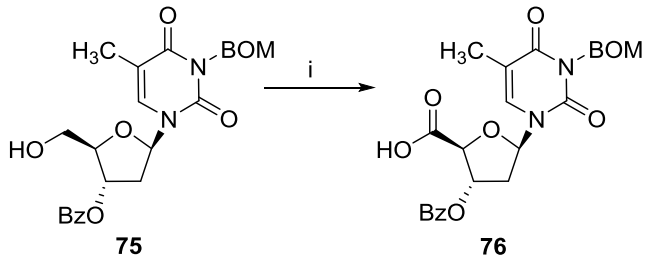
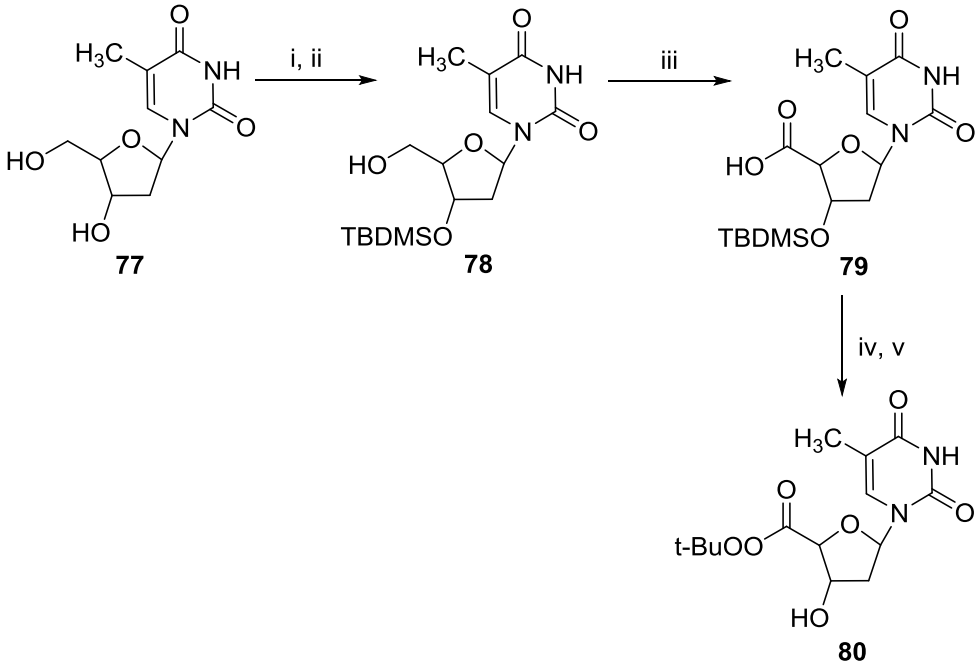


Figure 18. Structures of phosphonates ddTP and d4TP.



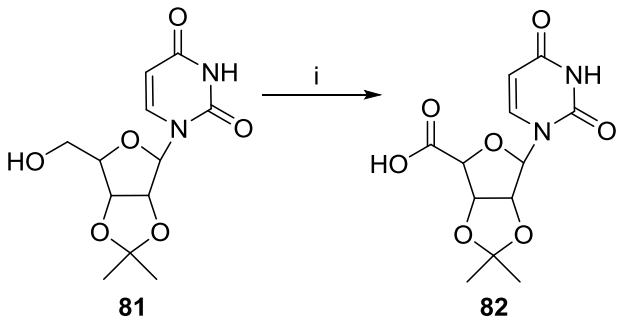
Scheme 13. Reaction conditions: i) TEMPO, BAIB, CH_3CN/H_2O, r.t., 16 h, yield 76%.

Montevecchi et al.[84] employed the TEMPO/BAIB system for almost quantitative oxidation (98%) of protected thymidine **78** to compound **79**, a precursor in the synthesis of *tert*-butyl perester of thymidine-4'-carboxylic acid **80** (Scheme 14).



Scheme 14. Reaction conditions: i) THF/DMF, TBDMSCl, imidazole, $AgNO_3$; ii) MeOH, PPTS; iii) TEMPO, BAIB, CH_3CN/H_2O, r.t., yield 98%; iv) THF, CDI, t-BuOOH; v) THF, TBAF.

High yield (81%) oxidation of 2',3'-*O*-isopropylideneuridine **81** to the desired carboxylic acid **82** using the TEMPO/BAIB system was achieved by Meurillon et al.[85] (Scheme 15).



Scheme 15. Reaction conditions: i) TEMPO, BAIB, CH_3CN/H_2O, r.t., yield 81%.

1.2.4. Oxidations of primary alcohols using TEMPO and $NaClO_2$/NaClO

1.2.4.1. Oxidation of primary alcohols using TEMPO/NaClO system

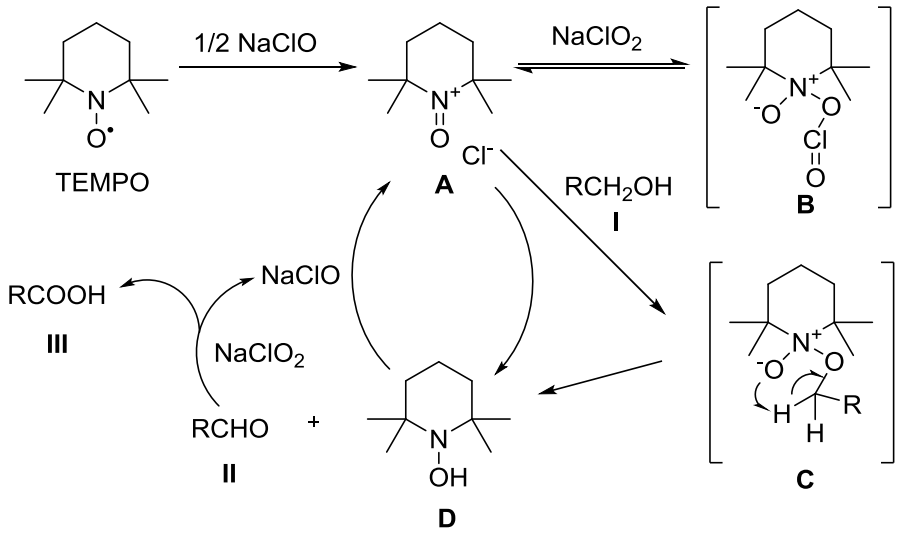
Oxoammonium salts are able to oxidize primary and secondary alcohols to the corresponding carbonyl derivatives.[86] Both stoichiometric and catalytic procedures have been described.[87] Using of TEMPO/sodium hypochlorite system was firstly developed by Anelli et al.[81,88] This TEMPO/bleach (aqueous solution of sodium hypochlorite) oxidation procedure under aqueous/organic two-phase reaction conditions is useful for the large-scale oxidations. It is not very effective for unsaturated alcohols, probably owing to the competitive addition of hypohalogenous acids to the double bond.[88] Another disadvantage of this reaction is potential chlorination of the aromatic rings that dramatically reduces yields.[89] Other oxidants (H_2O_2, CH_3COOOH, *t*-BuOOH, etc.) examined for the TEMPO-catalyzed oxidations did not give reasonable results. Use of sodium chlorite ($NaClO_2$) as the oxidant resulted in a very slow reaction.[89]

1.2.4.2. Oxidation of primary alcohols using Swern oxidation/$NaClO_2$ system

Swern oxidation[90] to the aldehyde followed by sodium chlorite oxidation to the corresponding acid was described by Lindgren et al.[91] Although this method gives reasonably good yields, its limitation consists in epimerization of the neighboring chiral center.[91]

1.2.4.3. Oxidation of primary alcohols using TEMPO/$NaClO_2$/NaClO system

TEMPO-catalyzed oxidation of primary alcohols to the corresponding carboxylic acids using sodium chlorite as the stoichiometric oxidant was reported by Zhao et al.[89] in 1999. The catalytic cycle for the TEMPO/$NaClO_2$/NaClO-catalyzed oxidation is shown in Scheme 16.



Scheme 16. Catalytic cycle for the TEMPO/$NaClO_2$/NaClO-catalyzed oxidation.[89]

A catalytic amount of NaClO oxidizes TEMPO radical to the *N*-oxoammonium ion **A**.[78] The intermediate **A**, in turn rapidly oxidizes the primary alcohol **I** to the aldehyde **II** and gives a molecule of the hydroxylamine **D**.[78] The aldehyde **II** is then oxidized by $NaClO_2$ to the carboxylic acid **III**[90], and a molecule of NaClO is regenerated. The hydroxylamine **D** can either be converted directly to the oxoammonium ion **A** or undergo a *syn*-proportionation with a molecule of the oxoammonium ion **A** to give two molecules of TEMPO radical.[78] The exact mechanism of TEMPO-catalyzed oxidation of alcohols is still not completely clear, but the previous report[78] has confirmed that oxoammonium ion **A** and hydroxylamine **D** are involved. It's also known that $NaClO_2$ can readily oxidize aldehydes to the carboxylic acids without presence of TEMPO.[91] Long induction period of the reaction without a catalytic amount of bleach is presumably due to the relatively slow oxidation of TEMPO radical or the hydroxylamine **D** by $NaClO_2$. Once the reaction is initiated, it becomes self-sustaining as NaClO is continuously regenerated. The chlorination problem (part 1.2.4.1.) is exceedingly suppressed due to remaining low concentration of NaClO during the reaction. Opportunities for epimerization of the neighboring chiral center (part 1.2.4.2.) are also reduced since the labile aldehyde intermediate is rapidly oxidized to the corresponding carboxylic acid by sodium chlorite.[89]

This methodology is mild and efficient and has been demonstrated on variety of primary alcohols.[89,92] The typical procedure consists of dissolution of the substrate in acetonitrile and mixing with phosphate buffer (pH = 6.7). A catalytic amount

(2-7 mol %) of TEMPO is added, followed by simultaneous addition of $NaClO_2$ and a catalytic amount (1-4 mol %) of bleach at 35 °C.[89]

Medicinal chemistry provides many opportunities for employment of this practical, economical and environmentally benign oxidative method. It was shown that this methodology also plays a key role in the synthesis of various intermediates and final compounds with potential biological activity within this thesis.

2. Aims of the Thesis

- Development of an efficient synthesis of novel ANPs derived from 2-(phosphonomethoxy)propanoic acid and evaluation of their antiviral activity.

- Development of a novel strategy for the synthesis of prodrugs of (*S*)-3-(adenin-9-yl)-2-(phosphonomethoxy)propanoic acid [(*S*)-CPMEA] and evaluation of antiviral activity of these compounds.

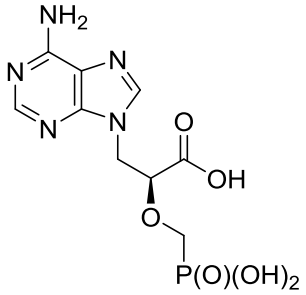
- Synthesis of novel ANPs containing 6-oxopurine bases and an elongated phosphonate linker [carboxyphosphonoethoxyethyl (CPEE) and hydroxy-phosphonoethoxypropyl (HPEP) derivatives] as potential inhibitors of plasmodial phosphoribosyltransferases.

- Development of novel synthesis of HPEP monomers for preparation of model oligonucleotides – nonamers serving for the measurement of thermal characteristics of the complexes (duplexes) – and for their potential use in antisense oligonucleotides and/or in siRNA field.

3. Results and Discussion

3.1. Novel types of ANPs derived from 2-(phosphono-methoxy)propanoic acid

One of the first aims of the presented work was to develop a synthesis of a novel acyclic nucleoside analogue, (*S*)-CPMEA **83a** [(*S*)-3-(adenin-9-yl)-2-(phosphono-methoxy)propanoic acid, Fig. 19]. This compound can be considered to be a direct structural modification of (*R*)-PMPA **31** (Fig. 11) or (*S*)-HPMPA **27** (Fig. 10) with oxidized 2'-methyl or 2'-hydroxymethyl functions, respectively. (*S*)-CPMEA can also be looked upon as an *O*-phosphonomethyl derivative of (*S*)-AHPA **33** (Fig. 12). (*S*)-CPMEA has been designed as a compound with potential anti-HIV activity.



83a

Figure 19. Structure of (*S*)-CPMEA.

3.1.1. Docking studies on (*S*)-CPMEA

The X-ray structure of HIV-1 RT-DNA complex after incorporation of tenofovir diphosphate is an ideal starting point for further rational and targeted drug design (Fig. 20a)[93]. In the complex, two amino acid residues (Arg 72 and Gln 151) in the binding site of the HIV-1 RT are oriented towards the C-2' methyl group of tenofovir diphosphate (Fig. 20a). Apparent incapability of the 2'-methyl group to essentially interact with the two polar amino acids of the HIV-1 RT led to a following suggestion: the replacement of the C-2' methyl with a more polar substituent which can interact with Arg 72 and/or Gln 151 would increase the binding affinity of such an inhibitor to HIV-1 RT. Our docking studies indicated that replacement of the C-2'

methyl group of tenofovir diphosphate (Fig. 20a) with a carboxyl group could, theoretically, lead to the formation of up-to four new hydrogen bonds with Arg 72 and Gln 151 (Fig. 20b).

a) b)

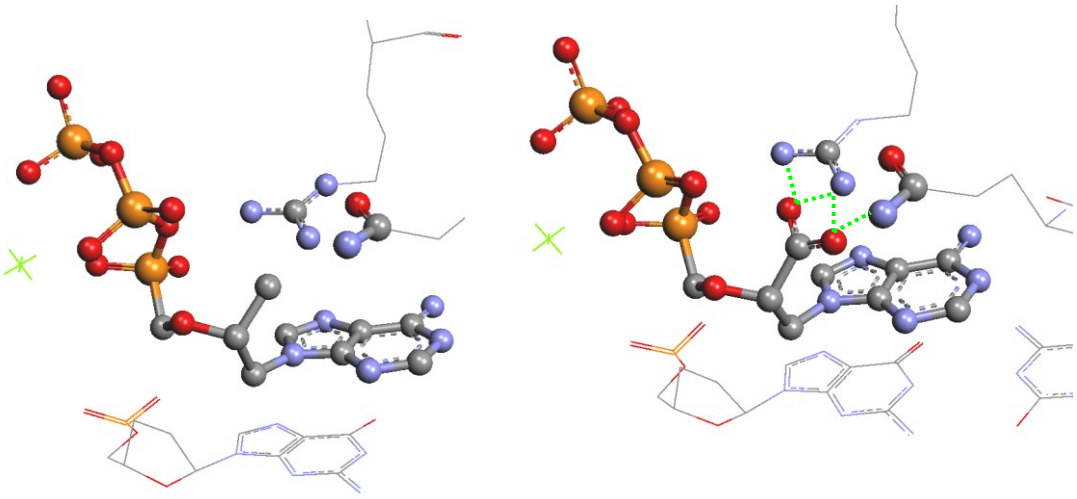


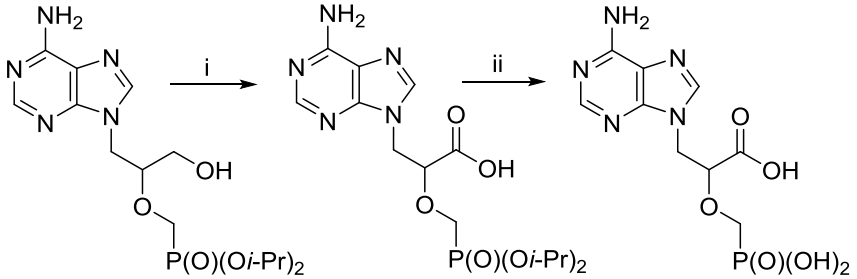
Figure 20. a) Immediate binding site of the comlex of HIV-1 RT and tenofovir diphosphate. b) Docking of the complex of HIV-1 RT and (*S*)-CPMEA **83a** showing possible hydrogen bonding (dotted green lines) between the carboxyl group of (*S*)-CPMEA diphosphate and Arg 72 and Gln 151.

Thus, the initial docking studies indicated that (*S*)-CPMEA **83a** (Fig. 19), an analogue of PMEA **30** (Fig. 11) bearing a carboxyl group at the C-2' position of the aliphatic chain, is a good candidate for strong binding affinity in the enzyme pocket of HIV-1 RT. Compound **83a**, as well as the other CPME analogues, was expected to display a wide range of biological properties.

3.1.2. Synthesis of (*S*)-CPMEA

The direct oxidation of the properly protected (*S*)-HPMPA followed by the removal of the protecting groups was used for the synthesis of target analogue **83a**. Based on that fact the diisopropyl (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl] adenine **84a** (Scheme 17) was chosen as the suitable starting material. This compound can be prepared in sufficient amounts according to the previously described procedures.[94] Currently, there is a considerable number of oxidizing agents, which can be used under relatively mild conditions and which tolerate

number of functional groups.[95] It was necessary to develop a simple, mild and clean oxidation of diisopropyl (*S*)-HPMPA **84a**, which would tolerate the presence of the unprotected amino group at the C-6 position of the purine moiety.



84a (*S*)-
84b (*R*)-

85a (*S*)-
85b (*R*)-

83a (*S*)-
83b (*R*)-

Scheme 17. Reaction conditions: i) see Table 1; ii) Me_3SiBr, MeCN, r.t., overnight.

Oxidation of the 5'-hydroxymethylene group of 2',3'-protected purine nucleosides to the corresponding 5'-uronic acid is often attained by using an excess of potassium permanganate in aqueous KOH solution (see part 1.2.3.2.). An attempted oxidation of compound **84a** under analogous conditions led only to complex reaction mixtures (monitored by TLC, Table 1, entry 1).

Mori and Togo[96] reported a simple and efficient oxidative conversion of primary alcohols to the corresponding methyl esters using iodine/potassium carbonate in methanol. Nevertheless, an attempt to prepare the methyl ester of derivative **85a** by the direct oxidative esterification of compound **84a** under the above described reaction conditions failed (Table 1, entry 2).

Table 1. Oxidation of diisopropyl (*S*)-HPMPA **84a** to diisopropyl (*S*)-CPMEA **85a**.

Entry	**Oxidative agent**	**Desired product**	**Isolated Yield**
1	$KMnO_4$/KOH	**85a**	complex mixture
2	I_2/K_2CO_3/MeOH	methyl ester of **85a**	complex mixture
3	$RuO_2/NaIO_4$	**85a**	63%
4	TEMPO/BAIB	**85a**	71%
5	TEMPO/$NaClO_2$/NaClO	**85a**	87%

Ruthenium tetroxide (RuO_4), another widely used oxidizing agent, is ideal when a very vigorous oxidizing agent is needed but mild conditions must be maintained (see part 1.2.3.4.). Since RuO_4 can decompose explosively, it is mostly prepared in situ by oxidation of $RuCl_3$ or RuO_2. In view of the fact that $RuCl_3$ is considered to be a very hygroscopic reagent, not easy to handle, it was decided to use RuO_2 as the oxidizing agent. Oxidation of diisopropyl (*S*)-HPMPA **84a** with $RuO_2/NaIO_4$ afforded derivative **85a** in a satisfactory yield (63%, Table 1, entry 3). The oxidation was carried out in a mixture of acetonitrile, chloroform, and water (1:1:2) according to the described procedure used for the preparation of adenosine 5'-uronamides,[77] with an addition of concentrated HCl to adjust the pH to 2.5. In spite of the good isolated yield, purification was very tedious and column chromatography had to be repeated several times to obtain pure (*S*)-CPMEA derivative **85a**, probably owing to the fact that phosphonates strongly bind metal ions. Thus, a cleaner and less laborious procedure was still desirable.

Commercially available TEMPO belongs to the class of stable organic nitroxyl radicals commonly used for the oxidation of primary and secondary alcohols.[95] In our case, TEMPO/BAIB (see part 1.2.3.5.) was used as a system of choice for mild and efficient oxidation of (*S*)-HPMPA derivative **84a** to afford the expected product **85a** in 71% yield (Table 1, entry 4).

When BAIB was replaced by $NaClO_2/NaClO$ (sodium hypochlorite is a readily available and inexpensive oxidant, household bleach) an even higher yield (87%, Table 1, entry 5) of (*S*)-CPMEA derivative **85a** was achieved. This oxidizing system (TEMPO/$NaClO_2$/NaClO, see part 1.2.4.3.) offers a clean reaction and easy isolation of the product by flash chromatography on silica gel followed by crystallization. This efficient procedure gave compound **85a** in very high purity.

Eventually, the desired product (*S*)-CPMEA (**83a**, Scheme 17) was obtained in a 76% yield by cleavage of the ester moiety of compound **85a** under the standard conditions (TMSBr in MeCN).[97]

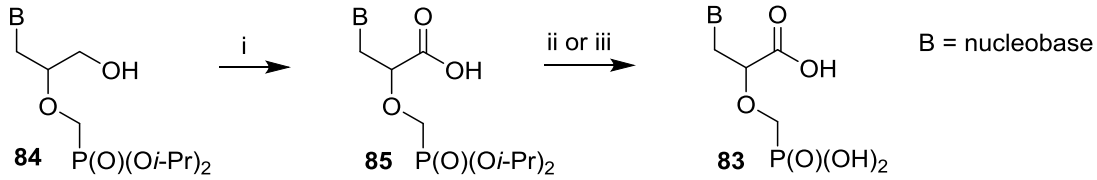
Analogously, oxidation of enantiomeric diisopropyl (*R*)-HPMPA **84b** utilizing the TEMPO/$NaClO_2$/NaClO system afforded the derivative **85b** in a 76% yield. Subsequent deprotection of compound **85b** gave free phosphonic acid **83b** in a 50% yield.

Unfortunately, none of the oxidizing systems listed in Table 1 afforeded (*S*)-CPMEA **83a** by direct oxidation of free (*S*)-HPMPA **27** (Fig. 10), confirming

the importance of the diester protection of the phosphonate group during the procedure, and synthetic transformations in general.

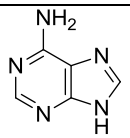
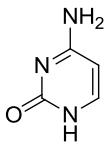
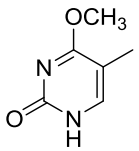
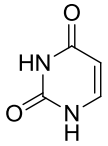
3.1.3. Synthesis of other CPME derivatives

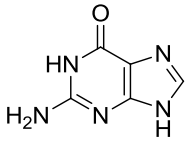
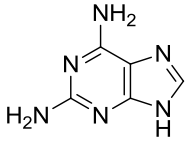
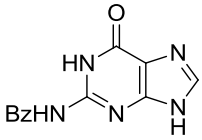
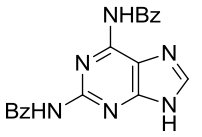
It was decided to verify the more general applicability of the optimized oxidation protocol. Therefore, it was utilized for the preparation of the CPME derivatives bearing other nucleobases (Table 2, Scheme 18). The desired diisopropyl CPME analogues **85** were obtained by the oxidation of the corresponding HPMP derivatives **84** with TEMPO/$NaClO_2$/NaClO in high yields (76-87%).



Scheme 18. General synthesis of CPME analogues **83**. Reaction conditions: i) TEMPO, $NaClO_2$, NaClO, MeCN, phosphate buffer, r.t., 24 h; ii) TMSBr, MeCN, r.t., overnight; iii) 0.5 M HCl (aq), microwave irradiation, 140 °C, 30 min.

Table 2. Oxidation of HPMP analogues **84** with TEMPO/$NaClO_2$/NaClO to give **85**, followed by diester removal to yield **83**.

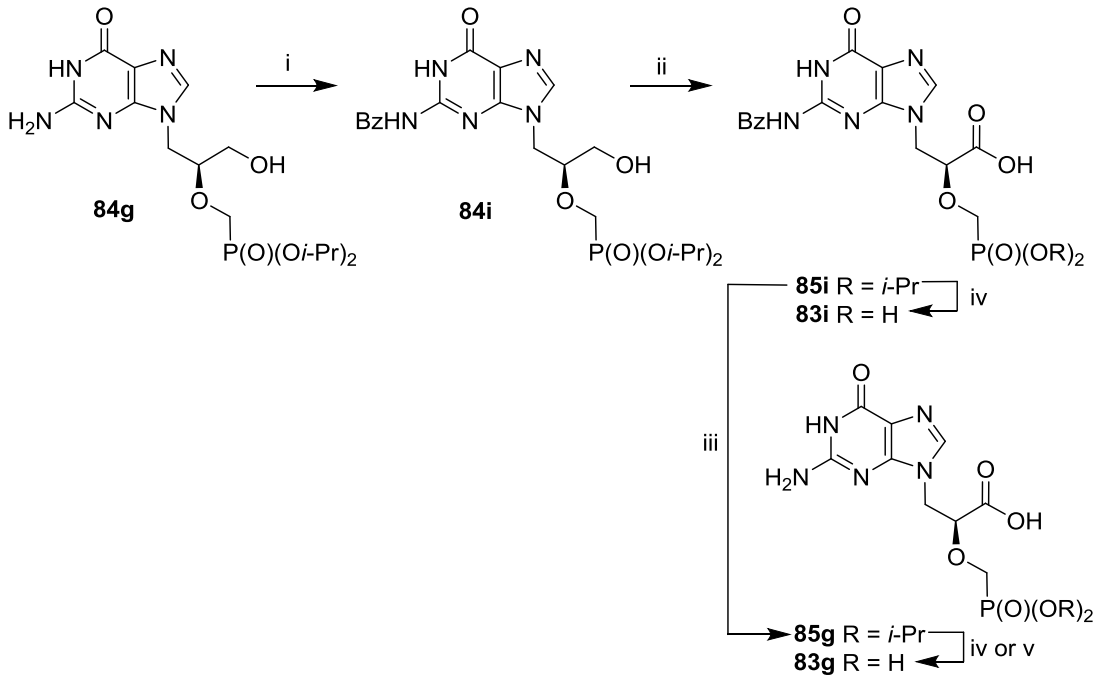
Entry	Nucleobase B	Starting compound	Product (yield)[a] of oxidation	Product (yield)[a] of the phosphonate deprotection
1		**84a** (*S*)-	**85a** (87%)	**83a** (68%)[b], (46%)[c]
2		**84a** (*R*)-	**85a** (76%)	**83b** (50%)[b]
3		**84c** (*S*)-	**85c** (83%)	**83c** (62%)[b], (39%)[c]
4		**84d** (*S*)-	**85d** (82%)	**83d** (64%)[b], (44%)[c]
5		**84e** (*S*)-	**85e** (81%)	**83e** (42%)[c]
6		**84f** (*R*)-	**85f** (77%)	**83f** (48%)[c]

7		**84g** (*S*)-	complex mixture (no **85g** isolated)[d]	**83g** (61%)[b], (37%)[c]
8		**84h** (*S*)-	complex mixture (no **85h** isolated)	**83h** (38%)[c,e]
9		**84i** (*S*)-	**85i** (78%)	**83i** (52%)[b]
10		**84j** (*S*)-	**85j** (80%)	N.A.[f]

[a]Isolated yields; [b]Procedure A; [c]Procedure B; [d]Compound **85g** prepared by other way (Scheme 19); [e]Prepared from compound **85k** (Scheme 20); [f]N.A. not applicable

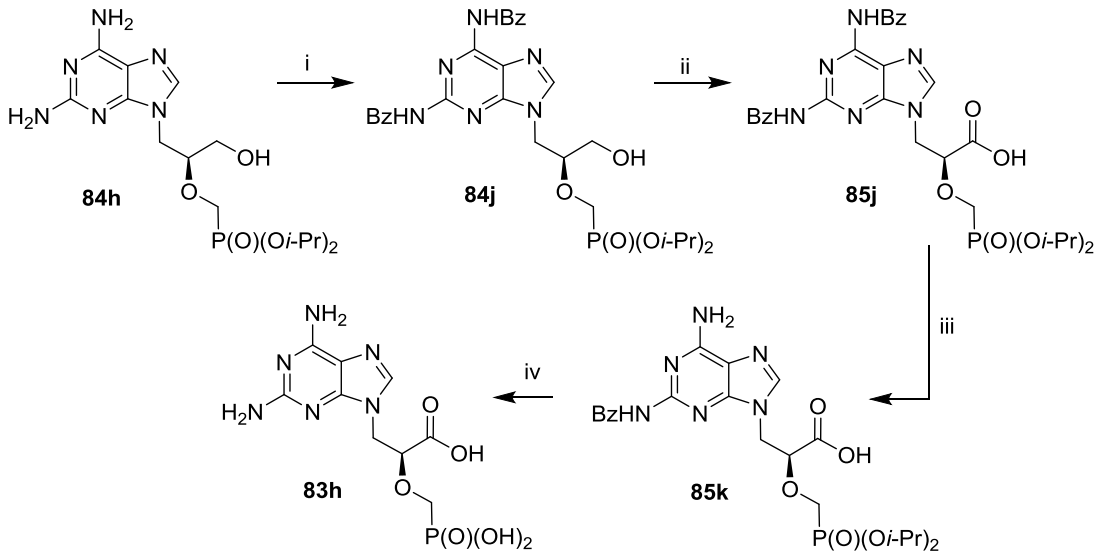
Functional groups of the unprotected nucleobases are usually well tolerated during the oxidation process (Table 2, entries 1-6). The only problem was observed with compounds bearing 2-aminopurine bases containing a guanidine motif (guanine and 2,6-diaminopurine). Treatment of the HPMPG derivative **84g**[94b] and HPMPDAP derivative **84h**[94b] with TEMPO/$NaClO_2$/NaClO resulted in complex reaction mixtures and no desired products were isolated (Table 2, entries 7 and 8).

To overcome the undesirable reactivity of the 2-aminopurine bases, it was decided to protect the amino groups of compounds **84g** and **84h** with the benzoyl group. Therefore, N^2-benzoylguanine derivative **84i**[94b] was prepared in a 69% yield from compound **84g** in pyridine by sequential treatment with TMSCl, BzCl, water and aqueous ammonia (Scheme 19). Benzoyl derivative **84i** was subsequently oxidized with TEMPO/$NaClO_2$/NaClO to give carboxy analogue **85i** in a 78% yield (Table 2, entry 9). Direct deprotection of the phosphonate moiety of the compound **85i** using TMSBr in acetonitrile[97] afforded N^2-benzoylguanine derivative **83i** in 52% yield (Table 2, entry 9, Scheme 19). For obtaining CPMEG **83g**, the benzoyl group of derivative **85i** was removed first, using MeONa methanolic solution, followed by the removal of the isopropyl groups from intermediate **85g** (Scheme 19).



Scheme 19. Reaction conditions: i) 1. TMSCl, pyridine, r.t., 2. BzCl, 3. H_2O, 4. NH_3 (aq); ii) TEMPO, $NaClO_2$, NaClO, MeCN, phosphate buffer, r.t., 24 h; iii) MeONa, MeOH, r.t., 24 h; iv) TMSBr, MeCN, r.t., overnight; v) 0.5 M HCl (aq), microwave irradiation, 140 °C, 30 min.

Analogously, the HPMPDAP analogue **84h** was protected as dibenzoyl derivative **84j** (70% yield), which was then oxidized using the TEMPO/$NaClO_2$/NaClO system to compound **85j** in a 80% yield (Table 2, entry 10, Scheme 20). The attempt to remove the benzoyl groups of derivative **85j** by MeONa in methanol resulted in compound **85k** (72% yield), with only N^6-position deprotected. Finally, CPMEDAP **83h** was obtained in a 38% yield from compound **85k** by simultaneous hydrolysis of the benzoyl group and diisopropyl esters using 0.5 M aqueous HCl under microwave irradiation (Scheme 20), a general method for the convenient removal of phosphonate diesters recently developed in our laboratory.[98]



Scheme 20. Reaction conditions: i) 1. TMSCl, pyridine, 2. BzCl, 3. H_2O, 4. NH_3 (aq); ii) TEMPO, $NaClO_2$, NaClO, MeCN, phosphate buffer, r.t., 24 h; iii) MeONa, MeOH, r.t., 24 h; iv) 0.5 M HCl (aq), microwave irradiation, 140 °C, 30 min.

All prepared diisopropyl CPME phosphonates **85** were deprotected to yield final free phosphonic acids **83** (Table 2, Scheme 18). As already mentioned above, two general methods for the removal of alkyl esters from the phosphonate group were employed: a) commonly used deprotection with TMSBr in acetonitrile followed by hydrolysis (procedure A);[97] b) recently described microwave-assisted hydrolysis using aqueous HCl (procedure B).[98] In our case, the procedure A gave better yields (50-68%) of the free phosphonic acids compared to the proceure B (37-46%). Diisopropyl phosphonate **85d**, with the methoxy group in the position C-4 of the pyrimidine ring, afforded under the deprotection conditions (by both procedures A and B) the corresponding thymine derivative **83d** (Table 2).

All CPME analogues **83** were tested for their antiviral properties. Although completely nontoxic in all assays, none of the compounds, including promising (*S*)-CPMEA **83a**, exhibited any activity against the HIV virus or any other viruses tested (HSV-1 (KOS), HSV-1 (KOS TK^-), HSV-2 (G), RSV, Vaccinia virus, Vesicular stomatitis virus, Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie B4 virus, Punta Toro virus, Feline Corona virus, and Feline Herpes virus). One of the most obvious reasons for the lack of the biological properties of CPME derivatives **83** may be their poor bioavailability caused by their high polarity (CPME derivatives are even more polar that the corresponding PME, PMP, and HPMP analogues). The majority of the therapeutically successful ANPs have to be

administered in the form of their prodrugs. The preparation of various prodrugs of compound (*S*)-CPMEA **83a** will be discussed in part 3.2.

3.1.4. Conclusion

Novel acyclic nucleoside analogue, (*S*)-3-(adenin-9-yl)-2-(phosphonomethoxy) propanoic acid **83a** [(*S*)-CPMEA, Fig. 19], was designed as an inhibitor of HIV-1 RT. Its synthesis was developed and optimized. The key step of the (*S*)-CPMEA **83a** synthesis is oxidation of the (*S*)-HPMPA derivative **84a** with TEMPO/$NaClO_2$/ NaClO to give compound **85a** in a high yield (87%). Subsequently, the whole series of CPME derivatives **85** was prepared in good to high yields from the corresponding HPMP analogues **84** by the optimized oxidative methodology using TEMPO. The oxidation process tolerates the functional groups present on the common nucleobases (A, C, T, U), with the exception of compounds containing the amino group in the position C-2 of the purine moiety (i.e. **84g** and **84h**) which were oxidized as their benzoyl derivatives **84i** and **84j**, respectively. Finally, two methods were employed for the deprotection of phosphonate diesters **85**: a) treatment with TMSBr in acetonitrile followed by hydrolysis; b) microwave-assisted hydrolysis with HCl. Unfortunately, none of the newly synthesized CPME compounds, including the most promising (*S*)-CPMEA, did show any interesting activity against the viruses tested.

3.2. Prodrugs of (*S*)-CPMEA

3.2.1. Introduction – prodrugs of ANPs

Several ANPs are used for treatment of various diseases in current medicine. To exploit their therapeutic potential, it was necessary to solve the problem caused by negative charge on the phosphonate moiety at physiological pH, which is responsible for limited penetration of ANPs to the cells, as well as for their low oral bioavailability (5-10%).[99] To overcome this obstacle intensive research was initiated to develop suitable lipophilic prodrugs of ANPs bearing only one negative charge or no charge at all. For this purpose the transformation of the free phosphonic acid to the corresponding ester or amide (mono, bis or mix ester-amide) is used. As already mentioned above, adefovir **30** (Fig. 11) and tenofovir **31** (Fig. 11) are utilized for oral application in form of their diesters[100] as adefovir dipivoxil **86** and tenofovir disoproxil fumarate **87** (Fig. 20).

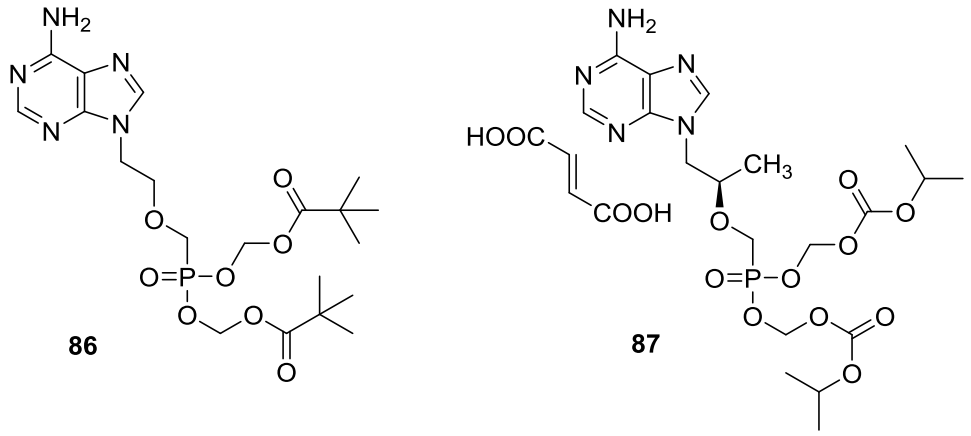


Figure 20. Prodrugs of adefovir and tenofovir.

Other significant class of prodrugs of ANPs is represented by highly lipophilic monoesters derived from linear aliphatic alcohols with 16-20 carbons, where at least one methylene group is replaced with oxygen atom (alkoxyalcohols). This class of prodrugs was designed as an analogy to natural phospholipids.[101] 3-(Hexadecyloxy)propyl ester of (*R*)-PMPA **88** (Fig. 21) is a typical example of such prodrug. This compound is currently in Phase I human trials as CMX157.[102] It exhibits, compared to tenofovir, 100-fold more potent antiviral activity against HIV and HBV viruses.[103]

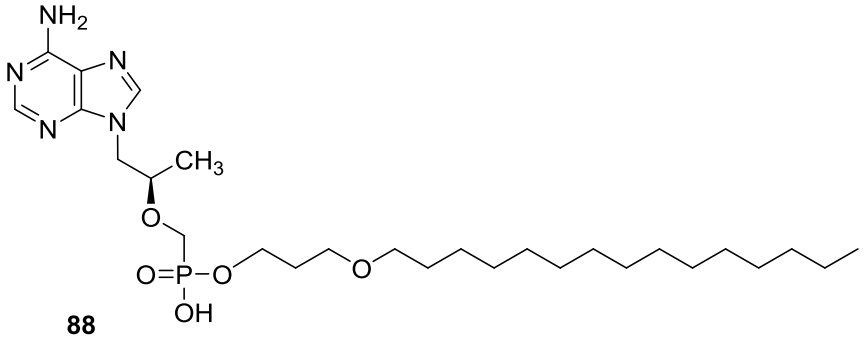


Figure 21. 3-(Hexadecyloxy)propyl ester of (*R*)-PMPA.

Bis-amidates (symmetrical diamides), derived from encoded amino acids, represent a very important class of prodrugs of ANPs. In this case, after metabolic activation of the prodrug to the active species, only nontoxic amino acid is released. Double prodrugs of PMEG (guanine analogue of adefovir, see part 3.3.2.) were considered to be the most promising compounds with high potential for treatment of cancer and leukemic diseases. The antiproliferative bis-amidate agents GS-9219[104] **89** and GS-9191[105] **90** (Fig. 22) were auspicious candidates for the treatment of non-Hodgkins lymphomas and human papillomavirus-associated proliferative disorders.

Ballatore et al.[106] prepared series of phosphoramidate prodrugs of adefovir **30** (Fig. 11) and tenofovir **31** (Fig. 11). These compounds exhibited improved anti-HIV activity compared to the corresponding parent ANPs.

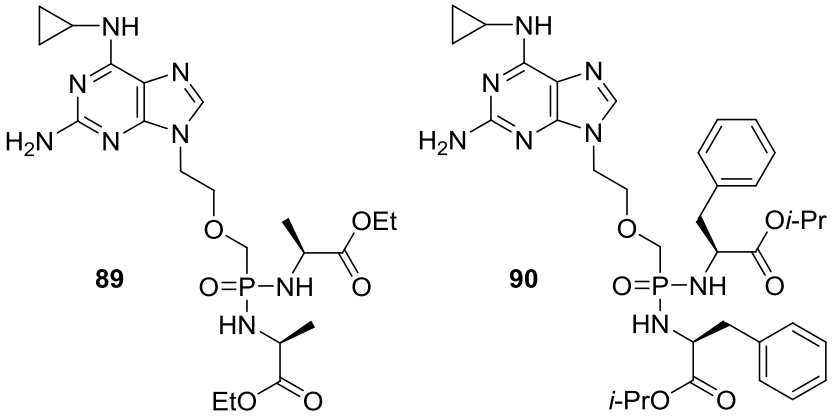


Figure 22. The antiproliferative bis-amidate agents GS-9219 and GS-9191.

Assymetrical phenyloxy amidates (mixed monoester mono-amidates) are another important class of prodrugs of ANPs. Agent GS-7340[107] **91** (Fig. 23) is an excellent example of such prodrugs. This compound (prodrug of tenofovir) is currently in Phase III human trials for treatment of HIV infections. In comparison with the parent

molecule (tenofovir), prodrug **91** is approximately 1000-fold more potent in inhibiting HIV replication *in vitro*.[108]

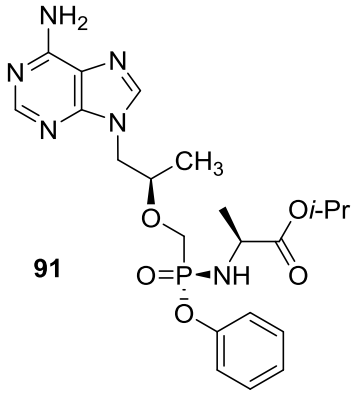


Figure 23. GS-7340 - the example of assymetrical phenyloxy amidate.

3.2.2. Preparation of (*S*)-CPMEA prodrugs

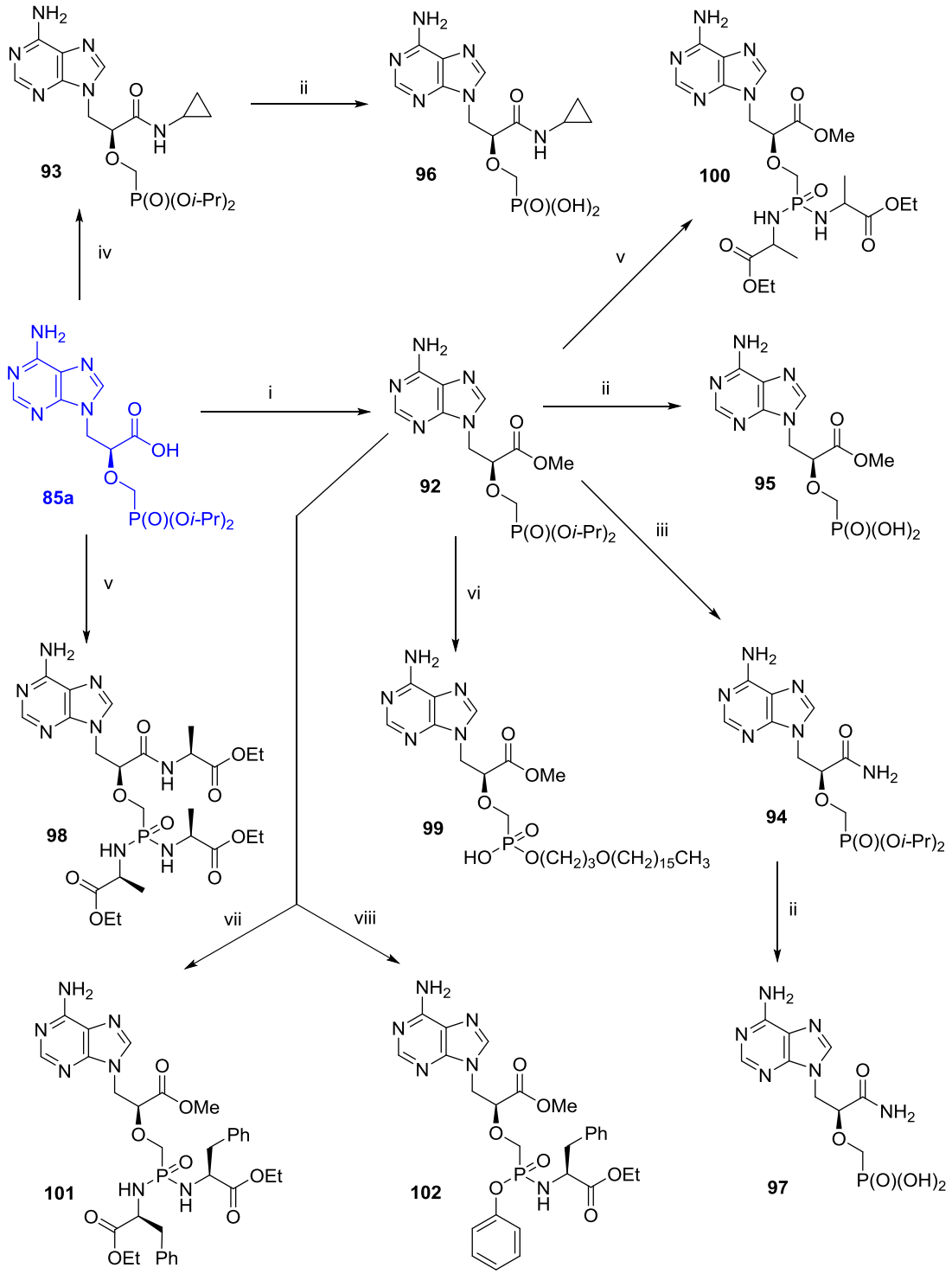
Compared to other types of ANPs, (*S*)-CPMEA has even more polar character due to the presence of both free phosphonic acid function and free carboxylic group. Thus, unfavorable pharmacological properties can be expected. In order to improve bioavailability of (*S*)-CPMEA, its transformation to appropriate prodrug was highly desirable.[103]

Thus, it was decided to convert (*S*)-CPMEA into several structurally different types of prodrugs, which had been designed to mask the phosphonate and/or carboxylic groups in the molecule.[109] The first class includes prodrugs containing groups masking the carboxylic moiety (esters and amides). Methyl ester of diisopropyl (*S*)-CPMEA **92** (Scheme 21) was synthesized by conversion of **85a** to a lithium salt followed by the reaction with methyliodide in DMF.[48] Cyclopropylamide **93** (Scheme 21) was prepared by the reaction of **85a** with cyclopropyl amine, EDAC and *N*-hydroxysuccinimide in DMF.[110] Finally, the treatment of compound **92** in ethanolic ammonia solution under microwave conditions was used for the preparation of amide derivative **94** (Scheme 21). Prepared diisopropylesters **92-94** were consequently treated with TMSBr to give the corresponding free phosphonic acids **95-97** (Scheme 21) in satisfactory yields (68-76%).

Tris-amidate analogue **98** (Scheme 21) was successfully prepared from compound **85a** according to a procedure developed originally in our laboratory:[109] appropriate phosphonate diester (ethyl or diisopropyl) is treated with TMSBr, followed by reaction with the corresponding amino acid ester hydrochloride in a presence of

triphenyl phosphine and Aldrithiol-2 in pyridine under basic conditions. In this case L-alanine ethylester hydrochloride was used to prepare prodrug **98**.

Compound **92** was further used for the synthesis of four other types of lipophilic prodrugs. First of them, 3-(hexadecyloxy)propyl monoester **99** (Scheme 21), was synthesized by the reaction of free phosphonic acid **95** (prepared *in situ* by ester cleavage of compound **92** with TMSBr) which was then heated with 3-(hexadecyloxy)propan-1-ol and DCC in dry pyridine. Synthesis of bis-amidate **100** (Scheme 21) was carried out analogously to the methodology used for compound **98**. Preparation of bis-amidate **101** (Scheme 21) was analogous to the above described method, nevertheless, due to lower reactivity of L-phenylalanine ethylester hydrochloride, it was necessary to heat the reaction mixture for 3 days to reach the full conversion. The last prodrug, monoester mono-amidate **102** (Scheme 21), was prepared by deprotection of the phosphonate moiety of compound **92** with TMSBr, followed by treatment of the silylated intermediate with L-phenylalanine ethylester hydrochloride and phenol under basic conditions in dry pyridine, and finally by heating with Aldrithiol-2 and triphenyl phosphine to complete the conversion. Prodrugs **98**-**102** were isolated in good to high yields (59-84%).



Scheme 21. Reaction conditions: i) 1. LiOH.H_2O/MeOH, 2. MeI/DMF, r.t., 1 h; ii) TMSBr, MeCN, r.t., overnight; iii) 3.5 M NH_3 in EtOH, 120 °C, 1 h, microwave irradiation; iv) EDAC, *N*-hydroxysuccinimide, cyclopropylamine, DMF, r.t., 2 days; v) 1. TMSBr, MeCN, r.t., overnight, 2. L-alanine ethylester hydrochloride, Et_3N, pyridine, 50 °C, 15 min, 3. Aldrithiol-2, PPh_3, pyridine, 50 °C, 5 h; vi) 1. TMSBr, MeCN, r.t., overnight, 2. 3-(hexadecyloxy)propan-1-ol, DCC, pyridine, 60 °C, 30 h; vii) 1. TMSBr, MeCN, r.t., overnight, 2. L-phenylalanine

ethylester hydrochloride, Et_3N, pyridine, 60 °C, 7 min, 3. Aldrithiol-2, PPh_3, pyridine, 70 °C, 3 days; viii) 1. TMSBr, MeCN, r.t., overnight, 2. L-phenylalanine ethylester hydrochloride, phenol, Et_3N, pyridine, 60 °C, 7 min, 3. Aldrithiol-2, PPh_3, pyridine, 65 °C, 5 h.

3.2.3. Biological results

The newly prepared prodrugs were evaluated for their antiviral properties. Compared to the parent molecule, (*S*)-CPMEA **83a** (Fig. 19), prodrugs **101** and **102** (Scheme 21) displayed submicromolar anti-HCV activity (Table 3). To the best of our knowledge, these are the first examples of ANP derivatives to exhibit potent activity against HCV. None of the prodrugs exhibited any interesting activity against the HIV-1 virus. All of the prodrugs were also tested for inhibitory activity of adenylate cyclase toxin from *Bordetella pertussis* (CyaA), but they were shown to be only weak inhibitors of this enzyme. The best CyaA inhibitor was compound **101**, which decreased the formation of cAMP to 68% compared to the full (100%) activity of CyaA.

Table 3. Anti-HCV and cytotoxic properties of (*S*)-CPMEA and its prodrugs.

Compound	HCV Replicon EC50 1A RLUC-384 (μM)	HCV Rep CC50 1A-Calcein-384 (μM)	HCV Replicon EC50 1B RLUC-384 (μM)	HCV Rep CC50 1B-Calcein-384 (μM)	HCV Replicon EC50 2A RLUC-384 (μM)	HCV Rep CC50 2A-Calcein-384 (μM)
83a	>44.4	>44.4	>44.4	>44.4	>44.4	>44.4
98	>44.4	>44.4	>44.4	>44.4	>44.4	>44.4
99	20.6	>44.4	30.9	>44.4	9.6	>44.4
100	>44.4	>44.4	>44.4	>44.4	39.2	>44.4
101	0.6	18.3	1.1	30.4	0.6	12.6
102	0.8	42.6	1.5	>44.4	0.6	25.3

3.2.4. Conclusion

Synthesis of various prodrugs of (*S*)-CPMEA has been developed and their biological activities have been evaluated. The compounds were shown to be only weak inhibitors of adenylate cyclase toxin from *Bordetella pertussis* (CyaA). Prodrugs **101** (bis-amidate) and **102** (monoester mono-amidate) exhibited submicromolar anti-HCV activity. No interesting anti-HIV activity was observed within this class of compounds.

3.3. ANPs with elongated CPEE and HPEP acyclic chain as inhibitors of plasmodial HG(X)PRTs

The synthesis of novel ANPs with a potential antimalarial activity was another project on which I participated during my Ph.D. studies. Malaria remains a recurrent problem in the modern world. The WHO (World Malaria Report 2011) have accounted for over 200 million new cases reported worldwide, 80% of which are in Africa, and almost 1 million deaths, 86% of which occur in group of children below the age of five.[111] Due to increasing resistance of the plasmodial parasites to current medications, there is a need to develop new classes of antimalarial drugs.[112] *Plasmodium falciparum (Pf)* and *Plasmodium vivax (Pv)* are the most widespread species (parasitic protozoans) that cause malaria in humans. *Pf* is responsible for approximately three quarters of the total infections,[113] but other plasmodial strains (e.g. *Pv*) can also cause serious illness.[114]

3.3.1. Short history of treatments for malaria[115]

The name ‚malaria' arose from the Italian term for ‚bad air' (*mala aria*). Its first effective treatment came from the bark of cinchona tree containing quinine. This tree grows on the slopes of Andes, especially in Peru. Historically, the Incas used infusions of cinchona bark powder to combat malarial fevers. This fact was later also known to Jesuits living in former spanish colonies in South America. The cinchona bark powder was firstly sent to Spain around 1640 and hereby Jesuits introduced the antimalarial treatment in Europe. Quinine was isolated from cinchona bark in 1820 and is still used especially for severe malaria treatment.

Around 1900 Paul Ehrlich observed that methylene blue had a specific antimalarial effect. He treated patients suffering from a mild form of malaria with this dye and obtained significant improvements. However, methylene blue was not effective against more severe tropical forms of malaria and moreover the dye was found to be highly toxic.

In the 1940s, quinine was replaced by chloroquine, firstly prepared in 1934. Chloroquine has been at the front line of the treatment of both mild and severe malaria for more than 50 years.

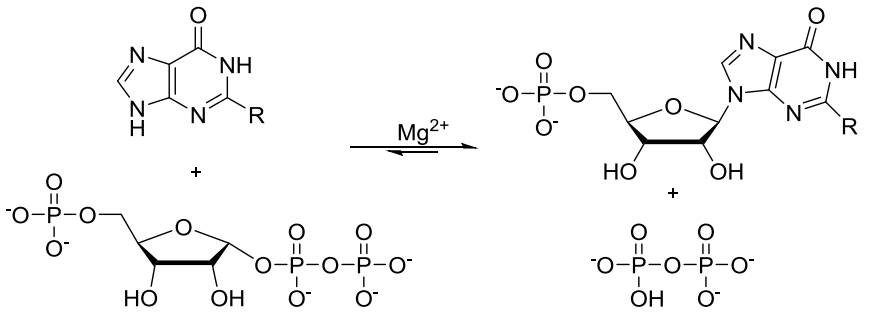
The problem of chloroquine-resistant *Plasmodium* has iniciated the search for novel alternative synthetic drugs effective against resistant strains. Mefloquine was one of them. This compound, structurally similar to quinine, was firstly introduced in

1985 and approved by FDA in 1989. Mefloquine has become a preventive antimalarial drug in the case of travelling to endemic areas.

The increasing resistance of *Plasmodium falciparum* to the most prescribed antimalarial drugs (chloroquine) became major reason for new research of antimalarial drugs. In the 1970s, artemisinins were discovered in the plant *Artemisia annua*, a Chinese traditional remedy for fevers, including malaria. In 2001, the WHO recommended the use of four artemisinin-combination therapies (ACTs) to treat malaria: artemether-lamifantrine; artesunate–mefloquine; artesunate–amodiaquine; and artesunate–sulfadoxine/pyrimethamine. Despite of increasing resistance to ACTs, it is still the most effective class of antimalarial treatment.[111]

3.3.2. Selective inhibitors of HG(X)PRT – ANPs

The previous studies[116] reported that some ANPs can inhibit hypoxanthine-guanine-(xanthine) phosphoribosyltransferase (HG(X)PRT),[114,117] which is a key enzyme of the purine salvage pathway of the malarial parasites. This enzyme catalyzes the formation of an *N*-glycosidic bond between the N^9 atom of purine base and the C^1 atom of α-D-5-phosphoribosyl-1-pyrophosphate (Fig. 24).[118]



The naturally occurring bases are:
a) guanine R = NH_2; b) hypoxanthine R = H; c) xanthine R = OH.

Figure 24. Reaction catalyzed by HG(X)PRT.

One significant difference in the metabolic pathways of *Plasmodium* and its human host cell is in the ability to synthesize the purine nucleoside monophosphates essential for the production of DNA/RNA. Mammalian cells are able to produce these metabolites either by *de novo* synthesis or by salvage. In contrast, *Plasmodium* parasites are unable to synthetize the purine ring and therefore, they are dependent on less energy utilizing salvage pathway. It implies that presence of HG(X)PRTs is

crucial in the replication and survival of the parasite and therefore, it is a promising target for the design of antiparasitic drug.

Keough et al.[116a] reported that several ANPs bearing the hypoxanthine or guanine bases revealed good inhibitory effects on *Pf*HGXPRT (Fig. 25). These compounds are considered to be the first inhibitors which selectively discriminate between the human and *Pf* enzymes. Intensive research of such type of ANPs resulted in a broader library of compounds with potent and/or selective inhibitory activities towards human, *Pf* and *Pv*HG(X)PRTs.[119]

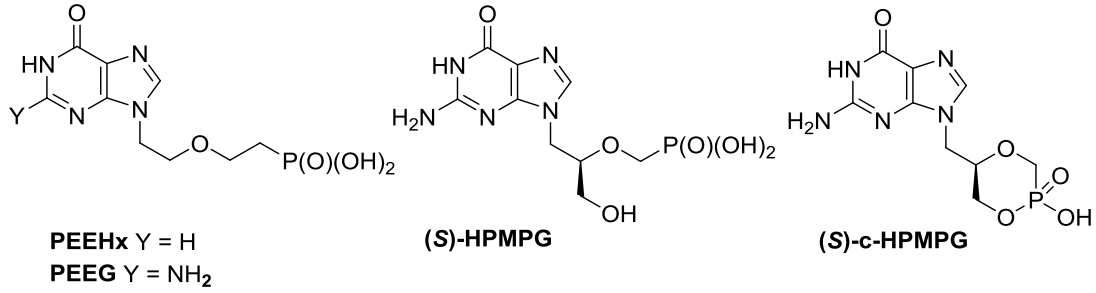


Figure 25. PEE and HPMP inhibitors of plasmodial phosphoribosyltransferases.

PEE derivatives (Fig. 25) possess sufficiently low K_i (~ 0.1 μM) and significantly higher affinity for *Pf*HGXPRT compared to human HGPRT. (*S*)-HPMPG or cyclic (*S*)-HPMPG derivatives have also their K_i values in a low micromolar range and display a favorable affinity for the *Pf* enzyme (Table 4).

Table 4. K_i values of selected ANPs for *Pf*HG(X)PRT and human HGPRT at pH 7.4.[116a]

Compound	K_i (μM)		R
	*Pf*HGXPRT	human HGPRT	K_i(h)/K_i(*Pf*)
PEEHx	0.3 ± 0.04	3.6 ± 0.20	12
PEEG	0.1 ± 0.02	1.0 ± 0.50	10
(*S*)-HPMPG[a]	28.4 ± 2.20	176.8 ± 10	6.2
(*S*)-*c*-HPMPG	8.0 ± 1.00	90.0 ± 10	11

[a]Measured at pH 8.5

From the crystal structures of three ANPs in complex with human HGPRT it is apparent that further chemical modifications could lead to inhibitors with increased potency. Česnek et al.[119a] reported that compounds with too short (1-3 atoms) or too long (>5 atoms) linker between the nucleobase and the phosphonate group do not inhibit *Plasmodium* enzymes. Optimal binding of the inhibitors to the malarial

enzymes occurs: a) when the linear linker contains five atoms; b) when the oxygen atom is located in the 3-position distal from the N^9 atom in the purin ring. The influence of a carboxylic group attached to the linker on potency and/or selectivity for the parasite enzymes has not been evaluated yet. To contribute to the structure-activity relatioship study, CPME derivatives bearing guanine and hypoxanthine were synthesized. The fact that both PEEG(Hx) and (*S*)-HPMPG derivatives showed to be good inhibitors of the *Plasmodium* enzymes led to the idea to combine these structural motifs which resulted in HPEP analogs bearing 6-oxopurine bases (Fig. 26). Furthermore, CPEE derivatives ("oxidized" forms of the HPEP analogues) and their methyl esters were prepared.

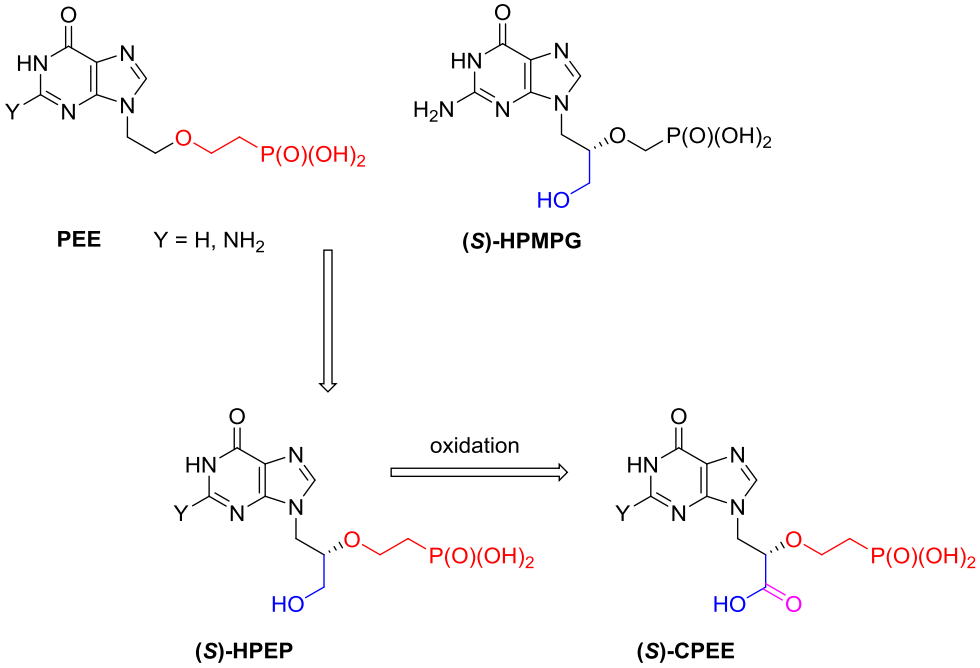
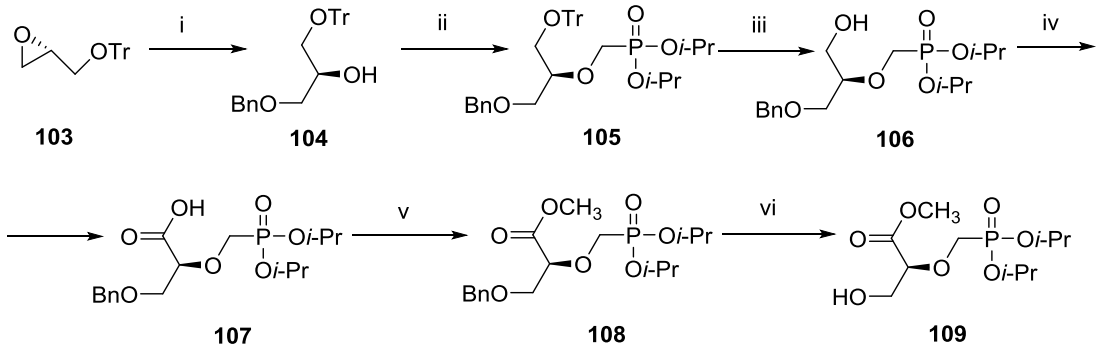


Figure 26. Design of new ANPs, namely (*S*)-HPEP and (*S*)-CPEE derivatives.

3.3.3. Synthesis of (*S*)-CPME derivatives bearing 6-oxopurine bases

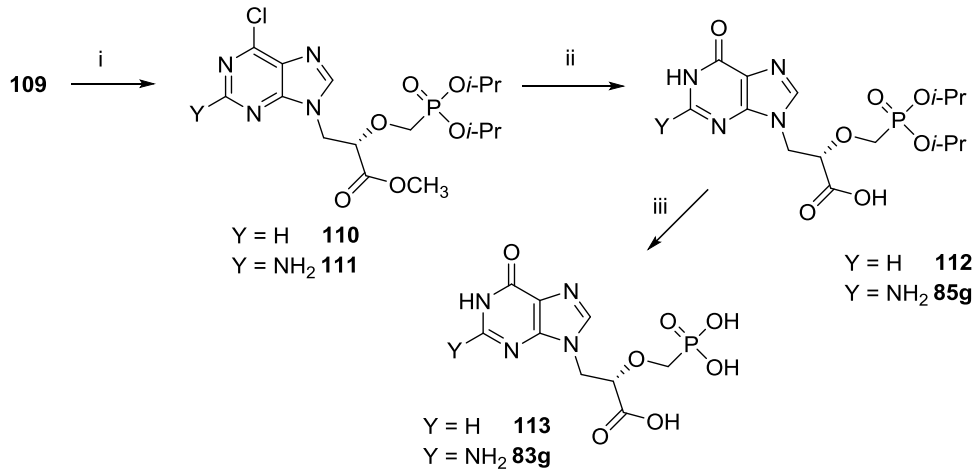
The synthon approach starting from (*S*)-tritylglycidol was used for the synthesis of the desired CPME compounds. The first step in the preparation of the key intermediate **109** (Scheme 22) was nucleophilic opening of the epoxide ring with benzylalcohol to afford product **104** in quantitative yield. Introduction of a methylphosphonate moiety to the secondary hydroxyl group was performed using diisopropyl (bromomethyl)phosphonate under the basic conditions in DMF to give compound **105**. The crude intermediate **105** was refluxed in 80% acetic acid to afford

deprotected alcohol **106**, which was subsequently oxidized under the mild conditions[92] (TEMPO/$NaClO_2$/NaClO) to the corresponding carboxylic acid **107**. Compound **107** was converted to methyl ester **108** with diazomethane, and after deprotection of the benzyl group the desired intermediate **109** was obtained.



Scheme 22. Reaction conditions: i) BnOH, NaH, DMF, 100 °C, 2 h; ii) 1. NaH, DMF, 0 °C, 15 min, 2. $BrCH_2P(O)(O$*i*$-Pr)_2$, DMF, r.t., 48 h; iii) 80% AcOH, reflux, 2 h; iv) TEMPO, $NaClO_2$, NaClO, phosphate buffer, MeCN, 40 °C, 48 h; v) CH_2N_2, EtOAc, r.t., 0.5 h; vi) H_2/Pd/C, MeOH, r.t., 72 h.

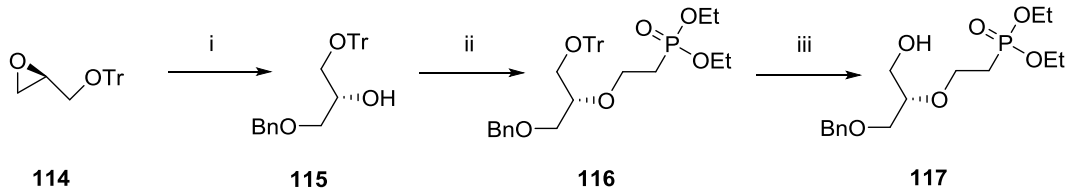
The Mitsunobu reaction[120] was applied for introduction of the acyclic moiety to the N^9-position of 6-chloropurine or 2-amino-6-chloropurine. In the former case the resulting 6-chloropurine derivative **110** (Scheme 23) was then transformed to hypoxanthine derivative **112** by hydrolysis under the basic conditions (DABCO/K_2CO_3) and at the same time the methyl ester was cleaved. In the case of 2-amino-6-chloropurine derivative **111** the Mitsunobu reaction was quenched by adding of water followed by heating to decompose the triphenylphosphoranylidene by-product[121] formed due to the presence of the free amino group. The chlorine atom was next displaced with hydroxyl, as described above, to form guanine derivative **85g**. To prepare free phosphonic acids **113** and **83g**, the phosphonate moiety was deprotected under standard conditions using TMSBr/MeCN followed by hydrolysis (Scheme 23).



Scheme 23. Reaction conditions: i) Mitsunobu reaction: 1. 6-chloro or 2-amino-6-chloropurine, compound **109**, PPh_3, dioxane, 2. DIAD, r.t., 24 h; ii) DABCO, K_2CO_3, dioxane/H_2O (4:1), 90 °C, 3-5 h; iii) TMSBr, MeCN, r.t., overnight.

3.3.4. Synthesis of (*S*)-HPEP derivatives bearing 6-oxopurine bases

The HPEP derivatives were obtained *via* the synthon approach starting from the (*R*)-tritylglycidol. Previously developed methodology for oxa-Michael additions of secondary alcohols to diethyl vinylphosphonate (DEVP) was employed. Treatment of compound **115** (Scheme 24) with DEVP and catalytic amount of KOH[122] afforded product **116** which was used for the next step as a crude intermediate (it was not possible to get rid of by-products with almost the same polarity using silica gel column chromatography; the product was purified and fully characterized after detrytilation step). The trityl group was then removed by treatment of compound **116** with 80% acetic acid under reflux to obtain desired alcohol **117** in a high yield (Scheme 24).

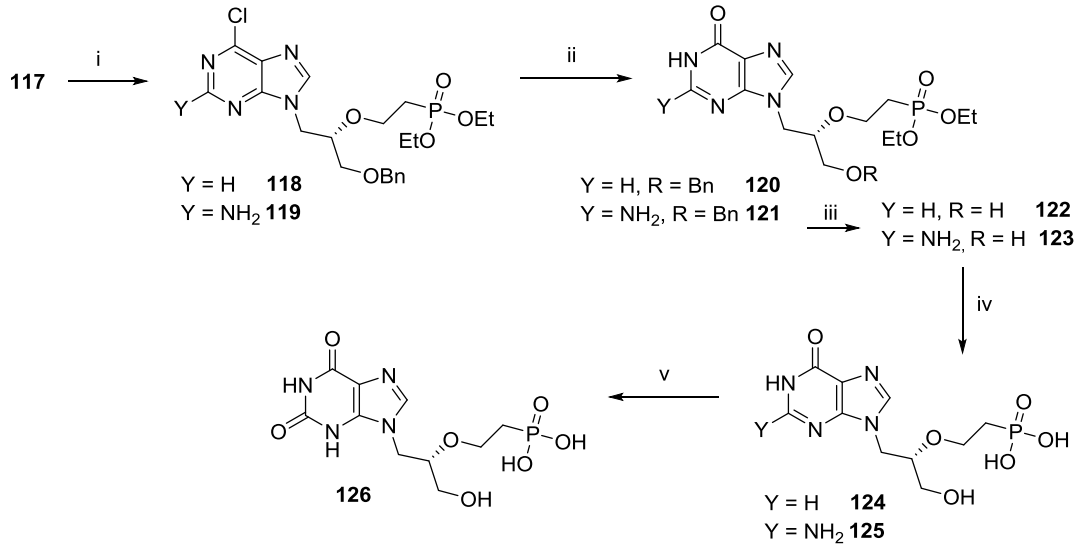


114 **115** **116** **117**

Scheme 24. Reaction conditions: i) BnOH, NaH, DMF, 100 °C, 2h; ii) KOH, CH_2=CHP(O)$(OEt)_2$, dioxane, r.t., 70 h; iii) 80% AcOH, reflux, 2 h.

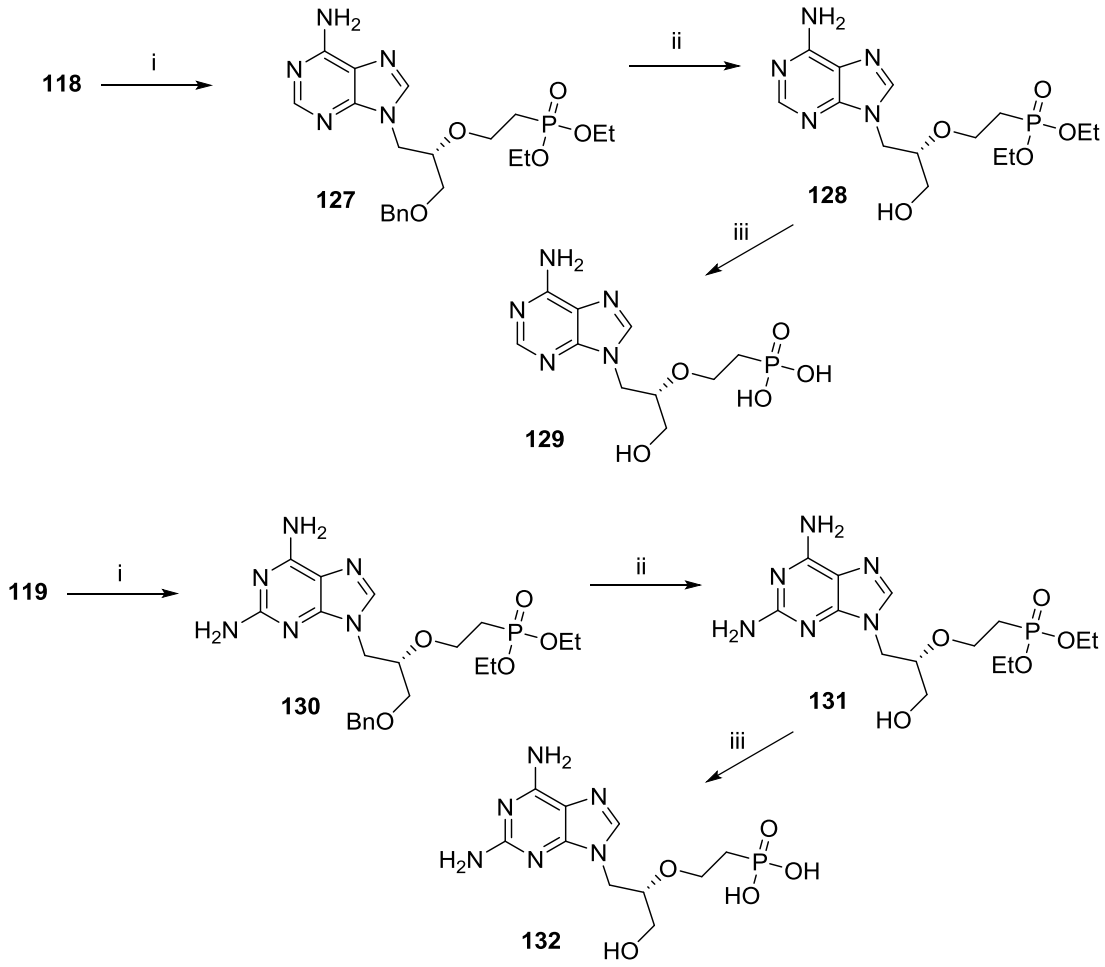
The treatment of alcohol **117** with 6-chloropurine and 2-amino-6-chloropurine under the Mitsunobu reaction conditions (Scheme 25) gave phosphonate diesters **118**

and **119**, respectively, in acceptable yields (80% and 55%). The triphenylphosphine adduct, which was formed in the case of the 2-amino-6-chloropurine base, was decomposed by the same manner as described above. Compounds **118** and **119** were treated with DABCO in the presence of potassium carbonate to give products **120** and **121**. Compounds **122** and **123** were obtained after removal of the benzyl groups and were subsequently converted to the corresponding free phosphonic acids **124** and **125**, respectively. Guanine derivative **125** was also converted to the corresponding xanthine analogue **126** by treatment with *iso*-pentyl nitrite in 80% acetic acid.



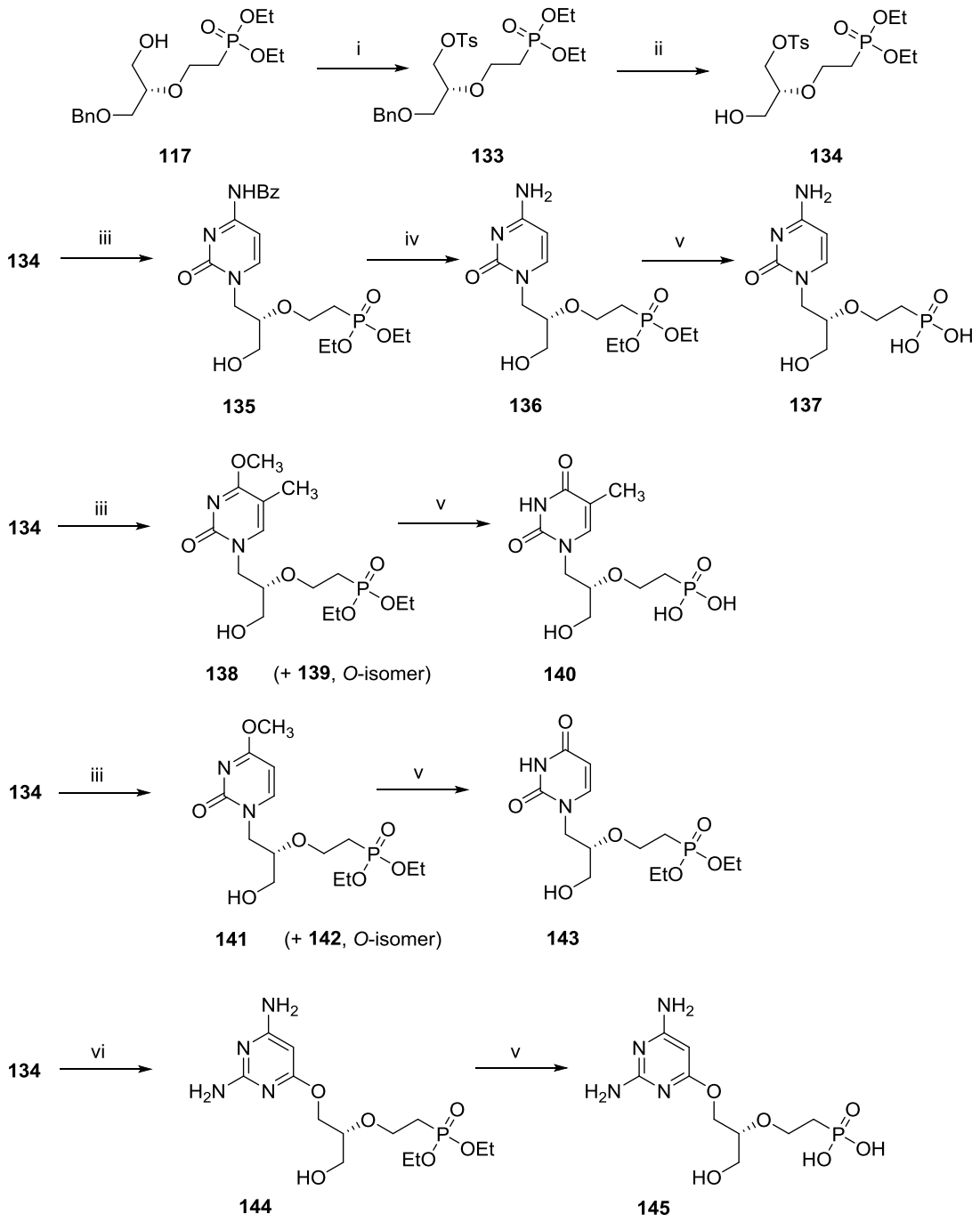
Scheme 25. Reaction conditions: i) Mitsunobu reaction 1. 6-chloropurine or 2-amino-6-chloropurine, compound **117**, PPh_3, dioxane, 2. DIAD, r.t., 24 h; ii) DABCO, K_2CO_3, dioxane/H_2O (4:1), 90 °C, 3 h; iii) H_2/Pd/C, AcOH, r.t., 72 h; iv) TMSBr, MeCN, r.t., overnight; v) *iso*-pentyl nitrite, 80% AcOH, r.t., overnight.

Subsequently, the (*S*)-HPEP analogues bearing other nucleobases (as adenine, 2,6-diaminopurine, cytosine, thymine, and uracil) were prepared to explore the potential antiviral, antineoplastic, and immunomodulatory properties within this novel class of ANPs. Synthesis of adenine and 2,6-diaminopurine derivatives **127** and **130** started from the corresponding 6-chloropurine or 2-amino-6-chloropurine derivatives **118** and **119**, respectively. The microwave-assisted reaction with ethanolic ammonia solution, followed by deprotection of the benzyl group *via* H_2/Pd/C in acetic acid, afforded compounds **128** and **131** in moderate yields. The standard deprotection with TMSBr/MeCN gave the desired free phosphonic acids **129** and **132** (Scheme 26).



Scheme 26. Reaction conditions: i) 3.5 M NH_3 in EtOH, microwave irradiation, 120 °C, 1 h; ii) H_2/Pd/C, AcOH, r.t., 72 h; iii) TMSBr, MeCN, r.t., overnight.

Synthesis of other HPEP derivatives (C, T, U, DAPy) was based on synthon approach starting from compound **117**. Compound **117** was treated with *p*-toluensulfonyl chloride in pyridine to give compound **133** and removal of the benzyl moiety afforded alkylating agent **134** in a high yield (95%). Compound **134** was subsequently used for alkylation for the corresponding protected bases to give products **135**, **138**, **139**, **141**, **142**, and **144**. The benzoyl group from compound **135** was removed by the reaction with MeONa/MeOH to give **136**. The final products **137**, **140**, **143**, and **145** were obtained by silylation of compounds **136**, **138**, **141**, and **144** with TMSBr, followed by hydrolysis (Scheme 27).

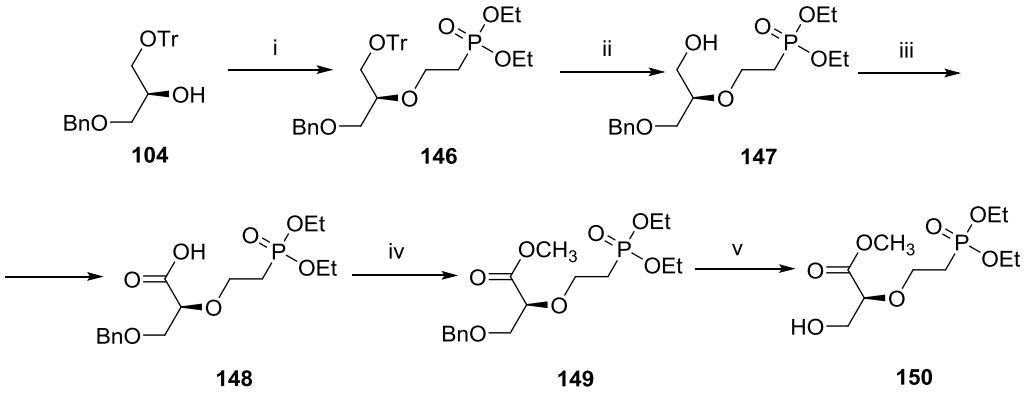


Scheme 27. Reaction conditions: i) TsCl, DMAP, pyridine, r.t., overnight; ii) H_2/Pd/C, MeOH/AcOH, r.t., 3 days; iii) base (BzC, T, U), compound **134**, NaH, DMF, 100 °C, 3 h; iv) MeONa, MeOH, r.t., overnight; v) TMSBr, MeCN, r.t., overnight; vi) 2,4-diamino-6-hydroxypyrimidine, Cs_2CO_3, DMF, 100 °C, 10 h.

All final compounds were purified by reverse phase HPLC chromatography and/or by crystallization from the water-ethanol mixture. The final phosphonic acids were fully characterized by ^{1}H and ^{13}C NMR, MS spectroscopy, IR spectroscopy and optical rotation assignment.

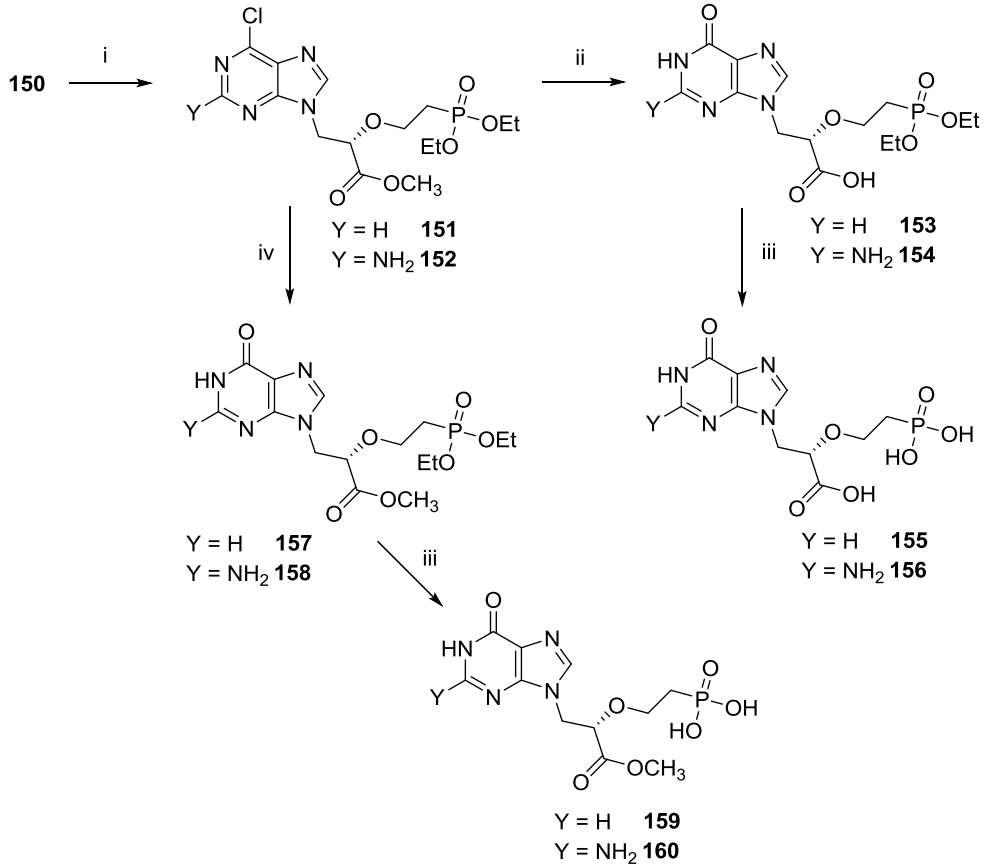
3.3.5. Synthesis of (*S*)-CPEE derivatives bearing 6-oxopurine bases

The synthesis of CPEE derivatives and their methyl esters combines previously mentioned synthetic steps. At first, (*S*)-tritylglycidol was opened with benzylalcohol to give compound **104** (Scheme 28). *O*-Alkylation of **104** with DEVP (described in part 3.3.4.) gave (after refluxing with 80% acetic acid) alcohol **147**. Subsequent steps leading to the desired synthon **150** were carried out in analogy with the methods described in part 3.3.3. (Scheme 28).



Scheme 28. Reaction conditions: i) KOH, CH_2=CHP(O)$(OEt)_2$, dioxane, r.t., 70 h; ii) 80% AcOH, reflux, 2 h; iii) TEMPO, $NaClO_2$, NaClO, phosphate buffer, MeCN, 40 °C, 48 h; iv) CH_2N_2, EtOAc, r.t., 0.5 h; v) H_2/Pd/C, MeOH, r.t., 72 h.

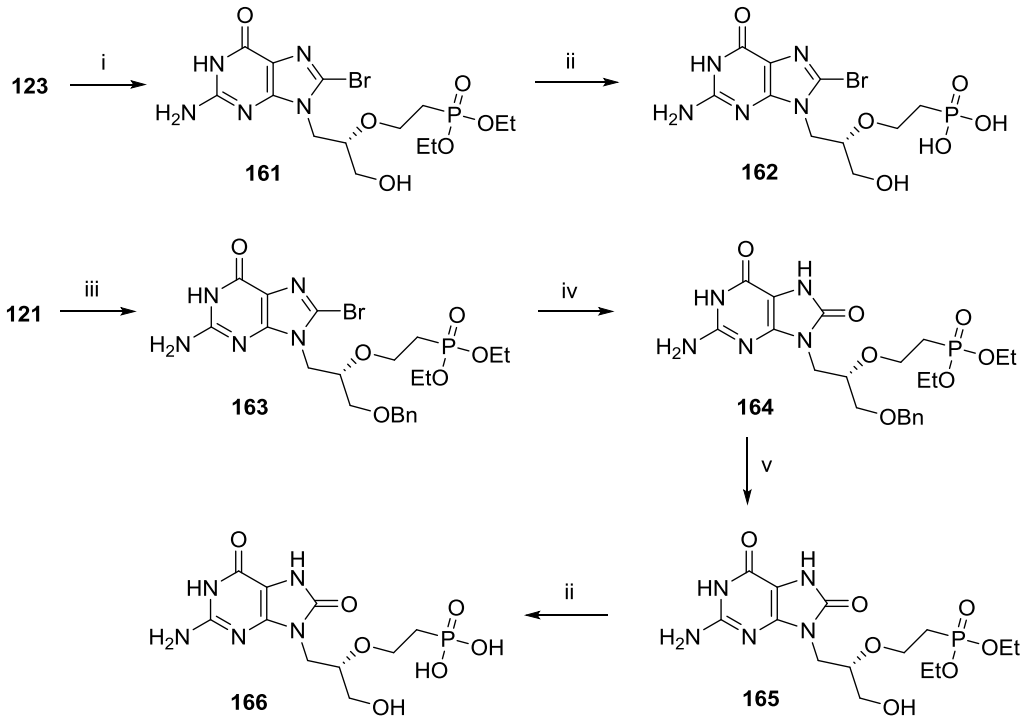
Alcohol **150** was treated with 6-chloropurine and 2-amino-6-chloropurine under the Mitsunobu reaction conditions to give compounds **151** and **152**, respectively (Scheme 29). Their treatment in basic (DABCO/K_2CO_3) or acidic (75% aqueous trifluoroacetic acid) conditions afforded derivatives **153** and **154** or **157** and **158**, respectively. Compounds **151** and **152** underwent under basic conditions simultaneous transformation of 6-chloro to 6-oxo and cleavage of methyl ester. Under acidic conditions, only the 6-chloro to 6-oxo transformation occurred and the methyl ester was preserved. Final free phosphonic acids **155**, **156** and **159**, **160** were prepared by ester cleavage of the corresponding diesters under the standard conditions (TMSBr in MeCN, Scheme 29).



Scheme 29. Reaction conditions: i) Mitsunobu conditions: 1. 6-chloropurine or 2-amino-6-chloropurine, compound **150**, PPh_3, dioxane, 2. DIAD, r.t., 24 h; ii) DABCO, K_2CO_3, dioxane/H_2O (4:1), 90 °C, 3-5 h; iii) TMSBr, MeCN, r.t., overnight; iv) 75% aqueous CF_3COOH, r.t., overnight.

3.3.6. Synthesis of 8-substituted derivatives of (*S*)-HPEPG

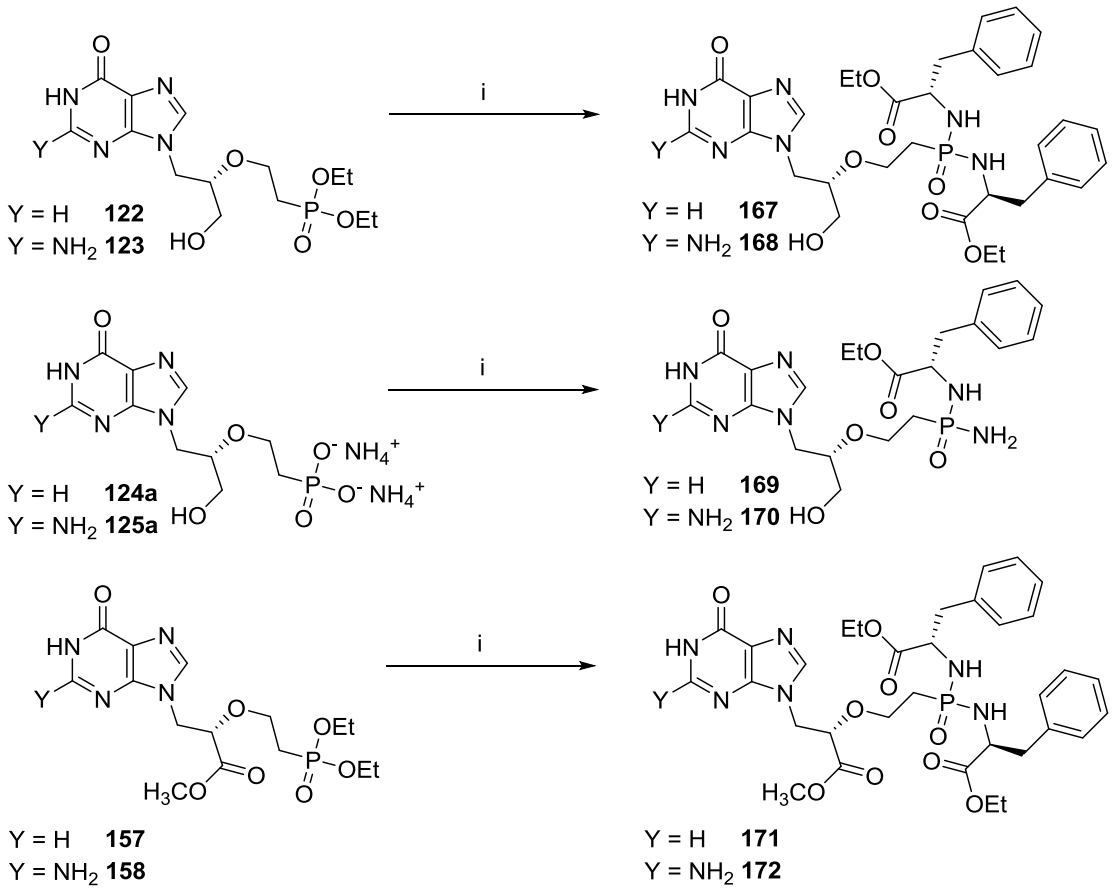
Two different methods were used for bromination of the C-8 position of the guanine base. The first method consist of direct bromination of compound **123** (Scheme 25) with bromine/CCl_4 in DMF and afforded product **161** (Scheme 30) in a good yield. The second method was expected to combine bromination ($NaBrO_3/Na_2S_2O_4$) with cleavage of the benzyl moiety of compound **121**,[123] nevertheless this reaction gave only 8-bromo product **163**. Compound **163** was subsequently transformed to 8-oxo derivative **164** by heating in AcONa/AcOH.[124] The desired compounds **162** and **166** were obtained after the deprotection of the benzyl and phosphonate moieties (Scheme 30).



Scheme 30. Reaction conditions: i) Br_2/CCl_4, DMF, r.t., 3 h; ii) TMSBr, MeCN, r.t., overnight; iii) $NaBrO_3$, $Na_2S_2O_4$, EtOAc/H_2O (5:4), r.t., 1 h; iv) AcONa/AcOH, 130 °C, 4 h; v) H_2/Pd/C, AcOH, r.t., 24 h.

3.3.7. Synthesis of prodrugs of (*S*)-HPEPG(Hx) and (*S*)-CPEEG (Hx)

For the erythrocyte cell-based assays, the direct synthesis of phosphoramidate prodrugs[125] **167**-**172** from corresponding phosphonate diesters **122**, **123**, **157** and **158,** and two ammonium salts of phosphonic acids **124a** and **125a** was performed by modification of our recently published method.[109] This one-pot reaction sequence consisted of two separate steps. The first step included *in situ* preparation of silyl esters from compounds **122**, **123**, **157**, **158**, **124a** and **125a** using TMSBr. In the second step, reaction of the silylated intermediates with L-phenylalanine ethylester hydrochloride in the presence of Aldrithiol-2 and triphenylphosphine afforded prodrugs **167**, **168**, **171**, **172**, **169** and **170** (Scheme 31).



Scheme 31. Reaction conditions: i) 1. TMSBr, MeCN, r.t., overnight, 2. L-phenylalanine ethylester hydrochloride, Et_3N, pyridine, 60 °C, 7 min., 3. Aldrithiol-2, PPh_3, pyridine, 70 °C, 3 days.

3.3.8. Biological results

The final 6-oxopurine analogues were tested for their affinity towards the human and plasmodial hypoxanthine-guanine-(xanthine)-phosphoribosyltransferases. These tests were performed by our collaborators Dr. Dianne T. Keough and Prof. Luke W. Guddat (The school of chemistry and molecular sciences, The University of Queensland, Brisbane, Australia).

Neither (*S*)-CPMEHx **113** (Scheme 23), nor (*S*)-CPMEG **83g** (Scheme 23) prepared in this series, were good inhibitors of the target enzymes. The possible explanation is that the linker between the nucleobase and the phosphonate moiety is too short (usually a linker of 5-6 atoms is required for the best antimalarial activity of ANPs).[119a]

Although the HPEP compounds were not found to be good inhibitors of the *Plasmodium* enzymes, they were potent inhibitors of the human enzyme, especially

(*S*)-HPEPG **125** (Scheme 25). The guanine compounds bound more tightly to all three enzymes studied. At a value of 5.6 μM, the K_i for (*S*)-HPEPHx **124** (Scheme 25) is comparable to that observed for the reaction products GMP (5.8 μM) and IMP (5.4 μM) with human HGPRT.[116a] However, with a K_i of 0.02 μM for human HGPRT, compound **125** is one of the most potent inhibitors of human 6-oxopurine PRTase discovered to date. Only the acyclic immucillin phosphonates, with K_i values of 10.6 nM and 0.65 nM for *Pf* HGXPRT,[126] the iminoribitols (1*S*)-1-(9-deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol 5-phosphate (immucillin HP) and (1*S*)-1-(9-deazaguanin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol 5-phosphate (immucillin GP) with K_i values of 1 nM for *Pf* HGXRPT[127] and a bisphosphonate with a K_i value of 10 nM for *E. coli* XGPRT[128] have lower K_i values for their most preferred enzyme.

(*S*)-CPEEHx **155** (Scheme 29) and (*S*)-CPEEG **156** (Scheme 29), and especially their methylesters **159** and **160** (Scheme 29), respectively, revealed potent inhibitory effects on human and *Plasmodium* enzymes. As in the case of HPEP derivatives, CPEE compounds were only weak inhibitors of *Plasmodium* enzymes, but showed interesting submicromolar activities on human enzymes.

Xanthine derivative **126** (Scheme 25) was not active (it's probably not able to bind to human or *Plasmodium* enzymes). Derivatives of the compound **125** modified in position C-8, namely compounds **162** and **166** (Scheme 30) also revealed no activity. It implies that 8-oxo derivative **166** is unlikely to bind because of the 8-oxo group. 8-Bromo derivative **162** displayed only weak activity on human enzyme.

Table 5. K_i values of 6-oxopurine ANPs for human HGPRT, *Pf*HGXPRT and *Pv*HGPRT at pH 7.4.

	Y	Compound	K_i (μM)		
			human	*Pf*HGXPRT	*Pv*HGPRT
HPEP	H	**124**	5.6	[a]NI	31
	NH_2	**125**	0.02	7.7	3.4
CPEE COOH	H	**155**	200	6	12
	NH_2	**156**	0.2	1.5	2.5
CPEE COOMe	H	**159**	0.9	[a]NI	32
	NH_2	**160**	0.1	2	0.7

[a]NI = no inhibition at that particular concentration of the compound used in the assay

All prepared prodrugs were tested on their antimalarial activity in erythrocyte cell culture assays. Masking the polar phosphonate group (compare mono-amidates with bis-amidates, Table 6) had a big effect on the antimalarial activity. The fact that guanine derivatives bound weakly to the *Pf* enzyme is reflected by the high IC_{50} values though cell permeability and hydrolysis had to be considered. The fact that some inhibition of the growth of *Pf* was observed showed that the prodrug did cross into the cell and was hydrolysed to the active form which, though weak, was still able to have an effect on inhibition of the growth of *Pf* (Table 6).

Table 6. Antimalarial activity of the prodrugs of the HPEP and CPEE analogues in erythrocyte cell culture assays.

	Y	Compound	IC_{50}(D6)[a] (μM)	IC_{90}(D6)[a] (μM)	IC_{50}(W2)[b] (μM)	IC_{90}(W2)[b] (μM)
HPEP (monoamidate)	H	**169**	695	799	523	1000
	NH_2	**170**	274±37	337±89	261±25	319±71
HPEP (bisamidate)	H	**167**	103	133	76	173
	NH_2	**168**	70	101	22	132
CPEE (bisamidate)	H	**171**	60±12	103±3	60±11	140±22
	NH_2	**172**	54±8	85±25	51±5	115±22
[c]CQ	-	-	0.012±0.004	0.015±0.005	0.169±0.075	0.228±0.041

[a]chloroquine sensitive [b]chloroquine resistant
[c]chloroquine

The HPEP analogues with other nucleobases (adenine, 2,6-diaminopurine, cytosine, thymine, uracil), and the open-ring derivative, namely compounds **129**, **132**, **137**, **140**, **143**, and **145**, respectively, were tested in standard antiviral assays (performed by Gilead Sciences, Inc.), but no interesting activity against HIV, HSV, and HCV was observed.

3.3.8.1. Crystal structures of (*S*)-HPEPHx and (*S*)-HPEPG in complex with human HGPRT

To determine the reasons for the difference in K_i values for compounds **124** and **125** with human HGPRT, crystal structures of the complexes were determined to 2.3 and 2.6 Å, respectively. Data collection and refinement statistics are presented in Table 9 (see part 5.1). In both crystal forms, the asymmetric unit is a tetramer of the enzyme. However, amino acid residues within the large mobile loop and at the *N*-terminus are not visible in the electron density (Table 9). Compounds **124** and **125**

could be built into their respective electron density maps in all four subunits of both complexes (Fig. 27). As expected the purine ring and phosphonate group occupy the guanine (or hypoxanthine) site and 5'-phosphate site as observed when GMP (or IMP) is bound to the enzyme.

In the complex with compound **124**, the purine ring is held in place by hydrogen bonds to the side-chain of K165 and to the backbone carbonyl of V187 in all four subunits. An additional hydrogen bond is observed subunit A, where the amide nitrogen of V187 and N-1 atom of the purine ring form a hydrogen bond (Fig. 28a). Compound **125** has similar hydrogen bond network at the purine binding site but with an additional hydrogen bond between the carbonyl oxygen of V187 and N-2 atom of the guanine base (Fig. 28b). The total contact area in comparing the conformation of the linker regions in the two compounds, the dihedral angle of the N9-C10-C11-O12 bond is on average, 27° for compound **124** and 49° for compound **125**. Thus, the flexibility of the linker is an important feature in optimizing the binding of compound **125** to human HGPRT. The equivalent dihedral angle N9-C1′-C2′-C3′ for GMP is 85° when bound to human HGPRT. However, in GMP this angle is restricted due to the participation of the C-2 and C-3 atoms in the ribose ring.

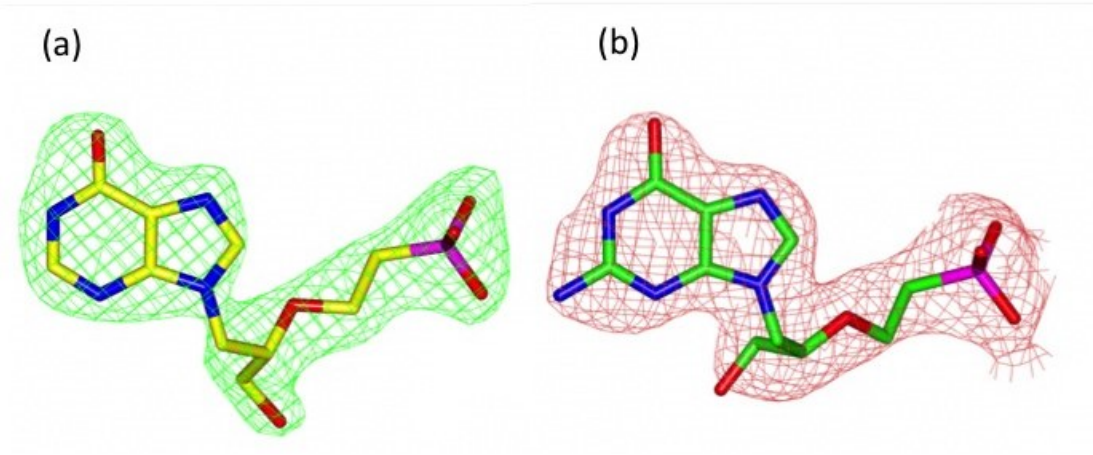


Figure 27. The $2F_o - F_c$ electron density for compounds **124** and **125** in complex with human HGPRT (subunit A) contoured at 1.1 σ.

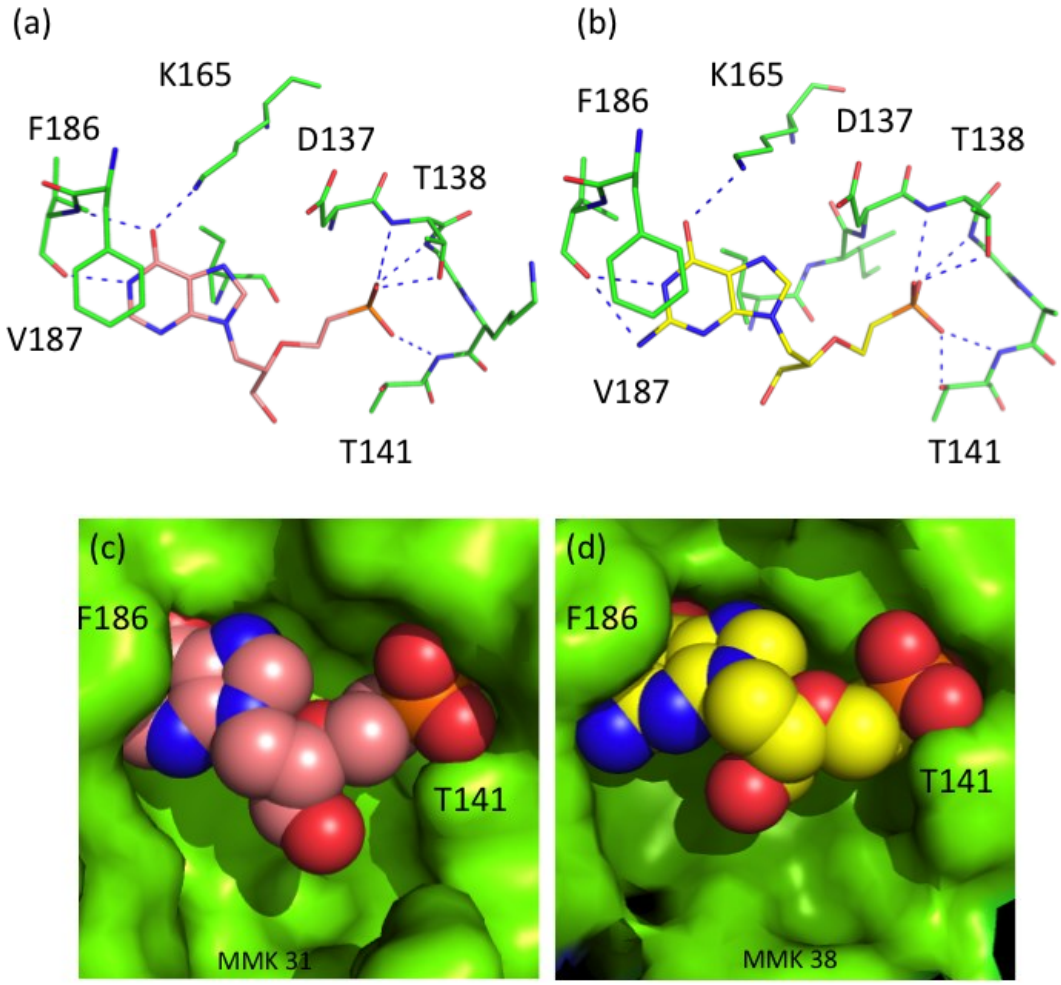


Figure 28. The interactions of compounds **124** and **125** with human HGPRT (a, b). Connolly surface of human HGPRT showing the location of compounds **124** and **125** (drawn as solid spheres) (c, d).

3.3.8.2. The metal binding site and its relationship to the position of the inhibitors

Magnesium ions are also present in the active site and located between E133 and D134 in all four subunits in both complexes. This is in contrast to the human HGPRT-GMP crystal structure, but in that study no Mg^{2+} was added to the enzyme preparation or to the crystallization buffer.[129] However, the crystal structure of human HGPRT in complex with immucillinGP and pyrophosphate does show two magnesium ions bound in the active site with one of these coordinating directly to carboxylate groups of E133 and D134, and to the hydroxyl groups on the iminorbitol ring, while the second Mg^{2+} coordinates with D193 and an oxygen atom from pyrophosphate. In the human HGPRT-**125** crystal structure there is no direct

coordination (distance < 2.5 Å) between the Mg^{2+} and the hydroxyl of compound **125**. However, compound **124** was found to interact directly with Mg^{2+} in one of the subunits of the complex HGPRT-**125**.

In the HGPRT-**124** complex, the magnesium ions are 6-coordinate with surrounding water molecules and OE2, OE1 atom of E133 and OD1 atom of D134 were observed in all four subunits (Fig. 29). It is noted that for subunit B of human HGPRT-**124** complex, the hydroxyl group of compound **124** interact directly with magnesium ion at the metal binding site causing distortion in magnesium coordination resulting in partial formation of octahedral geometry. In human HGPRT-**125** complex, partial coordination of magnesium ion with surrounding molecules was observed. This can be due to lower resolution of the crystal structure.

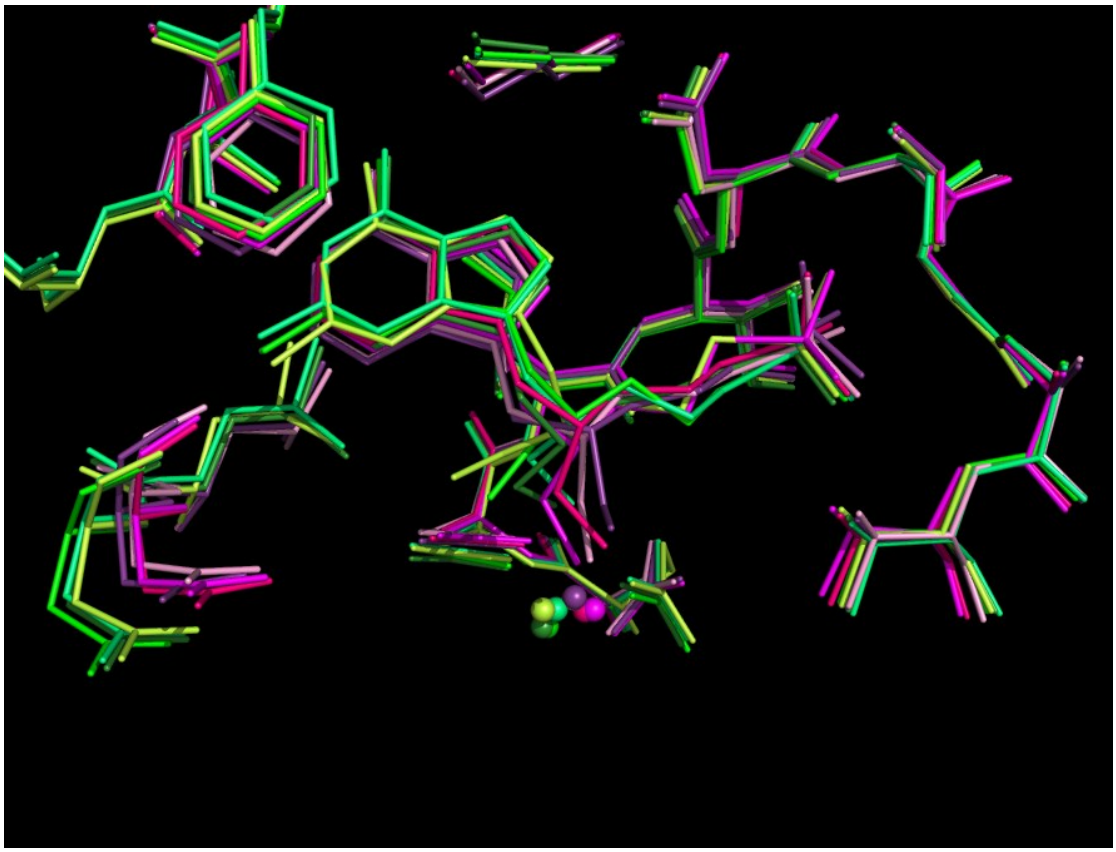


Figure 29. Superimposition of the four subunits of structures of compounds **124** (purple) and **125** (green).

3.3.9. Conclusion

For studying a binding mode to human and plasmodial 6-oxopurine phosphoribosyltransferases, series of 6-oxo-purine ANPs bearing the CPME side chain (compounds **113** and **83g**), the elongated HPEP side chain (compounds **124** and **125**) and the CPEE side chain (compounds **155**, **156** and **159**, **160**) were prepared.

Some of the target HPEP and CPEE analogues were found to be potent inhibitors of human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT with K_i ranging from 0.02 to 3.4 μM. The HPEP and CPEE derivatives possessing the hypoxanthine base (compounds **124** and **159**) exhibited no activity against *Pf*HGXPRT, while the guanine derivatives (compounds **125**, **156** and **160**) revealed activity against all three enzymes tested. Contrary to our expectations, the inhibitors were selective toward the human HGPRT compared to *Plasmodium* enzymes. All prepared prodrugs (compounds **167**-**172**) revealed poor antimalarial activity *in vitro* assay.

Neither (*S*)-CPMEHx nor (*S*)-CPMEG showed any significant activity in the given assays. All new ANPs with the elongated acyclic chain (HPEP) were screened for their antiviral activity however none of them exhibited any.

3.4. Potential inhibitors of Adenylate cyclase

3.4.1. Adenylate cyclase and PMEA analogues

Adenylate cyclase (AC) is an enzyme catalyzing the conversion of ATP to 3‘,5‘-cyclic AMP (cAMP) and pyrophosphate. AC constitues a family of membrane-bound enzymes central to one of the most important transduction systems and influence reagulation of cell function in virtually all cells.[130] Class II AC are toxins secreted by pathogenic bacteria such as *Bacillus anthracis* and *Bordetella pertussis* during infection. Adefovir diphosphate (PMEApp), the active metabolite of adefovir dipivoxil **86** (Fig. 20), was found to inhibit adenylate cyclase activity of edema factor (EF) and adenylyl cyclase toxin (ACT).[131] These are the key virulence factors which allows the bacterial (*Bacillus anthracis* and *Bordetella pertussis*, respectively) invasion into the mammalian body (Fig. 30).

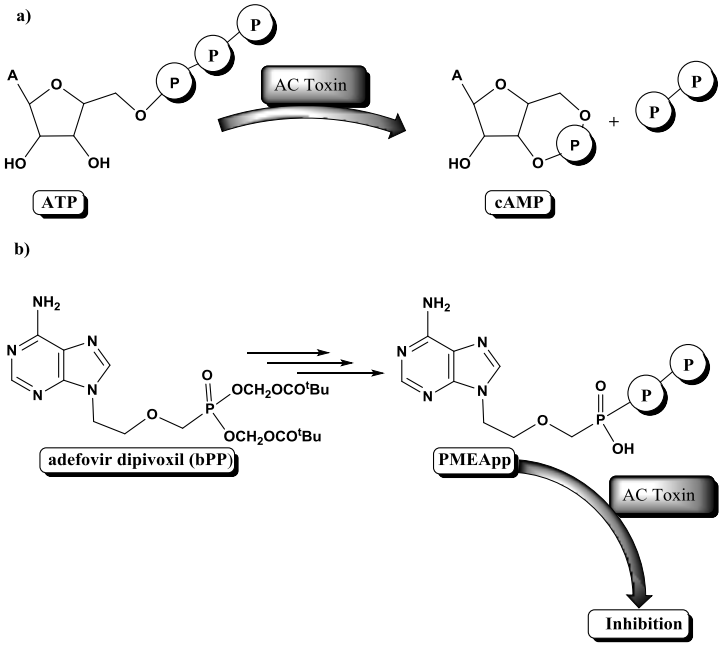
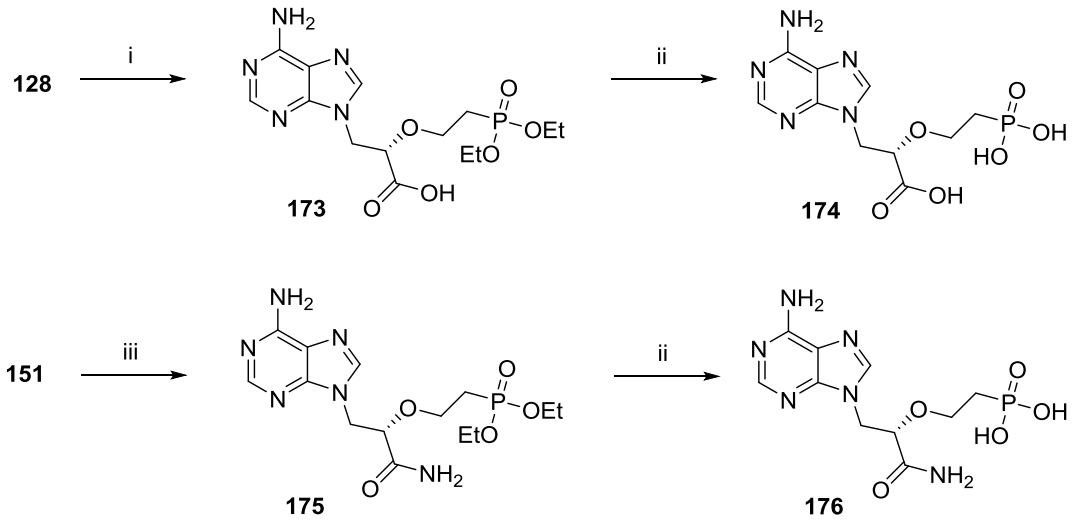


Figure 30. a) The catalysis of the transformation of ATP to cAMP by AC Toxin; b) The inhibition of AC Toxin by PMEApp.

Based on previous studies it was attempted to elucidate, whether the substitution at the position C-2’ of the acyclic chain of PMEA **30** (Fig. 11) and/or elongation of the phosphonate linker can improve the biological activity against adenylate cyclase toxin (ACT). All derivatives bearing adenine prepared within this thesis were used for the structure-activity relationship study. Synthesis of elongated PMEA analogues

with carboxyl moiety in the 2‘-position (*S*)-CPEEA **174** and the corresponding amide **176** is depicted in Scheme 32.



Scheme 32. Reaction conditions: i) TEMPO, $NaClO_2$, NaClO, phosphate buffer/MeCN (1:1), r.t., 24 h; ii) TMSBr, MeCN, r.t., overnight; iii) 3.5 M NH_3 in EtOH, microwave, 120 °C, 1 h.

3.4.2. Biological results - conclusion

Compounds **174** and **176** were tested on the adenylate cyclase toxin inhibition. As mentioned above, all of the tested 2’-substituted PMEA compounds (including (*S*)-CPMEA and its prodrugs) and 2’-substituted PEEA derivatives were shown to be only weak inhibitors of this enzyme.

3.5. Synthesis of oligonucleotides modified with HPEP units

3.5.1. Introduction

Oligomers of nucleotides (including antisense oligonucleotides,[132] small interfering RNAs (siRNA),[133] and aptamers[134]) are in the spotlight of scientists for more than thirty years. The potential of gene therapy was demonstrated for the first time in 1978 by using antisense oligonucleotides (AONs), complementary to the terminal sequence of RSV (Rous sarcoma Virus), to inhibit viral replication *in vitro*.[135] AONs in general are able to bind to the target mRNAs by Watson-Crick pairing. They either inhibit protein translation by blocking ribosome processing or, alternatively, activate the ubiquitous RNase H-mediated cleavage of the RNA strand of the formed heteroduplex. Chemical modifications of the natural phosphodiester linkage to increase the stability of the modified oligonucleotides against nucleases became a very important strategy in development of therapeutics based on the antisense principle. So far there is one approved drug, fomivirsen[136] (brand name Vitravene), a 21 nt oligonucleotide with phosphorothioate linkages (5'-GCG TTT GCT CTT CTT CTT GCG-3', FDA approved in 1998), which had been used in the treatment of HCMV-induced retinitis,[137,138] but there is also several promising drug candidates in various stages of clinical trials (e.g. mipomersen[139] for the treatment of familial hypercholesterolemia, which recently passed phase III; lexgenleucel-T (VRX-494)[140] for the treatment of HIV, which passed phase II or trabedersen[141] for the treatment of highly aggressive tumors, which is currently planned for phase II).

siRNAs are short (20-25 units) double stranded RNAs which interfere with a complementary sequence of a specific gene. Although the use of siRNAs in therapy lies in the distant future, as a proof-of-concept siRNA has been found to be very effective against lethal zaire ebola virus.[142]

Aptamers are oligonucleotide- or peptide-based molecules designed to specifically bind to a target. Pegaptanib[143] (FDA approved in 2004) is an anti-angiogenic aptamer used for the treatment of age-related macular degeneration. Aptamers can also be used for molecular recognition outside the field of clinical medicine.[144]

Another option how to increase the chemical and enzymatic stability of oligonucleotides is the replacement of phosphodiester internucleotide linkage with an isopolar phosphonate moiety. The fact that introduction of such phosphonate

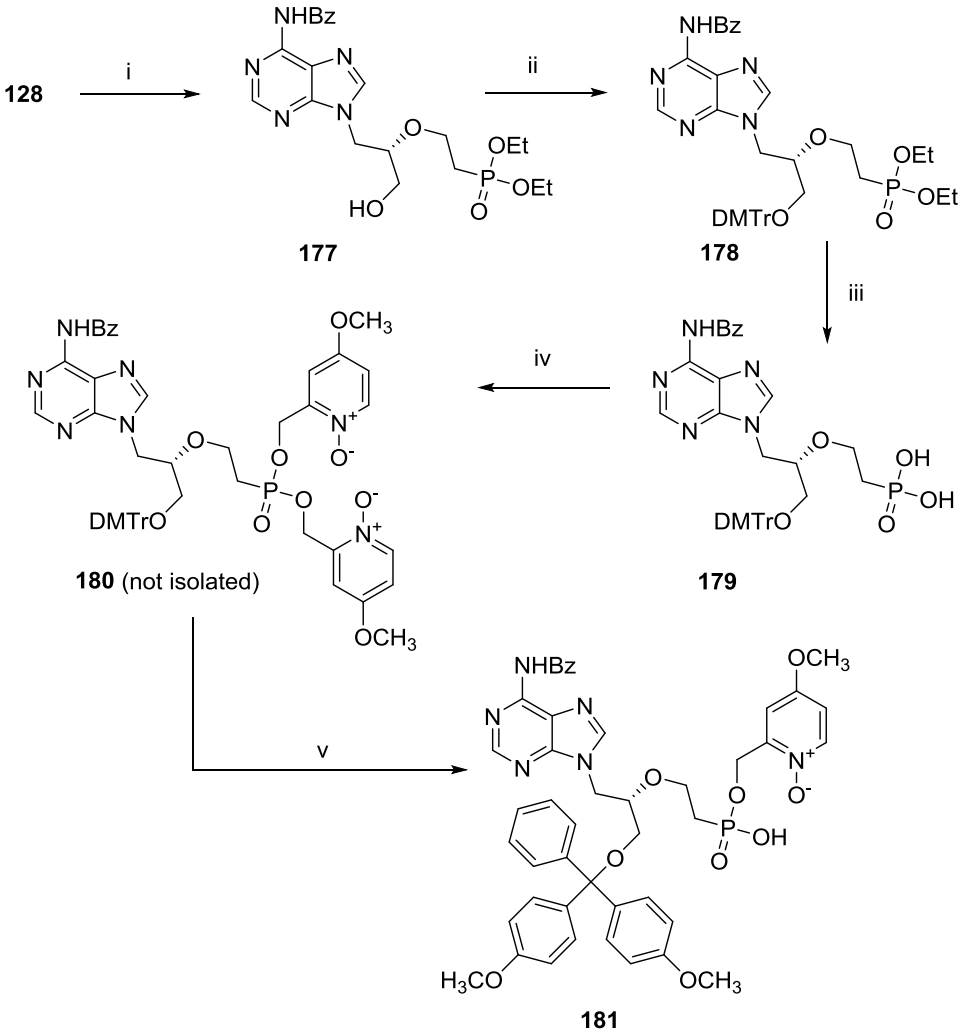
modification presumably does not block the formation of a duplex was substantiated by the hybridization experiments.[145,146] Furthemore, a series of oligonucleotide-based HIV-1 integrase inhibitors with phosphonate modification has been described.[147]

This part of my thesis deals with design and synthesis of building blocks based on ANPs with 9-[3-hydroxy-2-(phosphonoethoxy)propyl] (HPEP) moiety, the solid phase synthesis of chimeric oligonucleotides containing acyclic nucleoside phosphonate units, and study on their hybridization properties.

3.5.2. Synthesis of protected HPEP monomers

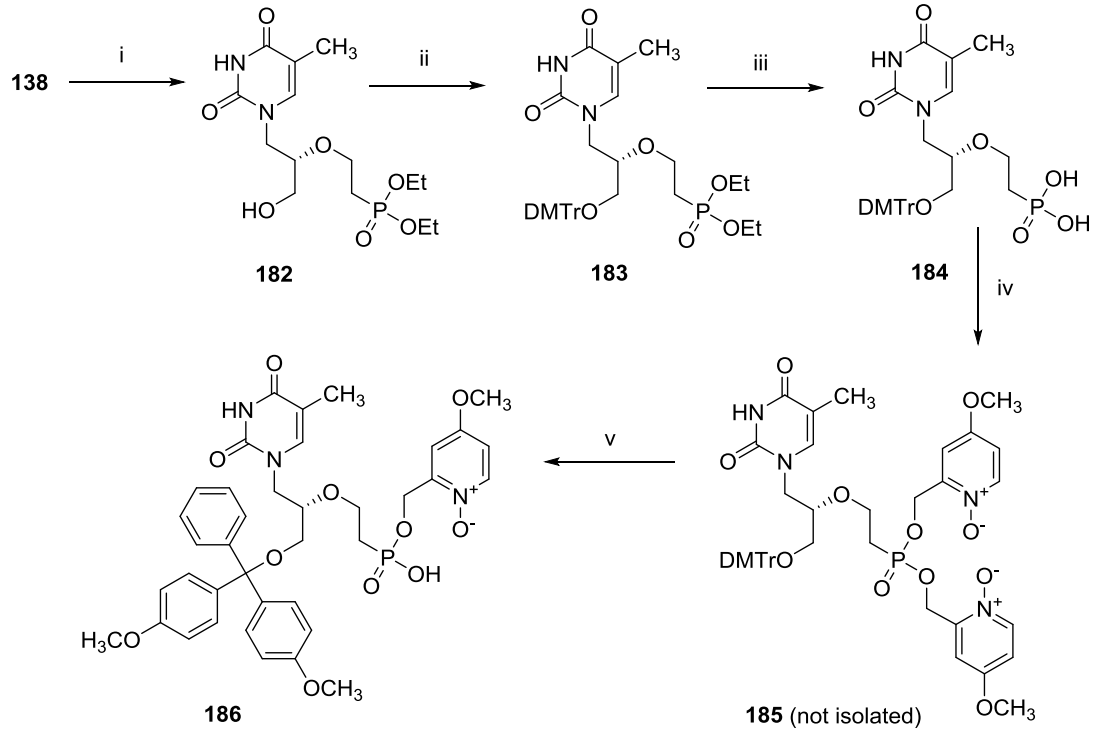
The desired suitably protected HPEP monomers with adenine, cytosine, guanine and thymine bases were prepared by multistep synthesis from the corresponding HPEP derivatives. The crucial synthetic steps included dimethoxytritylation of the 3'-hydroxyl group, removal of the ethyl ester groups with TMSBr and subsequent esterification of free phosphonic acid with 4-methoxy-1-oxido-2-pyridylmethanol (MOP-OH) in the presence of 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane-2-oxide as the condensing agent.

The adenine monomer **181** was prepared according to Scheme 33. In the first step, starting compound **128** [(*S*)-HPEPA diethylester, synthesis described in part 3.3.4.] was protected with benzoyl group to afford *N*-benzoyl derivative **177**. Its treatment with dimethoxytrityl chloride in pyridine resulted in fully protected phosphonate **178**. Compound **178** was subjected to the transesterification using TMSBr in the presence of 2,6-lutidine followed by neutralization with 1 M TEAB which provided protected phosphonic acid **179** in the form of triethylammonium salt. The final reaction consisted of two separate steps. The first step was condensation of compound **179** with MOP-OH in the presence of 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane-2-oxide under the catalysis of 4-methoxy pyridine 1-oxide (the formation of compound **180** was monitored only on TLC, the product was not isolated nor fully characterized). The second step included treatment with the mixture of thiophenol, triethylamine and DMF (1:1.4:2, respectively) to obtain (after purification by silica gel chromatography and lyophilisation from water/dioxane) mono(4-methoxy-1-*N*-oxido-2-pyridyl)methyl (MOP) phosphonate **181** in a reasonable yield (48%).



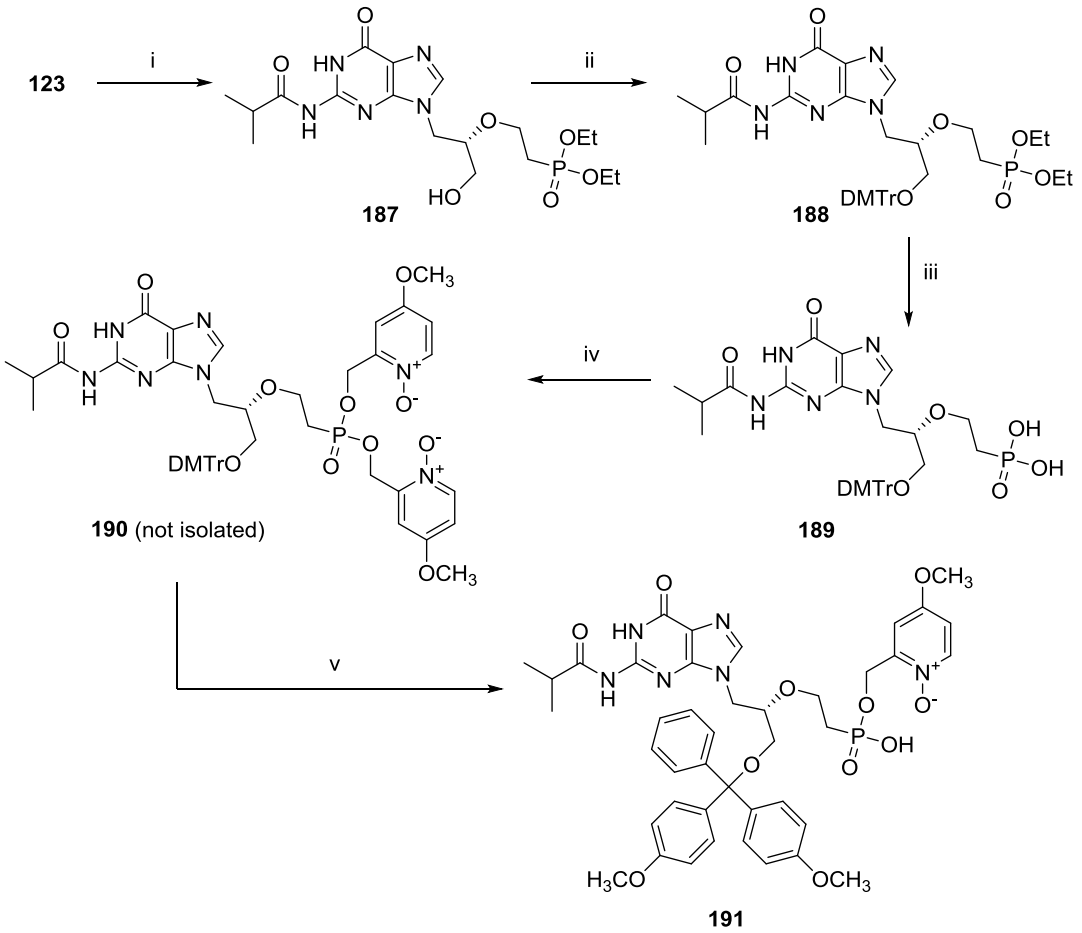
Scheme 33. Reaction conditions: i) 1. TMSCl, BzCl, 2. NH_3 (aq), pyridine, 0 °C, r.t., 2 h; ii) DMTrCl, pyridine, r.t., overnight; iii) 1. 2,6-lutidine, 2. TMSBr, MeCN, r.t., overnight; iv) 1. 2-(hydroxymethyl)-4-methoxypyridine 1-oxide, 4-methoxypyridine 1-oxide, 2. 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide, pyridine, r.t., overnight; v) PhSH, Et_3N, DMF, r.t., 1 h.

The thymine monomer **186** was synthesized by the analogous procedure as described above with the only difference. It was not necessary to protect the thymine base during the synthesis. Starting (*S*)-HPEPT derivative **138** (Scheme 27, the preparation is described in part 3.3.4) was heated in aqueous trifluoroacetic acid overnight and the demethylated product **182** was obtained in a good yield. Following steps were identical with those described above for the synthesis of compound **181**. The desired compound **186** (Scheme 34) was obtained in a satisfactory yield (51%).



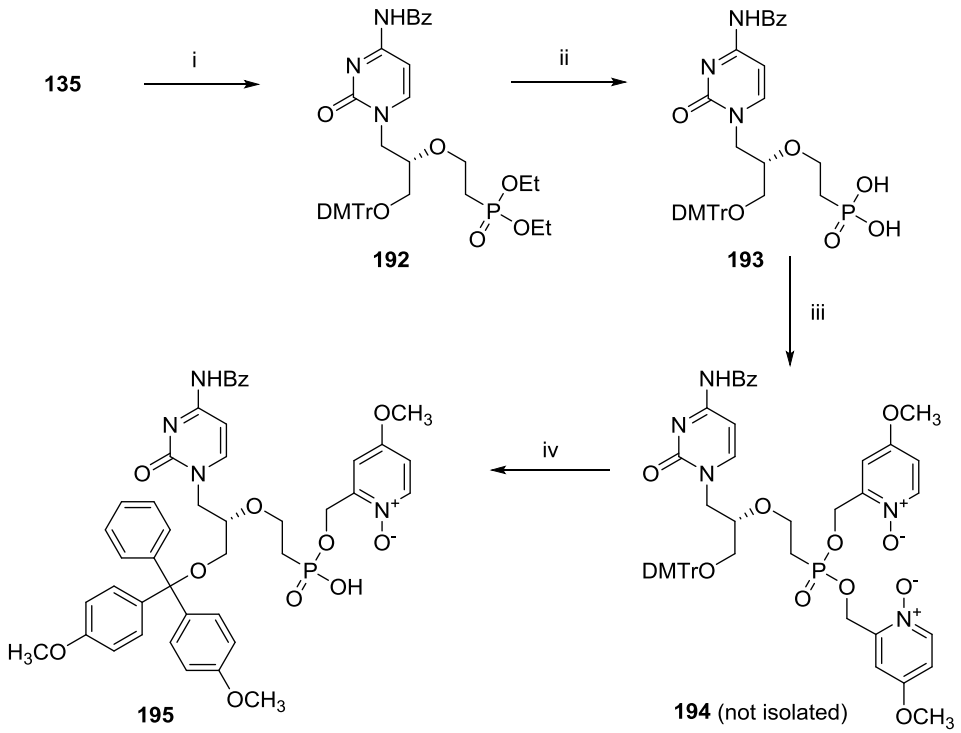
Scheme 34. Reaction conditions: i) 75 % aqueous CF_3COOH, 50 °C, overnight, ii) DMTrCl, pyridine, r.t., overnight; iii) 1. 2,6-lutidine, 2. TMSBr, MeCN, r.t., overnight; iv) 1. 2-(hydroxymethyl)-4-methoxypyridine 1-oxide, 4-methoxypyridine 1-oxide, 2. 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide, pyridine, r.t., overnight; v) PhSH, Et_3N, DMF, r.t., 1 h.

In the case of guanine monomer **191** (Scheme 35), *N-iso*-butyryl group was used (instead of the *N*-benzoyl group) for protection of *N*-2 position of the guanine base resulting in compound **187**. Reaction conditions were analogous to those described for *N*-benzoylation. Following reaction sequence (including dimethoxytrytilation, deprotection of phosphonate moiety and condensation with 2-(hydroxymethyl)-4-methoxypyridine 1-oxide) afforded compound **191** (Scheme 35) in an acceptable yield (47%).



Scheme 35. Reaction conditions: i) 1. TMSCl, *iso*-butyryl chloride, 2. NH_3 (aq), pyridine, 0 °C - r.t., 4 h; ii) DMTrCl, pyridine, r.t., overnight; iii) 1. 2,6-lutidine, 2. TMSBr, MeCN, r.t., overnight; iv) 1. 2-(hydroxymethyl)-4-methoxypyridine 1-oxide, 4-methoxypyridine 1-oxide, 2. 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide, pyridine, r.t., overnight; v) PhSH, Et_3N, DMF, r.t., 1 h.

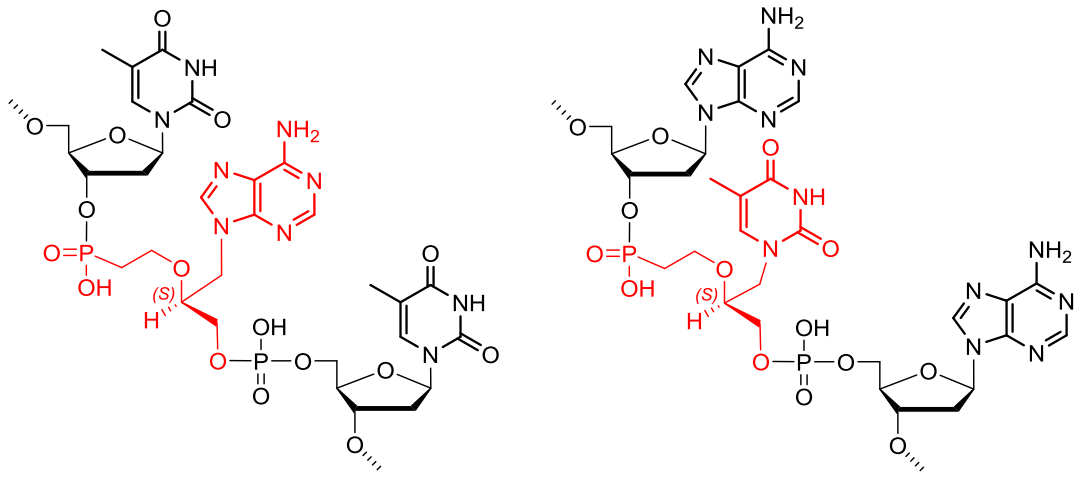
For the synthesis of cytosine monomer **195** (Scheme 36) *N*-benzoylcytosine derivative **135** was used as the starting material. The procedure was analogous to the previous paragraph and resulted in MOP phosphonate **195** in a satisfactory yield (51%). The reaction sequence is shown in Scheme 36.



Scheme 36. Reaction conditions: i) DMTrCl, pyridine, r.t., overnight; ii) 1. 2,6-lutidine, 2. TMSBr, MeCN, r.t., overnight; iii) 1. 2-(hydroxymethyl)-4-methoxy-pyridine 1-oxide, 4-methoxypyridine 1-oxide, 2. 2-chloro-5,5-dimethyl-1,3,2-dioxa-phosphinane 2-oxide, pyridine, r.t., overnight; iv) PhSH, Et_3N, DMF, r.t., 1 h.

3.5.3. Synthesis of oligonucleotides

The phosphotriester and phosphoramidite methods were used for the solid phase incorporation of nucleoside phosphonates and natural nucleotide units, respectively. The oligonucleotide assembly was performed from the 5' to the 3'-end using commercial reverse nucleoside phosphoramidites. The deprotection protocol included thiophenol and gaseous ammonia treatments to remove the MOP ester groups and all base-labeled protecting groups, respectively. All prepared oligonucleotides were purified by anion exchange chromatography. Thus, a series of DNA and RNA nonamers containing the units based on ANPs were successfully prepared (Fig. 31) and thermal stabilities of DNA-DNA, RNA-DNA, and RNA-RNA duplexes have been evaluated (Tables 7 and 8). The introduction of flexible acyclic units into the oligonucleotides resulted in destabilization of the duplexes (decreased *Tm* values).



5'-r(GCA UAU CAC)-3'

5'-r(GTG ATA TGC)-3'

5'-d(GCA TAT CAC)-3'

5'-d(GTG ATA TGC)-3'

Figure 31. Nonamers modified with HPEP unit.

Table 7. Melting points of the prepared oligonucleotides.

Data Name	T_m
Sample 1 5.00-60.00°C Ramp 2	19.0
Sample 2 5.00-60.00°C Ramp 2	13.0
Sample 3 5.00-60.00°C Ramp 2	19.1
Sample 4 5.00-60.00°C Ramp 2	15.1
Sample 5 5.00-60.00°C Ramp 2	10.3
Sample 6 5.00-60.00°C Ramp 2	10.0
Natural DNA-DNA	**36.9**
Natural DNA-RNA	**34.7**
Natural RNA-RNA	**46.1**

Table 8. Prepared nonamer sequences.

DNA/RNA	SEQUENCE	Sample
natural RNA	**5'-r(GUG AUA UGC)**	1
modified RNA	**5'-r(GCA UAU CAC)**	
natural RNA	**5'-r(GCA UAU CAC)**	2
modified RNA	**5'-r(GTG ATA TGC)**	
natural RNA	**5'-r(GUG AUA UGC)**	3
modified DNA	**5'-d(GCA TAT CAC)**	
natural DNA	**5'-d(GTG ATA TGC)**	4
modified DNA	**5'-d(GCA TAT CAC)**	
natural RNA	**5'-r(GCA UAU CAC)**	5
modified DNA	**5'-d(GTG ATA TGC)**	
natural DNA	**5'-d(GCA TAT CAC)**	6
modified DNA	**5'-d(GTG ATA TGC)**	

3.5.4. Conclusion

Two acyclic nucleoside phosphonates bearing HPEP moiety with adenine and thymine nucleobases were synthetically converted into a suitable building blocks for the subsequent automated solid phase synthesis of modified oligonucleotides. Phosphoramidite chemistry was used for the synthesis of a series of complementary nonamers where the modified acyclic monomers were incorporated using the phosphotriester method. Determination of thermal characteristics of the complexes of the modified nonamers with the complementary strands revealed a destabilizing effect of the introduced acyclic modifications.

In near future, the prepared monomers will be used for synthesis of HPEP modified siRNA and their biological properties will be studied in cooperation with Prof. Ronald Micura (Institute of Organic Chemistry, Leopold Franzens University, Innsbruck, Austria).

4. Resume

This thesis represents a part of systematic SAR research of acyclic nucleoside phosphonates (ANPs) carried out in the group of Nucleic acid chemistry (Prof. A. Holý) and later in the group of the Targeted analogues of nucleic acid components (Dr. Z. Janeba) at the IOCB. One of the main goals of this work was to synthesize (*S*)-CPMEA **83a** [(*S*)-3-(adenin-9-yl)-2-(phosphonomethoxy)propanoic acid, Fig. 19]. This compound has been designed as a compound with potential anti-HIV activity. The key step of the (*S*)-CPMEA synthesis was oxidation of (*S*)-HPMPA derivative **84a** using TEMPO/$NaClO_2$/NaClO to give compound **85a** in a high yield. Subsequently, the whole series of CPME derivatives **85** was prepared in good to high yields from the corresponding HPMP analogues **84** by the optimized oxidative methodology with TEMPO. Unfortunately, none of the newly synthesized CPME compounds, including the most promising (*S*)-CPMEA, did show any interesting activity against the viruses tested.

Synthetic strategies leading to prodrugs of (*S*)-CPMEA have been developed and optimized. The intention was to prepare several structurally different types of prodrugs, which had been designed to mask the phosphonate and/or carboxylic groups in the molecule. The prepared prodrugs were evaluated for their antiviral properties. Prodrugs **101** and **102** displayed submicromolar anti-HCV activity. To the best of our knowledge, these are the first examples of ANPs to exhibit potent activity against HCV. None of the prodrugs exhibited any interesting activity against the HIV-1 virus.

6-Oxopurine ANPs bearing either CPME (compounds **113** and **83g**) or elongated HPEP (compounds **124** and **125**) and CPEE (compounds **155**, **156** and **159**, **160**) side chains were prepared and studied for their inhibitory potential on human and plasmodial phosphoribosyltransferases. Some of HPEPG and CPEE analogues were found to be potent inhibitors of human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT with K_i ranging from 0.02 to 3.4 μM. The HPEP and CPEE derivatives bearing the hypoxanthine base (compounds **124** and **159**) exhibited no activity against *Pf*HGXPRT, while guanine derivatives (compounds **125**, **156** and **160**) revealed activity against the all three enzymes tested. The obtained results suggest that both

HPEP and CPEE derivatives have interesting antimalarial potential and should be further modified in order to obtain analogues with better activity and/or selectivity towards the plasmodial HG(X)PRTs. (*S*)-HPEPG **125** with a K_i of 0.02 μM for human HGPRT is one of the most potent inhibitors of the 6-oxopurine PRTase based on nucleotide analogues discovered to date. All prepared prodrugs (compounds **167**-**172**) revealed poor anatimalarial activity *in vitro*. Neither (*S*)-CPMEHx nor (*S*)-CPMEG showed any significant activity in the given assays. All new ANPs with the elongated acyclic chain (HPEP) were screened for their antiviral activity however none of them exhibited any.

All prepared compounds bearing the adenine base [(*S*)-CPMEA **83a** and its prodrugs, (*S*)-HPEPA **129**, (*S*)-CPEEA **174** and its amide **176**] were tested for inhibitory activity of adenylate cyclase toxin from *Bordetella pertussis* (CyaA), but were shown to be only weak inhibitors of this enzyme. The best inhibitor was bis-amidate **101**, which decreased the formation of cAMP to 68% compared to the full (100%) activity of CyaA.

Apart from the previous topics, it was also intended to employ the HPEP intermediates (**123**, **128**, **135**, and **138**) in the preparation of monomers and used these compounds in the preparation of novel oligonucleotides modified with HPEP units. Two acyclic nucleoside phosphonates bearing HPEP moiety with either adenine or thymine as nucleobase were synthetically converted into a suitably protected building blocks for the subsequent automated solid phase synthesis of modified oligonucleotides. Phosphoramidite chemistry was used for the synthesis of a series of complementary nonamers where the modified acyclic monomers were incorporated using the phosphotriester method. Determination of thermal characteristics of the complexes of the modified nonamers with the complementary strands revealed a destabilizing effect of the introduced acyclic modifications.

Remaining two prepared monomers bearing guanine and cytosine have been recently prepared a will also be used for the synthesis and study of modified oligonucleotides.

5. Experimental part

5.1. General – instrumentation and methods

Methods

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa, and compounds were dried in vacuum oven at 2 kPa. TLC was performed on TLC aluminium sheets – silica gel 60 F_{254} (Merck), chromatographic systems are described in the text. Column chromatography was performed on silica gel 230-400 mesh, 60 Å (Merck). Reverse phase HPLC separation were performed on a Waters Delta 600 instrument with a Waters 486 Tunable Absorbance Detector using column Phenomenex Gemini C-18 (10 μm, 250 x 21.2 mm, flow 10 mL/min preparative column). ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance 600 spectrometer (^{1}H at 600 MHz and ^{13}C at 151 MHz) and/or Avance 500 spectrometer (500 Mhz and 126 MHz) in $CDCl_3$, CD_3OD, DMSO-*d*6 or D_2O-(NaOD additive) and referred to TMS or residual solvent signal. Complete assignment is based on heteronuclear correlation experiments HSQC and H, C-HMBC. Chemical shifts (δ) are in ppm and coupling constants (J) in Hz. A numbering system for the assignment of NMR signals is given for the majority of the compound individually. GC/MS spectra were measured on Agilent 5975B MSD spectrometer coupled to 6890N gas chromatograph. Mass spectra were measured: a) on Q-Tof micro (Waters) using ESI technique; b) on LTQ Orbitrap XL (Thermofisher scientific) spectrometer using ESI technique; c) on a ZABEQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). Optical rotations were measured on an AUTOPOL IV polarimetr (Rudolph research analytical) at 20 °C; $[\alpha]_D$ values are given [10^{-1} deg cm^2 g^{-1}] and concentrations c are given in [g/100 mL]. Microwave experiments were carried out in 10 mL vial in CEM Discover (Explorer) microwave apparatus operated at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300W.

Materials and solvents

Unless stated otherwise, reagents and starting compounds were purchased from Sigma-Aldrich (Prague, Czech Republic).

Computational details

The docking studies (Figure 20) were performed with the ArgusLab[148] program with the use of the 'ArgusDock' algorithm. An exhaustive search was performed by enabling 'High precision' option in Docking precision menu, 'Dock' was chosen as the calculation type, 'flexible' for the ligand. Spacing of 0.2 Å between the grid points was used. The atomic co-ordinates for the X-ray structures of the HIV-1 RT-DNA complexes after incorporation of the anti-HIV drug tenofovir were downloaded from the Protein Data Bank (PDB code: 1T05).[93] The final inhibitor – protein complex was visualized using ArgusLab.[148]

Synthesis of oligonucleotides

The synthesis of oligonucleotides by method "trityl off" was carried out by Dr. Pavel Novák and thermal characteristics of prepared nonamers were measured by Dr. Šárka Rosenbergová (both members of group of Dr. Ivan Rosenberg at IOCB). Details of synthetic procedure and T_m experiments are described in the reference.[149]

Table 9

Data collection and refinement statiscics for human HGPRT in complex with compounds **124** and **125**.

compound	**124**	**125**
Data collection		
Temperature (K)	100	100
Unit cell length (Å)	*a* = 64.53 *b* = 94.31 *c* = 139.3	*a* = 74.27 *b* = 93.28 *c* = 129.88
Unit cell angle (°)	α = β = γ = 90	α = β = γ = 90
Space group	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$
Resolution range	53.3-2.30	46.64-2.61
Unique reflections [I> 0σ I]	34,663 (3,185)[a]	27,248 (3,277)
Total observations [I> 0σI]	143,334 (13,215)	153,376 (18,320)
Completeness (%)	90.4 (86.1)	97.3 (97.2)
R_{merge}	0.065 (0.491)	0.120 (0.729)

R_{pim}	x.xxx (x.xxx)	x.xxxx (x.xxx)
<I>/<σ(I)>	12.9 (2.2)	10.4 (2.2)
Subunits per asymmetric unit	4	4
Refinement		
Resolution range (Å)	47.33-2.30	46.64-2.61
R_{factor} [F> 0σ (F)]	22.28	20.86
R_{free} [F> 0σ (F)]	28.73	26.87
Average B-factor (Å)	42.57	45.96
RMS deviation from ideal		
Bond length (Å)	0.007	0.003
Bond angle (°)	1.15	0.844
Ramachandran plot statistics		
Residues in most favored regions (%)	95.6	95.1
Residues in disallowed regions (%)	0	1.5
Missing residues	A subunit: 1-3, 103-120 B subunit: 1-3, 102-119 C subunit: 1-3, 104-118 D subunit: 1-3, 102-118	A subunit: 1-3, 101-121 B subunit: 1-3, 101-122 C subunit: 1-3, 101-120 D subunit: 1-3, 101-121
Side chains modeled as alanine	B subunit: D89, I92, K140, Q151, T167, R169 C subunit: K140, T210, K212 D subunit: K140	A subunit: I23, R33, L67, K68 B subunit: R33, S91, K174, N202 C subunit: E29, D89, K214 D subunit: E29, D89

[a]Values in parentheses are for the outer resolution shell.

$$R_{merge} = \sum_{hkl}\sum_{i}\left|I_i(hkl) - \left(I(hkl)\right)\right| / \sum_{hkl}\sum_{i} I_i(hkl)$$

$$R_{p.i.m.} = \sum_{hkl}\left[\frac{1}{[N(hkl)-1]}\right]^{1/2}\sum_{i}\left|I_i(hkl) - \left(I(hkl)\right)\right| / \sum_{hkl}\sum_{i} I_i(hkl)$$

where $I_i(hkl)$ is the observed intensity and is the average intensity obtained from multiple observations of symmetry related reflections.

Table 10.

Dihedral angels between *N*-9 atom and oxygen linker of oxygen-linker acyclic nucleoside phosphonates in complex with human HGPRT (measured by UCSF Chimera program).

Ligand (Subunit)	Atom 1	Atom 2	Atom 3	Atom 4	Dihedral angle (°)
124 A	N9	C10	C11	O12	6.17
124 B	N9	C10	C11	O12	24.75
124 C	N9	C10	C11	O12	40.85
124 D	N9	C10	C11	O12	36.81
124 A	C10	C11	O12	C13	130.58
124 B	C10	C11	O12	C13	128.55
124 C	C10	C11	O12	C13	145.57
124 D	C10	C11	O12	C13	157.54
124 A	C11	O12	C13	C14	167.50
124 B	C11	O12	C13	C14	-136.35
124 C	C11	O12	C13	C14	151.34
124 D	C11	O12	C13	C14	178.96
124 A	O12	C13	C14	P17	162.55
124 B	O12	C13	C14	P17	179.86
124 C	O12	C13	C14	P17	157.77
124 D	O12	C13	C14	P17	166.03
125 A	N9	C10	C11	O12	30.97
125 B	N9	C10	C11	O12	59.13
125 C	N9	C10	C11	O12	47.34
125 D	N9	C10	C11	O12	60.03

125 A	C10	C11	O12	C13	93.23
125 B	C10	C11	O12	C13	157.85
125 C	C10	C11	O12	C13	139.03
125 D	C10	C11	O12	C13	90.96
125 A	C11	O12	C13	C14	157.15
125 B	C11	O12	C13	C14	142.32
125 C	C11	O12	C13	C14	148.64
125 D	C11	O12	C13	C14	-120.23
125 A	O12	C13	C14	P17	174.14
125 B	O12	C13	C14	P17	105.53
125 C	O12	C13	C14	P17	121.84
125 D	O12	C13	C14	P17	-149.82
PEEG A	N9	C10	C11	O12	52.05
PEEG B	N9	C10	C11	O12	48.33
PEEG A	C10	C11	O12	C13	78.69
PEEG B	C10	C11	O12	C13	89.10
PEEG A	C11	O12	C13	C14	131.95
PEEG B	C11	O12	C13	C14	165.84
PEEG A	O12	C13	C14	P15	144.18
PEEG B	O12	C13	C14	P15	163.57
PEEH A	N9	C10	C11	O12	48.36
PEEH B	N9	C10	C11	O12	66.41
PEEH A	C10	C11	O12	C13	91.31
PEEH B	C10	C11	O12	C13	51.82
PEEH A	C11	O12	C13	C14	123.72
PEEH B	C11	O12	C13	C14	176.69
PEEH A	O12	C13	C14	P15	143.45
PEEH B	O12	C13	C14	P15	-174.04
HPMPG A	N9	C10	C11	O12	42.62
HPMPG B	N9	C10	C11	O12	8.71
HPMPG A	C10	C11	O12	C13	151.59
HPMPG B	C10	C11	O12	C13	-165.11
HPMPG A	C11	O12	C13	P16	-151.63
HPMPG B	C11	O12	C13	P16	-114.63

Ligand (subunit)	Number of H bond with surrounding amino residues	Number H bond with surrounding water molecule	Total H bonds
124 A	9	5	14
124 B	7	2	9
124 C	8	2	10
124 D	9	2	11
125 A	8	2	10
125 B	10	1	11
125 C	10	2	12
125 D	9	0	9

5.2. General methods

Method A: Synthesis of the *N*-benzoyl derivatives

Starting HPMP or HPEP derivative (1.5 mmol) was dissolved in dry pyridine (20 mL) in a round-bottomed flask with a drying tube (protecting the reaction from moisture), and the reaction mixture was cooled in an ice bath. TMSCl (0.95 mL, 7.5 mmol) was added to the reaction mixture and after 30 min benzoyl chloride (0.87 mL, 7.5 mmol) was added. The reaction flask was removed from the ice bath and the mixture was stirred for 2 h at room temperature. Then the reaction mixture was cooled in an ice bath and cold water (4 mL) was added, followed after 15 min of stirring by concentrated aqueous ammonia (4 mL). The mixture was stirred at room temperature for another 30 min and solvents were removed *in vacuo* to give crude oil. This was subjected to silica gel chromatography (gradient from 0-5% methanol in chloroform) to afford (after evaporation) the corresponding products as a thick yellowish oil.

Method B: Oxidation of HPMP or HPEP diesters with TEMPO/$NaClO_2$/NaClO

The corresponding HPMP or HPEP diester (1.0 mmol), TEMPO (47 mg, 0.3 mmol), sodium chlorite (226 mg, 2 mmol) were dissolved in the mixture of acetonitrile (10 mL) and 0.67 M aqueous sodium phosphate buffer (10 mL, pH 6.7) and an aqueous solution of sodium hypochlorite (40.7 mg/mL), prepared by dilution of household bleach SAVO (1 mL) with water (1 mL), was added dropwise. The mixture was stirred at room temperature overnight. Solvents were evaporated and the residue was purified by silica gel chromatography (gradient from 2-25% methanol in chloroform) to afford (after evaporation) the crude product. Crystallization from ethyl acetate/methanol gave the corresponding CPME or CPEE derivatives in high yields.

Method C: Removal of benzoyl group

1.0 M sodium methanolate (2 mmol, 2 mL) was added to a solution of the *N*-benzoyl derivative (1 mmol) in methanol (10 mL). After stirring at room temperature for 24 h, the reaction mixture was neutralized with glacial acetic acid. Solvents were evaporated and the residue was codistilled with toluene (2 x 10 mL) and ethanol (2 x 10 mL). Chromatography on silica gel column (gradient 5 - 20% methanol in chloroform) afforded the corresponding derivatives in good yields.

Method D: Deprotection of diethyl or diisopropyl esters to free phosphonic acids

D1: *Deprotection with TMSBr:* The appropriate diester (0.5 mmol) was dissolved in acetonitrile (10 mL) and TMSBr (1 mL) was added dropwise. The mixture was stirred overnight and evaporated to dryness. Residue was codistilled with acetonitrile (3 x 20 mL), water (2 x 10 mL) and evaporated *in vacuo*. Crude product was crystallized from the ethanol/water mixture or further purified by **method E**.

D2: *Deprotection in microwave:* A solution of the appropriate diester (0.5 mmol) in 0.5 M aqueous hydrochloric acid (2 mL) was heated in a microwave at 140 °C for 30 min. Volatiles were evaporated, the residue was dissolved in water (1 mL) and the product was purified by **method E**.

Method E: Purification of free phosphonic acids

Crude phosphonic acid was dissolved in water and purified by preparative HPLC. Product was eluated by linear gradient from 2-80% methanol in water. UV absorbing fractions containing product were collected and evaporated *in vacuo*. Compounds were then crystallized from the water/ethanol mixture.

Method F: Synthesis of phosphoramidate prodrugs (L-alanine ethylester)

A mixture of corresponding diisopropyl ester (0.5 mmol), dry acetonitrile (10 mL), and TMSBr (0.5 mL) was stirred overnight at room temperature under argon. After evaporation (without any contact with air) *in vacuo* (40 °C, 2 mbar) and codistillation with dry acetonitrile (2 x 10 mL) (without any contact with air), the flask was resecurated with argon and L-alanine ethylester hydrochloride (2 mmol), dry triethylamine (1.5 mL), and dry pyridine (5 mL) were added and the mixture was heated at 50 °C for 15 min to obtain a homogenous solution. A solution of Aldrithiol-2 (3 mmol) and triphenylphosphine (3 mmol) in 6 mL of dry pyridine was added under argon. The resulting mixture was heated at 50 °C for 5 hours to reach the full conversion. After cooling, the bright yellow solution was evaporated (40 °C, 2 mbar), and the residue was purified by silica gel chromatography (0-7% methanol in chloroform) to afford (after evaporation and codistillation with dry acetone) the desired product in satisfactory yield.

Method G: Synthesis of the 6-chloropurine derivatives from phosphonate alcohols under Mitsunobu reaction conditions.

A solution of DIAD (2.95 mL, 15 mmol) in dioxane (50 mL) was added dropwise under argon atmosphere to a mixture of alcohol (**7, 9b, 12**) (10 mmol), 6-chloropurine (2.0 g, 13 mmol), and Ph_3P (3.41 g, 13 mmol) in dioxane (150 mL) and the reaction mixture was stirred at room temperature for 24 h. When the reaction was completed, solvents were removed *in vacuo* and the residue was purified by silica gel chromatography (gradient from 20-100% ethyl acetate in *iso*-hexanes and 0-6% methanol in ethyl acetate).

Method H: Synthesis of the 2-amino-6-chloropurine derivatives from phosphonate alcohols under the Mitsunobu reaction conditions.

The procedure is identical with the procedure E, only 2-amino-6-chloropurine was used (instead of 6-chloropurine). When the reaction was completed, water was added (50 mL) and the reaction mixture was stirred at 80 °C for 6 h (to remove triphenylphosphine aducts). The mixture was evaporated and the residue was purified by silica gel chromatography (gradient from 20-100% ethyl acetate in *iso*-hexanes and 0-12% methanol in ethyl acetate).

Method I: Sequential hydrolysis of the 6-chloropurine derivatives to the corresponding 6-oxopurine phosphonates using DABCO

The 6-chloropurine derivative (1 mmol) was dissolved in mixture of dioxane (100 mL) and water (20 mL), DABCO (0.8 mmol) and potassium carbonate (1 mmol) were added. The reaction mixture was heated at 90 °C for 3-5 h. After cooling down, volatiles were evaporated and the crude residue was purified by silica gel chromatography to give the corresponding product.

Method J: Sequential hydrolysis of the 6-chloropurine derivatives to the corresponding 6-oxopurine phosphonates using trifluoroacetic acid

The 6-chloropurine derivative (1 mmol) was dissolved in trifluoroacetic acid (75%, 15 mL) and the mixture was stirred overnight. Volatile materials were evaporated *in vacuo*, the residue codistilled with water (3 x 10 mL) and neutralized with ammonia (1:10 with water) to pH 6-7. Volatiles were evaporated and the crude

residue was purified by silica gel chromatography (gradient from 1-12% methanol in chloroform) to give the corresponding product.

Method K: Removal of benzyl group

The corresponding derivative (2 mmol) was dissolved in glacial acetic acid (120 mL) and a flask was purged with argon and evacuated (3 times). Catalytic amount of 10% palladium on carbon under argon atmosphere was added. The flask was evacuated and purged with hydrogen (3 times) and the mixture was vigorously stirred at room temperature until the reaction was completed (~ 3 days). The reaction mixture was filtered through short silica gel column and the filter pad was washed with acetic acid (50 mL) and methanol (50 mL). The filtrate was evaporated and a crude product was purified by silica gel chromatography (gradient from 2-12% methanol in chloroform) to afford the corresponding product.

Method L: Synthesis of phosphoramidate prodrugs (L-phenylalanine ethylester)

A mixture of an appropriate compound (0.5 mmol), dry acetonitrile (10 mL), and TMSBr (0.5 mL) was stirred overnight at room temperature under inert atmosphere. After evaporation (without any contact with air) *in vacuo* (40 °C, 2 mbar) and codistillation with dry acetonitrile (2 x 10 mL) (without any contact with air), the flask was securated with argon and L-phenylalanine ethylester hydrochloride (3 mmol), dry triethylamine (1.5 mL), and dry pyridine (4 mL) were added and the mixture was heated at 60 °C for 7 min. A solution of Aldrithiol-2 (4 mmol) and triphenylphosphine (4 mmol) in 5 mL of dry pyridine was added under argon. The resulting mixture was heated at 70 °C for 3 days to reach the full conversion. After cooling, the dark brown solution was evaporated (40 °C, 2 mbar), and the residue was purified by silica gel chromatography (methanol in chloroform) to afford (after evaporation) the crude product which was purified by flash chromatography on C_{18}-reversed phase silica gel (0-100% methanol in water) to give (after evaporation and codistillation with dry acetone) the corresponding compound as a yellowish foam.

Method M: Protecting of the hydroxyl moiety with 4,4'-dimethoxytrityl group

4,4'-Dimethoxytrityl chloride (1.02 g, 3 mmol) was added to the corresponding derivative (2 mmol) dissolved in dry pyridine (40 mL) and the reaction mixture was stirred at room temperature overnight. Solvent was evaporated and the residue was

purified by silica gel chromatography (gradient from 50-100% chloroform in *iso*-hexanes with 0.1% Et_3N) to give (after evaporation) the corresponding product as a white foam.

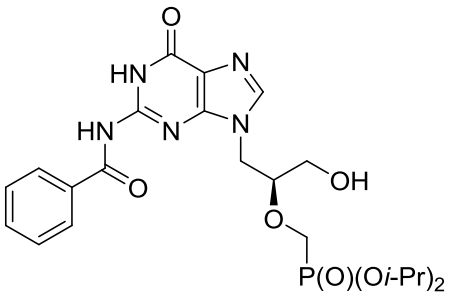
Method N: Selective deprotection of the phosphonate moiety

The corresponding derivative (1.6 mmol) was co-evaporated with acetonitrile (3 x 30 ml) and dissolved in dry acetonitrile (30 ml). 2,6-Lutidine (1.85 mL, 16 mmol) and TMSBr (1.06 mL, 8 mmol) were added under argon. The reaction mixture was stirred at room temperature under argon overnight. Acetonitrile was removed *in vacuo*, the residue was dissolved in the mixture of aqueous 2 M TEAB and methanol, and the volatiles were evaporated. The residue was purified by silica gel chromatography (linear gradient of H1 in ethyl acetate with 0.1% Et_3N) to give (after lyophilisation from dioxane) the corresponding product as a white solid.

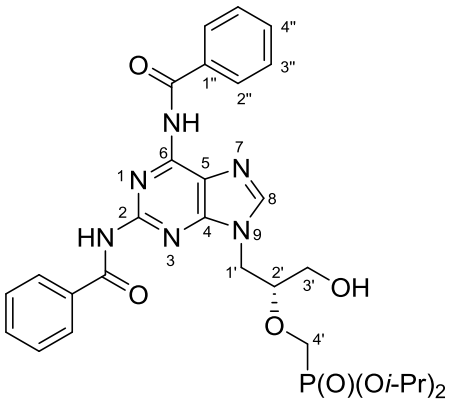
Method O: Condensation with 2-(hydroxymethyl)-4-methoxypyridine 1-oxide (MOP) followed by selective deprotection to monoester

A mixture of corresponding phosphonic acid (1 mmol), 2-(hydroxymethyl)-4-methoxypyridine 1-oxide (466 mg, 3 mmol) and 4-methoxypyridine 1-oxide (626 mg, 5 mmol) was co-distilled with dry pyridine (3 x 20 mL) and dissolved in dry pyridine (30 mL) under argon. Then 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphinane 2-oxide (923 mg, 5 mmol) was added, and the reaction mixture was stirred at room temperature under argon overnight. Pyridine was removed *in vacuo* and a residue was co-distilled with dry dioxane (3 x 20 mL). The crude residue was not further purified and the corresponding intermediate was not isolated and characterized. The the dark brown residue was dissolved in a mixture of thiophenol (2.3 mL), Et_3N (3.2 mL) and DMF (4.5 mL) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was subjected to silica gel chromatography (linear gradient of H1 in ethyl acetate with 0.1% Et_3N) to give (after lyophilisation from dioxane) the corresponding product as a white solid.

5.3. Synthesis of the *N*-benzoyl HPMP derivatives



Diisopropyl (*S*)-N^2-benzoyl-9-[3-hydroxy-2-(phosphonomethoxy)propyl]guanine (84i).[94b] From compound **84g** (605 mg, 1.5 mmol) according to **method A**; afforded 350 mg (yield 69%) of compound **84i** as a yellowish solid (recryst EtOAc/Hexane), m.p. 171-173 °C (m.p. 173-174 °C);[94b] MS-ESI$^+$ *m*/*z* (%) 508 (15, M+H$^+$), 530 (100, M+Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_{22}H_{31}O_7N_5P$ (M+H$^+$) 508.1956, found 508.1954. ^{1}H NMR (DMSO-d_6) spectrum is identical with the original spectroscopic data.[94b]



Diisopropyl (*S*)-N^2,N^6-dibenzoyl-9-[3-hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (84j)

From compound **84h** (604 mg, 1.5 mmol) according to **method A**; afforded 430 mg (yield 70%) of compound **84j** as a thick yellowish oil; ^{1}H NMR (DMSO-d_6) δ: 11.16 (s, 1H, NH), 11.01 (s, 1H, NH), 8.31 (s, 1H, H-8), 8.06 (m, 2H, H-2‘‘), 7.99 (m, 2H, H-2‘‘), 7.50 – 7.66 (m, 6H, H-3‘‘, H-4‘‘), 5.04 (t, $J_{OH\text{-}3'a}$ = $J_{OH\text{-}3'b}$ = 5.7 Hz, 3’-OH), 4.44 – 4.56 (m, 3H, H-1’a, **CH**-ipr), 4.35 (dd, J_{gem} = 14.7 Hz, $J_{1'b\text{-}2'}$ = 6.4 Hz, 1H, H-1’b), 3.89 – 3.94 (m, 3H, H-2‘, H-4’), 3.50 (m, 1H, H-3‘a), 3.43 (m, 1H, H-3‘b), 1.20 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.17 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.16 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.13 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d_6) δ: 165.86 (C=O), 165.74 (C=O), 153.90 (C-4), 152.30 (C-2), 150.68 (C-6), 145.28 (C-8), 134.46 (C-1‘‘), 133.56 (C-1‘‘), 132.64 (C-4‘‘), 132.20 (C-4‘‘), 128.70 (C-2‘‘, C-3‘‘), 128.63 (C-2‘‘, C-3‘‘), 128.59 (C-2‘‘, C-3‘‘), 128.28 (C-2‘‘, C-3‘‘), 123.08 (C-5), 79.91 (d, $J_{2'\text{-}P}$ = 11.5 Hz, C-2‘), 70.48 (d, $J_{C\text{-}O\text{-}P}$ = 6.3 Hz, **CH**-ipr), 63.57 (d, $J_{C\text{-}P}$ = 165.1 Hz, C-4’), 59.97 (C-3‘), 43.36 (C-1‘), 23.78 – 24.00 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m*/*z* (%) 611 (5, M+H$^+$), 633 (100, M+Na$^+$), 655 (12, M+2Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_{29}H_{36}O_7N_6P$ (M+H$^+$) 611.2378, found 611.2376.

5.4. Oxidations of HPMP to the corresponding CPME derivatives

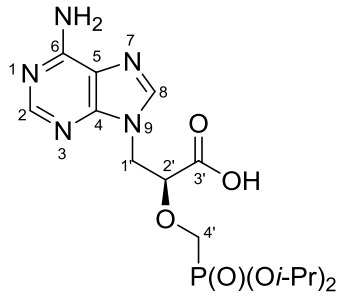
5.4.1. Oxidation of diisopropyl (*S*)-HPMPA with ruthenium tetroxide

Compound **84a** (387 mg, 1.0 mmol), RuO_2 (20 mg, 0.15 mmol), and $NaIO_4$ (1.1 g, 5.0 mmol) were vigorously stirred in a mixture of CH_3CN (15 mL), $CHCl_3$ (15 mL), and H_2O (25 mL). Concentrated aqueous HCl (~ 8 drops) was added and the system was adjusted to ~ pH 2.5. The heterogenous mixture was vigorously stirred at room temperature for 72 h. The solid was removed by filtration of the reaction mixture through a silica gel layer and washed with methanol. Solvents were evaporated and the residue was purified by column chromatography on silica gel ($CHCl_3$-MeOH-AcOH, 88:6:6). The purification was repeated two more times to remove colourful by-products and inorganic salts. Crystallization (EtOAc-MeOH) afforded 253 mg (yield 63%) of the desired compound **85a** as yellowish crystals.

5.4.2. Oxidation of diisopropyl (*S*)-HPMPA with TEMPO/BAIB

Compound **84a** (387 mg, 1.0 mmol), TEMPO (32 mg, 0.2 mmol), and BAIB (644 mg, 2.2 mmol) in a mixture of CH_3CN (2 mL) and H_2O (2 mL) were stirred at room temperature for 24 h and the solvents were evaporated. The flash chromatography on silica gel ($CHCl_3$-MeOH, 4:1) followed by crystallization (EtOAc-MeOH) afforded 285 mg (yield 71%) of compound **85a** as yellowish crystals.

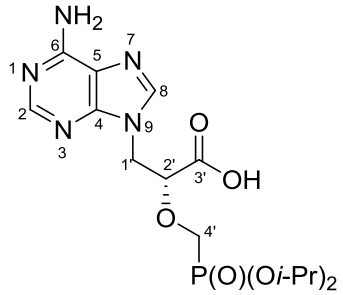
5.4.3. Oxidation of diisopropyl HPMP derivatives with TEMPO /$NaClO_2$ /NaClO



(*S*)-3-(6-Amino-*9H*-purin-9-yl)-2-[(diisopropoxy-phosphono)methoxy] propanoic acid (85a)

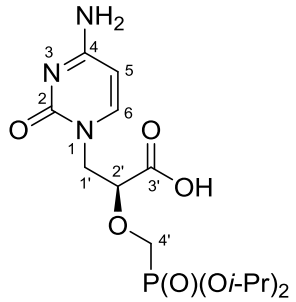
From compound **84a** (387 mg, 1 mmol) according to **method B**; afforded 349 mg (yield 87%) of compound **85a** as yellowish crystals, dec > 150 °C; ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, H-2), 8.03 (s, 1H, H-8), 7.28 (bs, 2H, NH_2), 4.37 – 4.55 (m, 5H, H-1‘, H-2‘, **CH**-ipr), 3.95 (dd, J_{gem} = 13.7 Hz,

$J_{4'a\text{-}P}$ = 8.7 Hz, 1H, H-4'a), 3.73 (dd, J_{gem} = 13.7 Hz, $J_{4'b\text{-}P}$ = 9.4 Hz, 1H, H-4'b), 1.18 (d, J = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.14 (d, J = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.13 (d, J = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.07 (d, J = 6.2 Hz, 3H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d$_6$) δ: 170.61 (C-3'), 155.93 (C-6), 152.37 (C-2), 149.75 (C-4), 141.47 (C-8), 118.55 (C-5), 77.99 (d, $J_{2'\text{-}P}$ = 12.7 Hz, C-2'), 70.58 (d, $J_{C\text{-}O\text{-}P}$ = 6.5 Hz, **CH**-ipr), 70.53 (d, $J_{C\text{-}O\text{-}P}$ = 6.5 Hz, **CH**-ipr), 64.08 (d, $J_{C\text{-}P}$ = 163.9 Hz, C-4'), 44.36 (C-1'), 23.92 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 4.0 Hz, **CH$_3$**-ipr), 23.88 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 3.9 Hz, **CH$_3$**-ipr), 23.78 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 4.5 Hz, **CH$_3$**-ipr), 23.67 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 4.5 Hz, **CH$_3$**-ipr); MS-ESI$^+$ *m*/*z* (%) 402 (60, M+H$^+$), 825 (100, 2M+Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_{15}H_{25}O_6N_5P$ (M+H$^+$) 402.1537, found 402.1536; FTIR (KBr, cm^{-1}) ν: 3396, 3181, 2981, 1688, 1651, 1599, 1233, 996. $[\alpha]^{20}_D$ = -26.2 (c = 0.302 g/100ml, MeOH).



(*R*)-3-(6-Amino-*9H*-purin-9-yl)-2-[(diisopropoxy-phosphono)methoxy]propanoic acid (85b)

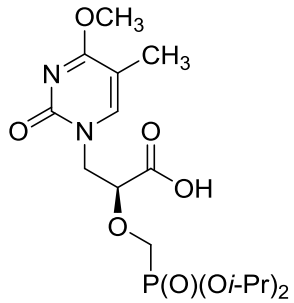
From compound **84b** (387 mg, 1 mmol) according to **method B**; afforded 305 mg (yield 76%) of compound **85b** as yellowish crystals, dec > 150 °C; ^{1}H NMR (DMSO-d$_6$), ^{13}C NMR (DMSO-d$_6$), MS, and FTIR spectra are identical with the compound **85a**. $[\alpha]^{20}_D$ = +32.4 (c = 0.339 g/100ml, MeOH).



(*S*)-3-(4-Amino-2-oxopyrimidin-1(*2H*)-yl)-2-[(diisopropoxyphosphono)methoxy]propanoic acid (85c)

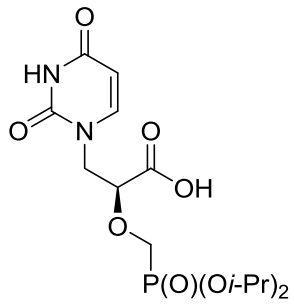
From compound **84c** (727 mg, 2 mmol) according to **method B**; afforded 626 mg (yield 83%) of compound **85c** as yellowish crystals, dec > 150 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.43 (d, $J_{6\text{-}5}$ = 7.3 Hz, 1H, H-6), 7.34 (bs, 1H, NH$_2$), 7.19 (bs, 1H, NH$_2$), 5.66 (d, $J_{5\text{-}6}$ = 7.3 Hz, 1H, H-5), 4.49 – 4.58 (m, 2H, **CH**-ipr), 4.26 (dd, $J_{2'\text{-}1'}$ = 8.7 Hz and 3.7 Hz, 1H, H-2'), 4.15 (dd, J_{gem} = 13.8 Hz, $J_{1'a\text{-}2'}$ = 3.7 Hz, 1H, H-1'a), 3.91 (dd, J_{gem} = 13.8 Hz, $J_{4'a\text{-}P}$ = 8.6 Hz, 1H, H-4'a), 3.64 – 3.71 (m, 2H, H-1'b, H-4'b), 1.17 – 1.23 (m, 12H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d$_6$) δ: 171.04 (C-3'), 165.84 (C-4), 155.22 (C-2), 147.29 (C-6), 93.24 (C-5), 77.77 (d, $J_{2'\text{-}P}$ = 12.8 Hz, C-2'), 70.50 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, **CH**-ipr), 64.15 (d, $J_{C\text{-}P}$ = 163.9 Hz, C-4'), 50.39 (C-1'), 23.77 – 23.98 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m*/*z* (%) 378 (20, M+H$^+$), 400 (85,

M+Na$^+$), 422 (100, M+2Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{14}H_{23}O_7N_3P$ (M-H$^+$) 376.1279, found 376.1280; FTIR (KBr, cm^{-1}) ν: 3357, 3183, 2979, 2901, 1723, 1652, 1376, 1238, 1002. $[\alpha]^{20}_D$ = -75.7 (c = 0.264 g/100ml, MeOH).



(*S*)-2-[(Diisopropoxyphosphono)methoxy]-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)propanoic acid (85d)

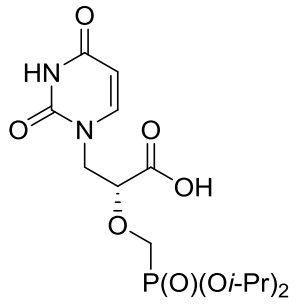
From compound **84d** (785 mg, 2 mmol) according to **method B**; afforded 667 mg (yield 82%) of compound **85d** as yellowish crystals, mp 82-90 °C, dec at 130 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.63 (q, $J_{6–CH3}$ = 1.0 Hz, 1H, H-6), 4.43 – 4.54 (m, 2H, **CH**-ipr), 4.27 (dd, J_{gem} = 13.7 Hz, $J_{1'a–2'}$ = 3.3 Hz, 1H, H-1'a), 4.03 (dd, J_{gem} = 13.8 Hz, $J_{4'a-P}$ = 7.8 Hz, 1H, H-4'a), 3.96 (dd, $J_{2'–1'}$ = 9.8 Hz and 3.3 Hz, 1H, H-2'), 3.82 (s, 3H, O-**CH$_3$**), 3.62 (dd, J_{gem} = 13.7 Hz, $J_{1'b–2'}$ = 9.7 Hz, 1H, H-1'b), 3.54 (dd, J_{gem} = 13.8 Hz, $J_{4'b-P}$ = 9.1 Hz, 1H, H-4'b), 1.84 (d, $J_{CH3–6}$ = 1.0 Hz, 3H, 5-**CH$_3$**), 1.20 (d, $J_{CH3–CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.17 (d, $J_{CH3–CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.15 (d, $J_{CH3–CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.12 (d, $J_{CH3–CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d$_6$) δ: 171.38 (C-3'), 169.99 (C-4), 155.45 (C-2), 147.59 (C-6), 101.43 (C-5), 79.65 (d, $J_{2'–P}$ = 12.5 Hz, C-2'), 70.16 (m, **CH**-ipr), 63.45 (d, $J_{C–P}$ = 163.6 Hz, C-4'), 53.95 (O-**CH$_3$**), 51.64 (C-1'), 23.66 – 23.90 (m, **CH$_3$**-ipr), 11.81 (5-**CH$_3$**); MS-ESI$^+$ *m/z* (%) 407 (5, M+H$^+$), 429 (15, M+Na$^+$), 451 (100, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{27}O_8N_2NaP$ (M+H$^+$) 429.1397, found 429.1397; FTIR (KBr, cm^{-1}) ν: 3440, 2981, 2935, 1670, 1541, 1480, 1374, 1244, 1106, 994. $[\alpha]^{20}_D$ = -84.4 (c = 0.399 g/100ml, MeOH).



(*S*)-2-[(Diisopropoxyphosphono)methoxy]-3-(2,4-dioxo-3,4-dihydro pyrimidin-1(*2H*)-yl)propanoic acid (85e)

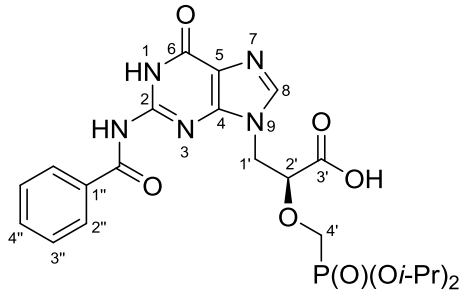
From compound **84e** (729 mg, 2 mmol) according to **method B**; afforded 613 mg (yield 81%) of compound **85e** as yellowish crystals, dec > 150 °C; ^{1}H NMR (DMSO-d$_6$) δ: 11.23 (bs, 1H, H-3), 7.49 (d, J_{6-5} = 7.9 Hz, 1H, H-6), 5.48 (dd, J_{5-6} = 7.9 Hz, J_{5-3} = 2.1 Hz, 1H, H-5), 4.49 – 4.59 (m, 2H, **CH**-ipr), 4.14 (dd, J_{gem} = 14.0 Hz, $J_{1'a-2'}$ = 3.5 Hz, 1H, H-1'a), 4.08 (dd, $J_{2'-1'}$ = 9.1 Hz and 3.5 Hz, 1H, H-2'), 3.97 (dd, J_{gem} = 13.9 Hz, $J_{4'a-P}$ = 8.3 Hz, 1H, H-4'a), 3.69 (dd, J_{gem} = 14.0 Hz,

$J_{1'b\text{-}2'}$ = 9.2 Hz, 1H, H-1'b), 3.65 (dd, J_{gem} = 13.9 Hz, $J_{4'b\text{-}P}$ = 8.5 Hz, 1H, H-4'b), 1.17 - 1.22 (m, 12H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d$_6$) δ: 171.22 (C-3'), 164.01 (C-4), 151.06 (C-2), 146.76 (C-6), 100.42 (C-5), 78.92 (d, $J_{2'\text{-}P}$ = 11.9 Hz, C-2'), 70.39 (m, **CH**-ipr), 63.72 (d, $J_{C\text{-}P}$ = 163.7 Hz, C-4'), 49.63 (C-1'), 23.79 – 23.99 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m*/*z* (%) 379 (5, M+H$^+$), 401 (60, M+Na$^+$), 423 (100, M+2Na$^+$); HRMS-ESI$^-$: *m*/*z* calcd for $C_{14}H_{22}O_8N_2P$ (M-H$^+$): 377.1119, found: 377.1119; FTIR (KBr, cm^{-1}) ν: 3417, 2981, 1691, 1630, 1464, 1388, 1245, 1105, 1001. $[\alpha]^{20}_D$ = -53.0 (c = 0.322 g/100ml, MeOH).



(*R*)-2-[(Diisopropoxyphosphono)methoxy]-3-(2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)propanoic acid (85f)

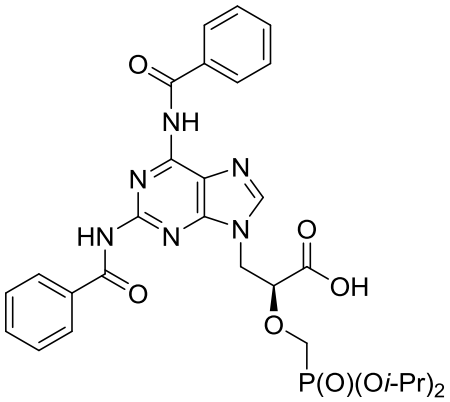
From compound **84f** (729 mg, 2 mmol) according to **method B**; afforded 580 mg (yield 77%) of compound **85f** as yellowish crystals, dec > 150 °C; ^{1}H NMR (DMSO-d$_6$), ^{13}C NMR (DMSO-d$_6$), MS, and FTIR spectra are identical with the compound **85e**. $[\alpha]^{20}_D$ = +68.1 (c = 0.584 g/100ml, MeOH).



(*S*)-3-(2-Benzamido-6-oxo-1H-purin-9(*6H*)-yl)-2-[(diisopropoxyphosphono)methoxy] propanoic acid (85i)

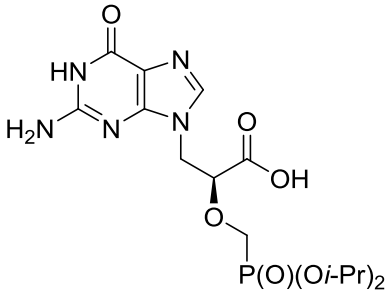
From compound **84i** (508 mg, 1 mmol) according to **method B**; afforded 407 mg (yield 78%) of compound **85i** as a colourless oil; ^{1}H NMR (DMSO-d$_6$) δ: 10.59 (bs, 1H, NH), 8.01 (s, 1H, H-8), 7.95 (m, 2H, H-2''), 7.58 (m, 1H, H-4''), 7.49 (m, 2H, H-3''), 7.43 (bs, 2H, NH$_2$), 4.35 – 4.54 (m, 5H, H-1', H-2', **CH**-ipr), 3.97 (dd, J_{gem} = 13.7 Hz, $J_{4'a\text{-}P}$ = 8.9 Hz, 1H, H-4'a), 3.77 (dd, J_{gem} = 13.7 Hz, $J_{4'b\text{-}P}$ = 9.7 Hz, 1H, H-4'b), 1.09 – 1.20 (m, 12H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d$_6$) δ: 170.59 (C-3'), 165.88 (C=O), 152.42 (C-2 or C-6), 150.61 (C-4), 141.37 (C-8), 134.65 (C-1''), 131.99 (C-4''), 128.50 (C-3''), 128.18 (C-2''), 116.23 (C-5), 77.98 (d, $J_{2'\text{-}P}$ = 13.5 Hz, C-2'), 70.54 – 70.63 (m, **CH**-ipr), 64.16 (d, $J_{C\text{-}P}$ = 164.4 Hz, C-4'), 44.25 (C-1'), 23.68 – 23.95 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m*/*z* (%) 522 (75, M+H$^+$), 544 (100, M+Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_{22}H_{29}O_8N_5P$ (M+H$^+$): 522.1748, found: 522.1747; FTIR (KBr, cm^{-1}) ν: 3318, 3143, 2981, 2500,

1674, 1602, 1529, 1453, 1443, 1387, 1255, 955. $[\alpha]^{20}_D$ = -40.7 (c = 0.194 g/100ml, MeOH).



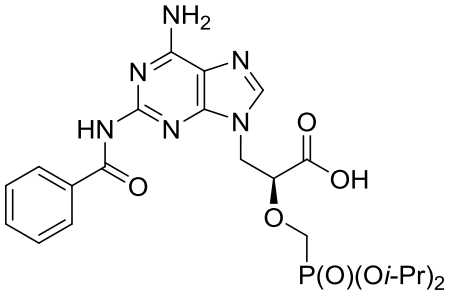
(*S*)-3-(*N*^{2},*N*^{6}-Dibenzoyl-2,6-diamino-*9H*-purin-9-yl)-2-[(diisopropoxyphosphono) methoxy]propanoic acid (85j)

From compound **84j** (611 mg, 1 mmol) according to **method B**; afforded 501 mg (yield 80%) of compound **85j** as a colourless oil which solidified; MS-ESI$^+$ *m*/*z* (%) 625 (7, M+H$^+$), 647 (15, M+Na$^+$) 669 (100, M+2Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_{29}H_{34}O_8N_6P$ (M+H$^+$): 625.2170, found: 625.2171.



(*S*)-3-(2-Amino-6-oxo-*1H*-purin-9(*6H*)-yl)-2-[(diisopropoxyphosphoryl)methoxy]propanoic acid (85g)

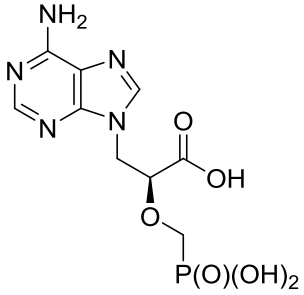
From compound **85i** (1 mmol, 521 mg) according to **method C**; afforded 338 mg (yield 81%) of compound **85g** as a yellowish solid; ^{1}H NMR (DMSO-d$_6$) δ: 12.32 (bs, 1H, H-1), 7.56 (s, 1H, H-8), 7.22 (bs, 2H, NH$_2$), 4.41 – 4.53 (m, 2H, **CH**-ipr), 4.33 (dd, J_{gem} = 13.5 Hz, $J_{1'a\text{-}2'}$ = 1.9 Hz, 1H, H-1'a), 4.10 (dd, J_{gem} = 13.7 Hz, $J_{4'a\text{-}P}$ = 8.1 Hz, 1H, H-4'a), 3.88 – 3.97 (m, 2H, H-1'b, H-2'), 3.56 (dd, J_{gem} = 13.7 Hz, $J_{4'b\text{-}P}$ = 9.0 Hz, 1H, H-4'b), 1.19 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.15 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.14 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.11 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d$_6$) δ: 171.53 (C-3'), 157.95 (C-6), 154.61 (C-2), 151.56 (C-4), 137.78 (C-8), 116.26 (C-5), 81.36 (d, $J_{2'\text{-}P}$ = 13.0 Hz, C-2'), 70.24 – 70.38 (m, **CH**-ipr), 63.28 (d, $J_{C\text{-}P}$ = 163.7 Hz, C-4'), 45.54 (C-1'), 23.74 – 23.98 (m, **CH$_3$**-ipr); MS-ESI$^-$ *m*/*z* (%) 416 (100, M-H$^+$), 438 (10, M+Na$^+$); HRMS-ESI$^-$: *m*/*z* calcd for $C_{15}H_{23}O_7N_5P$ (M-H$^+$) 416.1340, found 416.1339; FTIR (KBr, cm^{-1}) ν: 3423, 3163, 1686, 1630, 1579, 1485, 1410, 1242, 1178, 1105, 997. $[\alpha]^{20}_D$ = -35.6 (c = 0.407 g/100ml, MeOH).



(*S*)-3-(*N*^{2}-Benzoyl-2,6-diamino-*9H*-purin-9-yl)-2-[(diisopropoxyphosphono)methoxy] propanoic acid (85k)

From compound **85j** (468 mg, 0.75 mmol) according to **method C**; afforded 281 mg (yield 72%) of compound **85k** as a colourless oil which solidified; ^{1}H NMR (DMSO-d$_6$) δ: 10.59 (bs, 1H, 2-NH), 8.01 (s, 1H, H-8), 7.95 (m, 2H, H-2''), 7.58 (m, 1H, H-4''), 7.49 (m, 2H, H-3''), 7.43 (bs, 2H, NH$_2$), 4.35 – 4.54 (m, 5H, H-1', H-2', **CH**-ipr), 3.97 (dd, J_{gem} = 13.7 Hz, $J_{4'a\text{-}P}$ = 8.9 Hz, 1H, H-4'a), 3.77 (dd, J_{gem} = 13.7 Hz, $J_{4'b\text{-}P}$ = 9.7 Hz, 1H, H-4'b), 1.09 – 1.20 (m, 12H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d$_6$) δ: 170.59 (C-3'), 165.88 (C=O), 152.42 (C-2 or C-6), 150.61 (C-4), 141.37 (C-8), 134.65 (C-1''), 131.99 (C-4''), 128.50 (C-3''), 128.18 (C-2''), 116.23 (C-5), 77.98 (d, $J_{2'\text{-}P}$ = 13.5 Hz, C-2'), 70.54 – 70.63 (m, **CH**-ipr), 64.16 (d, $J_{C\text{-}P}$ = 164.4 Hz, C-4'), 44.25 (C-1'), 23.68 – 23.95 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m*/*z* (%) 521 (100, M+H$^+$), 543 (70, M+Na$^+$) 565 (20, M+2Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_{22}H_{30}O_7N_6P$ (M+H$^+$): 521.1908, found: 521.1906.

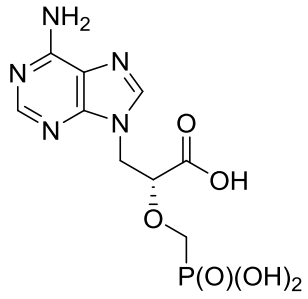
Free phosphonic acids:



(*S*)-3-(6-Amino-*9H*-purin-9-yl)-2-(phosphonomethoxy) propanoic acid (83a)

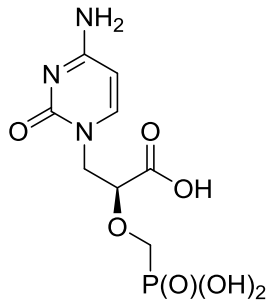
From compound **85a** (201 mg, 0.5 mmol) according to **method D1**; afforded 108 mg (yield 68%) of compound **83a** as a white solid, dec > 250 °C or according to **method D2**; afforded 73 mg (yield 46%) of compound **83a** as white crystals, dec > 250 °C; ^{1}H NMR (D$_2$O) δ: 8.17 (s, 1H, H-2), 8.16 (s, 1H, H-8), 4.55 (dd, J_{gem} = 14.8 Hz, $J_{1'a\text{-}2'}$ = 4.0 Hz, H-1'a), 4.48 (dd, J_{gem} = 14.8 Hz, $J_{1'b\text{-}2'}$ = 6.0 Hz, 1H, H-1'b), 4.20 (dd, $J_{2'\text{-}1'b}$ = 6.0 Hz, $J_{2'\text{-}1'a}$ = 4.0 Hz, 1H, H-2'), 3.71 (dd, J_{gem} = 12.8 Hz, $J_{4'a\text{-}P}$ = 8.6 Hz, 1H, H-4'a), 3.45 (dd, J_{gem} = 12.8 Hz, $J_{4'b\text{-}P}$ = 9.8 Hz, 1H, H-4'b); ^{13}C NMR (D$_2$O) δ: 177.20 (C-3'), 155.86 (C-6), 149.62 (C-4), 143.74 (C-8), 118.57 (C-5), 81.75 (d, $J_{2'\text{-}P}$ = 12.4 Hz, C-2'), 67.33 (d, $J_{C\text{-}P}$ = 155.0 Hz, C-4'), 46.12 (C-1'); MS-ESI$^+$ *m*/*z* (%) 318 (100, M+H$^+$), 340 (83, M+Na$^+$), 362 (65, M+2Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_9H_{13}O_6N_5P$ (M+H$^+$) 318.0598, found 318.0598; FTIR (KBr, cm^{-1}) ν: 3178, 3033, 2838, 1647, 1598, 1474, 1417, 1056; Anal. Calcd. for $C_9H_{12}N_5O_6P$

0.75H_2O: C, 32.69; H, 4.11; N, 21.18; P, 9.37. Found: C, 32.86; H, 3.99; N, 20.95; P, 9.61. $[\alpha]^{20}_D$ = -6.1 (c = 0.379 g/100ml, H_2O).



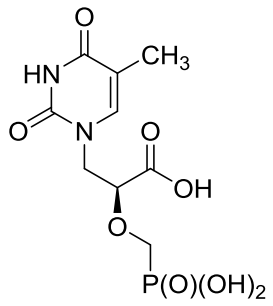
(*R*)-3-(6-Amino-*9H*-purin-9-yl)-2-(phosphonomethoxy) propanoic acid (83b)

From compound **85b** (201 mg, 0.5 mmol) according to **method D1**; afforded 80 mg (yield 50%) of compound **83b** as white crystals, dec > 250 °C; ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6), MS, and FTIR spectra are identical with the compound **83a**. $[\alpha]^{20}_D$ = +6.2 (c = 0.324 g/100ml, H_2O).



(*S*)-3-(4-Amino-2-oxopyrimidin-1(*2H*)-yl)-2-(phosphono-methoxy)propanoic acid (83c)

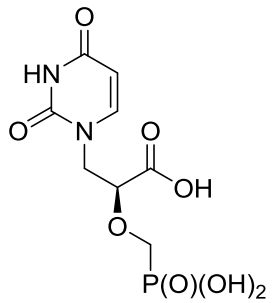
From compound **85c** (190 mg, 0.5 mmol) according to **method D1**; afforded 91 mg (yield 62%) of compound **83c** as yellowish crystals, dec > 250 °C or according to **method D2**; afforded 57 mg (yield 39%) of compound **83c** as yellowish crystals, dec > 250 °C; ^{1}H NMR (D_2O) δ: 7.64 (d, $J_{6\text{-}5}$ = 7.4 Hz, 1H, H-6), 5.98 (d, $J_{5\text{-}6}$ = 7.3 Hz, 1H, H-5), 4.21 (dd, J_{gem} = 14.0 Hz, $J_{1'a\text{-}2'}$ = 3.9 Hz, 1H, H-1'a), 4.05 (dd, $J_{2'\text{-}1'b}$ = 7.7 Hz, $J_{2'\text{-}1'a}$ = 3.9 Hz, 1H, H-2'), 3.89 (dd, J_{gem} = 14.0 Hz, $J_{1'b\text{-}2'}$ = 7.7 Hz, 1H, H-1'b), 3.71 (dd, J_{gem} = 12.9 Hz, $J_{4'a\text{-}P}$ = 8.7 Hz, 1H, H-4'a), 3.42 (dd, J_{gem} = 12.8 Hz, $J_{4'b\text{-}P}$ = 10.2 Hz, 1H, H-4'b); ^{13}C NMR (D_2O) δ: 177.57 (C-3'), 166.01 (C-4), 157.42 (C-2), 148.94 (C-6), 95.69 (C-5), 81.62 (d, $J_{2'\text{-}P}$ = 13.1 Hz, C-2'), 67.07 (d, $J_{C\text{-}P}$ = 156.4 Hz, C-4'), 51.87 (C-1'); MS-ESI$^-$ *m*/*z* (%) 292 (100, M-H$^+$), 314 (50, M+Na$^+$), 336 (25, M+2Na$^+$); HRMS-ESI$^-$: *m*/*z* calcd for $C_8H_{11}O_7N_3P$ (M-H$^+$) 292.0340, found 292.0340; FTIR (KBr, cm^{-1}) ν: 3385, 3171, 2795, 1650, 1613, 1494, 1400, 1051. $[\alpha]^{20}_D$ = -7.0 (c = 0.339 g/100ml, H_2O).



(*S*)-3-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-2-(phosphonomethoxy)propanoic acid (83d)

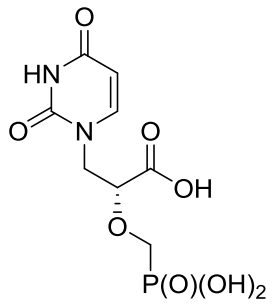
From compound **85d** (203 mg, 0.5 mmol) according to **method D1**; afforded 99 mg (yield 64%) of compound **83d** as white crystals, dec > 250 °C or according to **method D2**; afforded 68 mg (yield 44%) of compound **83d** as white

crystals, dec > 250 °C; ^{1}H NMR (D_2O) δ: 7.50 (q, $J_{6–CH3}$ = 1.2 Hz, 1H, H-6), 4.10 (dd, J_{gem} = 13.9 Hz, $J_{1'a–2'}$ = 4.1 Hz, 1H, H-1'a), 4.05 (dd, $J_{2'–1'}$ = 6.6 Hz and 4.1 Hz, 1H, H-2'), 3.96 (dd, J_{gem} = 13.9 Hz, $J_{1'b–2'}$ = 6.6 Hz, 1H, H-1'b), 3.69 (dd, J_{gem} = 12.8 Hz, $J_{4'a-P}$ = 8.7 Hz, 1H, H-4'a), 3.42 (dd, J_{gem} = 12.8 Hz, $J_{4'b-P}$ = 9.9 Hz, 1H, H-4'b), 1.87 (d, $J_{CH3–6}$ = 1.2 Hz, 3H, 5-**C**$\mathbf{H_3}$); ^{13}C NMR (D_2O) δ: 177.59 (C-3'), 167.78 (C-4), 152.84 (C-2), 144.62 (C-6), 110.71 (C-5), 81.61 (d, $J_{2'-P}$ = 12.6 Hz, C-2'), 67.24 (d, $J_{C–P}$ = 157.1 Hz, C-4'), 50.34 (C-1'), 11.93 (5-**C**$\mathbf{H_3}$); MS-ESI$^-$ *m*/*z* (%) 307 (100, M-H$^+$), 329 (60, M+Na$^+$), 351 (15, M+2Na$^+$); HRMS-ESI$^-$: *m*/*z* calcd for $C_9H_{12}O_8N_2P$ (M-H$^+$) 307.0326, found 307.0327; FTIR (KBr, cm^{-1}) ν: 3191, 3036, 2833, 1691, 1601, 1475, 1432, 1359, 1110, 1062. $[\alpha]^{20}_{D}$ = -2.7 (c = 0.401 g/100ml, H_2O).



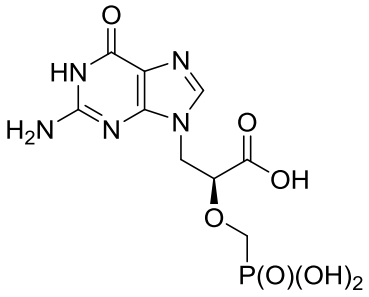
(*S*)-3-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-2-(phosphonomethoxy)propanoic acid (83e)

From compound **85e** (189 mg, 0.5 mmol) according to **method D2**; afforded 62 mg (yield 42%) of compound **83e** as white crystals, dec > 250 °C; ^{1}H NMR (D_2O + NaOD) δ: 7.59 (d, $J_{6–5}$ = 7.5 Hz, 1H, H-6), 5.74 (d, $J_{5–6}$ = 7.5 Hz, 1H, H-5), 3.97 – 4.05 (m, 3H, H-1', H-2'), 3.53 (dd, J_{gem} = 11.8 Hz, $J_{4'a-P}$ = 9.1 Hz, 1H, H-4'a), 3.30 (dd, J_{gem} = 11.8 Hz, $J_{4'b-P}$ = 10.2 Hz, 1H, H-4'b); ^{13}C NMR (D_2O + NaOD) δ: 178.47 (C-3'), 174.43 (C-4), 158.01 (C-2), 147.85 (C-6), 102.05 (C-5), 81.99 (d, $J_{2'-P}$ = 13.6 Hz, C-2'), 68.91 (d, $J_{C–P}$ = 150.7 Hz, C-4'), 51.03 (C-1'); MS-ESI$^-$ *m*/*z* (%) 293 (100, M-H$^+$); HRMS-ESI$^-$: *m*/*z* calcd for $C_8H_{10}O_8N_2P$ (M-H$^+$): 293.0180, found: 293.0180; FTIR (KBr, cm^{-1}) ν: 3428, 3168, 2976, 1692, 1617, 1402, 1356, 1070, 1050. $[\alpha]^{20}_{365}$ = -50.0 (c = 0.346 g/100ml, H_2O).



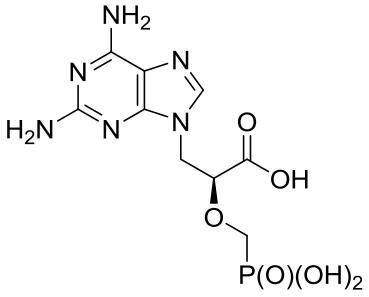
(*R*)-3-(2,4-Dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-2-(phosphonomethoxy)propanoic acid (83f)

From compound **85f** (189 mg, 0.5 mmol) according to **method D2**; afforded 71 mg (yield 48%) of compound **83f** as white crystals, dec > 250 °C; ^{1}H NMR (D_2O + NaOD), ^{13}C NMR (D_2O + NaOD), MS and FTIR spectra are identical with the compound **83e**. $[\alpha]^{20}_{365}$ = +46.0 (c = 0.336 g/100ml, H_2O).



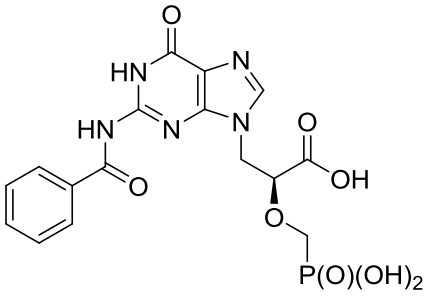
(*S*)-3-(2-Amino-6-oxo-*1H*-purin-9(*6H*)-yl)-2-(phosphonomethoxy)propanoic acid (83g)

From compound **85g** (209 mg, 0.5 mmol) according to **method D1**; afforded 102 mg (yield 61%) of compound **83g** as yellowish crystals, dec > 250 °C or according to **method D2**; afforded 62 mg (yield 37%) of compound **83g** as white solid, dec > 250 °C; ^{1}H NMR (D_2O) δ: 7.84 (s, 1H, H-8), 4.40 (dd, J_{gem} = 14.7 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, H-1'a), 4.32 (dd, J_{gem} = 14.7 Hz, $J_{1'b\text{-}2'}$ = 6.1 Hz, H-1'b), 4.17 (dd, $J_{2'\text{-}1'}$ = 6.1 Hz and 4.1 Hz, H-2'), 3.73 (dd, J_{gem} = 13.0 Hz, $J_{4'a\text{-}P}$ = 8.7 Hz, H-4'a), 3.51 (dd, J_{gem} = 13.0 Hz, $J_{4'b\text{-}P}$ = 9.8 Hz, H-4'b); ^{13}C NMR (D_2O) δ: 177.21 (C-3'), 159.57 (C-6), 154.24 (C-2), 152.37 (C-4), 141.38 (C-8), 116.08 (C-5), 81.73 (d, $J_{2'\text{-}P}$ = 12.5 Hz, C-2'), 66.85 (d, $J_{C\text{-}P}$ = 156.1 Hz, C-4'), 45.82 (C-1'); MS-ESI$^-$ *m*/*z* (%) 332 (100, M-H$^+$), 354 (50, M+Na$^+$), 376 (15, M+2Na$^+$); HRMS-ESI$^-$: *m*/*z* calcd for $C_9H_{11}O_7N_5P$ (M-H$^+$): 332.0402, found: 332.0404; FTIR (KBr, cm^{-1}) ν: 3405, 3128, 1693, 1607, 1540, 1481, 1409, 1108, 1069, 914. $[\alpha]^{20}_D$ = -20.2 (c = 0.316 g/100ml, H_2O).



(*S*)-3-(2,6-Diamino-*9H*-purin-9-yl)-2-(phosphonomethoxy)propanoic acid (83h)

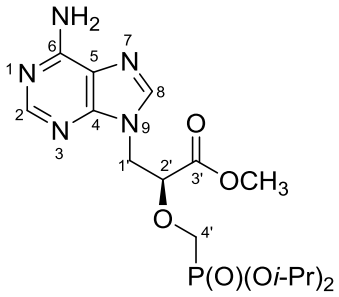
From compound **85k** (260 mg, 0.5 mmol) according to **method D2**; afforded 68 mg (yield 41%) of compound **83h** as white crystals, dec > 250 °C; ^{1}H NMR (D_2O + NaOD) δ: 7.94 (s, 1H, H-8), 4.33 – 4.39 (m, 2H, H-1'), 4.13 (t, $J_{2'\text{-}1'}$ = 5.1 Hz, 1H, H-2'), 3.58 (dd, J_{gem} = 12.1 Hz, $J_{4'a\text{-}P}$ = 8.9 Hz, 1H, H-4'a), 3.32 (dd, J_{gem} = 12.1 Hz, $J_{4'b\text{-}P}$ = 9.8 Hz, 1H, H-4'b); ^{13}C NMR (D_2O + NaOD) δ: 178.02 (C-3'), 160.60 (C-2), 456.66 (C-6), 151.92 (C-4), 141.68 (C-8), 113.20 (C-5), 81.90 (d, $J_{2'\text{-}P}$ = 12.7 Hz, C-2'), 68.82 (d, $J_{C\text{-}P}$ = 150.5 Hz, C-4'), 45.75 (C-1'); MS-ESI$^+$ *m*/*z* (%) 333 (100, M+H$^+$), 355 (25, M+Na$^+$), 377 (15, M+2Na$^+$); HRMS-ESI$^+$: *m*/*z* calcd for $C_9H_{14}O_6N_6P$ (M+H$^+$) 333.0707, found 333.0707; FTIR (KBr, cm^{-1}) ν: 3194, 2470, 1605, 1560, 1551, 1482, 1407, 1119, 1087, 916. $[\alpha]^{20}_D$ = -17.4 (c = 0.259 g/100ml, H_2O).



(*S*)-3-(2-Benzamido-6-oxo-*1H*-purin-9(*6H*)-yl)-2-(phosphonomethoxy)propanoic acid (83i)

From compound **85i** (261 mg, 0.5 mmol) according to **method D1**; afforded 114 mg (yield 52%) of compound **83i** as white crystals, dec > 250 °C; ^{1}H NMR (D_2O) δ: 8.11 (s, 1H, H-8), 7.97 (m, 2H, H-2‘‘), 7.62 (m, 1H, H-4‘‘), 7.53 (m, 2H, H-3‘‘), 4.45 – 4.52 (m, 2H, H-1‘), 4.16 (m, 1H, H-2‘), 3.59 (m, 1H, H-4’a), 3.40 (m, 1H, H-4’b); ^{13}C NMR (D_2O) δ: 177.97 (C-3‘), 172.77 (C=O), 162.48 (C-6), 153.12 (C-2), 151.42 (C-4), 142.76 (C-8), 135.04 (C-1‘‘), 133.13 (C-4‘), 129.28 (C-3‘), 128.54 (C-2‘), 119.73 (C-5), 81.83 (d, $J_{2‘\text{-}P}$ = 12.9 Hz, C-2‘), 68.76 (d, $J_{C\text{-}P}$ = 150.8 Hz, C-4’), 45.76 (C-1‘); MS-ESI$^+$ *m*/*z* (%) 438 (100, M+H$^+$), 460 (78, M+Na$^+$); HRMS-ESI$^-$: *m*/*z* calcd for $C_{16}H_{15}O_8N_5P$ (M-H$^+$) 436.0664, found 436.0664; FTIR (KBr, cm^{-1}) ν: 3408, 3135, 3033, 1665, 1604, 1402, 1269, 1154, 1061. $[\alpha]^{20}_D$ = -31.9 (c = 0.244 g/100ml, H_2O).

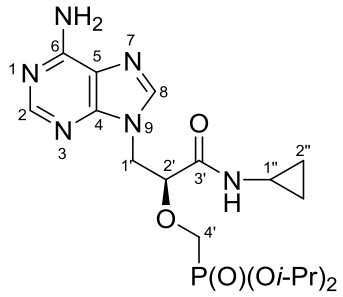
5.5. Synthesis of (*S*)-CPMEA prodrugs



Methyl (*S*)-3-(6-amino-*9H*-purin-9-yl)-2-[(diisopropoxyphosphoryl)methoxy]propanoate (92)

Compound **92** was prepared according to a modified procedure.[48] Compound **85a** (401 mg, 1 mmol) was dissolved in dry methanol (25 mL) and lithium hydroxide hydrate (42 mg, 1 mmol) was added. Solvent was evaporated and the residue was codistilled with DMF (2 x 20 mL). Then the residue was dissolved in dry DMF (25 mL) and methyl iodide (0.15 mL, 2.5 mmol) was added. The resulting solution was stirred at room temperature for 1 h. The reaction mixture was neutralized with 5 drops of glacial acetic acid and solvents were evaporated. A residue was codistilled with xylene (3 x 20 mL), absorbed on silica gel and purified by column chromatography (0-6% methanol in chloroform). Solvents were evaporated and the residue was crystalized from EtOAc-MeOH to give 145 mg (yield 35%) of compound **92** as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2), 7.99 (s, 1H, H-8), 7.21 (s, 2H, NH_2), 4.59 (dd, $J_{2‘\text{-}1’a}$ = 7.2 Hz, $J_{2‘\text{-}1’b}$ = 3.8 Hz, 1H, H-2‘), 4.53 (dd, J_{gem} = 14.6 Hz, $J_{1’b\text{-}2‘}$ = 3.8 Hz, 1H, H-1‘b), 4.41 – 4.53 (m,

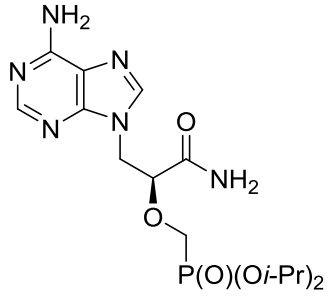
3H, H-1'a), **CH**-iPr), 3.91 (dd, J_{gem} = 13.8 Hz, $J_{4'b\text{-}P}$ = 8.9 Hz, 1H, H-4'b), 3.77 (dd, J_{gem} = 13.8 Hz, $J_{4'a\text{-}P}$ = 9.4 Hz, 1H, H-4'a), 3.67 (s, 3H, O-**CH$_3$**), 1.19 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-iPr), 1.16 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-iPr), 1.15 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-iPr), 1.10 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-iPr); ^{13}C NMR (DMSO-d$_6$) δ: 169.44 (C-3'), 156.11 (C-6), 152.60 (C-2), 149.71 (C-4), 141.34 (C-8), 118.52 (C-5), 77.83 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 12.8 Hz, C-2'), 70.55 (d, $J_{C\text{-}O\text{-}P}$ = 6.3 Hz, **CH**-iPr), 70.53 (d, $J_{C\text{-}O\text{-}P}$ = 6.3 Hz, **CH**-iPr), 64.10 (d, $J_{C\text{-}P}$ = 164.0 Hz, C-4'), 53.37 (O-**CH$_3$**), 44.25 (C-1'), 23.89 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 3.3 Hz, **CH$_3$**-iPr), 23.87 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 3.5 Hz, **CH$_3$**-iPr), 23.74 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 4.6 Hz, **CH$_3$**-iPr), 23.66 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 4.8 Hz, **CH$_3$**-iPr); MS-ESI$^+$ *m/z* (%): 416 (70, M+H$^+$), 438 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{27}O_6N_5P$ (M+H$^+$) 416.1694, found 416.1692; FTIR (KBr, cm^{-1}) ν: 3305, 3150, 2983, 2960, 1730, 1672, 1603, 1572, 1479, 1421, 1390, 1373, 1329, 1303, 1292, 1258, 1242, 1223, 1180, 1140, 1035, 1006, 995, 980. $[\alpha]^{20}_D$ = -35.7 (c = 0.281 g/100mL, DMSO).



Diisopropyl (*S*)-{[(3-[6-amino-*9H*-purin-9-yl]-1-(cyclopropylamino)-1-oxopropan-2-yl)oxy]methyl} phosphonate (93)

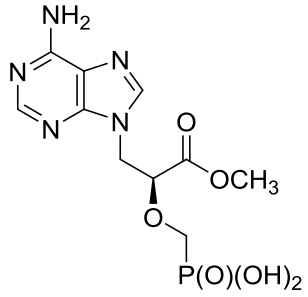
Compound **85a** (200 mg, 0.5 mmol), EDAC (192 mg, 1 mmol) and *N*-hydroxysuccinimide (115 mg, 1 mmol) were dissolved in dry DMF (10 mL), and cyclopropylamine (43 mg, 0.75 mmol) was added. The reaction mixture was stirred at room temperature for 2 days. Volatiles were evaporated, the residue was codistilled with toluene (3 x 30 mL) and purified by silica gel chromatography (2-6% methanol in chloroform) to afford (after evaporation) 165 mg (yield 75%) of compound **93** as a colorless hygroscopic oil which solidified; ^{1}H NMR (DMSO-d$_6$) δ: 8.13 (s, 1H, H-2), 8.07 (d, $J_{NH\text{-}1''}$ = 4.2 Hz, 1H, NH), 7.94 (s, 1H, H-8), 7.20 (bs, 2H, NH$_2$), 4.49 (dsept, $J_{CH\text{-}P}$ = 7.6 Hz, $J_{CH\text{-}CH3}$ = 6.2 Hz, 1H, **CH**-iPr), 4.45 (dsept, $J_{CH\text{-}P}$ = 7.6 Hz, $J_{CH\text{-}CH3}$ = 6.2 Hz, 1H, **CH**-iPr), 4.41 (dd, J_{gem} = 14.3 Hz, $J_{1'b\text{-}2'}$ = 4.3 Hz, 1H, H-1'b), 4.36 (dd, J_{gem} = 14.3 Hz, $J_{1'a\text{-}2'}$ = 7.1 Hz, 1H, H-1'a), 4.31 (dd, $J_{2'\text{-}1'a}$ = 7.1 Hz, $J_{2'\text{-}1'b}$ = 4.3 Hz, 1H, H-2'), 3.80 (dd, J_{gem} = 13.9 Hz, $J_{4'b\text{-}P}$ = 9.0 Hz, 1H, H-4'b), 3.64 (dd, J_{gem} = 13.9 Hz, $J_{4'a\text{-}P}$ = 8.3 Hz, 1H, H-4'a), 2.65 (m, 1H, H-1''), 1.19 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-iPr), 1.14 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 2x3H, **CH$_3$**-iPr), 1.10 (d, $J_{CH3\text{-}CH}$ = 6.2 Hz, 3H, **CH$_3$**-iPr), 0.62 (m, 2H, H-2''b), 0.39 (m, 2H, H-2''a); ^{13}C NMR (DMSO-d$_6$) δ: 169.25 (C-3'), 156.12 (C-6), 152.59 (C-2), 149.84 (C-4),

141.18 (C-8), 118.56 (C-5), 79.72 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 11.1 Hz, C-2‘), 70.71 (d, $J_{C\text{-}O\text{-}P}$ = 6.4 Hz, **C**H-iPr), 70.69 (d, $J_{C\text{-}O\text{-}P}$ = 6.4 Hz, **C**H-iPr), 64.05 (d, $J_{C\text{-}P}$ = 164.4 Hz, C-4‘), 44.37 (C-1‘), 23.90 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 3.8 Hz, **C**$\mathbf{H_3}$-iPr), 23.87 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 3.8 Hz, **C**$\mathbf{H_3}$-iPr), 23.77 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 4.7 Hz, **C**$\mathbf{H_3}$-iPr), 23.69 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 4.7 Hz, **C**$\mathbf{H_3}$-iPr), 22.39 (C-1‘‘), 5.80 (C-2‘‘); MS-ESI$^+$ *m/z* (%): 441 (100, M+H$^+$), 463 (30, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{18}H_{30}O_5N_6P$ (M+H$^+$) 441.2010, found 441.2011; FTIR (KBr, cm^{-1}) ν: 3404, 3321, 3191, 3260, 2981, 2932, 1710, 1671, 1645, 1598, 1418, 1387, 1244, 1178, 1105, 1012, 993. $[\alpha]^{20}_D$ = -27.6 (c = 0.348 g/100mL, DMSO).



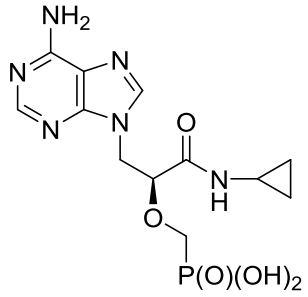
Diisopropyl (*S*)-{[(1-amino-3-[6-amino-*9H*-purin-9-yl]-1-oxopropan-2-yl)oxy]methyl}phosphonate (94)

Compound **92** (208 mg, 0.5 mmol) was dissolved in solution of 3.5 M ammonia in ethanol (5 mL). The mixture was microwave irradiated at 120 °C for 1 h. Volatiles were evaporated, the residue was purified by silica gel chromatography (1-10% methanol in chloroform) to afford (after evaporation) 166 mg (yield 83%) of compound **94** as a yellowish solid; ^{1}H NMR (DMSO-d$_6$) δ: 8.13 (s, 1H, H-2), 7.97 (s, 1H, H-8), 7.55 (d, J_{gem} = 1.7 Hz, 1H, 3‘-NH$_2$a), 7.38 (d, J_{gem} = 1.6 Hz, 1H, 3‘-NH$_2$b), 7.18 (s, 2H, 6-NH$_2$), 4.32 – 4.54 (m, 5H, H-2‘, H-4‘,**C**H-iPr), 3.86 (dd, J_{gem} = 14.0 Hz, $J_{1'b\text{-}2'}$ = 9.1 Hz, 1H, H-1‘b), 3.70 (dd, J_{gem} = 14.0 Hz, $J_{1'a\text{-}2'}$ = 8.2 Hz, 1H, H-1‘a), 1.09 – 1.19 (m, 12H, **C**$\mathbf{H_3}$-iPr); ^{13}C NMR (DMSO-d$_6$) δ: 170.47 (C-3‘), 156.10 (C-6), 152.57 (C-2), 149.87 (C-4), 141.13 (C-8), 118.55 (C-5), 79.92 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 11.0 Hz, C-2‘), 70.64 (m, **C**H-iPr), 64.27 (d, $J_{C\text{-}P}$ = 164.7 Hz, C-4‘), 44.26 (C-1‘), 23.64 – 23.89 (m, **C**$\mathbf{H_3}$-iPr); MS-ESI$^+$ *m/z* (%): 401 (30, M+H$^+$), 423 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{26}O_5N_6P$ (M+H$^+$) 401.1697, found 401.1698; FTIR (KBr, cm^{-1}) ν: 3374, 3324, 3264, 3193, 2979, 2929, 1687, 1649, 1603, 1577, 1481, 1469, 1420, 1387, 1330, 1304, 1245, 1234, 1106, 1029, 997. $[\alpha]^{20}_D$ = -4.9 (c = 0.286 g/100mL, MeOH).



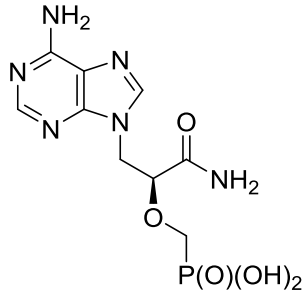
(*S*)-{[(3-(6-Amino-*9H*-purin-9-yl)-1-methoxy-1-oxopropan-2-yl)oxy]methyl}phosphonic acid (95)

From compound **92** (208 mg, 0.5 mmol) according to **method D1**; afforded 113 mg (yield 68%) of compound **95** as a white solid; ^{1}H NMR (D_2O) δ: 8.42 (s, 1H, H-2), 8.41 (s, 1H, H-8), 4.80 (dd, J_{gem} = 15.0 Hz, $J_{1'b\text{-}2'}$ = 3.9 Hz, 1H, H-1'b), 4.73 (dd, J_{gem} = 15.0 Hz, $J_{1'a\text{-}2'}$ = 5.0 Hz, 1H, H-1'a), 4.66 (dd, $J_{2'\text{-}1'a}$ = 5.0 Hz, $J_{2'\text{-}1'b}$ = 3.9 Hz, 1H, H-2'), 3.85 (dd, J_{gem} = 13.2 Hz, $J_{4'b\text{-}P}$ = 8.8 Hz, 1H, H-4'b), 3.75 (s, 3H, O-**C**$\mathbf{H_3}$), 3.64 (dd, J_{gem} = 13.2 Hz, $J_{4'a\text{-}P}$ = 9.3 Hz, 1H, H-4'a); ^{13}C NMR (D_2O) δ: 172.35 (C-3'), 150.64 (C-6), 149.13 (C-4), 146.25 (C-8), 145.31 (C-2), 118.40 (C-5), 78.39 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 11.6 Hz, C-2'), 67.34 (d, $J_{C\text{-}P}$ = 156.5 Hz, C-4'), 53.70 (O-**C**$\mathbf{H_3}$), 46.00 (C-1'); MS-ESI$^+$ *m/z* (%): 332 (100, M+H$^+$), 354 (30, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{15}O_6N_5P$ (M+H$^+$) 332.0755, found 332.0754; FTIR (KBr, cm^{-1}) ν: 3358, 3308, 1736, 1601, 1577, 1515, 1329, 1301, 1242, 1220, 1034, 931. $[\alpha]^{20}_D$ = -10.1 (c = 0.278 g/100mL, H_2O).



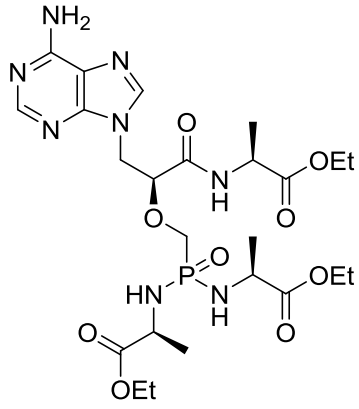
(*S*)-{[(3-(6-Amino-*9H*-purin-9-yl)-1-(cyclopropylamino)-1-oxopropan-2-yl)oxy]methyl}phosphonic acid (96)

From compound **93** (220 mg, 0.5 mmol) according to **method D1**; afforded 126 mg (yield 71%) of compound **96** as a yellowish solid; ^{1}H NMR (D_2O) δ: 8.21 (s, 1H, H-2), 8.18 (s, 1H, H-8), 4.56 – 4.67 (m, 2H, H-1'), 4.34 (m, 1H, H-2'), 3.72 (d, $J_{4'\text{-}P}$ = 9.4 Hz, 2H, H-4'), 2.37 (m, 1H, H-1''), 0.58 (m, 1H, H-2''b), 0.54 (m, 1H, H-2''b), 0.20 (m, 1H, H-2''a), -0.12 (m, 1H, H-2''a); ^{13}C NMR (D_2O) δ: 173.22 (C-3'), 154.96 (C-6), 151.56 (C-2), 149.51 (C-4), 144.23 (C-8), 118.28 (C-5), 80.59 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 12.4 Hz, C-2'), 67.38 (d, $J_{C\text{-}P}$ = 156.6 Hz, C-4'), 45.33 (C-1'), 22.10 (C-1''), 5.71 (C-2''), 5.24 (C-2''); MS-ESI$^+$ *m/z* (%): 357 (100, M+H$^+$), 379 (50, M+Na$^+$), 401 (15, 2M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{12}H_{18}O_5N_6P$ (M+H$^+$) 357.1071, found 357.1072; FTIR (KBr, cm^{-1}) ν: 3396, 3184, 1706, 1654, 1606, 1578, 1542, 1519, 1418, 1331, 1303, 1209, 1182, 1158, 1120, 1063, 1028, 921. $[\alpha]^{20}_D$ = -15.3 (c = 0.297 g/100mL, H_2O).



(*S*)-{[(1-Amino-3-(6-amino-*9H*-purin-9-yl)-1-oxopropan-2-yl)oxy]methyl}phosphonic acid (97)

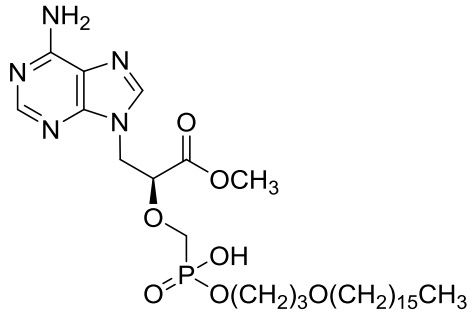
From compound **94** (200 mg, 0.5 mmol) according to **method D1**; afforded 120 mg (yield 76%) of compound **97** as a white solid; ^{1}H NMR (D_2O) δ: 8.183 (s, 1H, H-8), 8.178 (s, 1H, H-2), 4.62 (dd, J_{gem} = 14.9 Hz, $J_{1'b\text{-}2'}$ = 4.2 Hz, 1H, H-1'b), 4.59 (dd, J_{gem} = 14.9 Hz, $J_{1'a\text{-}2'}$ = 5.0 Hz, 1H, H-1'a), 4.36 (bt, $J_{2'\text{-}1'a}$ = $J_{2'\text{-}1'b}$ = 4.6 Hz, H-2'), 3.74 (dd, J_{gem} = 12.9 Hz, $J_{4'b\text{-}P}$ = 9.4 Hz, 1H, H-4'b), 3.68 (dd, J_{gem} = 12.9 Hz, $J_{4'a\text{-}P}$ = 9.4 Hz, 1H, H-4'a); ^{13}C NMR (D_2O) δ: 174.88 (C-3'), 155.66 (C-6), 152.55 (C-2), 149.56 (C-4), 144.03 (C-8), 118.45 (C-5), 80.96 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 12.5 Hz, C-2'), 67.74 (d, $J_{C\text{-}P}$ = 156.3 Hz, C-4'), 45.34 (C-1'); MS-ESI$^+$ *m/z* (%): 317 (100, M+H$^+$), 339 (15, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_9H_{14}O_5N_6P$ (M+H$^+$) 317.0758, found 317.0759; FTIR (KBr, cm^{-1}) ν: 3437, 3333, 1674, 1644, 1608, 1576, 1490, 1419, 1326, 1165, 1119, 1031, 938. $[\alpha]^{20}_{D}$ = -9.6 (c = 0.219 g/100mL, H_2O).



Diethyl 2,2'-{[({[(*S*)-3-(6-amino-*9H*-purin-9-yl)-1-{[(*S*)-1-ethoxy-1-oxopropan-2-yl]amino}-1-oxopropan-2-yl]oxy}methyl)phosphoryl]bis(azanediyl)}(*2S,2S'*)-dipropionate (98)

From compound **85a** (201 mg, 0.5 mmol) according to **method F**; afforded 188 mg (yield 61%) of compound **98** as a colorless oil which solidified; ^{1}H NMR (DMSO-d_6) δ: 8.46 (bd, $J_{NH\text{-}CH}$ = 7.4 Hz, 1H, 3'-NH), 8.13 (s, 1H, H-2), 8.01 (s, 1H, H-8), 7.20 (bs, 2H, NH_2), 4.78 (dd, $J_{NH\text{-}P}$ = 12.8 Hz, $J_{NH\text{-}CH}$ = 10.4 Hz, 1H, P-NH), 4.63 (dd, $J_{NH\text{-}P}$ = 12.0 Hz, $J_{NH\text{-}CH}$ = 10.6 Hz, 1H, P-NH), 4.51 (m, 1H, H-1'b), 4.35 – 4.40 (m, 2H, H-1'a, H-2'), 4.26 (p, $J_{CH\text{-}NH}$ = $J_{CH\text{-}CH3}$ = 7.3 Hz, 1H, 3'-NH-**CH**), 3.98 – 4.11 (m, 6H, O-**CH$_2$**-CH$_3$), 3.83 (m, 2H, P-NH-**CH**), 3.63 – 3.71 (m, 2H, H-4'), 1.14 – 1.24 (m, 18H, O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d_6) δ: 174.13 – 174.28 (m, C=O), 172.19 (C=O), 168.82 (C-3'), 156.06 (C-6), 152.52 (C-2), 149.97 (C-4), 141.44 (C-8), 118.50 (C-5), 80.04 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 10.1 Hz, C-2'), 67.38 (d, $J_{C\text{-}P}$ = 133.4 Hz, C-4'), 60.83 (O-**CH$_2$**-CH$_3$), 60.67 (O-**CH$_2$**-CH$_3$), 60.62 (O-**CH$_2$**-CH$_3$), 48.31 (NH-**CH**), 48.17 (NH-**CH**), 47.71 (NH-**CH**), 44.22 (C-1'), 20.62 – 20.78 (m, P-NH-CH-**CH$_3$**), 16.71 (3'-NH-CH-**CH$_3$**), 14.16 – 14.19

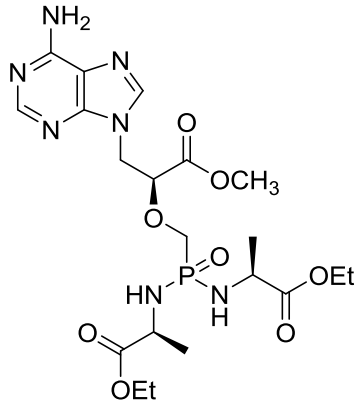
(O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 615 (100, M+H^+), 637 (50, M+Na^+); HRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{40}N_8O_9P$ (M+H^+) 615.2657, found 615.2658; FTIR (KBr, cm^{-1}) ν: 3426, 2981, 2940, 1741, 1598, 1574, 1477, 1436, 1419, 1369, 1325, 1244, 1201, 1155, 1051.



Methyl (*2S*)-3-(6-amino-*9H*-purin-9-yl)-2-{[(3-hexadecyloxy)propoxy)(hydroxy) phosphoryl]methoxy}propanoate (99)

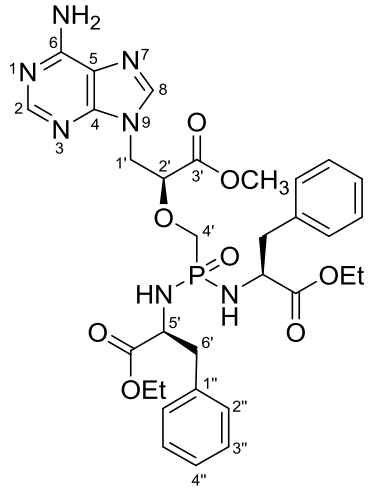
Compound **92** (104 mg, 0.25 mmol) was codistilled with dry acetonitrile (3 x 10 mL), dissolved in acetonitrile and TMSBr (0.5 mL) was added. The resulting solution was stirred at room temperature overnight. Volatiles were evaporated, and the residue was codistilled with dry acetonitrile (3 x 10 mL) and water (1 x 5 mL), and then dried in a high *vacuo* overnight. The residue was dissolved in dry pyridine (10 mL) under argon, 3-(hexadecyloxy)propan-1-ol (90 mg, 0.3 mmol) and dicyclohexylcarbodiimide (103 mg, 0.5 mmol) were added. The reaction mixture was heated at 60 °C for 30 h. Solvente were evaporated, the residue was codistilled with toluene (3 x 20 mL) and subjected to silica gel chromatography (10-60% methanol in chloroform) to afford (after evaporation) 102 mg (yield 66%) of compound **99** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 8.14 (bs, 1H, H-8), 8.10 (s, 1H, H-2), 7.17 (bs, 2H, NH_2), 4.69 (bs, 1H, H-2‘), 4.51 (bdd, J_{gem} = 13.5 Hz, $J_{1'b\text{-}2'}$ = 4.3 Hz, 1H, H-1‘b), 4.43 (m, 1H, H-1’a), 3.63 (bs, 2H, P-O-**CH_2**-CH_2-CH_2-O), 3.56 (s, 3H, O-**CH_3**), 3.55 (vbm, 1H, H-4’b), 3.32 (vbm, 1H, H-4’a), 3.31 (t, $J_{CH2\text{-}CH2}$ = 6.6 Hz, 2H, P-O-CH_2-CH_2-**CH_2**-O), 3.27 (t, $J_{CH2\text{-}CH2}$ = 6.4 Hz, 2H, O-**CH_2**-$(CH_2)_{14}$-CH_3), 1.62 (m, 2H, P-O-CH_2-**CH_2**-CH_2-O), 1.46 (m, 2H, O-CH_2-(**CH_2**)$_{14}$-CH_3), 1.17 – 1.29 (m, 26H, O-CH_2-(**CH_2**)$_{14}$-CH_3), 0.84 (t, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 3H, O-CH_2-$(CH_2)_{14}$-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 170.40 (C-3‘), 156.05 (C-6), 152.50 (C-2), 149.74 (C-4), 141.80 (C-8), 118.34 (C-5), 76.96 (C-2‘), 70.23 (O-**CH_2**-$(CH_2)_{14}$-CH_3), 67.35 (P-O-CH_2-CH_2-**CH_2**-O), 66.60 (C-4‘), 60.80 (P-O-**CH_2**-CH_2-CH_2-O), 51.96 (O-**CH_3**), 44.43 (C-1‘), 31.49 (O-CH_2-(**CH_2**)$_{14}$-CH_3), 29.49 (O-CH_2-(**CH_2**)$_{14}$-CH_3), 29.24 (7x) (O-CH_2-(**CH_2**)$_{14}$-CH_3), 29.20 (O-CH_2-(**CH_2**)$_{14}$-CH_3), 29.15 (O-CH_2-(**CH_2**)$_{14}$-CH_3), 28.90 (O-CH_2-(**CH_2**)$_{14}$-CH_3), 25.92 (O-CH_2-(**CH_2**)$_{14}$-CH_3), 22.29 (O-CH_2-(**CH_2**)$_{14}$-CH_3), 14.14 (O-CH_2-$(CH_2)_{14}$-**CH_3**); MS-ESI$^+$ *m/z* (%): 614 (95, M+H^+), 636 (100, M+Na^+), 658 (40, 2M+Na^+); HRMS-ESI$^+$: *m/z* calcd for $C_{29}H_{53}O_7N_5P$ (M+H^+)

614.3677, found 614.3679; FTIR (KBr, cm^{-1}) ν: 3325, 3171, 2923, 2852, 1749, 1598, 1574, 1469, 1439, 1418, 1364, 1328, 1297, 1240, 1215, 1169, 1082, 924, 883, 828, 799, 720.



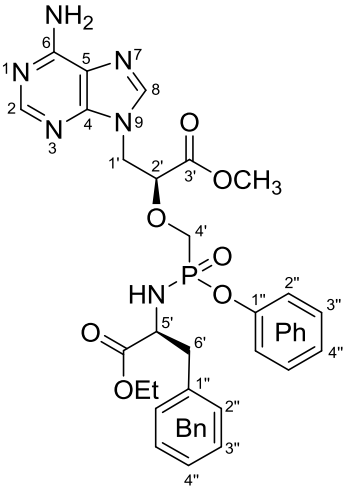
Diethyl 2,2'-{[({[(*S*)-3-(6-amino-*9H*-purin-9-yl)-1-methoxy-1-oxopropan-2-yl]oxy}methyl)phosphoryl] bis(azanediyl)}(*2S,2S'*)-dipropionate (100)

From compound **92** (208 mg, 0.5 mmol) according to **method F**; afforded 223 mg (yield 84%) of compound **100** as a white foam; ^{1}H NMR (DMSO-d_6) δ: 8.12 (s, 1H, H-2), 8.07 (s, 1H, H-8), 7.19 (s, 2H, NH_2), 4.60 (dd, J_{NH-P} = 12.3 Hz, J_{NH-CH} = 10.3 Hz, 1H, P-NH), 4.58 (dd, $J_{2'-1'a}$ = 5.8 Hz, $J_{2'-1'b}$ = 4.0 Hz, 1H, H-2'), 4.54 (dd, J_{gem} = 14.4 Hz, $J_{1'b-2'}$ = 4.0 Hz, 1H, H-1'b), 4.52 (dd, J_{NH-P} = 11.6 Hz, J_{NH-CH} = 10.3 Hz, 1H, P-NH), 4.46 (dd, J_{gem} = 14.4 Hz, $J_{1'a-2'}$ = 5.9 Hz, 1H, H-1'a), 4.00 – 4.13 (m, 4H, O-**CH_2**-CH_3), 3.78 – 3.89 (m, 2H, P-NH-**CH**), 3.76 (dd, J_{gem} = 13.4 Hz, $J_{4'b-P}$ = 7.2 Hz, 1H, H-4'b), 3.63 (s, 3H, O-**CH_3**), 3.59 (dd, J_{gem} = 13.4 Hz, $J_{4'a-P}$ = 8.7 Hz, 1H, H-4'a), 1.24 (d, J_{CH3-CH} = 7.2 Hz, 3H, P-NH-CH-**CH_3**), 1.23 (d, J_{CH3-CH} = 7.2 Hz, 3H, P-NH-CH-**CH_3**), 1.19 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**), 1.17 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 174.20 (d, J_{C-P} = 3.8 Hz, C=O), 174.12 (d, J_{C-P} = 5.5 Hz, C=O), 169.88 (C-3'), 156.10 (C-6), 152.59 (C-2), 149.78 (C-4), 141.58 (C-8), 118.44 (C-5), 77.90 (d, $J_{C-O-C-P}$ = 10.5 Hz, C-2'), 66.98 (d, J_{C-P} = 133.0 Hz, C-4'), 60.59 (O-**CH_2**-CH_3), 60.57 (O-**CH_2**-CH_3), 52.26 (O-**CH_3**), 48.25 (P-NH-**CH**), 48.19 (P-NH-**CH**), 44.46 (C-1'), 20.88 (d, J_{C-P} = 4.6 Hz, P-NH-CH-**CH_3**), 20.65 (d, J_{C-P} = 5.7 Hz, O-CH-**CH_3**), 14.17 (O-CH_2-**CH_3**), 14.16 (O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 530 (70, M+H$^+$), 552 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{20}H_{33}O_8N_7P$ (M+H$^+$) 530.2123, found 530.2124; FTIR (KBr, cm^{-1}) ν: 3424, 2982, 2938, 1738, 1600, 1577, 1476, 1439, 1418, 1375, 1328, 1245, 1207, 1148, 1062.



Diethyl 2,2'-{[({[(*S*)-3-(6-amino-*9H*-purin-9-yl)-1-methoxy-1-oxopropan-2-yl]oxy}methyl)phosphoryl]bis(azanediyl)}(*2S*,*2S'*)-bis(3-phenyl propanoate) (101)

From compound **92** (208 mg, 0.5 mmol) according to **method L** (chromatography 0-8% methanol in chloroform); afforded 201 mg (yield 59%) of compound **101** as a yellowish foam; ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, H-2), 8.01 (s, 1H, H-8), 7.15 – 7.28 (m, 10H, H-2‘‘, H-3‘‘, H-4‘‘, NH_2), 7.08 – 7.11 (m, 2H, H-2‘‘), 4.57 (dd, J_{NH-P} = 11.7 Hz, J_{NH-CH} = 10.9 Hz, 1H, P-NH), 4.39 – 4.50 (m, 3H, H-1‘, H-2‘), 4.32 (dd, J_{NH-P} = 12.6 Hz, J_{NH-CH} = 10.8 Hz, 1H, P-NH), 3.94 – 4.07 (m, 5H, H-5‘, O-**C**$\mathbf{H_2}$-CH_3), 3.90 (m, 1H, H-5‘), 3.59 (s, 3H, O-**C**$\mathbf{H_3}$), 3.44 (dd, J_{gem} = 13.3 Hz, $J_{4'b-P}$ = 7.6 Hz, 1H, H-4‘b), 3.22 (dd, J_{gem} = 13.3 Hz, $J_{4'a-P}$ = 8.6 Hz, 1H, H-4‘a), 2.91 (dd, J_{gem} = 13.4 Hz, $J_{6'-5'}$ = 6.7 Hz, 1H, H-6‘), 2.86 (dd, J_{gem} = 13.4 Hz, $J_{6'-5'}$ = 7.4 Hz, 1H, H-6‘), 2.82 (dd, J_{gem} = 13.4 Hz, $J_{6'-5'}$ = 6.6 Hz, 1H, H-6‘), 2.77 (dd, J_{gem} = 13.4 Hz, $J_{6'-5'}$ = 6.9 Hz, 1H, H-6‘), 1.10 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$), 1.05 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 172.98 (d, J_{C-P} = 2.8 Hz, C=O), 172.72 (d, J_{C-P} = 5.3 Hz, C=O), 169.74 (C-3‘), 156.12 (C-6), 152.64 (C-2), 149.78 (C-4), 141.60 (C-8), 137.35 (C-1‘‘), 137.15 (C-1‘‘), 129.58 (C-2‘‘), 129.57 (C-2‘‘), 128.29 (C-3‘‘), 128.24 (C-3‘‘), 126.65 (C-4‘‘), 126.59 (C-4‘‘), 118.44 (C-5), 77.93 (d, $J_{C-O-C-P}$ = 10.2 Hz, C-2‘), 66.91 (d, J_{C-P} = 134.0 Hz, C-4‘), 60.58 (O-**C**$\mathbf{H_2}$-CH_3), 60.54 (O-**C**$\mathbf{H_2}$-CH_3), 54.06 (C-5‘), 52.23 (O-**C**$\mathbf{H_3}$), 44.39 (C-1‘), 41.10 (C-6‘), 41.00 (C-6‘), 15.02 (O-CH_2-**C**$\mathbf{H_3}$), 14.96 (O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 682 (10, M+H$^+$), 704 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{32}H_{40}O_8N_7NaP$ (M+Na$^+$) 704.2568, found 704.2564; FTIR (KBr, cm^{-1}) ν: 3377, 3177, 1737, 1641, 1599, 1577, 1475, 1438, 1418, 1369, 1328, 1201, 1079, 1030, 974, 744, 701.



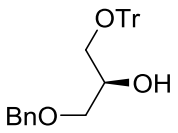
Ethyl {[([(*S*)-3-(6-amino-*9H*-purin-9-yl)-1-methoxy-1-oxopropan-2-yl]oxy)methyl](phenoxy)phosphoryl}-L-phenylalaninate (102)

A mixture of compound **92** (104 mg, 0.25 mmol), dry acetonitrile (10 mL), and TMSBr (0.25 mL) was stirred overnight at room temperature under argon. After evaporation (without any contact with air) *in vacuo* (40 °C, 2 mbar) and codistillation with dry acetonitrile (2 x 10 mL) (without any contact with air), the flask was securated with argon and L-phenylalanine ethyl ester hydrochloride (0.5 mmol), phenol (1.25 mmol), dry triethylamine (1 mL), and dry pyridine (4 mL) were added. The mixture was heated at 60 °C for 7 min. A solution of Aldrithiol-2 (2 mmol) and triphenylphosphine (2 mmol) in 4 mL of dry pyridine was added under argon. The resulting mixture was heated at 65 °C for 5 hours to reach the full conversion. After cooling, the dark yellow solution was evaporated (40 °C, 2 mbar), and the residue was purified by silica gel chromatography (0-5% methanol in chloroform) to afford (after evaporation) the crude product which was purified by flash chromatography on C_{18}-reverse phase silica gel (0-100% methanol in water) to give (after evaporation and codistillation with dry acetone) 105 mg (yield 72%) of compound **102** as a white foam; ^{1}H NMR (DMSO-d_6) δ: (2 diastereomers cca 1:0.9) 8.13 (s, 1H, H-2), 8.11 (s, 1H, H-2), 8.01 (s, 1H, H-8), 8.00 (s, 1H, H-8), 7.08 – 7.30 (m, 20H, Bn-H-2‘‘, Bn-H-3‘‘, Bn-H-4‘‘, Ph-H-3‘‘, Ph-H-4‘‘, NH_2), 6.97 (m, 2H, Ph-H-2‘‘), 6.89 (m, 2H, Ph-H-2‘‘), 5.77 (dd, $J_{NH\text{-}P}$ = 13.0 Hz, $J_{NH\text{-}CH}$ = 10.8 Hz, 1H, P-NH), 5.68 (dd, $J_{NH\text{-}P}$ = 11.8 Hz, $J_{NH\text{-}CH}$ = 10.4 Hz, 1H, P-NH), 4.37 – 4.57 (m, 6H, H-1‘, H-2‘), 3.89 – 4.09 (m, 6H, H-5‘, O-**C**$\mathbf{H_2}$-CH_3), 3.83 (dd, J_{gem} = 13.6 Hz, $J_{4'b\text{-}P}$ = 7.0 Hz, 1H, H-4‘b), 3.71 (dd, J_{gem} = 13.9 Hz, $J_{4'b\text{-}P}$ = 7.4 Hz, 1H, H-4‘b), 3.67 (dd, J_{gem} = 13.6 Hz, $J_{4'a\text{-}P}$ = 9.4 Hz, 1H, H-4‘a), 3.65 (s, 3H, O-**C**$\mathbf{H_3}$), 3.63 (s, 3H, O-**C**$\mathbf{H_3}$), 3.23 (dd, J_{gem} = 13.9 Hz, $J_{4'a\text{-}P}$ = 7.8 Hz, 1H, H-4‘a), 2.89 – 2.95 (m, 2H, H-6’b), 2.70 – 2.78 (m, 2H, H-6’a), 1.05 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$), 1.03 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 172.65 (d, $J_{C\text{-}P}$ = 2.7 Hz, C=O), 172.37 (d, $J_{C\text{-}P}$ = 2.8 Hz, C=O), 169.49 (C-3‘), 169.39 (C-3‘), 156.14 (C-6), 156.13 (C-6), 152.67 (C-2), 152.65 (C-2), 150.14 (d, $J_{C\text{-}P}$ = 9.4 Hz, Ph-C-1‘‘), 150.07 (d, $J_{C\text{-}P}$ = 9.4 Hz, Ph-C-1‘‘), 149.73 (C-4), 149.70 (C-4), 141.51 (C-8), 141.37 (C-8), 137.20 (Bn-C-1‘‘), 137.12 (Bn-C-1‘‘), 129.59 (Bn-C-2‘‘), 129.59 (Ph-

C-3‘‘), 129.51 (Bn-C-2‘‘), 129.51 (Ph-C-3‘‘), 128.35 (Bn-C-3‘‘), 128.34 (Bn-C-3‘‘), 126.72 (Bn-C-4‘‘), 126.71 (Bn-C-4‘‘), 124.57 (Ph-C-4‘‘), 124.53 (Ph-C-4‘‘), 120.81 (d, $J_{C\text{-}P}$ = 4.4 Hz, Ph-C-2‘‘), 120.72 (d, $J_{C\text{-}P}$ = 4.4 Hz, Ph-C-2‘‘), 118.48 (C-5), 118.45 (C-5), 78.19 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 12.2 Hz, C-2‘), 78.88 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 10.4 Hz, C-2‘), 65.76 (d, $J_{C\text{-}P}$ = 153.8 Hz, C-4‘), 65.62 (d, $J_{C\text{-}P}$ = 154.3 Hz, C-4‘), 60.76 (O-$\mathbf{CH_2}$-CH_3), 60.63 (O-$\mathbf{CH_2}$-CH_3), 55.25 (C-5‘), 55.22 (C-5‘), 52.36 (O-$\mathbf{CH_3}$), 52.34 (O-$\mathbf{CH_3}$), 44.41 (C-1‘), 44.37 (C-1‘), 39.60 (C-6‘), 14.04 (O-CH_2-$\mathbf{CH_3}$), 14.03 (O-CH_2-$\mathbf{CH_3}$); MS-ESI$^+$ *m/z* (%): 583 (25, M+H$^+$), 605 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{27}H_{32}O_7N_6P$ (M+H$^+$) 583.2065, found 583.2066; FTIR (KBr, cm^{-1}) ν: 3329, 3257, 3183, 2982, 1739, 1641, 1597, 1575, 1491, 1477, 1437, 1328, 1298, 1240, 1205, 1166, 1122, 1079, 1027, 799, 692, 649.

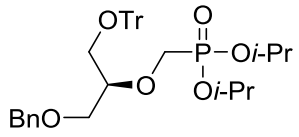
5.6. Synthesis of (*S*)-CPME, (*S*)-HPEP and (*S*)-CPEE compounds using synthon approach

5.6.1. Synthesis of (*S*)-CPMEHx and (*S*)-CPMEG



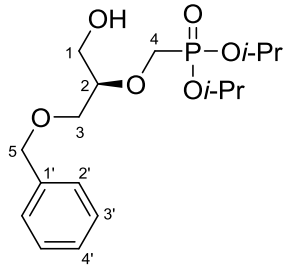
(*S*)-1-(Benzyloxy)-3-trityloxypropan-2-ol (104)

Compound **104** was prepared according to the modified procedure.[150] NaH (60% suspension in mineral oil, 5.8 g, 144 mmol) was suspended in anhydrous DMF (120 mL) and benzyl alcohol (15.6 g, 144 mmol) was added dropwise. The reaction mixture was stirred at r.t. for 20 min and then trityl glycidol **103** (38.0 g, 120 mmol) in DMF (100 mL) was added. The solution was stirred at 100 °C for 2 h. After cooling down, 100 mL of water was added and extracted with EtOAc (3 x 400 mL). The combined organics were washed with brine (2 x 200 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a crude oil. This was subjected to silica gel chromatography (gradient from 5-20% ethylacetate in *iso*-hexanes with 0.1% Et_3N) to afford (after evaporation) 49.4 g, yield 97%) of product **104** as a yellowish oil.



Diisopropyl (*S*)-{[(1-(benzyloxy)-3-(trityloxy)propan-2-yl)oxy]methyl}phosphonate (105)

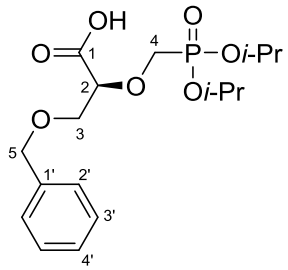
This compound was prepared according to the modified procedure.[146] Compound **104** (42.5 g, 100 mmol) was dissolved in anhydrous DMF (300 mL) and NaH (60% suspension in mineral oil, 6 g, 150 mmol) was added. The reaction mixture was stirred at r.t. for 20 min and then diisopropyl (bromomethyl)phosphonate (38 mL, 150 mmol) was added. The reaction mixture was stirred at r.t. for 24 h. The reaction was diluted with water (150 mL) and extracted with ethyl acetate (3x 500 mL). The combined organic layers were washed with water (2 x 200 mL), brine (2 x 200 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give the yellowish oil (78 g of the crude product, theoretical yield 60.3 g). The crude product was used in the next reaction step (iii) without any further purification.



Diisopropyl (*R*)-{[(1-(benzyloxy)-3-hydroxypropan-2-yl) oxy]methyl}phosphonate (106)

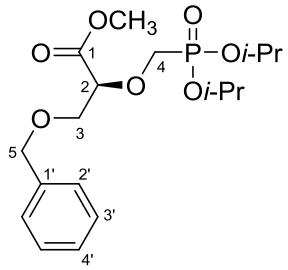
Compound **105** (78 g of the crude product) was dissolved in 80% acetic acid (400 mL) and heated to reflux for 2 h. After cooling down, the reaction mixture was diluted with 300 mL of water/dioxane (1:1) and white crystals of trityl alcohol were filtered off. The solution was extracted with ethyl acetate (3 x 400 mL), the combined organics were washed with water (2 x 150 mL), brine (2 x 150 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give a crude oil. This was subjected to silica gel chromatography (gradient from 20-100% ethyl acetate in *iso*-hexanes) to afford (after evaporation) 14.5 g (yield 40%) of compound **106** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 7.19 – 7.28 (m, 5H, H-2‘, H-3‘, H-4‘), 4.63 – 4.73 (m, 2H, **CH**-ipr), 4.46 (s, 2H, H-5), 3.97 (dd, J_{gem} = 14.1 Hz, $J_{4a\text{-}P}$ = 7.5 Hz, 1H, H-4a), 3.76 (dd, J_{gem} = 14.1 Hz, $J_{4b\text{-}P}$ = 8.4 Hz, 1H, H-4b), 3.65 (dd, J_{gem} = 12.8 Hz, $J_{1a\text{-}2}$ = 3.3 Hz, 1H, H-1a), 3.65 (m, 1H, H-2), 3.56 (dd, J_{gem} = 12.8 Hz, $J_{1b\text{-}2}$ = 6.9 Hz, 1H, H-1b), 3.46 – 3.52 (m, 2H, H-3), 3.43 (bs, 1H, OH), 1.24 – 1.27 (m, 12H, **CH$_3$**-ipr); ^{13}C NMR ($CDCl_3$) δ: 137.85 (C-1‘), 128,30 (C-3‘), 127.60 (C-4‘), 127.50 (C-2‘), 82.46 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 8.4 Hz, C-2), 73.36 (C-5), 71.45 (d, $J_{C\text{-}O\text{-}P}$ = 6.6 Hz, **CH**-ipr), 71.11 ((d, $J_{C\text{-}O\text{-}P}$ = 6.8 Hz, **CH**-ipr), 70.17 (C-3), 65.16 (d, $J_{C\text{-}P}$ = 168.0 Hz, C-4), 62.29 (C-1); MS-ESI$^+$ *m/z* (%): 361 (15, M+H$^+$), 383 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for

$C_{17}H_{29}O_6NaP$ ($M+Na^+$) 383.1594, found 383.1592. $[\alpha]^{20}_D$ = -13.7 (c = 0.270 g/100mL, $CHCl_3$).



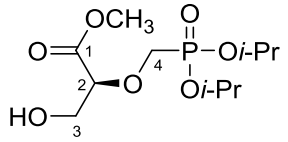
(*S*)-3-(Benzyloxy)-2-[(diisopropoxyphosphoryl)methoxy] propanoic acid (107)

The compound **107** was prepared according to the modified procedure.[92] To a solution of compound **106** (14.4 g, 40 mmol), TEMPO (625 mg, 4 mmol), sodium chlorite (80%) (9.0 g, 80 mmol), acetonitrile (200 mL) and 0.67 M sodium phosphate buffer (160 mL, pH 6.7) was added dropwise a solution of dilute sodium hypochlorite (40 mL of household bleach SAVO (40.72 mg/mL) with 80 mL of water). The reaction mixture was heated at 40 °C for 48 h. After cooling the reaction to r.t., water (300 mL) was added and the pH was adjusted to 8.0 by addition 48 mL of 2 M NaOH. Then the reaction mixture was cooled to 0 °C (ice bath) and solution of sodium sulfite (12 g Na_2SO_3 in 200 mL H_2O) was added. After stirring at r.t. for 0.5 h, the solution was acidified with 2 M HCl to pH 3-4 and extracted with ethyl acetate (3 x 300 mL). The combined organic phases were washed with water (2 x 200 mL), brine (2 x 200 mL), dried over $MgSO_4$ overnight, and then concentrated to give the crude product as yellowish oil. The oil was subjected to silica gel chromatography (gradient from 0-10% methanol in ethyl acetate) to afford (after evaporation) 13.2 g (yield 88%) of compound **107** as a colorless oil which solidifies; ^{1}H NMR (MeOD) δ: 7.25 – 7.35 (m, 5H, H-2‘, H-3‘, H-4‘), 4.70 – 4.79 (m, 2H, **CH**-ipr), 4.60 (d, J_{gem} = 12.0 Hz, 1H, H-5a), 4.55 (d, J_{gem} = 12.0 Hz, 1H, H-5b), 4.30 (m, 1H, H-2), 4.11 (dd, J_{gem} = 13.7 Hz, $J_{4a\text{-}P}$ = 8.8 Hz, 1H, H-4a), 3.84 (dd, J_{gem} = 13.7 Hz, $J_{4b\text{-}P}$ = 9.2 Hz, 1H, H-4b), 3.82 (m, 2H, H-3), 1.31 – 1.34 (m, 12 H, **CH$_3$**-ipr); ^{13}C NMR (MeOD) δ: 172.73 (C-1), 139.35 (C-1‘), 129.32 (C-3‘), 128.86 (C-2‘), 128.70 (C-4‘), 81.55 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 12.7 Hz, C-2), 74.36 (C-5), 73.33 – 73.46 (m, **CH**-ipr), 71.52 (C-3), 65.40 (d, $J_{C\text{-}P}$ = 167.5 Hz, C-4), 24.23 – 24.38 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m/z* (%): 375 (10, $M+H^+$), 397 (100, $M+Na^+$), 419 (40, $M+2Na^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{17}H_{27}O_7NaP$ ($M+Na^+$) 397.1387, found 397.1388. Anal. Calcd. for: $C_{17}H_{27}O_7P$: C, 54.54; H, 7.27; P, 8.27. Found: C, 54.37; H, 7.32; P, 8.09. $[\alpha]^{20}_D$ = -14.2 (c = 0.435 g/100mL, $CHCl_3$).



Methyl (*S*)-3-(benzyloxy)-2-[(diisopropoxyphosphoryl) methoxy]propanoate (108)

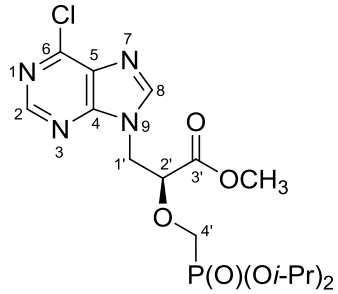
Compound **107** (11.2 g, 30 mmol) was dissolved in ethyl acetate (150 mL) and solution of diazomethane (1 M CH_2N_2 in dry Et_2O) was added dropwise until the solution was not yellowish. The reaction was stirred at r.t. for 0.5 h. Excess of diazomethane was decomposed by adding of several drops of ethanol. The solution was concentrated *in vacuo* and a crude product was purified by silica gel chromatography (gradient from 40-60% ethyl acetate in *iso*-hexanes) to give (after evaporation) 11.4 g (yield 98%) of compound **108** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 7.26 – 7.36 (m, 5H, H-2‘, H-3‘, H-4‘), 4.72 – 7.79 (m, 2H, **CH**-ipr), 4.61 (d, J_{gem} = 12.1 Hz, 1H, H-5a), 4.55 (d, J_{gem} = 12.1 Hz, 1H, H-5b), 4.38 (t, $J_{2\text{-}3}$ = 4.3 Hz, 1H, H-2), 4.06 (dd, J_{gem} = 13.9 Hz, $J_{4a\text{-}P}$ = 8.7 Hz, 1H, H-4a), 3.76 – 3.80 (m, 3H, H-3, H-4b), 3.76 (s, 3H, O-**CH$_3$**), 1.31 – 1.34 (m, 12 H, **CH$_3$**-ipr); ^{13}C NMR ($CDCl_3$) δ: 170.19 (C-1), 137.72 (C-1‘), 128.31 (C-3‘), 127.70 (C-2‘), 127.66 (C-4‘), 80.00 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 9.6 Hz, C-2), 73.40 (C-5), 71.41 (d, $J_{C\text{-}O\text{-}P}$ = 6.5 Hz, **CH**-ipr), 71.20 (d, $J_{C\text{-}O\text{-}P}$ = 6.5 Hz, **CH**-ipr), 70.21 (C-3), 64.93 (d, $J_{C\text{-}P}$ = 166.4 Hz, C-4), 52.08 (O-**CH$_3$**), 23.89 – 24.10 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m/z* (%): 389 (100, M+H$^+$), 411 (95, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{18}H_{30}O_7P$ (M+H$^+$) 389.1535, found 389.1536. $[\alpha]^{20}_D$ = -20.3 (c = 0.370 g/100mL, $CHCl_3$).



Methyl (*S*)-2-[(diisopropoxyphosphoryl)methoxy]-3-hydroxypropanoate (109)

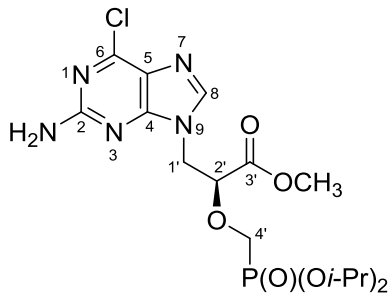
Compound **108** (9.7 g, 25 mmol) from the previous reaction step was dissolved in methanol (250 mL) and a flask was purged with argon and evacuated (3 times). Catalytic amount of 10% palladium on carbon under argon atmosphere was added. Then the flask was evacuated and purged with hydrogen (3 times) and the mixture was vigorously stirred at r.t. until the reaction was complete (~ 3 days). The reaction mixture was filtered through short silica gel column and the filter pad was washed with methanol (100 mL). The filtrate was evaporated and the crude product was purified by silica gel chromatography (gradient from 60-80% ethyl acetate in *iso*-hexanes) to afford (after evaporation) 7.1 g (yield 95%) of compound **109** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 4.72 – 4.82 (m, 2H, **CH**-ipr), 4.23 (dd, $J_{2\text{-}3b}$ = 6.4 Hz, $J_{2\text{-}3a}$ = 3.3 Hz, 1H, H-2), 4.11 (dd, J_{gem} = 14.0 Hz, $J_{4a\text{-}P}$ = 8.2

Hz, 1H, H-4a), 3.93 (dd, J_{gem} = 12.2 Hz, J_{3a-2} = 3.3 Hz, 1H, H-3a), 3.85 (dd, J_{gem} = 12.2 Hz, J_{3b-2} = 6.4 Hz, 1H, H-3b), 3.77 (s, 3H, O-**CH$_3$**), 3.77 (dd, J_{gem} = 14.0 Hz, J_{4b-P} = 7.7 Hz, 1H, H-4b), 3.22 (bs, 1H, OH), 1.31 – 1.37 (m, 12H, **CH$_3$**-ipr); ^{13}C NMR ($CDCl_3$) δ: 170.12 (C-1), 81.54 (d, $J_{C-O-C-P}$ = 8.7 Hz, C-2), 71.69 (d, J_{C-O-P} = 6.7 Hz, **CH**-ipr), 71.42 (d, J_{C-O-P} = 6.7 Hz, **CH**-ipr), 64.97 (d, J_{C-P} = 167.8 Hz, C-4), 62.84 (C-3), 52.08 (O-**CH$_3$**), 23.85 – 24.05 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m/z* (%): 299 (5, M+H$^+$), 321 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{24}O_7P$ (M+H$^+$) 299.1254, found 299.1255. $[\alpha]^{20}_D$ = -23.3 (c = 0.510 g/100mL, $CHCl_3$).



Methyl (*S*)-3-(6-chloro-*9H*-purin-9-yl)-2-[(diisopropoxyphosphoryl)methoxy]propanoate (110)

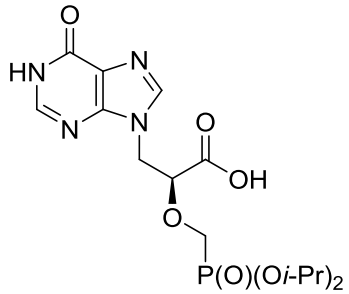
From alcohol **109** (1.19 g, 4 mmol) and 6-chloropurine (804 mg, 5.2 mmol), applied **method G**, obtained 835 mg (yield 48%) of compound **110** as a yellowish oil; ^{1}H NMR ($CDCl_3$) δ: 8.75 (s, 1H, H-2), 8.28 (s, 1H, H-8), 4.75 (dd, J_{gem} = 14.2 Hz, $J_{1'a-2'}$ = 3.3 Hz, 1H, H-1'a), 4.62 – 4.72 (m, 2H, **CH**-ipr), 4.61 (dd, J_{gem} = 14.2 Hz, $J_{1'b-2'}$ = 6.8 Hz, 1H, H-1'b), 4.57 (dd, $J_{2'-1'b}$ = 6.8 Hz, $J_{2'-1'a}$ = 3.3 Hz, 1H, H-2'), 4.06 (dd, J_{gem} = 14.0 Hz, $J_{4'a-P}$ = 8.8 Hz, 1H, H-4'a), 3.77 (s, 3H, O-**CH$_3$**), 3.67 (dd, J_{gem} = 14.0 Hz, $J_{4'b-P}$ = 7.9 Hz, 1H, H-4'b), 1.31 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.30 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.26 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.23 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr); ^{13}C NMR ($CDCl_3$) δ: 168.81 (C-3'), 151.97 (C-2), 151.82 (C-4), 151.06 (C-6), 146.34 (C-8), 131.26 (C-5), 77.50 (d, $J_{C-O-C-P}$ = 9.5 Hz, C-2'), 71.46 (d, J_{C-O-P} = 6.8 Hz, **CH**-ipr), 65.20 (d, J_{C-P} = 166.7 Hz, C-4'), 52.73 (O-**CH$_3$**), 45.31 (C-1'), 23.87 – 24.00 (m, **CH$_3$**-ipr); MS-ESI$^+$ m/z (%): 435 (40, M+H$^+$), 457 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{25}O_6N_4ClP$ (M+H$^+$) 435.1195, found 435.1194; FTIR ($CHCl_3$, cm^{-1}) ν: 3126, 3002, 1754, 1594, 1567, 1499, 1438, 1339, 1233, 1145, 998. $[\alpha]^{20}_D$ = -7.8 (c = 0.275 g/100mL, DMSO).



Methyl (*S*)-3-(2-amino-6-chloro-*9H*-purin-9-yl)-2-[(diisopropoxyphosphoryl)methoxy]propanoate (111)

From alcohol **109** (1.19 g, 4 mmol) and 2-amino-6-chloropurine (882 mg, 5.2 mmol), applied **method H** to give 722 mg (yield 40%) of compound **111** as a

yellowish oil which solidified; ^{1}H NMR (DMSO-d_6) δ: 7.99 (s, 1H, H-8), 6.93 (bs, 2H, NH_2), 4.56 (dd, $J_{2'-1'b}$ = 7.9 Hz, $J_{2'-1'a}$ = 3.6 Hz, 1H, H-2'), 4.40 – 4.52 (m, 3H, **CH**-ipr, H-1'a), 4.33 (dd, J_{gem} = 14.7 Hz, $J_{1'b-2'}$ = 7.9 Hz, 1H, H-1'b), 3.91 (dd, J_{gem} = 13.8 Hz, $J_{4'a-P}$ = 8.7 Hz, 1H, H-4'a), 3.76 (dd, J_{gem} = 13.8 Hz, $J_{4'b-P}$ = 9.3 Hz, 1H, H-4'b), 3.69 (s, 3H, O-**CH$_3$**), 1.18 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.15 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.14 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr), 1.09 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-ipr); ^{13}C NMR (DMSO-d_6) δ: 169.42 (C-3'), 160.04 (C-2), 154.32 (C-4), 149.49 (C-6), 143.77 (C-8), 123.15 (C-5), 77.56 (d, $J_{C-O-C-P}$ = 12.4 Hz, C-2'), 70.57 (m, **CH**-ipr), 64.21 (d, J_{C-P} = 164.2 Hz, C-4'), 52.52 (O-**CH$_3$**), 44.29 (C-1'), 23.66 – 23.91 (m, **CH$_3$**-ipr); MS-ESI$^+$ *m/z* (%): 450 (40, M+H$^+$), 472 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{26}O_6N_5ClP$ (M+H$^+$) 450.1304, found 450.1308; FTIR ($CHCl_3$, cm^{-1}) ν: 3532, 3424, 3205, 2987, 2958, 2939, 1753, 1612, 1568, 1438, 1388, 1234, 1180, 1104. $[\alpha]^{20}_D$ = -10.5 (c = 0.357 g/100mL, DMSO).

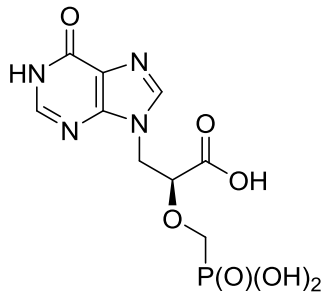


(*S*)-2-[(Diisopropoxyphosphoryl)methoxy)-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl]propanoic acid (112)

Treatment of compound **110** (435 mg, 1 mmol) by **method I** (chromatography: linear gradient of H1 in ethyl acetate) gave 237 mg (yield 59%) of compound **112** as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: 8.04 (s, 1H, H-2), 7.99 (s, 1H, H-8), 4.50 (dd, J_{gem} = 14.4 Hz, $J_{1'a-2'}$ = 3.1 Hz, 1H, H-1'a), 4.36 – 4.50 (m, 2H, **CH**-iPr), 4.21 (ddm, J_{gem} = 14.4 Hz, $J_{1'b-2'}$ = 9.2 Hz, 1H, H-1'b), 4.11 (bdd, J_{gem} = 14.4 Hz, $J_{4'a-P}$ = 8.1 Hz, 1H, H-4'a), 3.97 (dm, $J_{2'-1'b}$ = 9.2 Hz, 1H, H-2'), 3.57 (dd, J_{gem} = 13.7 Hz, $J_{4'b-P}$ = 8.8 Hz, 1H, H-4'b), 1.16 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-iPr), 1.11 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH(**CH$_3$**-iPr), 1.10 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-iPr), 1.07 (d, J_{CH3-CH} = 6.2 Hz, 3H, **CH$_3$**-iPr); ^{13}C NMR (DMSO-d_6) δ: 170.77 (C-3'), 157.06 (C-6), 148.73 (C-4), 145.44 (C-2), 140.72 (C-8), 123.77 (C-5), 81.15 (d, $J_{C-O-C-P}$ = 12.5 Hz, C-2'), 70.26 (d, J_{C-O-P} = 6.3 Hz, **CH**-iPr), 70.16 (d, J_{C-O-P} = 6.3 Hz, **CH**-iPr), 63.23 (d, J_{C-P} = 163.6 Hz, C-4'), 46.18 (C-1'), 23.65 – 23.94 (m, **CH$_3$**-iPr); MS-ESI$^-$ *m/z* (%): 401 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{15}H_{22}O_7N_4P$ (M-H$^+$) 401.1232, found 401.1231; FTIR (KBr, cm^{-1}) ν: 3423, 2981, 2936, 1699, 1611, 1550, 1465, 1386, 1376, 1243, 1126, 993. $[\alpha]^{20}_D$ = -14.9 (c = 0.356 g/100mL, MeOH).

(*S*)-3-(2-Amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-2-[(diisopropoxyphosphoryl) methoxy]propanoic acid (85g)

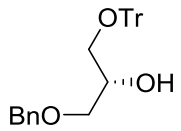
Treatment of compound **111** (450 mg, 1 mmol) by **method I** (chromatography: linear gradient of H1 in ethyl acetate) gave 234 mg (yield 56%) of the title compound as a yellowish solid. For spectroscopical data see part 5.4.3.



(*S*)-3-(6-Oxo-1,6-dihydro-*9H*-purin-9-yl)-2-(phospho-nomethoxy)propanoic acid (113)

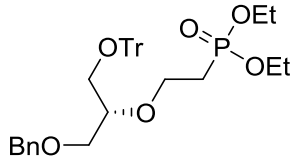
Treatment of compound **112** (201 mg, 0.5 mmol) by **method D1** followed by **method E** gave 110 mg (yield 69%) of compound **113** as a white solid; ^{1}H NMR (D_2O) δ: 8.17 (s, 2H, H-2, H-8), 4.60 (dd, J_{gem} = 14.8 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1'a), 4.54 (dd, J_{gem} = 14.8 Hz, $J_{1'b\text{-}2'}$ = 5.6 Hz, 1H, H-1'b), 4.21 (dd, $J_{2'\text{-}1'b}$ = 5.6 Hz, $J_{2'\text{-}1'a}$ = 4.1 Hz, 1H, H-2'), 3.72 (dd, J_{gem} = 12.9 Hz, $J_{4'a\text{-}P}$ = 8.8 Hz, 1H, H-4'a), 3.48 (dd, J_{gem} = 12.9 Hz, $J_{4'b\text{-}P}$ = 9.9 Hz, 1H, H-4'b); ^{13}C NMR (D_2O) δ: 177.04 (C-3'), 159.31 (C-6), 149.78 (C-4), 146.15 (C-2), 143.70, (C-8), 123.47 (C-5), 81.63 (d, $J_{C\text{-}O\text{-}C\text{-}P}$ = 12.4 Hz, C-2'), 67.06 (d, $J_{C\text{-}P}$ = 155.2 Hz, C-4'), 46.43 (C-1');MS-ESI$^-$ m/z (%): 317 (100, M-H$^+$), 339 (25, M+Na$^+$), 355 (15, M+2Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_9H_{10}O_7N_4P$ (M-H$^+$) 317.0293, found 317.0293; FTIR (KBr, cm^{-1}) ν: 1700, 1586, 1552, 1480, 1414, 1349, 1289, 1152, 1059, 968, 906, 788. $[\alpha]^{20}_D$ = -14.9 (c = 0.470 g/100mL, H_2O).

5.6.2. Synthesis of (*S*)-HPEP derivatives



(*R*)-1-(Benzyloxy)-3-trityloxypropan-2-ol (115)

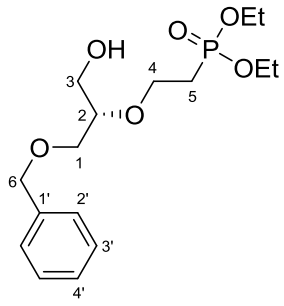
Synthesis and yield of this compound was identical to the preparation of compound **104** (see part 5.6.1.).



Diethyl (*R*)-{2-[(1-(benzyloxy)-3-(trityloxy)propan-2-yl)oxy]ethyl}phosphonate (116)

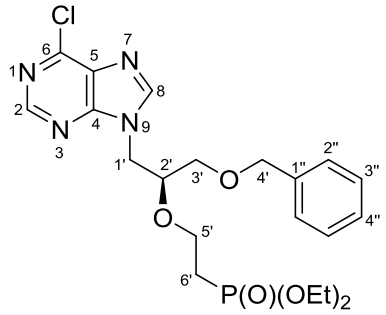
A mixture of compound **115** (42.5 g, 100 mmol) and catalytic amount of KOH (1.4 g, 25 mmol) in dry dioxane (500 mL) was stirred at r.t. for 15 min. Diethyl vinylphosphonate (20 mL, 130 mmol)

was added dropwise and the reaction was vigorously stirred at r.t. for 70 h. The mixture was poured into water and extracted with ethyl acetate (3 x 500 mL). The combined organic phases were washed with brine (2 x 400 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude mixture was subjected to silica gel chromatography (gradient from 20-70% ethylacetate in *iso*-hexanes with 0.1% Et_3N) to give (after evaporation) 33 g (yield 56%) of the compound **116** in the form of the crude product as a yellowish oil. This compound did not appear entirely pure. MS-ESI$^+$ *m/z* (%): 611 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{35}H_{41}O_6NaP$ (M+Na$^+$) 611.2533, found 611.2530.



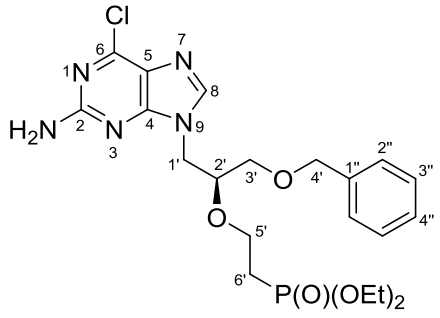
Diethyl (*S*)-{2-[(1-(benzyloxy)-3-hydroxypropan-2-yl)oxy]ethyl}phosphonate (117)

Compound **116** (33 g of the crude product) was dissolved in 80% acetic acid (400 mL) and heated to reflux for 2 h. After cooling down, the reaction mixture was diluted with 300 mL of water/dioxane (1:1) and white crystals of trityl alcohol were filtered off. The solution was extracted with ethyl acetate (3 x 400 mL), the combined organics were washed with water (2 x 150 mL), brine (2 x 150 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give a crude oil. This was subjected to silica gel chromatography (gradient from 40-100% ethylacetate in *iso*-hexanes and 0-5% methanol in ethyl acetate) to afford (after evaporation) 13.5 g (yield 39%) of compound **116** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 7.27 – 7.35 (m, 5H, H-2‘, 3‘, 4‘), 4.53 (s, 2H, H-6), 4.05 – 4.16 (m, 4H, P-O-**CH_2**-CH_3), 3.98 (m, 1H, H-4a), 3.81 (m, 1H, H-4b), 3.72 (dd, J_{gem} = 11.7 Hz, $J_{3a\text{-}2}$ = 2.8 Hz, H-3a), 3.62 (m, 1H, H-2), 3.49 – 3.59 (m, 3H, H-1, H-3b), 2.02 – 2.16 (m, 2H, H-5), 1.32 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**), 1.31 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**); ^{13}C NMR ($CDCl_3$) δ: 137.98 (C-1‘), 128.34 (C-3‘), 127.62 (C-4‘), 127.54 (C-2‘), 80.02 (C-2), 73.40 (C-6), 70.19 (C-1), 63.93 (d, $J_{C\text{-}C\text{-}P}$ = 5.9 Hz, C-4), 62.17 (C-3), 61.87 (d, $J_{C\text{-}O\text{-}P}$ = 6.4 Hz, P-O-**CH_2**-CH_3), 61.57 (d, $J_{C\text{-}O\text{-}P}$ = 6.5 Hz, P-O-**CH_2**-CH_3), 27.08 (d, $J_{C\text{-}P}$ = 141.5 Hz, C-5), 16.32 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 347 (20, M+H$^+$), 369 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{28}O_6P$ (M+H$^+$) 347.1618, found 347.1617. Anal. Calcd. for: $C_{16}H_{28}O_6P$ H_2O: C, 52.74; H, 8.02; P, 8.50. Found: C, 52.95; H, 7.98; P, 8.40. $[\alpha]^{20}_D$ = -7.4 (c = 0.298 g/100mL, $CHCl_3$).



Diethyl (*S*)-{2-[(1-(benzyloxy)-3-(6-chloro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl}phosphonate (118)

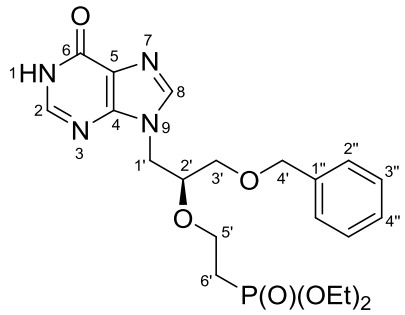
From alcohol **117** (3.46 g, 10 mmol) and 6-chloropurine (2.0 g, 13 mmol), applied **method G**, obtained 3.85 g (yield 80%) of compound **118** as a yellowish oil; ^{1}H NMR (DMSO-d$_6$) δ: 8.76 (s, 1H, H-2), 8.66 (s, 1H, H-8), 7.26 – 7.35 (m, 5H, H-2‘‘, H-3‘‘, H-4‘‘), 4.38 – 4.52 (m, 4H, H-1‘, H-4‘), 3.99 (m, 1H, H-2‘), 3.87 – 3.93 (m, 4H, P-O-**C**$\mathbf{H_2}$-CH$_3$), 3.68 (m, 1H, H-5’a), 3.53 (m, 1H, H-5’b), 3.50 (d, $J_{3'\text{-}2'}$ = 5.0 Hz, 2H, H-3‘), 1.89 (dt, $J_{6'\text{-}5'}$ = 7.4 Hz, $J_{6'\text{-}P}$ = 18.3 Hz, 2H, H-6‘), 1.15 (t, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 6H, P-O-CH$_2$-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d$_6$) δ: 152.42 (C-4), 151.68 (C-2), 149.12 (C-6), 148.33 (C-8), 138.22 (C-1‘‘), 130.78 (C-5), 128.42 (C-3‘‘), 127.72 (C-2‘‘), 127.71 (C-4‘‘), 76.12 (C-2‘), 72.63 (C-4‘), 69.46 (C-3‘), 63.99 (C-5‘), 61.15 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**C**$\mathbf{H_2}$-CH$_3$), 44.96 (C-1‘), 26.30 (d, $J_{C\text{-}P}$ = 136.9 Hz, C-6‘), 16.37 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.9 Hz, P-O-CH$_2$-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 483 (100, M+H$^+$), 505 (65, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_5N_4ClP$ (M+H$^+$) 483.1559, found 483.1558. $[\alpha]^{20}_D$ = -11.7 (c = 0.403 g/100mL, DMSO).



Diethyl (*S*)-{2-[(1-(2-amino-6-chloro-*9H*-purin-9-yl)-3-(benzyloxy)propan-2-yl)oxy]ethyl} phosphonate (119)

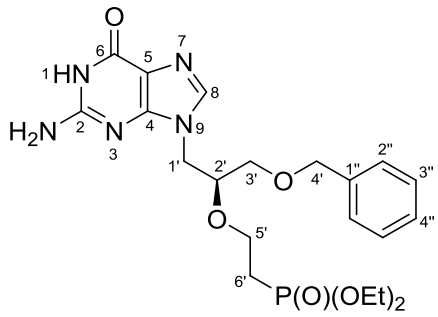
Compound **117** (1.73 g, 5 mmol) and 2-amino-6-chloropurine (1.1 g, 6.5 mmol), applied **method H** to give 1.4 g (yield 55%) of compound **119** as a yellowish oil; ^{1}H NMR (DMSO-d$_6$) δ: 8.07 (s, 1H, H-8), 7.27 – 7.36 (m, 5H, H-2‘‘, H-3‘‘, H-4‘‘), 6.91 (bs, 2H, NH$_2$), 4.49 (s, 2H, H-4‘), 4.22 (dd, J_{gem} = 14.5 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1’a), 4.12 (dd, J_{gem} = 14.5 Hz, $J_{1'b\text{-}2'}$ = 7.5 Hz, 1H, H-1‘b), 3.87 – 3.94 (m, 5H, H-2‘, P-O-**C**$\mathbf{H_2}$-CH$_3$), 3.66 (m, 1H, H-5’a), 3.45 – 3.52 (m, 3H, H-3‘, H-5‘b), 1.84 – 1.91 (m, 2H, H-6‘), 1.20 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-**C**$\mathbf{H_3}$), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d$_6$) δ: 159.99 (C-2), 154.53 (C-4), 149.48 (C-6), 144.01 (C-8), 138.24 (C-1‘‘), 128.47 (C-3‘‘), 127.77 (C-2‘‘), 127.59 (C-4‘‘), 123.28 (C-5), 76.18 (C-2‘), 72.67 (C-4‘), 69.66 (C-3‘), 64.00 (C-5‘), 61.19 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**C**$\mathbf{H_2}$-CH$_3$), 44.36 (C-1‘), 26.37 (d, $J_{C\text{-}P}$ = 136.2 Hz, C-6‘), 16.39 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.9 Hz, P-O-CH$_2$-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 498 (75,

M+H$^+$), 520 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_5N_5ClNaP$ (M+Na$^+$) 520.1487, found 520.1487. Anal. Calcd. for: $C_{21}H_{29}O_5N_5ClP$: C, 50.66; H, 5.87; N, 14.07; P, 6.22; Cl, 7.12. Found: C, 50.48; H, 5.99; N, 14.10; P, 5.97; Cl, 7.03. $[\alpha]^{20}_D$ = -19.0 (c = 0.415 g/100mL, DMSO).



Diethyl (*S*)-{2-[1-(benzyloxy)-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl} phosphonate (120)

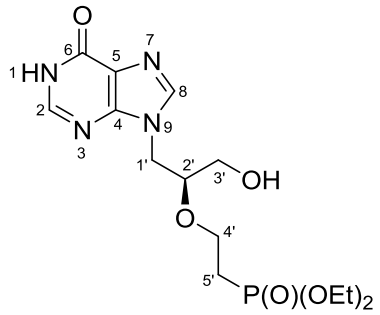
Treatment of compound **118** (966 mg, 2 mmol) by **method I** (chromatography: gradient from 1-5% methanol in chloroform) gave 761 mg (yield 82%) of compound **120** as a yellowish oil; ^{1}H NMR (DMSO-d$_6$) δ: 12.29 (s, 1H, NH), 8.04 (s, 2H, H-2), 8.04 (s, 2H, H-8), 7.27 – 7.36 (m, 5H, H-2‘‘, H-3‘‘, H-4‘‘), 4.49 (s, 2H, H-4‘), 4.32 (dd, J_{gem} = 14.3 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1’a), 4.21 (dd, J_{gem} = 14.3 Hz, $J_{1'b\text{-}2'}$ = 7.3 Hz, 1H, H-1‘b), 3.88 – 3.95 (m, 5H, H-2‘, P-O-**CH$_2$**-CH$_3$), 3.65 (m, 1H, H-5’a), 3.43 – 3.56 (m, 3H, H-3‘, H-5‘b), 1.88 (dt, $J_{6'\text{-}P}$ = 18.3 Hz, 2H, H-6‘), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 156.88 (C-6), 148.78 (C-4), 145.68 (C-2), 141.15 (C-8), 138.30 (C-1‘‘), 128.46 (C-3‘‘), 127.75 (C-2‘‘), 127.72 (C-4‘‘), 123.86 (C-5), 76.61 (C-2‘), 72.63 (C-4‘), 69.62 (C-3‘), 64.07 (C-5‘), 61.18 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 61.17 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 44.54 (C-1‘), 26.35 (d, $J_{C\text{-}P}$ = 136.5 Hz, C-6‘), 16.39 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.9 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 465 (30, M+H$^+$), 487 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_6N_4NaP$ (M+Na$^+$) 487.1717, found 487.1716. Anal. Calcd. for: $C_{21}H_{29}O_6N_4P$: C, 54.31; H, 6.29; N, 12.06; P, 6.67. Found: C, 54.11; H, 6.35; N, 11.89; P, 6.51. $[\alpha]^{20}_D$ = -10.3 (c = 0.455 g/100mL, DMSO).



Diethyl (*S*)-{2-[(1-amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-3-(benzyloxy)propan-2-yl)oxy] ethyl}phosphonate (121)

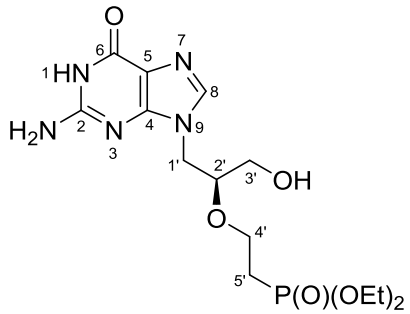
Treatment of compound **119** (996 mg, 2 mmol) by **method I** (chromatography: gradient from 1-7% methanol in chloroform) gave 631 mg (yield 66%) of compound **121** as a yellowish solid; ^{1}H NMR (DMSO-d$_6$) δ: 10.57 (s, 1H, NH), 7.63 (s, 1H, H-8), 7.27 – 7.37 (m, 5H, H-2‘‘, H-3‘‘, H-4‘‘), 6.44 (s, 2H, NH$_2$),

4.49 (s, 2H, H-4‘), 3,97 – 4.12 (m, 2H, H-1‘), 3.84 – 3.95 (m, 5H, H-2‘, P-O-**CH$_2$**-CH$_3$), 3.64 (m, 1H, H-5’a), 3.38 – 3.52 (m, 3H, H-3‘), 3.38 – 3.52 (m, 3H, H-5‘b), 1.88 (dt, $J_{6'\text{-}5'}$ = 7.5 Hz, $J_{6'\text{-}P}$ = 18.3 Hz, 2H, H-6‘), 1.17 (t, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 6H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 157.03 (C-6), 153.72 (C-2), 151.56 (C-4), 138.30 (C-1‘‘), 138.27 (C-8), 128.49 (C-3‘‘), 127.80 (C-2‘‘), 127.74 (C-4‘‘), 116.49 (C-5), 76.56 (C-2‘), 72.66 (C-4‘), 69.83 (C-3‘), 64.05 (C-5‘), 61.21 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 61.20 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 44.09 (C-1‘), 26.36 (d, $J_{C\text{-}P}$ = 136.2 Hz, C-6‘), 16.40 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 480 (55, M+H$^+$), 502 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{31}O_6N_5P$ (M+H$^+$) 480.2007, found 480.2006. Anal. Calcd. for: $C_{21}H_{31}O_6N_5P$: C, 52.61; H, 6.31; N, 14.61; P, 6.46. Found: C, 52.37; H, 6.32; N, 14.47; P, 6.37. $[\alpha]^{20}_D$ = -10.5 (c = 0.342 g/100mL, DMSO).



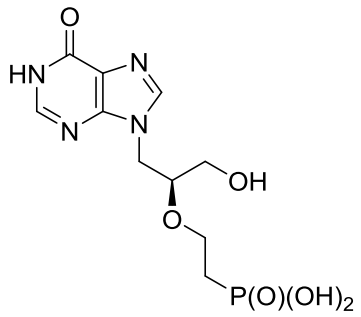
Diethyl (*S*)-{2-[(1-hydroxy-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl}phosphonate (122)

Compound **120** (720 mg, 1.55 mmol) was treated with **method K** to give 465 mg (yield 80%) of compound **122** as a yellowish oil which solidified; ^{1}H NMR (DMSO-d$_6$) δ: 8.04 (m, 2H, H-2), 8.04 (m, 2H, H-8), 4.90 (bs, 1H, OH), 4.30 (dd, J_{gem} = 14.3 Hz, $J_{1'a\text{-}2'}$ = 3.9 Hz, 1H, H-1’a), 4.14 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 7.6 Hz, 1H, H-1‘b), 3.88 – 3.96 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.58 – 3.71 (m, 2H, H-2‘, H-4‘a), 3.39 – 3.51 (m, 3H, H-3’, H-4’b), 1.85 – 1.92 (m, 2H, H-5’), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 156.90 (C-6), 148.77 (C-4), 145.68 (C-2), 141.19 (C-8), 123.87 (C-5), 78.64 (C-2‘), 63.86 (C-4‘), 61.22 (m, P-O-**CH$_2$**-CH$_3$), 60.91 (C-3‘), 44.48 (C-1‘), 26.34 (d, $J_{C\text{-}P}$ = 136.7 Hz, C-5‘), 16.41 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 6.0 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 375 (100, M+H$^+$), 397 (50, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{24}O_6N_4P$ (M+H$^+$) 375.1428, found 375.1427; FTIR (KBr, cm^{-1}) ν: 3052, 1696, 1593, 1551, 1414, 1341, 1221, 1118, 1100, 1054, 1042, 1028, 961, 789. Anal. Calcd. for: $C_{14}H_{24}O_6N_4P$: C, 44.92; H, 6.19; N, 14.97; P, 8.27. Found: C, 45.13; H, 6.33; N, 14.75; P, 8.13. $[\alpha]^{20}_D$ = -12.5 (c = 0.208 g/100mL, MeOH).



Diethyl (*S*)-{2-[(1-amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-3-hydroxypropan-2-yl)oxy]ethyl} phosphonate (123)

Compound **121** (580 mg, 1.21 mmol) was treated with **method K** to give 367 mg (yield 78%) of compound **123** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 7.62 (s, 1H, H-8), 4.10 (dd, J_{gem} = 14.3 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1'a), 3.91 – 3.99 (m, 5H, H-1'b, P-O-**CH_2**-CH_3), 3.61 – 3.68 (m, 2H, H-2', H-4'a), 3.53 (m, 1H, H-4'b), 3.38 – 3.41 (m, 2H, H-3'), 1.86 – 1.95 (m, 2H, H-5'), 1.21 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**); 1.20 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 156.82 (C-6), 153.58 (C-2), 151.38 (C-4), 138.10 (C-8), 116.40 (C-5), 78.45 (C-2'), 63.64 (C-4'), 61.07 (m, P-O-**CH_2**-CH_3), 60.87 (C-3'), 43.70 (C-1'), 26.36 (d, $J_{C\text{-}P}$ = 136.7 Hz, C-5'), 16.20 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 390 (100, M+H$^+$), 412 (15, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{25}O_6N_5P$ (M+H$^+$) 390.1537, found 390.1536; FTIR (KBr, cm^{-1}) ν: 3423, 3123, 2982, 1691, 1657, 1609, 1583, 1540, 1481, 1412, 1229, 1167, 1117, 1051, 1027, 961, 782. Anal. Calcd. for: $C_{14}H_{25}O_6N_5P$: C, 43.19; H, 6.21; N, 17.99; P, 7.96. Found: C, 42.95; H, 6.25; N, 17.91; P, 7.83. $[\alpha]^{20}_D$ = -22.6 (c = 0.261 g/100mL, MeOH).

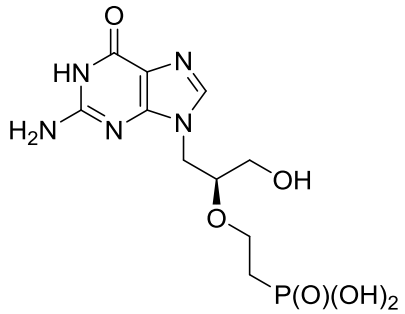


(*S*)-{2-[(1-Hydroxy-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl}phosphonic acid (124)

Treatment of compound **122** (187 mg, 0.5 mmol) by **method D1** followed by **method E** gave 116 mg (yield 73%) of compound **124** as a white solid; ^{1}H NMR (D_2O) δ: 8.12 (s, 1H, H-2), 8.10 (s, 1H, H-8), 4.40 (dd, J_{gem} = 14.7 Hz, $J_{1'a\text{-}2'}$ = 3.8 Hz, 1H, H-1'a), 4.26 (dd, J_{gem} = 14.8 Hz, $J_{1'b\text{-}2'}$ = 7.7 Hz, 1H, H-1'b), 3.81 (m, 1H, H-2'), 3.63 – 3.70 (m, 2H, H-3'a, H-4'a), 3.47 – 3.54 (m, 2H, H-3'b, H-4'b), 1.60 – 1.76 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 158.46 (C-6), 149.02 (C-4), 145.79 (C-2), 142.75 (C-8), 122.92 (C-5), 78.08 (C-2'), 65.90 (C-4'), 60.52 (C-3'), 44.68 (C-1'), 28.86 (d, $J_{C\text{-}P}$ = 129.9 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 319 (100, M+H$^+$), 341 (80, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{16}O_6N_4P$ (M+H$^+$) 319.0802, found 319.0802; FTIR (KBr, cm^{-1}) ν: 3050, 1689, 1592, 1552, 1521, 1414, 1343, 1211, 1097, 1055, 1041, 909, 789. $[\alpha]^{20}_D$ = -5.3 (c = 0.338 g/100mL, H_2O).

(*S*)-{2-[(1-Hydroxy-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl} phosphonic acid (ammonium salt) (124a)

Treatment of compound **122** (187 mg, 0.5 mmol) by **method D1**; the reaction mixture was evaporated, codistilled with acetonitrile (3 x 20 mL) and neutralized with excess of aqueous ammonia (1:10 with water). Volatiles were evaporated and crystallization from EtOAc-MeOH afforded the corresponding ammonium salt as a white solid (115 mg, yield 65%).

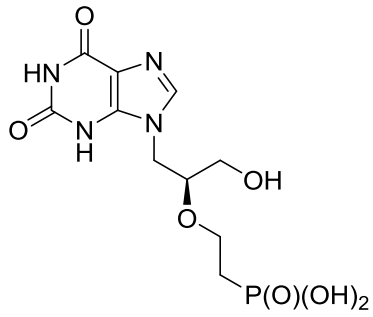


(*S*)-{2-[(1-Amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-3-hydroxypropan-2-yl)oxy]ethyl}phosphonic acid (125)

Treatment of compound **123** (195 mg, 0.5 mmol) by **method D1** followed by **method E** gave 127 mg (yield 76%) of compound **125** as a white solid; ^{1}H NMR (D_2O) δ: 7.74 (s, 1H, H-8), 4.19 (dd, J_{gem} = 14.8 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1'a), 4.04 (dd, J_{gem} = 14.8 Hz, $J_{1'b\text{-}2'}$ = 7.1 Hz, 1H, H-1'b), 3.75 (m, 1H, H-2), 3.52 – 3.69 (m, 3H, H-3'a), 3.52 – 3.69 (m, 3H, H-4'), 3.45 (dd, J_{gem} = 12.4 Hz, $J_{3'b\text{-}2'}$ = 5.5 Hz, 1H, H-3'b), 1.62 – 1.77 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 158.54 (C-6), 153.46 (C-2), 151.47 (C-4), 140.24 (C-8), 115.16 (C-5), 65.61 (d, $J_{C\text{-}C\text{-}P}$ = 1.8 Hz, C-4'), 60.22 (C-3'), 43.46 (C-1'), 28.61 (d, $J_{C\text{-}P}$ = 130.0 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 334 (100, M+H$^+$), 356 (20, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{17}O_6N_5P$ (M+H$^+$) 334.0911, found 334.0910; FTIR (KBr, cm^{-1}) ν: 3379, 3320, 3222, 1688, 1655, 1611, 1580, 1539, 1480, 1411, 1097, 1049, 909, 781. $[\alpha]^{20}_D$ = -15.4 (c = 0.240 g/100mL, H_2O).

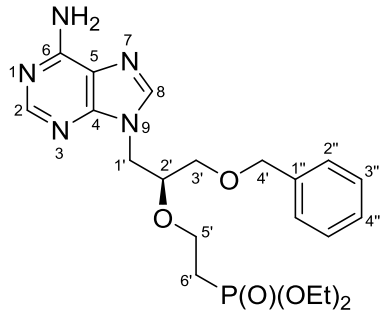
(*S*)-{2-[(1-Hydroxy-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl} phosphonic acid (ammonium salt) (125a)

Treatment of compound **123** (195 mg, 0.5 mmol) by **method D1**; the reaction was evaporated, codistilled with acetonitrile (3 x 20 ml) and neutralized with excess of aqueous ammonia (1:10 with water). Volatiles were evaporated and crystallization from EtOAc-MeOH afforded the corresponding ammonium salt as a white solid (111 mg, yield 60%).



(*S*)-{2-[(1-(2,6-Dioxo-1,2,3,6-tetrahydro-*9H*-purin-9-yl)-3-hydroxypropan-2-yl)oxy]ethyl}phosphonic acid (126)

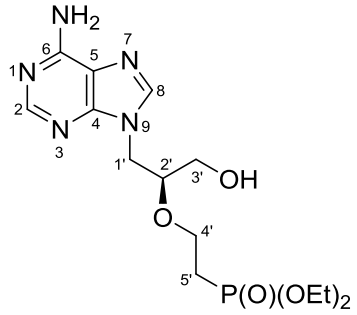
Compound **125** (250 mg, 0.75 mmol) was dissolved in 80% acetic acid (50 mL) and *iso*-pentyl nitrite (1.5 mL) was added. The reaction mixture was stirred at r.t. overnight. Volatiles were evaporated and a residue was codistilled with water (3 x 10 mL) and toluene (3 x 20 mL). The crude product was purified by **method E** to give 87 mg (yield 35%) of compound **126** as a white solid; ^{1}H NMR (D_2O + NaOD) δ: 7.73 (s, 1H, H-8), 4.23 (dd, J_{gem} = 14.8 Hz, $J_{1'b\text{-}2'}$ = 4.3 Hz, 1H, H-1'b), 4.10 (dd, J_{gem} = 14.8 Hz, $J_{1'a\text{-}2'}$ = 6.9 Hz, 1H, H-1'a), 3.85 (ddt, $J_{2'\text{-}1'a}$ = 6.9 Hz, $J_{2'\text{-}3'a}$ = 5.2 Hz, $J_{2'\text{-}1'b}$ = $J_{2'\text{-}3'b}$ = 4.4 Hz, 1H, H-2'), 3.78 (m, 1H, H-4'b), 3.69 (m, 1H, H-4'a), 3.59 (dd, J_{gem} = 12.4 Hz, $J_{3'b\text{-}2'}$ = 4.5 Hz, 1H, H-3'b), 3.52 (dd, J_{gem} = 12.4 Hz, $J_{3'a\text{-}2'}$ = 5.3 Hz, 1H, H-3'a), 1.68 – 1.81 (m, 2H, H-5'); ^{13}C NMR (D_2O + NaOD) δ: 161.65 (C-6), 160.15 (C-2), 154.03 (C-4), 140.63 (C-8), 115.07 (C-5), 78.40 (C-2'), 67.49 (C-4'), 60.99 (C-3'), 43.98 (C-1'), 30.46 (d, $J_{C\text{-}P}$ = 126.2 Hz, C-5'); MS-ESI$^-$ *m/z* (%): 333 (100, M-H$^+$), 355 (75, M+Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{14}O_7N_4P$ (M-H$^+$) 333.0606, found 333.0607; FTIR (KBr, cm^{-1}) ν: 3500, 3193, 3124, 2294, 1720, 1695, 1612, 1580, 1393, 1149, 1100, 1051, 1010, 747. $[\alpha]^{20}_D$ = -4.7 (c = 0.363 g/100mL, H_2O).



Diethyl (*S*)-{2-[(1-(6-amino-*9H*-purin-9-yl)-3-(benzyloxy)propan-2-yl)oxy]ethyl}phosphonate (127)

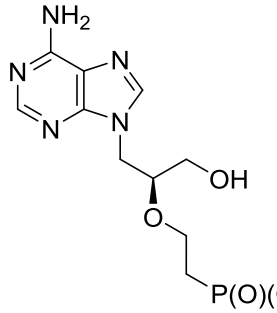
Compound **118** (966 mg, 2 mmol) was placed into 2 reaction vials and a solution of 3.5 M ammonia in ethanol (5 ml) was added to each of them. Each vial was microwave irradiated at 140 °C for 30 min. The mixture was evaporated and codistilled with water (2 x 10 mL) and ethanol (2 x 10 mL). The residue was purified by silica gel chromatography (gradient from 0-5% methanol in chloroform) to afford (after evaporation) 816 mg (yield 88%) of compound **127** as a colorless oil which solidified; ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, H-2), 8.07 (s, 1H, H-8), 7.27 – 7.36 (m, 5H, H-2'', H-3'', H-4''), 7.20 (bs, 2H, NH_2), 4.49 (s, 2H, H-4'), 4.32 (dd, J_{gem} = 14.3 Hz, $J_{1'a\text{-}2'}$ = 4.2 Hz, 1H, H-1'a), 4.21 (dd, J_{gem} = 14.3 Hz, $J_{1'b\text{-}2'}$ = 7.1 Hz, 1H, H-

1‘b), 3.86 - 3.96 (m, 5H, H-2‘, P-O-**C**$\mathbf{H_2}$-CH_3), 3.65 (m, 1H, H-5’a), 3.43 – 3.54 (m, 3H, H-3‘, H-5’b), 1.87 (dt, $J_{6‘\text{-}P}$ = 18.2 Hz, 2H, H-6‘), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 156.15 (C-6), 152.60 (C-2), 149.90 (C-4), 141.65 (C-8), 138.33 (C-1‘‘), 128.45 (C-3‘‘), 127.75 (C-2‘‘), 127.70 (C-4‘‘), 118.66 (C-5), 76.50 (C-2‘), 72.61 (C-4‘), 69.78 (C-3‘), 64.03 (C-5‘), 61.15 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 44.14 (C-1‘), 26.35 (d, $J_{C\text{-}P}$ = 136.4 Hz, C-6‘), 16.39 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 464 (100, M+H$^+$), 486 (75, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{31}O_5N_5P$ (M+H$^+$) 464.2057, found 464.2057. Anal. Calcd. for: $C_{21}H_{30}O_5N_5P$: C, 54.42; H, 6.52; N, 15.11; P, 6.68. Found: C, 54.63; H, 6.66; N, 14.95; P, 6.41. $[\alpha]^{20}_D$ = -11.3 (c = 0.358 g/100mL, DMSO).



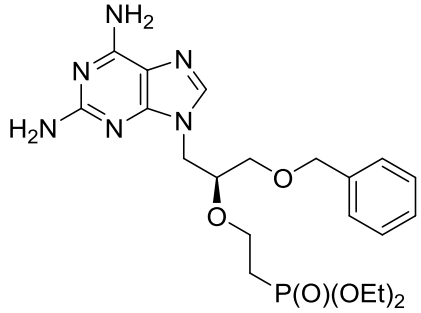
Diethyl (*S*)-{2-[(1-(6-amino-*9H*-purin-9-yl)-3-hydroxypropan-2-yl)oxy]ethyl}phosphonate (128)

Compound **127** (834 mg, 1.8 mmol) was treated with **method K** to give 605 mg (yield 90%) of compound **128** as a colorless oil; ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, H-2), 8.08 (s, 1H, H-8), 7.21 (bs, 2H, NH_2), 4.31 (dd, J_{gem} = 14.4 Hz, $J_{1‘a\text{-}2‘}$ = 4.3 Hz, 1H, H-1’a), 4.21 (dd, J_{gem} = 14.4 Hz, $J_{1‘b\text{-}2‘}$ = 7.3 Hz, 1H, H-1‘b), 3.87 – 3.95 (m, 4H, P-O-**C**$\mathbf{H_2}$-CH_3), 3.72 (m, $J_{2‘\text{-}1’a}$ = 4.2 Hz, $J_{2‘\text{-}1’b}$ = 7.2 Hz, $J_{2‘\text{-}3‘}$ = 5.3 Hz, 1H, H-2‘), 3.62 (m, 1H, H-4’a), 3.49 (m, 1H, H-4’b), 3.37 (m, 2H, H-3‘), 1.88 (m, 2H, H-5‘), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**C**$\mathbf{H_3}$), 1.17 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 156.16 (C-6), 152.57 (C-2), 149.89 (C-4), 141.76 (C-8), 118.66 (C-5), 78.49 (C-2‘), 63.81 (C-4‘), 61.21 (d, $J_{C\text{-}O\text{-}P}$ = 6.4 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 61.18 (d, $J_{C\text{-}O\text{-}P}$ = 6.4 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 60.98 (C-3‘), 44.03 (C-1‘), 26.33 (d, $J_{C\text{-}P}$ = 136.3 Hz, C-5‘), 16.40 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 374 (25, M+H$^+$), 396 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{25}O_5N_5P$ (M+H$^+$) 374.1588, found 374.1587; FTIR (KBr, cm^{-1}) ν: 3333, 3191, 1645, 1600, 1576, 1478, 1417, 1392, 1327, 1300, 1229, 1164, 1115, 1051, 1028, 962. Anal. Calcd. for: $C_{14}H_{24}O_5N_5P$: C, 45.04; H, 6.48; N, 18.76; P, 8.30. Found: C, 44.86; H, 6.59; N, 18.55; P, 8.18. $[\alpha]^{20}_D$ = -16.5 (c = 0.213 g/100mL, MeOH).



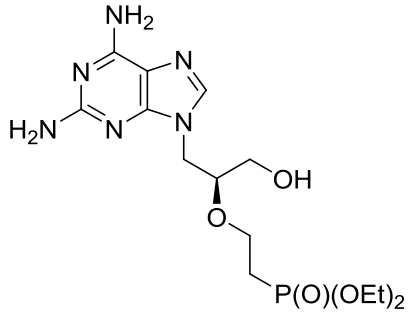
(*S*)-{2-[(1-(6-Amino-*9H*-purin-9-yl)-3-hydroxy propan-2-yl)oxy]ethyl}phosphonic acid (129)

Treatment of compound **128** (187 mg, 0.5 mmol) by **metod D1** followed by **method E** gave 125 mg (yield 79%) of compound **129** as a white solid; ^{1}H NMR (D_2O) δ: 8.07 (s, 1H, H-2), 8.02 (s, 1H, H-8), 4.30 (dd, J_{gem} = 14.7 Hz, $J_{1'a\text{-}2'}$ = 3.8 Hz, 1H, H-1'a), 4.17 (dd, J_{gem} = 14.7 Hz, $J_{1'b\text{-}2'}$ = 7.6 Hz, 1H, H-1'b), 3.75 (dsext, $J_{2'\text{-}1'a}$ = 3.9 Hz, $J_{2'\text{-}1'b}$ = 7.7 Hz, 1H, H-2'), 3.58 – 3.66 (m, 2H, H-3'a, H-4'a), 3.38 – 3.46 (m, 2H, H-3'b, H-4'b), 1.60 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 155.24 (C-6), 152.15 (C-2), 148.82 (C-4), 142.88 (C-8), 118.00 (C-5), 77.86 (C-2'), 65.92 (d, $J_{C\text{-}C\text{-}P}$ = 1.4 Hz, C-4'), 60.44 (C-3'), 44.18 (C-1'), 28.85 (d, $J_{C\text{-}P}$ = 129.6 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 318 (100, M+H$^+$), 340 (20, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{17}O_5N_5P$ (M+H$^+$) 318.0962, found 318.0962; FTIR (KBr, cm^{-1}) ν: 1645, 1605, 1577, 1515, 1479, 1306, 1100, 1047, 902, 797. $[\alpha]^{20}_D$ = -6.4 (c = 0.389 g/100mL, H_2O).



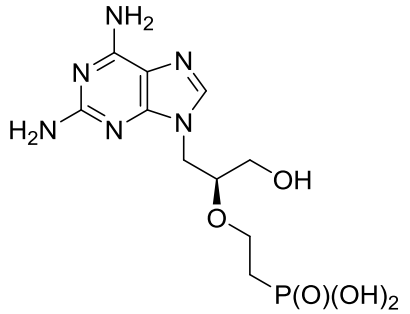
Diethyl (*S*)-{2-[1-(benzyloxy)-3-(2,6-diamino-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl} phosphonate (130)

Compound **119** (498 mg, 1 mmol) was placed into reaction vial and a solution of 3.5 M ammonia in ethanol (5 mL) was added. The vial was microwave irradiated at 120 °C for 1 h. The mixture was evaporated and codistilled with water (2 x 10 mL) and ethanol (2 x 10 mL). The residue was purified by silica gel chromatography (gradient from 0-7% methanol in chloroform) to afford (after evaporation) 407 mg (yield 85%) of compound **130** as a colorless oil; MS-ESI$^+$ *m/z* (%): 479 (100, M+H$^+$), 501 (80, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{31}O_5N_5P$ (M+H$^+$) 479.2093, found 479.2095.



Diethyl (*S*)-{2-[(1-(2,6-diamino-*9H*-purin-9-yl)-3-hydroxypropan-2-yl)oxy]ethyl}phosphonate (131)

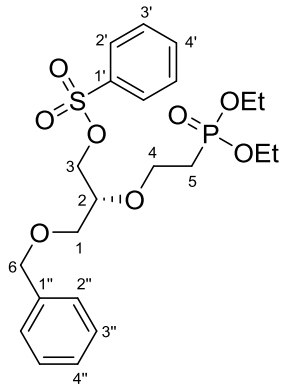
Compound **130** (383 mg, 0.8 mmol) was treated with **method K** to afford (after evaporation) 273 mg (yield 88%) of compound **131** as a yellowish oil; ^{1}H NMR (DMSO-d_6) δ: 7.65 (s, 1H, H-8), 6.67 (bs, 2H, 6-NH_2), 5.80 (bs, 2H, 2-NH_2), 4.99 (bs, 1H, 3'-OH), 4.11 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1'a), 3.89 – 3.99 (m, 5H, H-1'b, P-O-**CH_2**-CH_3), 3.61 – 3.67 (m, 2H, H-2', H-4'a), 3.54 (m, 1H, H-4'b), 3.33 – 3.39 (m, 2H, H-3'), 1.88 – 1.94 (dm, 2H, $J_{5'\text{-}P}$ = 18.1 Hz, 2H, H-5'), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 3H, P-O-CH_2-**CH_3**), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 3H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 160.41 (C-2), 156.33 (C-6), 152.09 (C-4), 138.41 (C-8), 113.03 (C-5), 78.45 (C-2'), 63.73 (C-4'), 61.22 (m, P-O-**CH_2**-CH_3), 60.80 (C-3'), 43.30 (C-1'), 26.35 (d, $J_{C\text{-}P}$ = 136.4 Hz, C-5'), 16.42 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.7 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 389 (35, M+H$^+$), 411 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{26}O_5N_6P$ (M+H$^+$) 389.1697, found 389.1697; FTIR (KBr, cm^{-1}) ν: 3336, 3201, 2983, 1637, 1596, 1477, 1410, 1392, 1344, 1279, 1223, 1165, 1123, 1100, 1047, 1029, 964, 792. $[\alpha]^{20}_D$ = -17.4 (c = 0.281 g/100mL, MeOH).



(*S*)-{2-[(1-(2,6-Diamino-*9H*-purin-9-yl)-3-hydroxypropan-2-yl)oxy]ethyl}phosphonic acid (132)

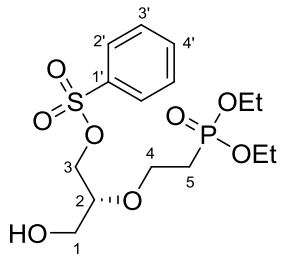
Treatment of compound **131** (194 mg, 0.5 mmol) by **method D1** followed by **method E** gave 125 mg (yield 75%) of compound **132** as a white solid; ^{1}H NMR (D_2O + NaOD) δ: 7.81 (s, 1H, H-8), 4.26 (dd, J_{gem} = 14.8 Hz, $J_{1'a\text{-}2'}$ = 4.3 Hz, 1H, H-1'a), 4.12 (dd, J_{gem} = 14.8 Hz, $J_{1'b\text{-}2'}$ = 6.9 Hz, 1H, H-1'b), 3.83 (m, 1H, H-2'), 3.75 (m, 1H, H-4'a), 3.68 (dd, J_{gem} = 12.3 Hz, $J_{3'a\text{-}2'}$ = 4.4 Hz, 1H, H-3'a), 3.67 (m, 1H, H-4'b), 3.50 (dd, J_{gem} = 12.3 Hz, $J_{3'b\text{-}2'}$ = 5.3 Hz, 1H, H-3'b), 1.59 – 1.70 (m, 2H, H-5'); ^{13}C NMR (D_2O + NaOD) δ: 160.56 (C-2), 156.64 (C-6), 151.83 (C-4), 141.31 (C-8), 113.25 (C-5), 78.23 (C-2'), 68.11 (d, $J_{C\text{-}C\text{-}P}$ = 3.3 Hz, C-4'), 61.07 (C-3'), 44.07 (C-1'), 30.96 (d, $J_{C\text{-}P}$ = 124.0 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 333 (100, M+H$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{18}O_5N_6P$ (M+H$^+$) 333.1071, found 333.1072; FTIR

(KBr, cm^{-1}) ν: 3330, 3184, 1653, 1601, 1409, 1222, 1122, 1050, 1037, 911. $[\alpha]^{20}_D$ = -11.8 (c = 0.279 g/100mL, H_2O).



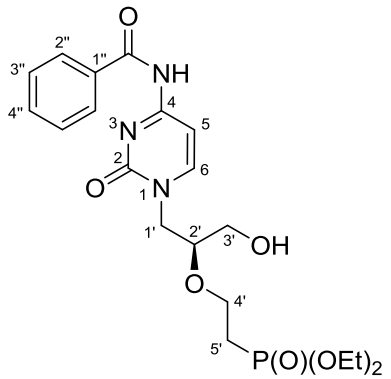
(*R*)-3-(Benzyloxy)-2-[2-(diethoxyphosphoryl)ethoxy] propyl 4-methylbenzenesulfonate (133)

Compound **117** (13 g, 37.5 mmol) was dissolved in dry pyridine (350 mL) in a round-bottomed flask with a drying tube (protecting the reaction from moisture). The solution was cooled to 0 °C (ice bath), *p*-toluenesulfonyl chloride (14.3 g, 75 mmol) and 4-(dimetylamino)pyridine (1.4 g, 11.3 mmol) were added. Ice bath was removed and the reaction mixture was stirred at r.t. overnight. The solution was poured into ice and extracted with ethyl acetate (3 x 300 mL). The combined organic layers were washed with saturated aqueous solution of sodium bicarbonate (2 x 200 mL), brine (2 x 200 mL), dried over $MgSO_4$ and concentrated to give the crude residue. The residue was purified by silica gel chromatography (gradient from 30-100% ethyl acetate in *iso*-hexanes and 0-4% methanol in ethyl acetate) to afford (after evaporation) 15.6 g (yield 83%) of compound **133** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 7.78 (m, 2H, H-2‘), 7.23 – 7.34 (m, 7H, H-3‘, H-2‘‘, H-3‘‘, H-4‘‘), 4.46 (m, 2H, H-6), 4.16 (dd, J_{gem} = 10.5 Hz, $J_{3a\text{-}2}$ = 4.2 Hz, 1H, H-3a), 4.02 – 4.10 (m, 5H, H-3b, P-O-**CH_2**-CH_3), 3.75 (m, 2H, H-4), 3.68 (m, 1H, H-2), 3.49 (m, 2H, H-1), 2.43 (s, 3H, 4‘-**CH_3**), 2.03 (dt, $J_{5\text{-}P}$ = 18.6 Hz, 2H, H-5), 1.30 (dt, $J_{CH3\text{-}P}$ = 3.0 Hz, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR ($CDCl_3$) δ: 144.82 (C-4‘), 137.62 (C-1‘‘), 132.78 (C-1‘), 129.80 (C-3‘), 128.34 (C-3‘‘), 127.89 (C-2‘), 127.71 (C-4‘‘), 127.54 (C-2‘‘), 76.46 (C-2), 73.41 (C-6), 69.22 (C-3), 68.50 (C-1), 64.51 (C-4), 61.60 (m, P-O-**CH_2**-CH_3), 27.14 (d, $J_{C\text{-}P}$ = 139.6 Hz, C-5), 21.57 (4‘-**CH_3**), 16.34 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 6.0 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 501 (55, M+H$^+$), 523 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{23}H_{34}O_8PS$ (M+H$^+$) 501.1707, found 501.1703. Anal. Calcd. for $C_{23}H_{33}O_8PS$: C, 55.19; H, 6.55; P, 6.19; S, 6.41. Found: C, 55.21; H, 6.68; P, 5.89; S, 6.11. $[\alpha]^{20}_D$ = +3.1 (c = 0.350 g/100mL, $CHCl_3$).



(*R*)-2-[2-(Diethoxyphosphoryl)ethoxy]-3-hydroxypropyl 4-methylbenzenesulfonate (134)

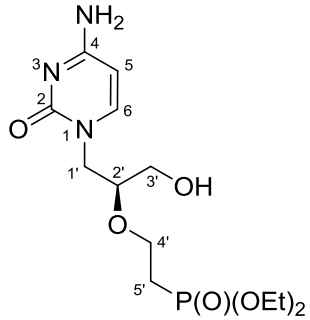
Compound **133** (11.0 g, 22.0 mmol) was dissolved in the mixture of methanol and acetic acid (5:1, 300 mL) and treated by **method K** (chromatography: gradient from 0-5% methanol in chloroform) to give (after evaporation) 8.58 g (yield 95%) of compound **134** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 7.78 (m, 2H, H-2‘), 7.35 (m, 2H, H-3‘), 4.00 – 4.14 (m, 6H, H-3, P-O-**C**$\mathbf{H_2}$-CH_3), 3.89 (m, 1H, H-4a), 3.76 (m, 1H, H-4b), 3.69 (dd, J_{gem} = 12.2 Hz, J_{1a-2} = 3.1 Hz, 1H, H-1a), 3.62 (m, 1H, H-2), 3.46 (dd, J_{gem} = 12.2 Hz, J_{1b-2} = 5.8 Hz, 1H, H-1b), 3.15 (bs, 1H, OH), 2.45 (s, 3H, 4‘-**C**$\mathbf{H_3}$), 2.04 (m, 2H, H-5), 1.32 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**C**$\mathbf{H_3}$), 1.30 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR ($CDCl_3$) δ: 144.95 (C-4‘), 132.75 (C-1‘), 129.87 (C-3‘), 127.91 (C-2‘), 78.52 (C-2), 69.35 (C-3), 64.02 (d, J_{C-C-P} = 6.8 Hz, C-4), 62.05 (d, J_{C-O-P} = 6.4 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 61.61 (d, J_{C-O-P} = 6.4 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 60.83 (C-1), 26.90 (d, J_{C-P} = 141.9 Hz, C-5), 21.61 (4‘-**C**$\mathbf{H_3}$), 16.33 (d, $J_{C-C-O-P}$ = 6.1 Hz, P-O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 411 (20, M+H$^+$), 433 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{28}O_8PS$ (M+H$^+$) 411.1237, found 411.1237. Anal. Calcd. for $C_{16}H_{27}O_8PS$: C, 46.82; H, 6.63; P, 7.55; S, 7.81. Found: C, 46.90; H, 6.73; P, 7.33; S, 7.54. $[\alpha]^{20}_D$ = +6.8 (c = 0.617 g/100mL, $CHCl_3$).



Diethyl (*S*)-{2-[(1-[4-benzamido-2-oxopyrimidin-1(*2H*)-yl]-3-hydroxypropan-2-yl)oxy]ethyl} phosphonate (135)

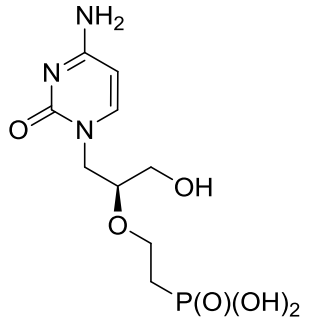
N-Benzoylcytosine (710 mg, 3.3 mmol) and sodium hydride (60% suspension in mineral oil) (150 mg, 3.6 mmol) were suspended in dry DMF (40 mL). The reaction mixture was heated at 80 °C for 1 h. Then a solution of compound **134** (1.23 g, 3 mmol) in dry DMF (20 mL) was added dropwise. The reaction mixture was heated at 80 °C and temperature was gradually elevated up to 110 °C during 3 h. After cooling down, the solution was neutralized with glacial acetic acid and volatiles were evaporated. A residue was codistilled with toluene (3 x 30 mL) and purified by silica gel chromatography (gradient from 0-4% methanol in chloroform) to afford (after evaporation) 459 mg (yield 34%) of compound **135** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 11.18 (bs, 1H, NH), 8.05 (d,

$J_{6\text{-}5}$ = 7.2 Hz, 1H, H-6), 8.00 (m, 2H, H-2''), 7.62 (m, 1H, H-4''), 7.51 (m, 2H, H-3''), 7.31 (d, $J_{5\text{-}6}$ = 7.2 Hz, 1H, H-5), 4.85 (t, $J_{OH\text{-}3'}$ = 5.8 Hz, 3'-OH), 4.11 (dd, J_{gem} = 13.2 Hz, $J_{1'a\text{-}2'}$ = 3.6 Hz, 1H, H-1'a), 3.91 – 4.00 (m, 4H, P-O-**CH_2**-CH_3), 3.61 – 3.74 (m, 3H, H-1'b, H-2', H-4'a), 3.39 – 3.54 (m, 3H, H-3', H-4'b), 1.96 (dt, $J_{5'\text{-}P}$ = 18.2 Hz, $J_{5'\text{-}4'}$ = 7.4 Hz, 2H, H-5'), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 167.50 (C=O), 163.20 (C-4), 155.90 (C-2), 151.60 (C-6), 133.38 (C-1''), 132.86 (C-4''), 128.63 (C-3''), 128.57 (C-2''), 95.60 (C-5), 77.79 (C-2'), 64.13 (C-4'), 61.21 (m, P-O-**CH_2**-CH_3), 61.05 (C-3'), 51.03 (C-1'), 26.46 (d, $J_{C\text{-}P}$ = 136.7 Hz, C-5'), 16.40 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 6.0 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 454 (25, M+H$^+$), 476 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{20}H_{29}O_7N_3P$ (M+H$^+$) 454.1738, found 454.1738; FTIR (KBr, cm^{-1}) ν: 3414, 1696, 1656, 1623, 1561, 1487, 1384, 1367, 1224, 1163, 1098, 1050, 1028, 961, 707. $[\alpha]^{20}_D$ = -65.4 (c = 0.286 g/100mL, MeOH).



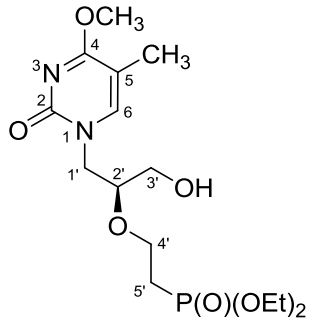
Diethyl (*S*)-{2-[(1-[4-amino-2-oxopyrimidin-1(*2H*)-yl]-3-hydroxypropan-2-yl)oxy]ethyl}phosphonate (136)

Compound **135** (453 mg, 1 mmol) was treated with **method C**. The crude product was purified by silica gel chromatography (gradient from 2-10% methanol in chloroform) to give (after evaporation) 332 mg (yield 95%) of compound **136** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 7.46 (d, $J_{6\text{-}5}$ = 7.2 Hz, 1H, H-6), 7.05 (bs, 2H, NH_2), 5.63 (d, $J_{5\text{-}6}$ = 7.2 Hz, 1H, H-5), 3.92 – 3.99 (m, 4H, P-O-**CH_2**-CH_3), 3.87 (dd, J_{gem} = 12.7 Hz, $J_{1'a\text{-}2'}$ = 3.2 Hz, 1H, H-1'a), 3.62 – 3.68 (m, 1H, H-2'), 3.48 – 3.56 (m, 3H, H-1'b, H-4'), 3.39 (dd, J_{gem} = 11.7 Hz, $J_{3'a\text{-}2'}$ = 4.5 Hz, 1H, H-3'a), 3.34 (dd, J_{gem} = 11.7 Hz, $J_{3'b\text{-}2'}$ = 5.3 Hz, 1H, H-3'b), 1.96 (dt, $J_{5'\text{-}P}$ = 18.2 Hz, $J_{5'\text{-}4'}$ = 7.3 Hz, 2H, H-5'), 1.21 (2xt, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 166.19 (C-4), 156.26 (C-2), 147.23 (C-6), 93.00 (C-5), 78.35 (C-2'), 63.97 (C-4'), 61.21 (C-3'), 61.14 (m, P-O-**CH_2**-CH_3), 49.86 (C-1'), 26.50 (d, $J_{C\text{-}P}$ = 136.4 Hz, C-5'), 16.37 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.9 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 350 (60, M+H$^+$), 372 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{24}O_6N_3NaP$ (M+Na$^+$) 372.1295, found 372.1295; FTIR (KBr, cm^{-1}) ν: 2987, 1647, 1523, 1494, 1388, 1279, 1227, 1163, 1101, 1049, 1027, 962, 790. $[\alpha]^{20}_D$ = -17.9 (c = 0.367 g/100mL, DMSO).



(*S*)-{2-[(1-[4-Amino-2-oxopyrimidin-1(*2H*)-yl]-3-hydroxypropan-2-yl)oxy]ethyl}phosphonic acid (137)

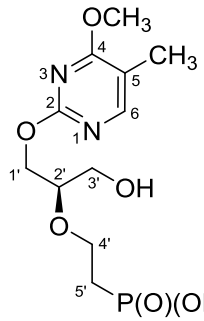
Treatment of compound **136** (175 mg, 0.5 mmol) by **method D1** followed by **method E** gave 113 mg (yield 77%) of compound **137** as a white solid; ^{1}H NMR (D_2O) δ: 7.56 (d, $J_{6\text{-}5}$ = 7.3 Hz, 1H, H-6), 5.99 (d, $J_{5\text{-}6}$ = 7.3 Hz, 1H, H-5), 4.05 (dd, J_{gem} = 13.9 Hz, $J_{1'a\text{-}2'}$ = 3.5 Hz, 1H, H-1'a), 3.71 (dd, J_{gem} = 13.9 Hz, $J_{1'b\text{-}2'}$ = 7.9 Hz, 1H, H-1'b), 3.60 – 3.82 (m, 4H, H-2', H-4', H-3'a), 3.55 (dd, J_{gem} = 12.0 Hz, $J_{3'b\text{-}2'}$ = 4.8 Hz, 1H, H-3'b), 1.75 – 1.84 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 167.13 (C-4), 159.05 (C-2), 148.48 (C-6), 96.04 (C-5), 78.26 (C-2'), 67.31 (d, $J_{C\text{-}C\text{-}P}$ = 1.9 Hz, C-4'), 61.43 (C-3'), 51.14 (C-1'), 30.06 (d, $J_{C\text{-}P}$ = 128.2 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 294 (100, M+H$^+$), 316 (40, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_9H_{17}O_6N_3P$ (M+H$^+$) 294.0850, found 294.0852; FTIR (KBr, cm^{-1}) ν: 3390, 3325, 1652, 1604, 1527, 1495, 1398, 1126, 1108, 1064, 906, 789, 815. $[\alpha]^{20}_D$ = -22.7 (c = 0.308 g/100mL, H_2O).



Diethyl (*S*)-{2-[(1-hydroxy-3-[4-methoxy-5-methyl-2-oxopyrimidin-1(*2H*)-yl]propan-2-yl)oxy]ethyl} phosphonate (138)

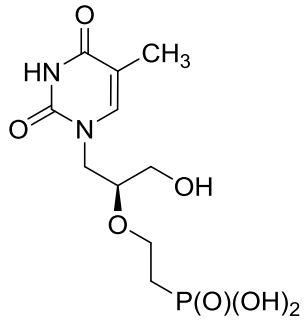
4-Methoxy-5-methylpyrimidin-2(*1H*)-one (632 mg, 4.51 mmol) and sodium hydride (60% suspension in mineral oil) (197 mg, 4.92 mmol) were suspended in dry DMF (50 mL) and the mixture was heated at 100 °C for 1 h. A solution of compound **134** (1.68 g, 4.10 mmol) in dry DMF (30 mL) was added dropwise. The reaction mixture was heated at 100 °C for 3 h. After cooling down, the mixture was neutralized with glacial acetic acid and volatiles were evaporated. The residue was codistilled with toluene (3 x 40 mL) and purified by silica gel chromatography (gradient from 0-5% methanol in chloroform) to afford (after evaporation) two products as a colorless oil: 819 mg (yield 53%) of compound **138** and 251 mg (yield 16%) of the corresponding *O*-isomer **139**; ^{1}H NMR (DMSO-d_6) δ: 7.70 (q, $J_{6\text{-}5\text{-}CH3}$ = 1.0 Hz, 1H, H-6), 3.92 – 3.99 (m, 5H, H-1'a, P-O-**CH_2**-CH_3), 3.84 (s, 3H, H-4-O-**CH_3**), 3.56 – 3.69 (m, 3H, H-1'b, H-2', H-4'a), 3.41 – 3.52 (m, 2H, H-3'a, H-4'b), 3.35 – 3.39 (m, 1H, H-3'b), 1.90 – 1.97 (dm, $J_{5'\text{-}P}$ = 18.2 Hz, 2H, H-5'), 1.87 (d, $J_{5\text{-}CH3\text{-}6}$ = 1.0 Hz, 3H, H-5-**CH_3**), 1.20 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 170.18 (C-

4), 155.72 (C-2), 147.41 (C-6), 102.10 (C-5), 77.96 (C-2'), 64.03 (C-4'), 61.18 (P-O-**CH_2**-CH_3, C-3'), 54.04 (4-O-**CH_3**), 50.30 (C-1'), 26.45 (d, $J_{C\text{-}P}$ = 136.1 Hz, C-5'), 16.36 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.6 Hz, P-O-CH_2-**CH_3**), 11.58 (5-**CH_3**); MS-ESI$^+$ *m/z* (%): 379 (25, M+H$^+$), 401 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{27}O_7N_2NaP$ (M+Na$^+$) 401.1448, found 401.1447; FTIR (KBr, cm^{-1}) ν: 2982, 2956, 1669, 1539, 1379, 1451, 1399, 1385, 1371, 1337, 1245, 1213, 1124, 1098, 1051, 1029, 961, 788. $[\alpha]^{20}_D$ = -56.6 (c = 0.242 g/100mL, MeOH).



Diethyl (*S*)-{2-[(1-hydroxy-3-[(4-methoxy-5-methylpyrimidin-2-yl)oxy]propan-2-yl)oxy]ethyl}phosphonate (139)

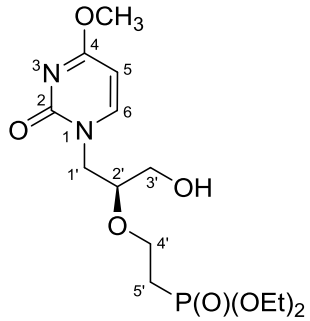
^{1}H NMR (DMSO-d_6) δ: 8.09 (q, $J_{6\text{-}5\text{-}CH3}$ = 0.9 Hz, 1H, H-6), 4.35 (dd, J_{gem} = 11.4 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1'a), 4.23 (dd, J_{gem} = 11.4 Hz, $J_{1'b\text{-}2'}$ = 5.9 Hz, 1H, H-1'b), 3.94 – 4.01 (m, 4H, P-O-**CH_2**-CH_3), 3.91 (s, 3H, H-4-O-**CH_3**), 3.75 (m, 2H, H-4'), 3.65 (m, 1H, H-2'), 3.51 (m, 2H, H-3'), 2.07 (dt, $J_{5'\text{-}P}$ = 18.2 Hz, $J_{5'\text{-}4'}$ = 7.1 Hz, 2H, H-5'), 2.00 (d, $J_{5\text{-}CH3\text{-}6}$ = 0.9 Hz, 3H, H-5-**CH_3**), 1.21 (2xt, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 169.16 (C-4), 163.32 (C-2), 157.39 (C-6), 110.72 (C-5), 78.50 (C-2'), 66.57 (C-1'), 63.70 (C-4'), 61.16 (m, P-O-**CH_2**-CH_3), 60.57 (C-3'), 53.90 (4-O-**CH_3**), 26.58 (d, $J_{C\text{-}P}$ = 136.7 Hz, C-5'), 16.39 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.6 Hz, P-O-CH_2-**CH_3**), 11.63 (5-**CH_3**); MS-ESI$^+$ *m/z* (%): identical to compound **138**; FTIR (KBr, cm^{-1}) ν: 2982, 1608, 1576, 1477, 1425, 1398, 1387, 1296, 1248, 1236, 1206, 1120, 1099, 1048, 1028, 963, 791. $[\alpha]^{20}_D$ = -11.3 (c = 0.212 g/100mL, MeOH).



(*S*)-{2-[(1-hydroxy-3-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl]propan-2-yl)oxy]ethyl}phosphonic acid (140)

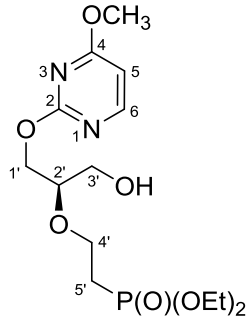
Treatment of compound **138** (189 mg, 0.5 mmol) by **method D1** followed by **method E** gave 113 mg (yield 73%) of compound **140** as a white solid; ^{1}H NMR (D_2O) δ: 7.47 (m, 1H, H-6), 3.98 (m, 1H, H-1'a), 3.72 – 3.85 (m, 4H, H-1'b, H-2', H-3'a, H-4'a), 3.70 (m, 1H, H-4'b), 3.56 (m, 1H, H-3'b), 1.89 (bd, $J_{CH3\text{-}6}$ = 1.0 Hz, 3H, H-5-**CH_3**), 1.81 – 1.90 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 167.65 (C-4), 153.03 (C-2), 144.41 (C-6), 111.10 (C-5), 78.36 (C-2'), 66.97 (C-4'), 61.27 (C-3'), 49.55 (C-1'),

29.96 (d, $J_{C\text{-}P}$ = 128.9 Hz, C-5'), 11.88 (5-**CH_3**); MS-ESI$^-$ *m/z* (%): 307 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{16}O_7N_2P$ (M-H$^+$) 307.0701, found 307.0702; FTIR (KBr, cm^{-1}) ν: 2931, 1681, 1472, 1434, 1386, 1361, 1254, 1099, 1046, 907. $[\alpha]^{20}_D$ = -21.0 (c = 0.247 g/100mL, H_2O).



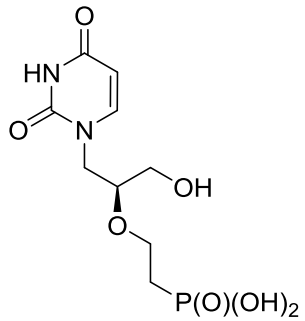
Diethyl (*S*)-{2-[(1-hydroxy-3-[4-methoxy-2-oxo-pyrimidin-1(*2H*)-yl]propan-2-yl)oxy]ethyl}phosphonate (141)

4-Methoxypyrimidin-2(1*H*)-one (416 mg, 3.3 mmol) and sodium hydride (60% suspension in mineral oil) (150 mg, 3.6 mmol) were suspended in dry DMF (40 mL) and heated at 90 °C for 1 h. A solution of compound **134** (1.23 g, 3 mmol) in dry DMF (20 mL) was added dropwise. The reaction mixture was heated at 100 °C for 3 h. After cooling down the solution was neutralized with glacial acetic acid and evaporated. The residue was codistilled with toluene (3 x 30 mL) and purified by silica gel chromatography (gradient from 0-5% methanol in chloroform) to afford (after evaporation) two products as a colorless oils: 415 mg (yield 38%) of compound **141** and 238 mg (yield 22%) of the corresponding *O*-isomer **142**; ^{1}H NMR (DMSO-d_6) δ: 7.85 (d, $J_{6\text{-}5}$ = 7.2 Hz, 1H, H-6), 5.96 (d, $J_{5\text{-}6}$ = 7.2 Hz, 1H, H-5), 4.81 (t, $J_{OH\text{-}3'a}$ = $J_{OH\text{-}3'b}$ = 5.8 Hz, 1H, 3'-OH), 4.02 (dd, J_{gem} = 13.2 Hz, $J_{1'a\text{-}2'}$ = 3.6 Hz, 1H, H-1'a), 3.90 – 4.00 (m, 4H, P-O-**CH_2**-CH_3), 3.81 (s, 3H, O-**CH_3**), 3.60 – 3.70 (m, 2H, H-1'b, H-4'a), 3.58 (m, 1H, H-2'), 3.42 – 3.51 (m, 2H, H-3'a, H-4'b), 3.38 (m, 1H, H-3'b), 1.89 – 1.97 (m, 2H, H-5'), 1.20 (2xt, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 171.31 (C-4), 155.73 (C-2), 150.45 (C-6), 93.78 (C-5), 77.84 (C-2'), 64.07 (C-4'), 61.23 (m, P-O-**CH_2**-CH_3), 61.20 (C-3'), 61.18 (m, P-O-**CH_2**-CH_3), 53.80 (O-**CH_3**), 50.66 (C-1'), 26.49 (d, $J_{C\text{-}P}$ = 128.3 Hz, C-5'), 16.39 (2xd, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 365 (10, M+H$^+$), 387 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{25}O_7N_2NaP$ (M+Na$^+$) 387.1292, found 387.1291; FTIR (KBr, cm^{-1}) ν: 1667, 1608, 1542, 1484, 1416, 1391, 1239, 1230, 1207, 1113, 1051, 1028, 988, 962, 788. $[\alpha]^{20}_D$ = -89.2 (c = 0.288 g/100mL, MeOH).



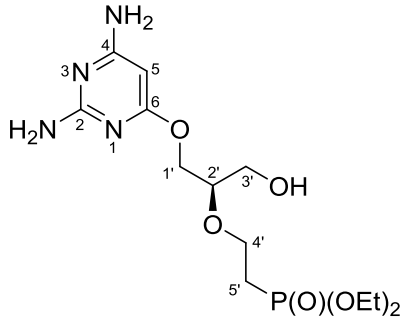
Diethyl (*S*)-{2-[(1-hydroxy-3-[(4-methoxypyrimidin-2-yl) oxy]propan-2-yl)oxy]ethyl}phosphonate (142)

^{1}H NMR (DMSO-d$_6$) δ: 8.27 (d, $J_{6\text{-}5}$ = 5.7 Hz, 1H, H-6), 6.56 (d, $J_{5\text{-}6}$ = 5.7 Hz, 1H, H-5), 4.78 (t, $J_{OH\text{-}3'a}$ = $J_{OH\text{-}3'b}$ = 5.7 Hz, 1H, 3'-OH), 4.38 (dd, J_{gem} = 11.4 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1'a), 4.26 (dd, J_{gem} = 11.4 Hz, $J_{1'b\text{-}2'}$ = 5.9 Hz, 1H, H-1'b), 3.93 – 4.03 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.89 (s, 3H, O-**CH$_3$**), 3.63 – 3.84 (m, 3H, H-2', H-3'a, H-4'a), 3.47 – 3.57 (m, 2H, H-3'b, H-4'b), 2.01 – 2.09 (m, 2H, H-5'), 1.21 (2xt, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 171.15 (C-4), 164.77 (C-2), 159.14 (C-6), 102.02 (C-5), 78.43 (C-2'), 66.73 (C-1'), 63.71 (d, $J_{C\text{-}C\text{-}P}$ = 1.2 Hz, C-4'), 61.20 (d, $J_{C\text{-}O\text{-}P}$ = 5.7 Hz, P-O-**CH$_2$**-CH$_3$), 61.15 (d, $J_{C\text{-}O\text{-}P}$ = 5.7 Hz, P-O-**CH$_2$**-CH$_3$), 60.51 (C-3'), 53.85 (O-**CH$_3$**), 26.60 (d, $J_{C\text{-}P}$ = 136.5 Hz, C-5'), 16.40 (2xd, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): identical to compound **16**; FTIR (KBr, cm^{-1}) ν: 2983, 2930, 1739, 1608, 1574, 1483, 1454, 1421, 1285, 1248, 1239, 1206, 1120, 1052, 1028, 985, 962, 790. $[\alpha]^{20}_D$ = -22.7 (c = 0.278 g/100mL, MeOH).



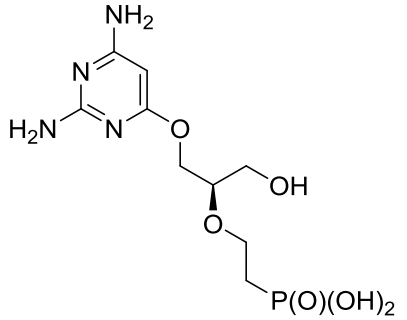
(*S*)-{2-[(1-(2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)-3-hydroxypropan-2-yl)oxy]ethyl}phosphonic acid (143)

Treatment of compound **141** (182 mg, 0.5 mmol) by **method D1** followed by **method E** gave 110 mg (yield 75%) of compound **143** as a white solid; ^{1}H NMR (D$_2$O) δ: 7.64 (d, $J_{6\text{-}5}$ = 7.9 Hz, 1H, H-6), 5.84 (d, $J_{5\text{-}6}$ = 7.9 Hz, 1H, H-5), 4.05 (bdd, J_{gem} = 13.1 Hz, $J_{1'a\text{-}2'}$ = 2.4 Hz, 1H, H-1'a), 3.65 – 3.86 (m, 5H, H-1'b, H-2', H-3'a, H-4'), 3.58 (m, 1H, H-3'b), 1.81 – 1.90 (m, 2H, H-5'); ^{13}C NMR (D$_2$O) δ: 167.49 (C-4), 153.01 (C-2), 148.69 (C-6), 101.87 (C-5), 78.23 (C-2'), 67.06 (d, $J_{C\text{-}C\text{-}P}$ = 1.8 Hz, C-4'), 61.21 (C-3'), 49.80 (C-1'), 30.09 (d, $J_{C\text{-}P}$ = 128.3 Hz, C-5'); MS-ESI$^-$ *m/z* (%): 293 (100, M-H$^+$), 315 (60, M+Na$^+$); HRMS-ESI$^-$: *m/z* calcd for C$_9$H$_{14}$O$_7$N$_2$P (M-H$^+$) 293.0544, found 293.0548; FTIR (KBr, cm^{-1}) ν: 1684, 1465, 1394, 1357, 1255, 1099, 1046, 902, 810, 765. $[\alpha]^{20}_D$ = -34.5 (c = 0.527 g/100mL, H$_2$O).



Diethyl (*R*)-{2-[(1-[(2,6-diaminopyrimidin-4-yl) oxy]-3-hydroxypropan-2-yl)oxy]ethyl} phosphonate (144)

2,6-Diamino-4-hydroxypyrimidine (858 mg, 6.8 mmol) and caesium carbonate (1.34 g, 4.1 mmol) were suspended in dry DMF (40 mL), the suspension was heated at 100 °C until the components were dissolved. A solution of compound **134** (1.35 g, 3.4 mmol) in dry DMF (20 mL) was added dropwise. The reaction mixture was heated at 100 °C for 10 h. Solvent was evaporated and the residue was codistilled with toluene (3 x 30 mL) and purified by silica gel chromatography (gradient from 2-10% methanol in chloroform) to afford (after evaporation) 434 mg (yield 35%) of compound **144** as a yellowish oil which solidified. The corresponding *N*-isomer was not isolated; ^{1}H NMR (DMSO-d$_6$) δ: 6.00 (bs, 2H, 4-NH$_2$), 5.85 (bs, 2H, 2-NH$_2$), 5.03 (s, 1H, H-5), 4.71 (bs, 1H, 3'-OH), 4.16 (dd, J_{gem} = 11.3 Hz, $J_{1'a\text{-}2'}$ = 4.2 Hz, 1H, H-1'a), 4.08 (dd, J_{gem} = 11.3 Hz, $J_{1'b\text{-}2'}$ = 6.0 Hz, 1H, H-1'b), 3.94 – 4.02 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.68 – 3.78 (m, 2H, H-4'), 3.56 (m, 1H, H-2'), 3.47 (dd, J_{gem} = 11.4 Hz, $J_{3'a\text{-}2'}$ = 4.9 Hz, 1H, H-3'a), 3.43 (dd, J_{gem} = 11.4 Hz, $J_{3'b\text{-}2'}$ = 5.8 Hz, 1H, H-3'b), 2.05 (dt, $J_{5'\text{-}P}$ = 18.1 Hz, $J_{5'\text{-}4'}$ = 7.3 Hz, 2H, H-5'), 1.22 (2xt, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 170.08 (C-6), 166.19 (C-4), 163.07 (C-2), 78.81 (C-2'), 76.30 (C-5), 64.68 (C-1'), 63.71 (C-4'), 61.19 (m, P-O-**CH$_2$**-CH$_3$), 60.78 (C-3'), 26.59 (d, $J_{C\text{-}P}$ = 136.7 Hz, C-5'), 16.42 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 365 (100, M+H$^+$), 387 (25, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{26}O_6N_4P$ (M+H$^+$) 365.1585, found 365.1585; FTIR (KBr, cm^{-1}) ν: 3464, 3337, 3212, 2982, 2958, 2908, 2876, 1625, 1591, 1546, 1521, 1480, 1425, 1311, 1216, 1163, 1117, 1098, 1049, 1029, 964. $[\alpha]^{20}_D$ = -8.0 (c = 0.126 g/100mL, DMSO).

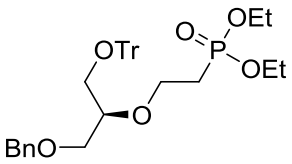


(*R*)-{2-[(1-[(2,6-Diaminopyrimidin-4-yl)oxy]-3-hydroxypropan-2-yl)oxy]ethyl}phosphonic acid (145)

Treatment of compound **144** (182 mg, 0.5 mmol) by **method D1** followed by **method E** gave 117 mg (yield 76%) of compound **145** as a yellowish solid; ^{1}H NMR (DMSO-d$_6$) δ: 6.03 (bs, 2H, 4-NH$_2$), 5.91 (bs, 2H, 2-NH$_2$), 5.05 (s, 1H, H-

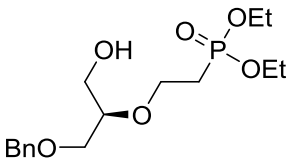
5), 4.09 (dd, J_{gem} = 11.1 Hz, $J_{1'a\text{-}2'}$ = 4.9 Hz, 1H, H-1'a), 4.06 (dd, J_{gem} = 11.1 Hz, $J_{1'b\text{-}2'}$ = 5.4 Hz, 1H, H-1'b), 3.63 – 3.75 (m, 2H, H-4'), 3.50 (m, 1H, H-2'), 3.45 (dd, J_{gem} = 11.4 Hz, $J_{3'a\text{-}2'}$ = 4.8 Hz, 1H, H-3'a), 3.39 (dd, J_{gem} = 11.4 Hz, $J_{3'b\text{-}2'}$ = 5.5 Hz, 1H, H-3'b), 1.62 – 1.69 (m, 2H, H-5'); ^{13}C NMR (DMSO-d_6) δ: 170.19 (C-6), 166.17 (C-4), 163.04 (C-2), 78.26 (C-2'), 76.28 (C-5), 66.21 (C-4'), 64.65 (C-1'), 60.78 (C-3'), 31.03 (d, $J_{C\text{-}P}$ = 130.9 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 309 (100, M+H$^+$), 331 (20, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_9H_{18}O_6N_4P$ (M+H$^+$) 309.0959, found 309.0959; FTIR (KBr, cm^{-1}) ν: 3400, 3324, 3192, 1680, 1657, 1615, 1561, 1520, 1468, 1446, 1399, 1313, 1238, 1114, 1049, 1029, 921. $[\alpha]^{20}_D$ = -2.1 (c = 0.322 g/100mL, H_2O).

5.6.3. Synthesis of (*S*)-CPEEHx and (*S*)-CPEEG derivatives



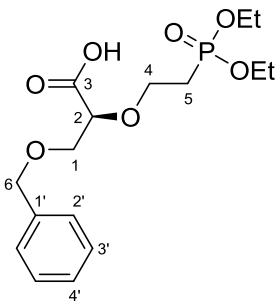
Diethyl (*S*)-{2-[(1-(benzyloxy)-3-(trityloxy)propan-2-yl) oxy]ethyl}phosphonate (146)

Compound **104** (17 g, 40 mmol) was treated analogously to compound **116** to give 13 g (yield 55%) of compound **146** as a yellowish oil (in the form of a crude product). MS spectrum is identical to compound **116**.



Diethyl (*R*)-{2-[(1-(benzyloxy)-3-hydroxypropan-2-yl) oxy]ethyl}phosphonate (147)

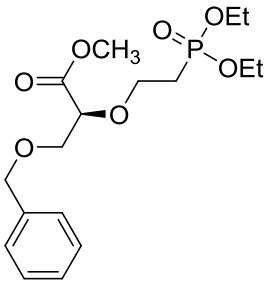
Compound **146** (13 g of the crude product) was treated analogously compound **117** to give 5.1 g (yield 37%) of compound **147** as a colorless oil; ^{1}H NMR, ^{13}C NMR and MS spectra are identical to compound **117**. $[\alpha]^{20}_D$ = +8.3 (c = 0.289 g/100mL, $CHCl_3$).



(*S*)-3-(Benzyloxy)-2-[2-(diethoxyphosphoryl)ethoxy] propanoic acid (148)

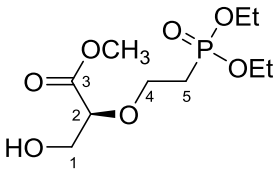
Compound **147** (4.2 g, 12 mmol) was treated analogously to compound **106** (chromatography: gradient from 0-3% methanol in chloroform) to give 3.6 g (yield 83%) of compound **148** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 7.27 – 7.36 (m, 5H, H-2', 3', 4'), 4.57 (s, 2H, H-6), 4.08 – 4.18 (m, 5H, H-2, P-O-**CH_2**-

CH_3), 3.99 (m, 1H, H-4a), 3.91 (dd, J_{gem} = 10.5 Hz, $J_{1a\text{-}2}$ = 2.7 Hz, 1H, H-1a), 3.85 (m, 1H, H-4b), 3.78 (dd, J_{gem} = 10.5 Hz, $J_{1b\text{-}2}$ = 6.6 Hz, 1H, H-1b), 2.25 (m, 2H, H-5a), 2.10 (m, 2H, H-5b), 1.33 (t, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 3H, P-O-CH_2-**CH_3**), 1.29 (t, $J_{CH3\text{-}CH2}$ = 7.0 Hz, 3H, P-O-CH_2-**CH_3**); ^{13}C NMR ($CDCl_3$) δ: 171.39 (C-3), 137.64 (C-1‘), 128.30 (C-3‘), 127.65 (C-4‘), 127.55 (C-2‘), 79.29 (C-2), 73.45 (C-6), 70.90 (C-1), 65.42 (d, $J_{C\text{-}C\text{-}P}$ = 6.4 Hz, C-4), 62.50 (d, $J_{C\text{-}O\text{-}P}$ = 6.3 Hz, P-O-**CH_2**-CH_3), 62.00 (d, $J_{C\text{-}O\text{-}P}$ = 6.7 Hz, P-O-**CH_2**-CH_3), 26.51 (d, $J_{C\text{-}P}$ = 141.7 Hz, C-5), 16.23 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.9 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 361 (45, M+H$^+$), 383 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{26}O_7P$ (M+H$^+$) 361.1411, found 361.1409. Anal. Calcd. for $C_{16}H_{26}O_7P$ $0.3H_2O$: C, 52.54; H, 7.06; P, 8.01. Found: C, 52.68; H, 7.08; P, 7.83. $[\alpha]^{20}_D$ = -23.4 (c = 0.295 g/100mL, $CHCl_3$).



Methyl (*S*)-3-(benzyloxy)-2-[2-(diethoxyphosphoryl) ethoxy]propanoate (149)

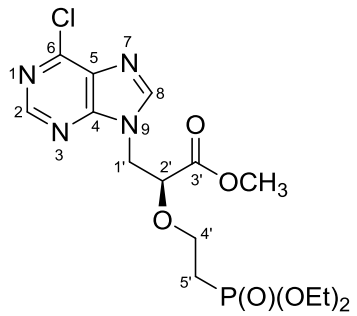
Compound **148** (3.6 g, 10 mmol) was treated analogously to compound **107** to give 3.7 g (yield 99%) of compound **149** as a colorless oil; MS-ESI$^+$ *m/z* (%): 375 (15, M+H$^+$), 397 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{17}H_{27}O_7NaP$ (M+Na$^+$) 397.1387, found 397.1386.



Methyl (*S*)-2-[2-(diethoxyphosphoryl)ethoxy]-3-hydroxy propanoate (150)

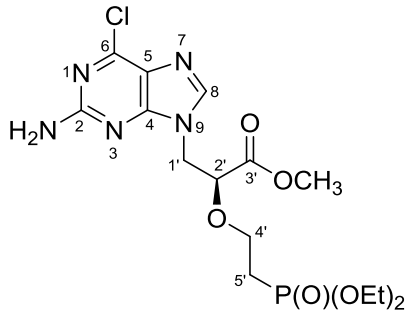
Compound **149** (3.56 g, 9.5 mmol) was dissolved in the mixture of methanol and acetic acid (3:1, 120 mL) and treated analogously to compound **108** (chromatography: gradient from 0-5% methanol in chloroform) to give 2.5 g (yield 93%) of compound **150** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ: 4.04 – 4.19 (m, 6H, H-2, H-4a, P-O-**CH_2**-CH_3), 3.90 (dd, J_{gem} = 12.0 Hz, $J_{1a\text{-}2}$ = 3.2 Hz, 1H, H-1a), 3.69 – 3.80 (m, 5H, H-1b, H-4b, O-**CH_3**), 2.05 – 2.24 (m, 2H, H-5), 1.34 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**), 1.33 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**); ^{13}C NMR ($CDCl_3$) δ: 170.63 (C-3), 80.49 (C-2), 64.79 (d, $J_{C\text{-}C\text{-}P}$ = 5.9 Hz, C-4), 62.76 (C-1), 62.09 (d, $J_{C\text{-}O\text{-}P}$ = 6.5 Hz, P-O-**CH_2**-CH_3), 61.73 (d, $J_{C\text{-}O\text{-}P}$ = 6.5 Hz, P-O-**CH_2**-CH_3), 52.08 (O-**CH_3**), 26.73 (d, $J_{C\text{-}P}$ = 142.1 Hz, C-5), 16.35 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 285 (5, M+H$^+$), 307 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{21}O_7NaP$ (M+Na$^+$)

307.0917, found 307.0916. Anal. Calcd. for: $C_{10}H_{21}O_7P\ H_2O$: C, 39.74; H, 7.67; P, 10.25. Found: C, 39.91; H, 7.69; P, 9.98. $[\alpha]^{20}_D$ = -41.7 (c = 0.281 g/100mL, $CHCl_3$).



Methyl (*S*)-3-(6-chloro-*9H*-purin-9-yl)-2-[2-(diethoxyphosphoryl)ethoxy]propanoate (151)

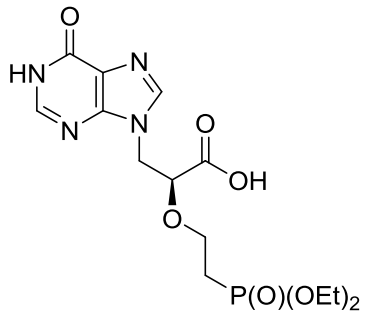
From alcohol **150** (1.71 g, 6 mmol) and 6-chloropurine (1.21 g, 7.8 mmol), applied **method G**, obtained 1.1 g (yield 44%) of compound **151** as a yellow oil; ^{1}H NMR (DMSO-d_6) δ: 8.75 (s, 1H, H-2), 8.32 (s, 1H, H-8), 4.72 (dd, J_{gem} = 14.5 Hz, $J_{1'a\text{-}2'}$ = 3.9 Hz, 1H, H-1'a), 4.58 (dd, J_{gem} = 14.6 Hz, $J_{1'b\text{-}2'}$ = 6.7 Hz, 1H, H-1'b), 4.31 (dd, $J_{2'\text{-}1'a}$ = 3.8 Hz, $J_{2'\text{-}1'b}$ = 6.8 Hz, 1H, H-2'), 4.03 – 4.14 (m, 4H, P-O-**C**$\mathbf{H_2}$-CH_3), 3.96 (m, 1H, H-4'a), 3.75 (s, 3H, O-**C**$\mathbf{H_3}$), 3.62 (m, 1H, H-4'b), 2.00 – 2.14 (m, 2H, H-5'), 1.31 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 169.32 (C-3'), 151.98 (C-2), 151.71 (C-4), 151.02 (C-6), 146.53 (C-8), 131.13 (C-5), 76.55 (C-2'), 65.43 (C-4'), 61.87 (m, P-O-**C**$\mathbf{H_2}$-CH_3), 52.67 (O-**C**$\mathbf{H_3}$), 45.43 (C-1'), 26.87 (d, $J_{C\text{-}P}$ = 140.8 Hz, C-5'), 16.37 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 6.0 Hz, P-O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 421 (35, M+H$^+$), 443 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{23}O_6N_4ClP$ (M+H$^+$) 421.1038, found 421.1037. Anal. Calcd. for: $C_{15}H_{22}ClN_4O_6P$: C, 42.82; H, 5.27; Cl, 8.43; N, 13.31; P, 7.36. Found: C, 42.65; H, 5.37; Cl, 8.25; N, 13.03; P, 7.15. $[\alpha]^{20}_D$ = -5.3 (c = 0.316 g/100mL, DMSO).



Methyl (*S*)-3-(2-amino-6-chloro-*9H*-purin-9-yl)-2-[2-(diethoxyphosphoryl)ethoxy]propanoate (152)

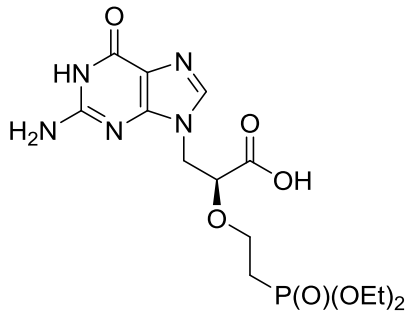
Compound **150** (1.99 g, 7 mmol) and 2-amino-6-chloropurine (1.54 g, 9.1 mmol), applied **method H** to give 1.16 g (yield 38%) of compound **152** as a yellowish oil; ^{1}H NMR (DMSO-d_6) δ: 8.05 (s, 1H, H-8), 6.93 (s, 2H, NH_2), 4.46 (dd, $J_{2'\text{-}1'a}$ = 4.1 Hz, $J_{2'\text{-}1'b}$ = 7.1 Hz, 1H, H-2'), 4.41 (dd, J_{gem} = 14.5 Hz, $J_{1'a\text{-}2'}$ = 4.0 Hz, 1H, H-1'a), 4.31 (dd, J_{gem} = 14.5 Hz, $J_{1'b\text{-}2'}$ = 7.1 Hz, 1H, H-1'b), 3.90 – 3.99 (m, 4H, P-O-**C**$\mathbf{H_2}$-CH_3), 3.72 (m, 1H, H-4'a), 3.66 (s, 3H, O-**C**$\mathbf{H_3}$), 3.54 (m, 1H, H-4'b), 1.91 – 2.05 (m, 2H, H-5'), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 170.01 (C-3'), 160.02 (C-2), 154.35 (C-4), 149.48 (C-6), 144.00 (C-

8), 123.08 (C-5), 76.03 (C-2‘), 64.50 (C-4‘), 61.29 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 52.40 (O-**C**$\mathbf{H_3}$), 44.46 (C-1‘), 26.11 (d, $J_{C\text{-}P}$ = 137.3 Hz, C-5‘), 16.41 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.7 Hz, P-O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 436 (40, M+H$^+$), 458 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{24}O_6N_5ClP$ (M+H$^+$) 436.1147, found 436.1146. $[\alpha]^{20}_D$ = -7.4 (c = 0.398 g/100mL, DMSO).



(*S*)-2-[2-(Diethoxyphosphoryl)ethoxy]-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propanoic acid (153)

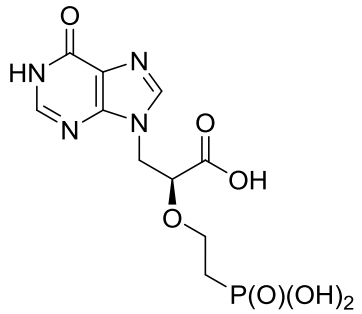
Treatment of compound **151** (421 mg, 1 mmol) by **method I** (chromatography: linear gradient of H1 in ethyl acetate) gave 221 mg (yield 57%) of compound **153** as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: 12.48 (bs, 1H, NH), 8.022 (s, 1H, H-2), 8.017 (s, 1H, H-8), 4.47 (dd, J_{gem} = 14.2 Hz, $J_{1'a\text{-}2'}$ = 3.5 Hz, 1H, H-1’a), 4.21 (dd, J_{gem} = 14.2 Hz, $J_{1'b\text{-}2'}$ = 8.5 Hz, 1H, H-1‘b), 3.88 – 3.97 (m, 4H, P-O-**C**$\mathbf{H_2}$-CH_3), 3.86 (dd, $J_{2'\text{-}1'a}$ = 3.5 Hz, $J_{2'\text{-}1'b}$ = 8.4 Hz, 1H, H-2‘), 3.80 (m, 1H, H-4’a), 3.39 (m, 1H, H-4’a), 1.86 – 1.97 (m, 2H, H-5‘), 1,17 (t, J_{CH3CH2} = 7.1 Hz, 6H, P-O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 172.00 (C-3‘), 157.01 (C-6), 148.73 (C-4), 145.53 (C-2), 141.06 (C-8), 123.70 (C-5), 79.92 (C-2‘), 63.17 (C-4‘), 61.23 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 61.22 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 46.07 (C-1‘), 26.19 (d, $J_{C\text{-}P}$ = 136.8 Hz, C-5‘), 16.39 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^-$ *m/z* (%): 387 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{14}H_{20}O_7N_4P$ (M-H$^+$) 387.1075, found 387.1074; FTIR (KBr, cm^{-1}) ν: 2982, 1698, 1608, 1549, 1522, 1390, 1350, 1229, 1124, 1052, 1028, 963. $[\alpha]^{20}_D$ = -14.0 (c = 0.377 g/100mL, MeOH).



(*S*)-3-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-2-[2-(diethoxyphosphoryl)ethoxy]propanoic acid (154)

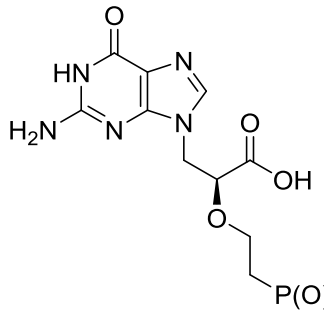
Treatment of compound **152** (436 mg, 1 mmol) by **method I** (chromatography: linear gradient of H1 in ethyl acetate) gave 222 mg (yield 55%) of compound **152** as a yellowish solid. **OR:** Compound **152** (436 mg, 1 mmol) was dissolved in 15 ml of 75% CF_3COOH and the solution was heated at 50 °C overnight. Volatiles were evaporated and a residue was purified by silica gel

chromatography (gradient from 10-50% methanol in chloroform) to afford (after evaporation) 314 mg (yield 78%) of compound **154** as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: 11.49 (bs, 1H, NH), 7.60 (s, 1H, H-8), 6.98 (bs, 2H, NH_2), 4.32 (dd, J_{gem} = 14.1 Hz, $J_{1'a\text{-}2'}$ = 3.2 Hz, 1H, H-1'a), 3.87 – 3.99 (m, 5H, H-1'b, P-O-**CH_2**-CH_3), 3.75 – 3.84 (m, 2H, H-2', H-4'a), 3.29 – 3.42 (m, 1H, H-4'b), 1.88 – 1.96 (m, 2H, H-5'), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 172.66 (C-3'), 157.46 (C-6), 154.18 (C-2), 151.50 (C-4), 138.21 (C-8), 116.26 (C-5), 80.02 (C-2'), 63.26 (C-4'), 61.24 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**CH_2**-CH_3), 45.57 (C-1'), 26.74 (d, $J_{C\text{-}P}$ = 136.5 Hz, C-5'), 16.41 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 404 (25, M+H$^+$), 426 (100, M+Na$^+$), 448 (85, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{23}O_7N_5P$ (M+H$^+$) 404.1330, found 404.1329; FTIR (KBr, cm^{-1}) ν: 3413, 3319, 3128, 2983, 2930, 2775, 1740, 1693, 1621, 1538, 1481, 1369, 1101. $[\alpha]^{20}_D$ = -49.8 (c = 0.283 g/100mL, DMSO).



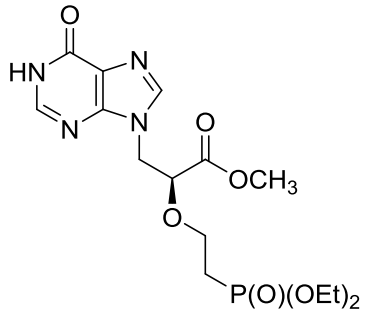
(*S*)-3-(6-Oxo-1,6-dihydro-*9H*-purin-9-yl)-2-(2-phosphonoethoxy)propanoic acid (155)

Treatment of compound **153** (194 mg, 0.5 mmol) by **method D1** followed by **method E** gave 125 mg (yield 75%) of compound **155** as a yellowish solid; ^{1}H NMR (D_2O) δ: 8.02 (s, 1H, H-2), 8.01 (s, 1H, H-8), 4.40 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 3.2 Hz, 1H, H-1'a), 4.19 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 8.4 Hz, 1H, H-1'b), 3.83 (dd, $J_{2'\text{-}1'a}$ = 3.2 Hz, $J_{2'\text{-}1'b}$ = 8.4 Hz, 1H, H-2'), 3.56 (m, 1H, H-4'a), 3.31 (m, 1H, H-4'b), 1.45 – 1.58 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 175.67 (C-3'), 158.67 (C-6), 150.00 (C-4), 146.78 (C-2), 143.17 (C-8), 124.16 (C-5), 80.58 (C-2'), 67.39 (C-4'), 47.30 (C-1'), 31.2 (C-5'), 30.2 (C-5'); MS-ESI$^-$ *m/z* (%): 331 (100, M-H$^+$), 353 (30, M+Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{12}O_7N_4P$ (M-H$^+$) 331.0449, found 331.0450; FTIR (KBr, cm^{-1}) ν: 3058, 1692, 1614, 1590, 1551, 1520, 1415, 1349, 1291, 1112, 1063, 900. $[\alpha]^{20}_D$ = -5.0 (c = 0.301 g/100mL, H_2O).



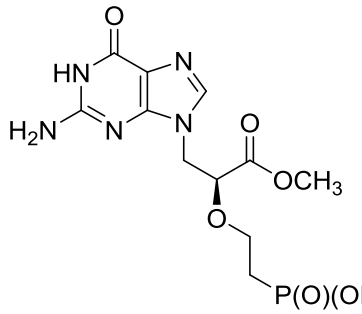
(*S*)-3-(2-Amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-2-(2-phosphonoethoxy)propanoic acid (156)

Treatment of compound **154** (202 mg, 0.5 mmol) by **method D1** followed by **method E** gave 127 mg (yield 73%) of compound **156** as a sallow solid; ^{1}H NMR (D_2O) δ: 7.69 (s, 1H, H-8), 4.32 (dd, J_{gem} = 14.7 Hz, $J_{1'a\text{-}2'}$ = 3.9 Hz, 1H, H-1'a), 4.16 (dd, J_{gem} = 14.7 Hz, $J_{1'b\text{-}2'}$ = 7.1 Hz, 1H, H-1'b), 4.03 (dd, $J_{2'\text{-}1'a}$ = 3.9 Hz, $J_{2'\text{-}1'b}$ = 7.0 Hz, 1H, H-2'), 3.65 (m, 1H, H-4'a), 3.50 (m, 1H, H-4'b), 1.68 – 1.81 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 176.79 (C-3'), 158.87 (C-6), 153.54 (C-2), 151.72 (C-4), 140.44 (C-8), 115.47 (C-5), 78.98 (C-2'), 65.86 (C-4'), 43.25 (C-1'), 28.54 (d, $J_{C\text{-}P}$ = 129.5 Hz, C-5'); MS-ESI$^-$ *m/z* (%): 346 (100, M-H$^+$), 368 (35, M+Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{13}O_7N_5P$ (M-H$^+$) 346.0558, found 346.0556; FTIR (KBr, cm^{-1}) ν: 3388, 3142, 1696, 1601, 1540, 1478, 1410, 1299, 1107, 1063, 906, 781. $[\alpha]^{20}_D$ = -23.7 (c = 0.207 g/100mL, H_2O).



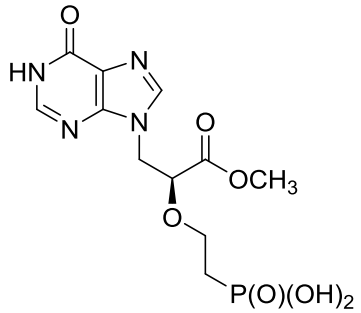
Methyl (*S*)-2-[2-(diethoxyphosphoryl)ethoxy]-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propanoate (157)

Treatment of compound **151** (421 mg, 1 mmol) by **method J** gave 338 mg (yield 84%) of compound **157** as a colorless oil which solidified; ^{1}H NMR (DMSO-d_6) δ: 8.04 (s, 1H, H-2), 8.01 (s, 1H, H-8), 4.38 – 4.52 (m, 3H, H-1', H-2'), 3.90 – 3.98 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.72 (m, 1H, H-4'a), 3.64 (s, 3H, O-**CH$_3$**), 3.53 (m, 1H, H-4'b), 1.91 – 2.05 (m, 2H, H-5'), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d_6) δ: 169.95 (C-3'), 156.83 (C-6), 148.64 (C-4), 145.81 (C-2), 141.16 (C-8), 123.72 (C-5), 76.38 (C-2'), 64.52 (C-4'), 61.29 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**CH$_2$**-CH$_3$), 52.35 (O-**CH$_3$**), 44.78 (C-1'), 26.08 (d, $J_{C\text{-}P}$ = 139.8 Hz, C-5'), 16.41 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 403 (30, M+H$^+$), 425 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{24}O_7N_4P$ (M+H$^+$) 403.1377, found 403.1376; FTIR (KBr, cm^{-1}) ν: 3056, 2980, 2955, 1752, 1695, 1587, 1550, 1441, 1415, 1391, 1220, 1121, 1090, 1045, 1029, 964. $[\alpha]^{20}_D$ = -8.0 (c = 0.263 g/100mL, MeOH).



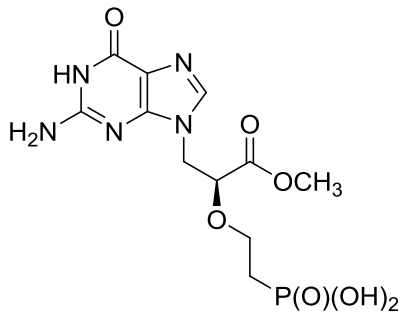
Methyl (*S*)-3-(2-amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-2-[2-(diethoxyphosphoryl)ethoxy] propanoate (158)

Treatment of compound **152** (436 mg, 1 mmol) by **method J** gave 338 mg (yield 81%) of compound **158** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 10.69 (bs, 1H, NH), 7.60 (s, 1H, H-8), 6.57 (bs, 2H, NH_2), 4.40 (dd, $J_{2'\text{-}1'a}$ = 4.1 Hz, $J_{2'\text{-}1'b}$ = 7.3 Hz, 1H, H-2'), 4.28 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1'a), 4.18 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 7.3 Hz, 1H, H-1'b), 3.89 – 4.00 (m, 4H, P-O-**CH_2**-CH_3), 3.71 (m, 1H, H-4'a), 3.66 (s, 3H, O-**CH_3**), 3.51 (m, 1H, H-4'b), 1.92 – 2.02 (m, 2H, H-5'), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 170.09 (C-3'), 156.99 (C-6), 153.90 (C-2), 151.46 (C-4), 138.26 (C-8), 116.28 (C-5), 76.43 (C-2'), 64.50 (C-4'), 61.32 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**CH_2**-CH_3), 52.31 (O-**CH_3**), 44.18 (C-1'), 26.13 (d, $J_{C\text{-}P}$ = 136.5 Hz, C-5'), 16.40 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**), 16.39 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 418 (50, M+H$^+$), 440 (100, M+Na$^+$), 462 (45, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{25}O_7N_5P$ (M+H$^+$) 418.1486, found 418.1485; FTIR (KBr, cm^{-1}) ν: 3434, 3140, 2756, 1681, 1605, 1539, 1470, 1459, 1414, 1358, 1301, 1212, 1143, 1026, 971, 801. $[\alpha]^{20}_D$ = -13.5 (c = 0.310 g/100mL, MeOH).



(*S*)-{2-[(1-Methoxy-1-oxo-3-[6-oxo-1,6-dihydro-*9H*-purin-9-yl]propan-2-yl)oxy]ethyl}phosphonic acid (159)

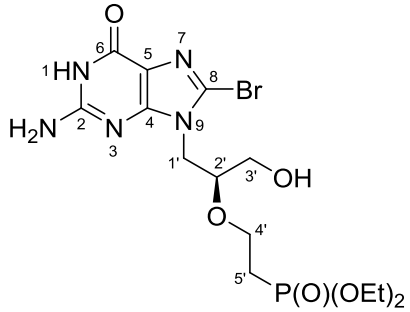
Treatment of compound **157** (201 mg, 0.5 mmol) by **method D1** followed by **method E** gave 123 mg (yield 71%) of compound **159** as a yellowish solid; ^{1}H NMR (D_2O) δ: 8.12 (s, 1H, H-2), 8.06 (s, 1H, H-8), 4.56 – 4.66 (m, 2H, H-1'), 4.50 (m, 1H, H-2'), 3.45 – 3.81 (m, 5H, H-4', O-**CH_3**), 1.72 – 1.99 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 172.23 (C-3'), 158.54 (C-6), 148.89 (C-4), 145.83 (C-2), 142.87 (C-8), 123.03 (C-5), 76.35 (C-2'), 66.95 (C-4'), 53.01 (O-**CH_3**), 45.27 (C-1'), 28.80 (d, $J_{C\text{-}P}$ = 129.4 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 347 (100, M+H$^+$), 369 (95, M+Na$^+$), 391 (55, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{16}O_7N_4P$ (M+H$^+$) 347.0751, found 347.0751; FTIR (KBr, cm^{-1}) ν: 1742, 1677, 1587, 1551, 1434, 1402, 1348, 1123, 1045, 900. $[\alpha]^{20}_D$ = -5.2 (c = 0.278 g/100mL, H_2O)



(*S*)-{2-[(3-[2-Amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl]-1-methoxy-1-oxopropan-2-yl)oxy]ethyl}phosphonic acid (160)

Treatment of compound **158** (209 mg, 0.5 mmol) by **method D1** followed by **method E** gave 125 mg (yield 69%) of compound **160** as a white solid; ^{1}H NMR (D_2O) δ: 7.77 (s, 1H, H-8), 4.48 – 4.53 (m, 2H, H-1'a), 4.48 – 4.53 (m, 2H, H-2'), 4.42 (dd, J_{gem} = 15.6 Hz, $J_{1'b\text{-}2'}$ = 6.0 Hz, 1H, H-1'b), 3.83 (m, 1H, H-4'a), 3.74 (s, 3H, O-**CH_3**), 3.73 (m, 1H, H-4'b), 1.85 – 1.95 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 173.03 (C-3'), 159.49 (C-6), 154.31 (C-2), 152.26 (C-4), 141.18 (C-8), 116.14 (C-5), 76.98 (C-2'), 67.44 (C-4'), 53.62 (O-**CH_3**), 45.20 (C-1'), 29.41 (d, $J_{C\text{-}P}$ = 129.9 Hz, C-5'); MS-ESI$^-$ *m/z* (%): 360 (100, M-H$^+$), 382 (60, M+Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{11}H_{15}O_7N_5P$ (M-H$^+$) 360.0715, found 360.0714; FTIR (KBr, cm^{-1}) ν: 3344, 3158, 2954, 1630, 1598, 1482, 1438, 1412, 1362, 1108, 1074, 900, 783. $[\alpha]^{20}_D$ = -3.1 (c = 0.285 g/100mL, H_2O).

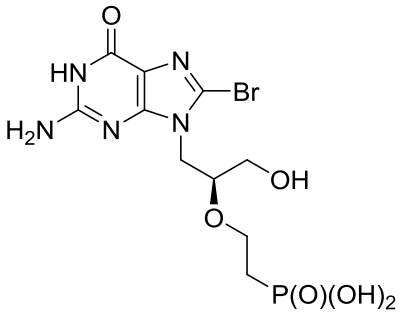
5.6.4. Synthesis of 8-substituted (*S*)-HPEPG derivatives



Diethyl (*S*)-{2-[(1-[2-amino-8-bromo-6-oxo-1,6-dihydro-*9H*-purin-9-yl]-3-hydroxypropan-2-yl) oxy]ethyl}phosphonate (161)

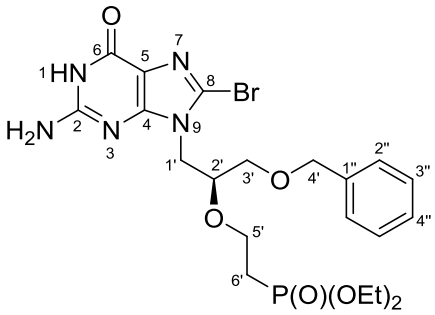
Compound **123** (779 mg, 2 mmol) was dissolved in DMF (10 mL) and a solution of bromine (0.15 mL, 3 mmol) in carbon tetrachloride (10 mL) was added. The reaction mixture was stirred at r.t. for 3 h. Volatiles were evaporated and the residue was codistilled with toluene (3 x 20 mL). The residue was subjected to silica gel chromatography (gradient from 5-12% methanol in chloroform) to afford (after evaporation) the crude product. Crystallization from ethyl acetate gave 800 mg (yield 85%) of compound **161** as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: 10.71 (s, 1H, NH), 6.63 (bs, 2H, NH_2), 3.84 - 4.05 (m, 6H, H-1', P-O-**CH_2**-CH_3), 3.71 (m, 1H, H-2'), 3.57 (m, 1H, H-4'a), 3.47 (dd, J_{gem} = 11.8 Hz, $J_{3'a\text{-}2'}$ = 4.5 Hz, 1H, H-3'a), 3.40 (dd, J_{gem} = 11.8 Hz, $J_{3'b\text{-}2'}$ = 5.5 Hz, 1H, H-3'b), 3.39 (m, 1H, H-4'b), 1.73 – 1.91 (m,

2H, H-5‘), 1.18 (t, $J_{CH3-CH2}$ = 7.0 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 155.72 (C-6), 154.02 (C-2), 152.81 (C-4), 121.74 (C-8), 116.88 (C-5), 78.26 (C-2‘), 64.18 (C-4‘), 61.45 (C-3‘), 61.30 (d, J_{C-O-P} = 6.2 Hz, P-O-**CH_2**-CH_3), 61.22 (d, J_{C-O-P} = 6.2 Hz, P-O-**CH_2**-CH_3), 45.20 (C-1‘), 26.38 (d, J_{C-P} = 135.9 Hz, C-5‘), 16.39 (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH_2-**CH_3**), 16.38 (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 468 (10, M+H$^+$), 470 (9, M+H$^+$), 490 (100, M+Na$^+$), 492 (97, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{23}O_6N_5BrNaP$ (M+Na$^+$) 490.0462, found 490.0461; FTIR (KBr, cm^{-1}) ν: 3417, 3311, 3215, 3149, 2983, 2933, 1690, 1633, 1599, 1523, 1465, 1365, 1221, 1117, 1029, 964. $[\alpha]^{20}_D$ = -19.9 (c = 0.371 g/100mL, DMSO).



(*S*)-{2-[(1-[2-Amino-8-bromo-6-oxo-1,6-dihydro-*9H*-purin-9-yl]-3-hydroxypropan-2-yl)oxy]ethyl} phosphonic acid (162)

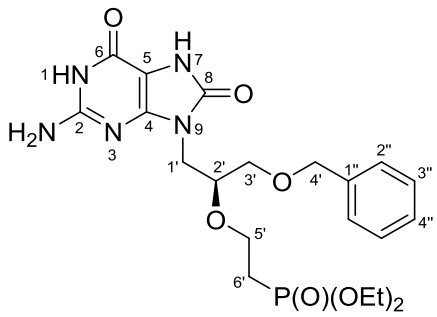
Treatment of compound **161** (234 mg, 0.5 mmol) by **method D1** followed by **method E** gave 159 mg (yield 77%) of compound **162** as a yellowish solid; ^{1}H NMR (D_2O) δ: 4.13 – 4.20 (m, 2H, H-1‘), 3.88 (m, 1H, H-2‘), 3.72 – 3.80 (m, 2H, H-3’a), 3.72 – 3.80 (m, 2H, H-4’a), 3.60 (dd, J_{gem} = 12.4 Hz, $J_{3‘b-2‘}$ = 5.3 Hz, 1H, H-3’b), 3.57 (m, 1H, H-4’b), 1.79 – 1.96 (m, 2H, H-5‘); ^{13}C NMR (D_2O) δ: 158.07 (C-6), 154.30 (C-2), 153.14 (C-4), 125.48 (C-8), 116.67 (C-5), 78.52 (C-2‘), 65.90 (C-4‘), 61.38 (C-3‘), 45.37 (C-1‘), 28.61 (d, J_{C-P} = 132.6 Hz, C-5‘); MS-ESI$^-$ *m/z* (%): 410 (100, M-H$^+$), 412 (97, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{14}O_6N_5BrP$ (M-H$^+$) 409.9871, found 409.9871; FTIR (KBr, cm^{-1}) ν: 3561, 3463, 3328, 3162, 2311, 1689, 1636, 1599, 1410, 1236, 1117, 1043, 931, 776. $[\alpha]^{20}_D$ = -4.3 (c = 0.257 g/100mL, H_2O).



Diethyl (*S*)-{2-[(1-[2-amino-8-bromo-6-oxo-1,6-dihydro-*9H*-purin-9-yl]-3-(benzyloxy) propan-2-yl)oxy]ethyl}phosphonate (163)

Compound **121** (1.92 g, 4 mmol) was dissolved in ethyl acetate (20 mL) and aqueous solution (15 mL) of sodium bromate (1.81 g, 12 mmol) was added. A solution of sodium hydrosulfite (2.6 g, 12 mmol) in water (30 mL) was

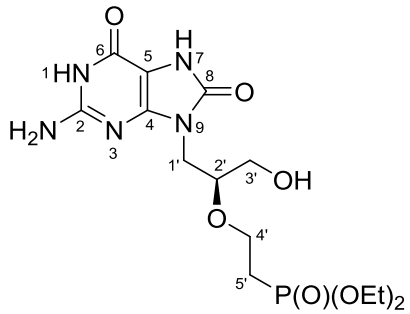
added dropwise to the vigorously stirred two-phase system. The mixture was vigorously stirred at r.t. for 2 h. The reaction was diluted with water and extracted with ethyl acetate (3 x 150 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography (gradient from 1-8% methanol in chloroform) to give (after evaporation) 1.50 g (yield 67%) of compound **163** as a yellow foam; ^{1}H NMR (DMSO-d_6) δ: 10.71 (s, 1H, NH-1), 7.27 – 7.37 (m, 5H, H-2‘‘, H-3‘‘, H-4‘‘), 6.59 (bs, 2H, NH_2), 4.51 (s, 2H, H-4‘), 3.99 – 4.07 (m, 2H, H-1‘), 3.83 – 3.95 (m, 5H, H-2‘), 3.83 – 3.95 (m, 5H, P-O-**CH_2**-CH_3), 3.61 (m, 1H, H-5’a), 3.55 (dd, J_{gem} = 10.5 Hz, $J_{3‘a\text{-}2‘}$ = 4.2 Hz, 1H, H-3’a), 3.49 (dd, J_{gem} = 10.5 Hz, $J_{3‘b\text{-}2‘}$ = 5.4 Hz, 1H, H-3‘b), 3.38 (m, 1H, H-5’b), 1.72 – 1.88 (m, 2H, H-6‘), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 155.75 (C-6), 154.02 (C-2), 152.84 (C-4), 138.27 (C-1‘‘), 128.46 (C-3‘‘), 127.75 (C-2‘‘), 127.71 (C-4‘‘), 121.61 (C-8), 116.94 (C-5), 76.19 (C-2‘), 72.67 (C-4‘), 70.22 (C-3‘), 64.35 (C-5‘), 61.23 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH_2**-CH_3), 61.18 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH_2**-CH_3), 45.41 (C-1‘), 26.42 (d, $J_{C\text{-}P}$ = 135.8 Hz, C-6‘), 16.36 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**), 16.35 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 558 (100, M+H$^+$), 560 (95, M+H$^+$), 580 (45, M+Na$^+$), 582 (43, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{30}O_6N_5BrP$ (M+H$^+$) 558.1112, found 558.1114. $[\alpha]^{20}_D$ = -20.7 (c = 0.304 g/100mL, DMSO).



Diethyl (*S*)-{2-[(1-[2-amino-6,8-oxo-1,6,7,8-tetrahydro-*9H*-purin-9-yl]-3-(benzyloxy) propan-2-yl)oxy]ethyl}phosphonate (164)

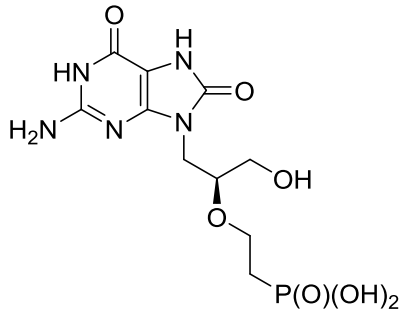
Compound **163** (860 mg, 1.54 mmol) was dissolved in a solution of sodium acetate (1.26 g, 15.4 mmol) in glacial acetic acid (100 mL). The reaction mixture was heated at 130 °C for 4 h. Acetic acid was evaporated and the residue was codistilled with water (1 x 20 mL), toluene (3 x 20 mL) and purified by silica gel chromatography (gradient from 2-8% methanol in chloroform) to afford (after evaporation) 608 mg (yield 80%) of compound **164** as a white foam; ^{1}H NMR (DMSO-d_6) δ: 10.72 (bs, 1H, NH-1), 10.56 (s, 1H, NH-7), 7.25 – 7.36 (m, 5H, H-2‘‘, H-3‘‘, H-4‘‘), 6.48 (bs, 2H, NH_2), 4.47 (bs, 2H, H-4‘), 3.86 – 3.98 (m, 5H, P-O-**CH_2**-CH_3), 3.74 (dd, J_{gem} = 14.0 Hz, $J_{1‘a\text{-}2‘}$ = 7.2 Hz, 1H, H-1’a), 3.67 (m, 1H, H-5’a), 3.63 (dd, J_{gem} = 14.0 Hz, $J_{1‘b\text{-}2‘}$ = 5.6 Hz, 1H, H-1‘b), 3.58 (m, 1H, H-5’b), 3.52 (dd, J_{gem} =

10.6 Hz, $J_{3'a\text{-}2'}$ = 3.8 Hz, 1H, H-3'a), 3.44 (dd, J_{gem} = 10.6 Hz, $J_{3'b\text{-}2'}$ = 5.9 Hz, 1H, H-3'b), 1.81 – 1.96 (m, 2H, H-6'), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**), 1.17 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 153.71 (C-6), 152.57 (C-8), 151.36 (C-2), 148.35 (C-4), 138.44 (C-1''), 128.42 (C-3''), 127.71 (C-2''), 127.64 (C-4''), 98.45 (C-5), 75.67 (C-2'), 72.58 (C-4'), 70.81 (C-3'), 63.90 (C-5'), 61.22 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**CH_2**-CH_3), 61.19 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**CH_2**-CH_3), 40.64 (C-1'), 26.52 (d, $J_{C\text{-}P}$ = 135.6 Hz, C-6'), 16.41 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 496 (15, M+H$^+$), 518 (100, M+Na$^+$), 540 (15, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{30}O_7N_5NaP$ (M+Na$^+$) 518.1775, found 518.1775; FTIR (KBr, cm^{-1}) ν: 3423, 3318, 3025, 1716, 1682, 1596, 1455, 1387, 1365, 1219, 1099, 1027, 962, 736. $[\alpha]^{20}_D$ = -14.6 (c = 0.274 g/100mL, MeOH).



Diethyl (*S*)-{2-[(1-[2-amino-6,8-oxo-1,6,7,8-tetrahydro-*9H*-purin-9-yl]-3-hydroxypropan-2-yl)oxy]ethyl}phosphonate (165)

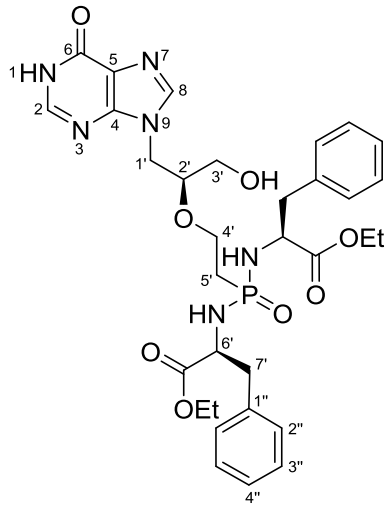
Compound **164** (533 mg, 1.1 mmol) was treated with **method K** to give 361 mg (yield 82%) of compound **165** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 10.67 (bs, 1H, NH-1), 10.60 (s, 1H, NH-7), 6.49 (bs, 2H, NH_2), 4.75 (t, $J_{OH\text{-}3'}$ = 6.0 Hz, OH), 3.88 – 4.01 (m, 4H, P-O-**CH_2**-CH_3), 3.57 – 3.72 (m, 5H, H-1'), 3.57 – 3.72 (m, 5H, H-2'), 3.57 – 3.72 (m, 5H, H-4'), 3.42 (ddd, J_{gem} = 12.0 Hz, $J_{3'a\text{-}OH}$ = 6.1 Hz, $J_{3'a\text{-}2'}$ = 3.9 Hz, 1H, H-3'a), 3.34 (dt, J_{gem} = 12.0 Hz, $J_{3'b\text{-}OH}$ = $J_{1'b\text{-}2'}$ = 5.9 Hz, 1H, H-3'b), 1.85 – 1.97 (m, 2H, H-5'), 1.20 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 153.63 (C-6), 152.69 (C-8), 151.21 (C-2), 148.36 (C-4), 98.41 (C-5), 77.77 (C-2'), 63.61 (C-4'), 61.75 (C-3'), 61.28 (d, $J_{C\text{-}O\text{-}P}$ = 6.3 Hz, P-O-**CH_2**-CH_3), 61.22 (d, $J_{C\text{-}O\text{-}P}$ = 6.3 Hz, P-O-**CH_2**-CH_3), 40.14 (C-1'), 26.46 (d, $J_{C\text{-}P}$ = 135.9 Hz, C-5'), 16.42 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 406 (20, M+H$^+$), 428 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{25}O_7N_5P$ (M+H$^+$) 406.1486, found 406.1486; FTIR (KBr, cm^{-1}) ν: 3169, 1712, 1685, 1593, 1539, 1460, 1382, 1360, 1248, 1226, 1100, 1050, 960. $[\alpha]^{20}_D$ = -4.0 (c = 0.276 g/100mL, MeOH).



(*S*)-{2-[(1-[2-amino-6,8-oxo-1,6,7,8-tetrahydro-*9H*-purin-9-yl]-3-hydroxypropan-2-yl)oxy]ethyl} phosphonic acid (166)

Treatment of compound **165** (203 mg, 0.5 mmol) by **method D1** followed by **method E** gave 135 mg (yield 77%) of compound **166** as a yellowish solid; ^{1}H NMR (D_2O) δ: 3.91 (dd, J_{gem} = 14.6 Hz, $J_{1'a\text{-}2'}$ = 5.1 Hz, 1H, H-1'a), 3.87 (dd, J_{gem} = 14.6 Hz, $J_{1'b\text{-}2'}$ = 6.0 Hz, 1H, H-1'b), 3.73 – 3.87 (m, 3H, H-2'), 3.73 – 3.87 (m, 3H, H-4'), 3.71 (dd, J_{gem} = 12.3 Hz, $J_{3'a\text{-}2'}$ = 3.9 Hz, 1H, H-3'a), 3.55 (dd, J_{gem} = 12.3 Hz, $J_{3'b\text{-}2'}$ = 6.0 Hz, 1H, H-3'b), 1.74 – 1.91 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 154.55 (C-8), 154.21 (C-6), 153.39 (C-2), 150.09 (C-4), 99.77 (C-5), 78.23 (C-2'), 66.44 (d, $J_{C\text{-}C\text{-}P}$ = 1.6 Hz, C-4'), 61.75 (C-3'), 40.59 (C-1'), 29.57 (d, $J_{C\text{-}P}$ = 129.8 Hz, C-5'); MS-ESI$^+$ *m/z* (%): 350 (100, M+H$^+$), 372 (65, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{17}O_7N_5P$ (M+H$^+$) 350.0860, found 350.0860; FTIR (KBr, cm^{-1}) ν: 3444, 3366, 3205, 2646, 1684, 1652, 1607, 1538, 1461, 1222, 1126, 1047, 1014, 978, 964. $[\alpha]^{20}_D$ = -7.3 (c = 0.399 g/100mL, H_2O).

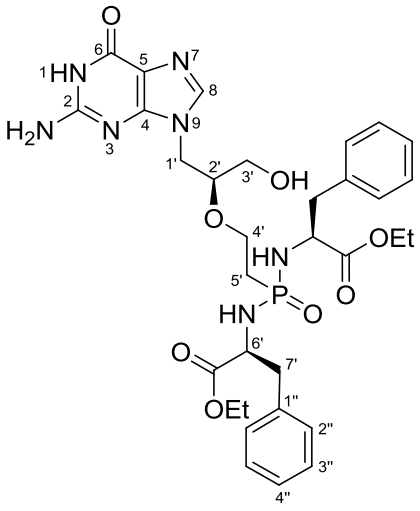
5.6.5. Synthesis of (*S*)-HPEPG(Hx) and (*S*)-CPEEG(Hx) prodrugs



Diethyl 2,2'-{[(2-{[(*S*)-1-hydroxy-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl} phosphoryl]bis(azanediyl)}(2*S*,2'*S*)-bis(3-phenyl propanoate) (167)

Treatment of compound **122** (187 mg, 0.5 mmol) by **method L** (chromatography: gradient from 0-10% methanol in chloroform) gave 103 mg (yield 31%) of compound **167** as a yellowish foam; ^{1}H NMR (DMSO-d_6) δ: 12.29 (bs, 1H, NH-1), 8.04 (s, 1H, H-2), 7.96 (s, 1H, H-8), 7.15 – 7.27 (m, 8H, H-2'', H-3'', H-4''), 7.08 – 7.11 (m, 2H, H-2''), 4.98 (t, $J_{OH\text{-}3'}$ = 5.7 Hz, 1H, OH-3'), 4.49 (dd, $J_{NH\text{-}P}$ = 12.7 Hz, $J_{NH\text{-}6'}$ = 10.8 Hz, 1H, NH), 4.27 (dd, J_{gem} = 14.3 Hz, $J_{1'a\text{-}2'}$ = 3.9 Hz, 1H, H-1'a), 4.10 (dd, $J_{NH\text{-}P}$ = 12.8 Hz, $J_{NH\text{-}6'}$ = 10.5 Hz, 1H, NH), 4.06 (dd, J_{gem} = 14.3 Hz, $J_{1'b\text{-}2'}$ = 7.4 Hz, 1H, H-1'b), 4.01 (q, 4H, O-**CH$_2$**-CH$_3$), 3.89 – 4.01 (m, 2H, H-3'a, H-6'), 3.83 (m, 1H, H-

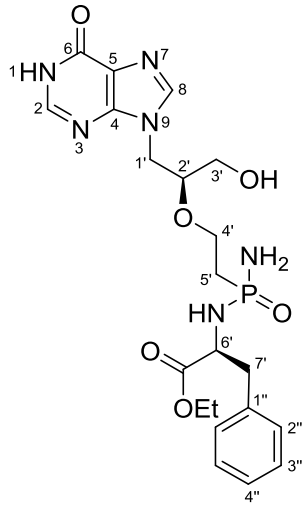
6‘), 3.54 (m, 1H, H-2‘), 3.43 (m, 1H, H-4’a), 3.36 (m, 1H, H-3’b), 3.26 (m, 1H, H-4’b), 2.89 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 6.6 Hz, 1H, H-7‘), 2.83 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 6.3 Hz, 1H, H-7‘), 2.82 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 7.8 Hz, 1H, H-7‘), 2.73 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 7.3 Hz, 1H, H-7‘), 1.40 – 1.55 (m, 2H, H-5‘), 1.10 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**), 1.05 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 173.25 (d, $J_{C\text{-}P}$ = 2.5 Hz, C=O), 173.03 (d, $J_{C\text{-}P}$ = 5.4 Hz, C=O), 156.84 (C-6), 148.69 (C-4), 145.59 (C-2), 141.07 (C-8), 137.48 (C-1‘‘), 137.33 (C-1‘‘), 129.56 (C-2‘‘), 129.52 (C-2‘‘), 128.28 (C-3‘‘), 128.24 (C-3‘‘), 126.64 (C-4‘‘), 126.59 (C-4‘‘), 123.84 (C-5), 78.38 (C-2‘), 63.99 (C-4‘), 60.50 (O-**CH_2**-CH_3), 60.46 (C-3‘), 60.42 (O-**CH_2**-CH_3), 54.30 (C-6‘), 54.11 (C-6‘), 44.38 (C-1‘), 40.0 (C-7‘), 39.9 (C-7‘), 30.15 (d, $J_{C\text{-}P}$ = 111.2 Hz, C-5‘), 14.10 (O-CH_2-**CH_3**), 14.04 (O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 669 (5, M+H$^+$), 691 (100, M+Na$^+$), 713 (20, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{32}H_{41}O_8N_6NaP$ (M+Na$^+$) 691.2616, found 691.2615; FTIR (KBr, cm^{-1}) ν: 3382, 3121, 1978, 1736, 1695, 1603, 1586, 1473, 1414, 1386, 1273, 1198, 1181, 1080, 1031, 746, 701.



Diethyl 2,2’-{[(2-{[(*S*)-1-(2-amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-3-hydroxypropan-2-yl)oxy]ethyl}phosphoryl]bis(azanediyl)}(2*S*,2’*S*)-bis(3-phenylpropanoate) (168)

Treatment of compound **123** (195 mg, 0.5 mmol) by **method L** (chromatography: gradient from 0-12% methanol in chloroform) gave 99 mg (yield 29%) of compound **168** as a yellowish foam; ^{1}H NMR (DMSO-d_6) δ: 10.55 (bs, 1H, NH-1), 7.55 (s, 1H, H-8), 7.15 – 7.28 (m, 8H, H-2‘‘, H-3‘‘, H-4‘‘), 7.09 – 7.12 (m, 2H, H-2‘‘), 6.47 (bs, 1H, NH_2), 4.96 (t, $J_{OH\text{-}3'a}$ = $J_{OH\text{-}3'b}$ = 5.8 Hz, 1H, OH-3‘), 4.52 (dd, $J_{NH\text{-}P}$ = 12.8 Hz, $J_{NH\text{-}6'}$ = 10.8 Hz, 1H, NH), 4.12 (dd, $J_{NH\text{-}P}$ = 12.9 Hz, $J_{NH\text{-}6'}$ = 10.5 Hz, 1H, NH), 4.06 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 4.1 Hz, 1H, H-1‘a), 4.02 (q, 4H, O-**CH_2**-CH_3), 3.90 – 4.01 (m, 2H, H-3’a, H-6‘), 3.85 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 7.1 Hz, 1H, H-1’b), 3.84 (m, 1H, H-6‘), 3.46 (m, 1H, H-2‘), 3.40 (m, 1H, H-4’a), 3.27 – 3.39 (m, 2H, H-3’b, H-4’b), 2.89 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 6.7 Hz, 1H, H-7‘), 2.84 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 6.4 Hz, 1H, H-7‘), 2.83 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 7.7 Hz, 1H, H-7‘), 2.73 (dd, J_{gem} = 13.4 Hz, $J_{7'\text{-}6'}$ = 7.4 Hz, 1H, H-

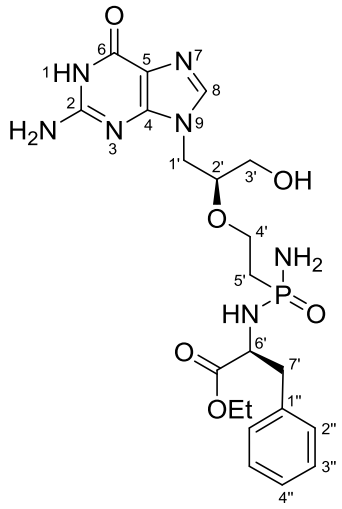
7‘), 1.43 – 1.58 (m, 2H, H-5‘), 1.11 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**), 1.06 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 173.25 (d, $J_{C\text{-}P}$ = 2.4 Hz, C=O), 173.06 (d, $J_{C\text{-}P}$ = 5.0 Hz, C=O), 157.01 (C-6), 153.70 (C-2), 151.49 (C-4), 138.23 (C-8), 137.46 (C-1‘‘), 137.35 (C-1‘‘), 129.57 (C-2‘‘), 129.54 (C-2‘‘), 128.29 (C-3‘‘), 128.25 (C-3‘‘), 126.65 (C-4‘‘), 126.60 (C-4‘‘), 116.41 (C-5), 78.28 (C-2‘), 63.86 (C-4‘), 60.52 (O-**CH_2**-CH_3), 60.45 (C-3‘), 60.43 (O-**CH_2**-CH_3), 54.35 (C-6‘), 54.09 (C-6‘), 43.51 (C-1‘), 40.0 (C-7‘), 30.19 (d, $J_{C\text{-}P}$ = 112.3 Hz, C-5‘), 14.10 (O-CH_2-**CH_3**), 14.04 (O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 684 (20, M+H$^+$), 706 (100, M+Na$^+$), 728 (20, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{32}H_{43}O_8N_7P$ (M+H$^+$) 684.2905, found 684.2907; FTIR (KBr, cm^{-1}) ν: 3337, 3209, 3110, 1735, 1689, 1629, 1600, 1539, 1485, 1443, 1414, 1194, 1181, 1115, 1081, 1031, 961.



Ethyl {amino[2-([(*S*)-1-hydroxy-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl]oxy)ethyl]phosphoryl}-L-phenylalaninate (169)

Treatment of compound **124a** (176 mg, 0.5 mmol) by **method L** (chromatography: gradient from 0-16% methanol in chloroform) gave 69 mg (yield 28%) of compound **169** as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: (2 diastereoisomers in a ratio of 1:0.6) 12.26 (bs, 2H, NH-1), 8.031 (s, 1H, H-2), 8.029 (s, 1H, H-2), 7.984 (s, 1H, H-8), 7.979 (s, 1H, H-8), 7.22 – 7.27 (m, 4H, H-3‘‘), 7.15 – 7.21 (m, 6H, H-2‘‘, H-4‘‘), 5.06 (bs, 2H, OH-3‘), 4.20 – 4.31 (m, 2H, H-1‘), 4.07 – 4.15 (m, 2H, H-1‘), 3.87 – 4.01 (m, 6H, O-**CH_2**-CH_3, H-6‘), 3.81 (d, $J_{H\text{-}P}$ = 2.0 Hz, 2H, NH_2), 3.75 (d, $J_{H\text{-}P}$ = 2.0 Hz, 2H, NH_2), 3.27 – 3.61 (m, 10H, H-2‘, H-3‘, H-4‘), 2.81 – 2.93 (m, 4H, H-7‘), 1.49 – 1.66 (m, 2H, H-5‘), 1.07 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**), 1.06 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 173.38 (d, $J_{C\text{-}P}$ = 2.8 Hz, C=O), 173.26 (d, $J_{C\text{-}P}$ = 4.3 Hz, C=O), 156.90 (C-6), 148.72 (C-4), 145.65 (C-2), 141.08 (C-8), 129.50 (C-2‘‘), 128.28 (C-3‘‘), 126.58 (C-4‘‘), 126.57 (C-4‘‘), 123.85 (C-5), 123.84 (C-5), 78.39 (C-2‘), 78.36 (C-2‘), 64.47 (C-4‘), 64.40 (C-4‘), 60.57 (C-3‘), 60.51 (C-3‘), 60.37 (O-**CH_2**-CH_3), 60.35 (O-**CH_2**-CH_3), 55.28 (C-6‘), 54.97 (C-6‘), 44.47 (C-1‘), 44.44 (C-1‘), 40.03 (C-7‘), 30.95 (d, $J_{C\text{-}P}$ = 111.0 Hz, C-5‘), 30.72 (d, $J_{C\text{-}P}$ = 111.0 Hz, C-5‘), 14.10 (O-CH_2-**CH_3**), 14.09 (O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 493 (10, M+H$^+$), 515 (100, M+Na$^+$), 537 (25,

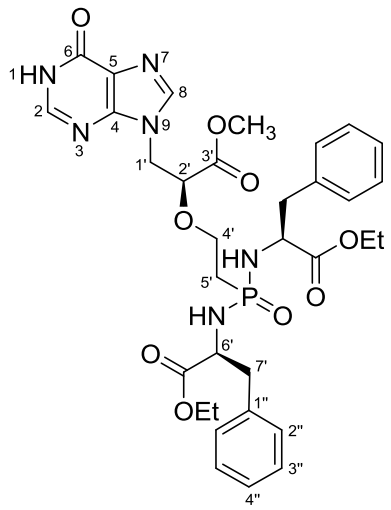
M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_6N_6NaP$ (M+Na$^+$) 515.1778, found 515.1776; FTIR (KBr, cm^{-1}) ν: 3392, 3273, 3118, 2904, 2873, 1733, 1694, 1587, 1559, 1548, 1414, 1345, 1198, 1178, 1121, 1042, 1031, 960, 702. From this reaction was also isolated compound **167** (90 mg, yield 27%).



Ethyl {amino[2-([(*S*)-1-(2-amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-3-hydroxypropan-2-yl]oxy)ethyl] phosphoryl}-L-phenylalaninate (170)

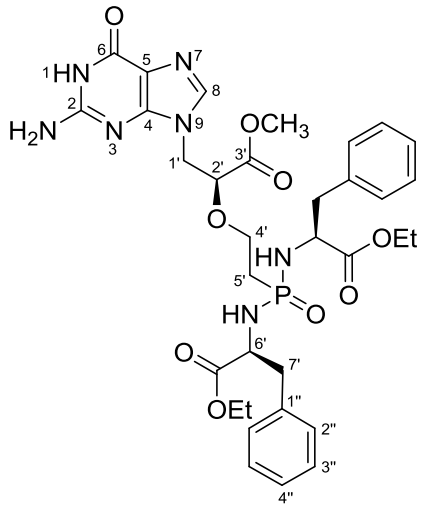
Treatment of compound **125a** (184 mg, 0.5 mmol) by **method L** (chromatography: gradient from 0-24% methanol in chloroform) gave 45 mg (yield 18%) of compound **170** as a yellowish solid; ^{1}H NMR (DMSO-d_6) δ: (2 diastereoisomers in a ratio of 0.4:0.6) 10.61 (bs, 2H, NH-1), 7.58 (s, 1H, H-8), 7.57 (s, 1H, H-8), 7.22 – 7.28 (m, 4H, H-3‘‘), 7.16 – 7.21 (m, 6H, H-2‘‘, H-4‘‘), 6.53 (bs, 4H, NH_2-2), 5.09 (t, $J_{OH\text{-}3‘}$ = 5.8 Hz, 1H, OH-3‘), 5.04 (t, $J_{OH\text{-}3‘}$ = 5.8 Hz, 1H, OH-3‘), 4.33 (t, $J_{NH\text{-}P}$ = $J_{NH\text{-}6‘}$ = 10.5 Hz, 1H, NH-6‘), 4.26 (t, $J_{NH\text{-}P}$ = $J_{NH\text{-}6‘}$ = 10.5 Hz, 1H, NH-6‘), 4.07 (dd, J_{gem} = 14.3 Hz, $J_{1‘a\text{-}2‘}$ = 4.1 Hz, 1H, H-1’a), 4.06 (dd, J_{gem} = 14.3 Hz, $J_{1‘a\text{-}2‘}$ = 4.1 Hz, 1H, H-1’a), 3.99 (q, $J_{CH2\text{-}CH3}$ = 7.1 Hz, 2H, O-**C**$\mathbf{H_2}$-CH_3), 3.98 (q, $J_{CH2\text{-}CH3}$ = 7.1 Hz, 2H, O-**C**$\mathbf{H_2}$-CH_3), 3.90 – 3.98 (m, 2H, H-6‘), 3.90 2x(dd, J_{gem} = 14.3 Hz, $J_{1‘b\text{-}2‘}$ = 7.1 Hz, 1H, H-1‘b), 3.84 (d, $J_{H\text{-}P}$ = 2.2 Hz, 2H, NH_2), 3.78 (d, $J_{H\text{-}P}$ = 2.0 Hz, 2H, NH_2), 3.29 – 3.57 (m, 10H, H-2‘, H-3‘, H-4‘), 2.81 – 2.94 (m, 4H, H-7‘), 1.52 – 1.70 (m, 4H, H-5‘), 1.07 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$), 1.06 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 173.41 (d, $J_{C\text{-}P}$ = 2.7 Hz, C=O), 173.30 (d, $J_{C\text{-}P}$ = 4.0 Hz, C=O), 157.03 (C-6), 153.77 (C-2), 151.54 (C-4), 151.52 (C-4), 138.23 (C-8), 137.63 (C-1‘‘), 137.60 (C-1‘‘), 129.52 (C-2‘‘), 128.31 (C-3‘‘), 126.60 (C-4‘‘), 126.59 (C-4‘‘), 116.40 (C-5), 116.39 (C-5), 78.31 (C-2‘), 78.26 (C-2‘), 64.31 (C-4‘), 64.21 (C-4‘), 60.53 (C-3‘), 60.40 (O-**C**$\mathbf{H_2}$-CH_3), 60.38 (O-**C**$\mathbf{H_2}$-CH_3), 55.33 (C-6‘), 54.99 (C-6‘), 43.64 (C-1‘), 43.58 (C-1‘), 40.10 (C-7‘), 30.97 (d, $J_{C\text{-}P}$ = 110.5 Hz, C-5‘), 30.74 (d, $J_{C\text{-}P}$ = 109.4 Hz, C-5‘), 14.12 (O-CH_2-**C**$\mathbf{H_3}$), 14.11 (O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 508 (15, M+H$^+$), 530 (100, M+Na$^+$), 552 (25, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{30}O_6N_7NaP$ (M+Na$^+$) 530.1887, found 530.1885; FTIR (KBr, cm^{-1}) ν: 3335, 3190, 3115, 2905, 1736, 1690,

1631, 1600, 1541, 1415, 1180, 1118, 1033, 961, 703. From this reaction was also isolated compound **168** (85 mg, yield 25%).



Diethyl 2,2'-{[(2-{[(*S*)-1-methoxy-1-oxo-3-(6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl)oxy]ethyl} phosphoryl]bis(azanediyl)}(2*S*,2'*S*)-bis(3-phenyl propanoate) (171)

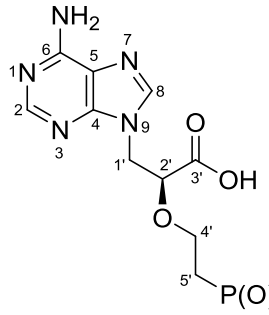
Treatment of compound **157** (201 mg, 0.5 mmol) by **method L** (chromatography: gradient from 0-8% methanol in chloroform) gave 98 mg (yield 28%) of compound **171** as a yellowish foam; ^{1}H NMR (DMSO-d_6) δ: 12.33 (bs, 1H, NH-1), 8.05 (s, 1H, H-2), 7.93 (s, 1H, H-8), 7.10 – 7.28 (m, 8H, H-2‘‘, H-3‘‘, H-4‘‘), 7.07 – 7.11 (m, 2H, H-2‘‘), 4.51 (dd, $J_{NH\text{-}P}$ = 12.8 Hz, $J_{NH\text{-}6‘}$ = 10.8 Hz, 1H, NH), 4.44 (dd, J_{gem} = 14.4 Hz, $J_{1‘a\text{-}2‘}$ = 4.2 Hz, 1H, H-1'a), 4.33 (dd, J_{gem} = 14.4 Hz, $J_{1‘b\text{-}2‘}$ = 6.8 Hz, 1H, H-1‘b), 4.26 (dd, $J_{2‘\text{-}1'a}$ = 4.2 Hz, $J_{2‘\text{-}1'b}$ = 6.8 Hz, 1H, H-2‘), 4.12 (dd, $J_{NH\text{-}P}$ = 13.0 Hz, $J_{NH\text{-}6‘}$ = 10.5 Hz, 1H, NH), 4.01 (q, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 2H, O-**C**$\mathbf{H_2}$-CH_3), 3.97 (q, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 2H, O-**C**$\mathbf{H_2}$-CH_3), 3.94 (m, 1H, H-6‘), 3.82 (m, 1H, H-6‘), 3.65 (s, 3H, O-**C**$\mathbf{H_3}$), 3.49 (m, 1H, H-4'a), 3.22 (m, 1H, H-4'b), 2.88 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 6.4 Hz, 1H, H-7‘), 2.822 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 6.2 Hz, 1H, H-7‘), 2.816 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 7.9 Hz, 1H, H-7‘), 2.70 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 7.5 Hz, 1H, H-7‘), 1.45 – 1.57 (m, 2H, H-5‘), 1.11 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$), 1.06 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 173.28 (d, $J_{C\text{-}P}$ = 2.3 Hz, C=O), 173.05 (d, $J_{C\text{-}P}$ = 5.1 Hz, C=O), 170.08 (C-3‘), 156.84 (C-6), 148.59 (C-4), 145.84 (C-2), 141.01 (C-8), 137.49 (C-1‘‘), 137.36 (C-1‘‘), 129.57 (C-2‘‘), 129.53 (C-2‘‘), 128.29 (C-3‘‘), 128.23 (C-3‘‘), 126.65 (C-4‘‘), 126.58 (C-4‘‘), 123.76 (C-5), 76.41 (C-2‘), 65.33 (C-4‘), 60.52 (O-**C**$\mathbf{H_2}$-CH_3), 60.44 (O-**C**$\mathbf{H_2}$-CH_3), 54.28 (C-6‘), 54.06 (C-6‘), 52.32 (O-**C**$\mathbf{H_3}$), 44.79 (C-1‘), 40.09 (C-7‘), 29.91 (d, $J_{C\text{-}P}$ = 112.0 Hz, C-5‘), 14.11 (O-CH_2-**C**$\mathbf{H_3}$), 14.05 (O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 697 (25, M+H$^+$), 719 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{33}H_{42}O_9N_6P$ (M+H$^+$) 697.2745, found 697.2745; FTIR (KBr, cm^{-1}) ν: 3122, 2953, 2903, 1737, 1698, 1586, 1547, 1520, 1455, 1438, 1416, 1342, 1201, 1180, 1118, 1078, 1030, 702.



Diethyl 2,2'-{[(2-{[(*S*)-3-(2-amino-6-oxo-1,6-dihydro-*9H*-purin-9-yl)-1-methoxy-1-oxopropan-2-yl)oxy]ethyl}phosphoryl]-bis(azanediyl)} (2*S*,2'*S*)-bis(3-phenylpropanoate) (172)

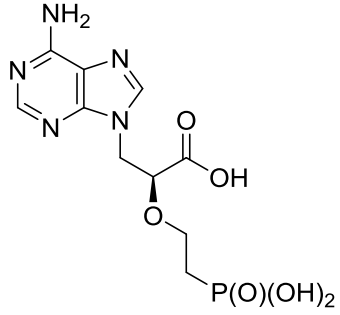
Treatment of compound **158** (209 mg, 0.5 mmol) by **method L** (chromatography: gradient from 0-12% methanol in chloroform) gave 89 mg (yield 25%) of compound **172** as a yellowish foam; ^{1}H NMR (DMSO-d_6) δ: 10.56 (bs, 1H, NH-1), 7.53 (s, 1H, H-8), 7.15 – 7.28 (m, 8H, H-2‘‘, H-3‘‘, H-4‘‘), 7.07 – 7.11 (m, 2H, H-2‘‘), 6.47 (bs, 2H, NH_2), 4.51 (dd, $J_{NH\text{-}P}$ = 12.8 Hz, $J_{NH\text{-}6‘}$ = 10.8 Hz, 1H, NH), 4.23 (dd, J_{gem} = 13.7 Hz, $J_{1‘a\text{-}2‘}$ = 3.7 Hz, 1H, H-1'a), 4.16 (dd, $J_{2‘\text{-}1’a}$ = 3.7 Hz, $J_{2‘\text{-}1’b}$ = 7.0 Hz, 1H, H-2‘), 4.12 (dd, $J_{NH\text{-}P}$ = 12.8 Hz, $J_{NH\text{-}6‘}$ = 10.5 Hz, 1H, NH), 4.10 (dd, J_{gem} = 13.7 Hz, $J_{1‘b\text{-}2‘}$ = 7.0 Hz, 1H, H-1‘b), 4.02 (q, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 2H, O-**C**$\mathbf{H_2}$-CH_3), 3.97 (q, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 2H, O-**C**$\mathbf{H_2}$-CH_3), 3.94 (m, 1H, H-6‘), 3.82 (m, 1H, H-6‘), 3.66 (s, 3H, O-**C**$\mathbf{H_3}$), 3.45 (m, 1H, H-4'a), 3.21 (m, 1H, H-4'b), 2.88 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 6.5 Hz, 1H, H-7‘), 2.83 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 6.3 Hz, 1H, H-7‘), 2.82 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 7.8 Hz, 1H, H-7‘), 2.70 (dd, J_{gem} = 13.4 Hz, $J_{7‘\text{-}6‘}$ = 7.6 Hz, 1H, H-7‘), 1.48 – 1.58 (m, 2H, H-5‘), 1.11 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$), 1.06 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 173.26 (d, $J_{C\text{-}P}$ = 2.4 Hz, C=O), 173.06 (d, $J_{C\text{-}P}$ = 5.1 Hz, C=O), 170.22 (C-3‘), 156.96 (C-6), 153.80 (C-2), 151.41 (C-4), 138.11 (C-8), 137.47 (C-1‘‘), 137.37 (C-1‘‘), 129.58 (C-2‘‘), 129.53 (C-2‘‘), 128.29 (C-3‘‘), 128.24 (C-3‘‘), 126.65 (C-4‘‘), 126.59 (C-4‘‘), 116.30 (C-5), 76.50 (C-2‘), 65.24 (C-4‘), 60.53 (O-**C**$\mathbf{H_2}$-CH_3), 60.44 (O-**C**$\mathbf{H_2}$-CH_3), 54.31 (C-6‘), 54.04 (C-6‘), 52.28 (O-**C**$\mathbf{H_3}$), 44.12 (C-1‘), 40.00 (C-7‘), 29.94 (d, $J_{C\text{-}P}$ = 112.1 Hz, C-5‘), 14.11 (O-CH_2-**C**$\mathbf{H_3}$), 14.05 (O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^+$ *m/z* (%): 712 (15, M+H$^+$), 734 (100, M+Na$^+$), 756 (25, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{33}H_{43}O_9N_7P$ (M+H$^+$) 712.2854, found 712.2853; FTIR (KBr, cm^{-1}) ν: 3172, 2902, 1737, 1690, 1600, 1538, 1495, 1487, 1455, 1388, 1368, 1296, 1198, 1180, 1120, 1079, 702.

5.7. Synthesis of (*S*)-CPEEA derivatives



(*S*)-3-(6-Amino-*9H*-purin-9-yl)-2-[2-(diethoxyphosphoryl)ethoxy]propanoic acid (173)

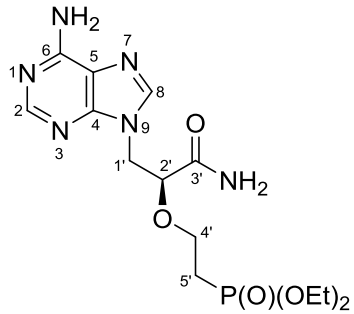
Compound **128** (373 mg, 1 mmol) was treated by **method B** (chromatography: gradient from 2-25% methanol in chloroform) to afford (after evaporation) the crude product. Crystallization from EtOAc-MeOH gave 321 mg (yield 83%) of compound **173** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 8.12 (s, 1H, H-2), 8.06 (s, 1H, H-8), 7.12 (s, 2H, NH_2), 4.48 (dd, J_{gem} = 14.2 Hz, $J_{1'a\text{-}2'}$ = 3.5 Hz, 1H, H-1'a), 4.23 (dd, J_{gem} = 14.2 Hz, $J_{1'b\text{-}2'}$ = 8.1 Hz, 1H, H-1'b), 3.88 – 3.95 (m, 5H, P-O-**C**$\mathbf{H_2}$-CH_3, H-2'), 3.78 (m, 1H, H-4'a), 3.29 – 3.46 (m, 1H, H-4'b), 1.89 – 1.96 (m, 2H, H-5'), 1.17 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**C**$\mathbf{H_3}$), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**C**$\mathbf{H_3}$); ^{13}C NMR (DMSO-d_6) δ: 172.38 (C-3'), 156.02 (C-6), 152.38 (C-2), 149.87 (C-4), 141.62 (C-8), 118.56 (C-5), 79.70 (C-2'), 63.19 (C-4'), 61.29 (d, $J_{C\text{-}O\text{-}P}$ = 5.6 Hz, P-O-**C**$\mathbf{H_2}$-CH_3), 45.39 (C-1'), 26.60 (C-5'), 25.50 (C-5'), 16.35 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-CH_2-**C**$\mathbf{H_3}$); MS-ESI$^-$ *m/z* (%): 386 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{14}H_{21}O_6N_5P$ (M-H$^+$) 386.1235, found 386.1234; FTIR (KBr, cm^{-1}) ν: 1732, 1634, 1603, 1575, 1395, 1230, 1123, 1050, 1028, 965. $[\alpha]^{20}_D$ = -9.4 (c = 0.308 g/100mL, MeOH).



(*S*)-3-(6-Amino-*9H*-purin-9-yl)-2-(2-phosphonoethoxy)propanoic acid (174)

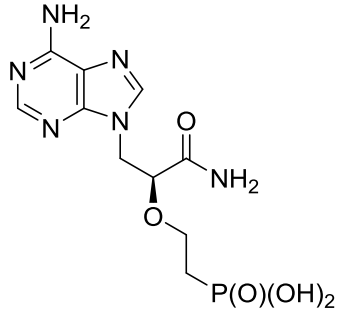
Treatment of compound **146** (194 mg, 0.5 mmol) by **method D1** followed by **method E** gave 124 mg (yield 75%) of compound **174** as a white solid; ^{1}H NMR (D_2O) δ: 8.23 (s, 1H, H-2), 8.13 (s, 1H, H-8), 4.58 (dd, J_{gem} = 14.7 Hz, $J_{1'a\text{-}2'}$ = 3.9 Hz, 1H, H-1'a), 4.42 (dd, J_{gem} = 14.7 Hz, $J_{1'b\text{-}2'}$ = 7.0 Hz, 1H, H-1'b), 4.16 (dd, $J_{2'\text{-}1'a}$ = 3.9 Hz, $J_{2'\text{-}1'b}$ = 7.0 Hz, 1H, H-2'), 3.71 (m, 1H, H-4'a), 3.54 (m, 1H, H-4'b), 1.76 – 1.83 (m, 2H, H-5'); ^{13}C NMR (D_2O) δ: 177.48 (C-3'), 156.02 (C-6), 152.93 (C-2), 149.79 (C-4), 143.61 (C-8), 118.78 (C-5), 79.87 (C-2'), 66.78 (C-4'), 43.39 (C-1'), 29.60 (C-5'), 28.90 (C-5'); MS-ESI$^-$ *m/z* (%): 330 (100, M-H$^+$), 352 (80, M+Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{13}O_6N_5P$ (M-H$^+$) 330.0755, found

330.0754; FTIR (KBr, cm^{-1}) ν: 3315, 3120, 1732, 1637, 1612, 1575, 1418, 1105, 1071, 899. $[\alpha]^{20}_D$ = -13.6 (c = 0.213 g/100mL, H_2O).



Diethyl (*S*)-{2-[(1-amino-3-[6-amino-*9H*-purin-9-yl]-1-oxopropan-2-yl)oxy]ethyl}phosphonate (175)

Compound **151** (421 mg, 1 mmol) was placed into reaction vial and a solution of 3.5 M ammonia in ethanol (5 mL) was added. The vial was microwave irradiated at 120 °C for 1 h. The mixture was evaporated and codistilled with water (2 x 10 mL) and ethanol (2 x 10 mL). The residue was purified by silica gel chromatography (gradient from 1-6% methanol in chloroform) to afford (after evaporation) 297 mg (yield 77%) of compound **175** as a colorless oil which solidified; ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, H-2), 8.00 (s, 1H, H-8), 7.71 (d, J_{gem} = 2.0 Hz, 1H, H-3‘-NH_a), 7.47 (d, J_{gem} = 2.0 Hz, 1H, H-3‘-NH_b), 7.19 (bs, 2H, H-6-NH_2), 4.48 (dd, J_{gem} = 14.3 Hz, $J_{1'a\text{-}2'}$ = 3.8 Hz, 1H, H-1’a), 4.32 (dd, J_{gem} = 14.3 Hz, $J_{1'b\text{-}2'}$ = 7.1 Hz, 1H, H-1‘b), 4.18 (dd, $J_{2'\text{-}1'a}$ = 3.8 Hz, $J_{2'\text{-}1'b}$ = 7.1 Hz, 1H, H-2‘), 3.89 – 3.97 (m, 4H, P-O-**CH_2**-CH_3), 3.60 (m, 1H, H-4‘a), 3.50 (m, 1H, H-4‘b), 1.96 – 2.04 (m, 2H, H-5’a, H-5‘b), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 171.49 (C-3‘), 156.14 (C-6), 152.65 (C-2), 149.99 (C-4), 141.32 (C-8), 118.54 (C-5), 78.22 (C-2‘), 64.87 (d, $J_{C\text{-}C\text{-}P}$ = 2.9 Hz, C-4‘), 61.33 (m, P-O-**CH_2**-CH_3), 44.37 (C-1‘), 25.78 (d, $J_{C\text{-}P}$ = 137.6 Hz, C-5‘), 16.40 (m, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 387 (10, M+H^+), 409 (100, M+Na^+); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{24}O_5N_6P$ (M+H^+) 387.1540, found 387.1540; FTIR (KBr, cm^{-1}) ν: 3372, 3320, 3195, 2929, 1687, 1651, 1603, 1577, 1481, 1467, 1422, 1388, 1330, 1304, 1234, 1105, 1031, 995. $[\alpha]^{20}_D$ = -14.7 (c = 0.285 g/100mL, MeOH).



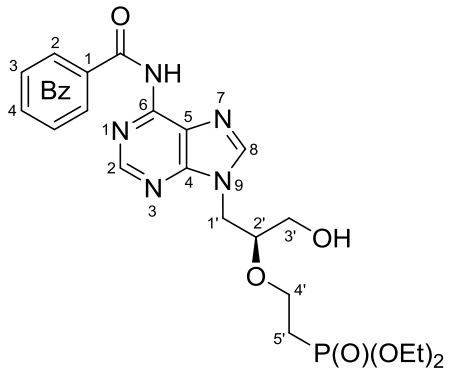
(*S*)-{2-[(1-Amino-3-[6-amino-*9H*-purin-9-yl]-1-oxopropan-2-yl)oxy]ethyl}phosphonic acid (176)

Treatment of compound **175** (193 mg, 0.5 mmol) by **method D1** followed by **method E** gave 125 mg (yield 76%) of compound **176** as a white solid; ^{1}H NMR (D_2O) δ: 8.08 (s, 1H, H-2), 8.03 (s, 1H, H-8), 4.48 (dd, J_{gem} = 14.9 Hz, $J_{1'a\text{-}2'}$ = 4.0 Hz, 1H, H-1’a), 4.40 (dd, J_{gem} = 14.9 Hz, $J_{1'b\text{-}2'}$ = 6.0 Hz, 1H, H-

1‘b), 4.15 (dd, $J_{2'\text{-}1'a}$ = 4.0 Hz, $J_{2'\text{-}1'b}$ = 6.0 Hz, 1H, H-2‘), 3.63 (m, 1H, H-4‘a), 3.51 (m, 1H, H-4‘b), 1.63 – 1.80 (m, 2H, H-5’); ^{13}C NMR (D_2O) δ: 174.54 (C-3‘), 154.93 (C-6), 151.79 (C-2), 148.86 (C-4), 142.8 (C-8), 117.74 (C-5), 77.52 (C-2‘), 66.65 (C-4‘), 44.79 (C-1‘), 28.31 (d, $J_{C\text{-}P}$ = 131.7 Hz, C-5‘);MS-ESI$^-$ *m/z* (%): 329 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{14}O_5N_6P$ (M-H$^+$) 329.0769, found 329.0769; FTIR (KBr, cm^{-1}) ν: 1673, 1646, 1606 1579, 1510, 1478, 1122, 1054, 901. $[\alpha]^{20}_D$ = -13.3 (c = 0.305 g/100mL, H_2O).

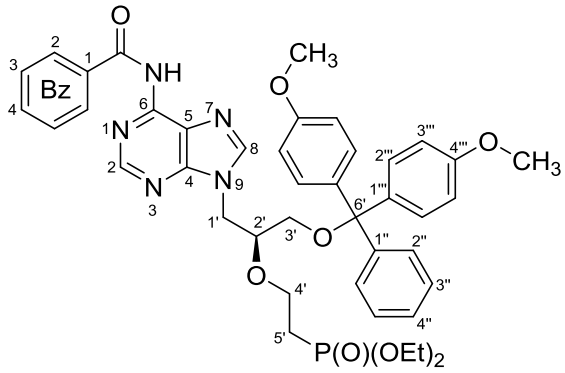
5.8. Synthesis of (*S*)-HPEP monomers

5.8.1. Synthesis of the adenine monomer



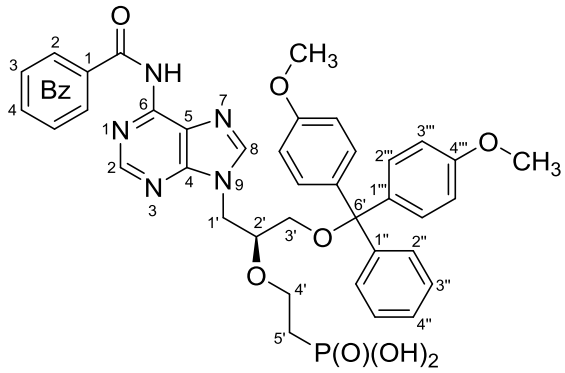
Diethyl (*S*)-{2-[(1-[6-benzamido-*9H*-purin-9-yl]-3-hydroxypropan-2-yl)oxy]ethyl} phosphonate (177)

Compound **128** (1.12 g, 3 mmol) was treated by **method A** to give 977 mg (yield 68%) of compound **177** as a yellowish oil; ^{1}H NMR (DMSO-d_6) δ: 11.16 (bs, 1H, NH), 8.73 (s, 1H, H-2), 8.44 (s, 1H, H-8), 8.04 (m, 2H, H-2Bz), 7.64 (m, 1H, H-4Bz), 7.55 (m, 2H, H-3Bz), 4.94 (bs, 1H, OH), 4.47 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 4.0 Hz, 1H, H-1’a), 4.29 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 7.3 Hz, 1H, H-1‘b), 3.88 – 3.96 (m, 4H, P-O-**CH_2**-CH_3), 3.79 (m, 1H, H-2’), 3.67 (m, 1H, H-4’a), 3.53 (m, 1H, H-4’b), 3.41 – 3.49 (m, 2H, H-3’), 1.84 – 1.97 (m, 2H, H-5’), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 166.20 (C=O), 152.85 (C-4), 151.50 (C-2), 150.23 (C-6), 145.56 (C-8), 133.68 (C-1Bz), 132.60 (C-4Bz), 128.67 (C-3Bz), 128.64 (C-2Bz), 125.59 (C-5), 78.33 (C-2’), 63.74 (C-4’), 61.23 (m, $J_{C\text{-}O\text{-}P}$ = 6.6 Hz, P-O-**CH_2**-CH_3), 60.88 (C-3’), 44.32 (C-1’), 26.37 (d, $J_{C\text{-}P}$ = 136.4 Hz, C-5’), 16.40 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.9 Hz, P-O-CH_2-**CH_3**); MS-ESI$^+$ *m/z* (%): 478 (15, M+H$^+$), 500 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_6N_5P$ (M+H$^+$) 478.1850, found 478.1848. $[\alpha]^{20}_D$ = -20.8 (c = 0.302 g/100ml, DMSO).



Diethyl (*S*)-{2-[(1-[6-benzamido-*9H*-purin-9-yl]-3-[bis(4-methoxyphenyl)(phenyl)methoxy]propan-2-yl)oxy]ethyl}phosphonate (178)

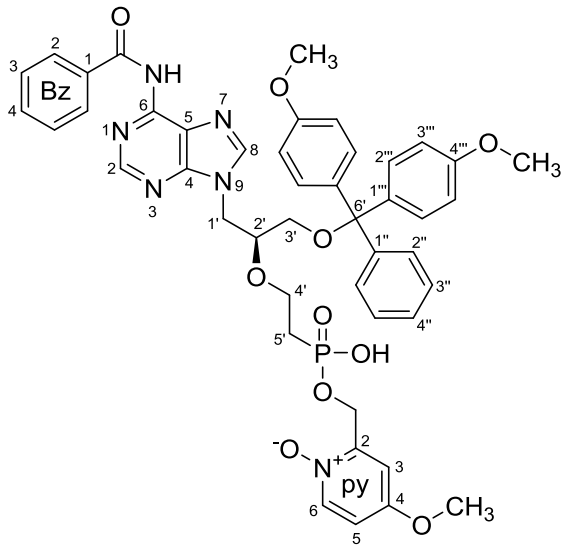
Compound **177** (955 mg, 2 mmol) was treated by **method M** to give 1.29 g (yield 83%) of compound **178** as a white foam; ^{1}H NMR (DMSO-d$_6$) δ: 11.12 (bs, 1H, NH), 8.68 (s, 1H, H-2), 8.38 (s, 1H, H-8), 8.04 (m, 2H, H-2Bz), 7.64 (m, 1H, H-4Bz), 7.55 (m, 2H, H-3Bz), 7.37 (m, 2H, H-2''), 7.30 (m, 2H, H-3''), 7.19 – 7.23 (m, 5H, H-4'', H-2'''), 6.85 – 6.88 (m, 4H, H-3'''), 4.44 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 4.7 Hz, 1H, H-1'a), 4.40 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 6.5 Hz, 1H, H-1'b), 3.87 – 3-97 (m, 5H, H-2', P-O-**CH$_2$**-CH$_3$), 3.73 (s, 6H, O-**CH$_3$**), 3.69 (m, 1H, H-4'a), 3.53 (m, 1H, H-4'b), 2.99 – 3.10 (m, 2H, H-3'), 1.88 – 1.96 (m, 2H, H-5'), 1.15 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 165.70 (C=O), 158.28 (C-4'''), 152.74 (C-4), 151.51 (C-2), 150.17 (C-6), 145.24 (C-8), 144.88 (C-1''), 135.55 (C-1'''), 133.70 (C-1Bz), 132.55 (C-4Bz), 129.80 (C-2'''), 128.63 (C-3Bz), 128.61 (C-2Bz), 128.00 (C-3''), 127.79 (C-2''), 126.89 (C-4''), 125.08 (C-5), 113.36 (C-3'''), 85.90 (C-6'), 76.78 (C-2'), 64.25 (C-4'), 63.38 (C-3'), 61.20 (d, $J_{C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-**CH$_2$**-CH$_3$), 55.20 (O-**CH$_3$**), 44.50 (C-1'), 26.42 (d, $J_{C\text{-}P}$ = 136.6 Hz, C-5'), 16.37 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.6 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 780 (10, M+H$^+$), 802 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{42}H_{46}O_8N_5NaP$ (M+Na$^+$) 802.2976, found 802.2980. $[\alpha]^{20}_D$ = -10.3 (c = 0.278 g/100ml, DMSO).



(*S*)-{2-[(1-[6-Benzamido-*9H*-purin-9-yl]-3-[bis(4-methoxyphenyl)(phenyl)methoxy]propan-2-yl)oxy]ethyl} phosphonic acid [bis(triethyl-ammonium) salt] (179)

Compound **178** (1.25 g, 1.6 mmol) was treated by **method N** to give 1.05 g (as a salt) (yield 71%) of compound **179** as a white solid; ^{1}H NMR (DMSO-d$_6$) δ: 8.68 (s, 1H, H-2), 8.38 (s, 1H, H-8), 8.04 (m, 2H, H-2Bz), 7.64 (m, 1H, H-4Bz), 7.55 (m, 2H, H-3Bz), 7.38 (m, 2H, H-2''), 7.30 (m, 2H, H-3''), 7.19 – 7.25 (m, 5H, H-4'', H-

2’’’), 6.85 – 6.88 (m, 4H, H-3’’’), 4.43 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 4.5 Hz, 1H, H-1’a), 4.37 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 7.2 Hz, 1H, H-1‘b), 3.85 – 3.89 (m, 1H, H-2’), 3.73 (s, 6H, O-**CH$_3$**), 3.65 (m, 1H, H-4’a), 3.42 – 3.50 (m, 1H, H-4’b), 3.01 (q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 12H, **CH$_2$**-Et$_3$N), 2.91 (m, 2H, H-3’), 1.52 (m, 2H, H-5’), 1.12 t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 18H, **CH$_3$**-Et$_3$N); ^{13}C NMR (DMSO-d$_6$) δ: 172.30 (C=O), 156.24 (C-4’’’), 152.66 (C-4), 151.37 (C-2), 150.09 (C-6), 145.33 (C-8), 144.90 (C-1’’), 135.61 (C-1’’’), 133.70 (C-1Bz), 132.53 (C-4Bz), 129.76 (C-2’’’), 128.60 (C-3Bz), 128.59 (C-2Bz), 128.00 C-3’’), 127.79 (C-2’’), 126, 85 (C-4’’), 125.09 (C-5), 113.37 (C-3’’’), 85.76 (C-6’), 76.31 (C-2’), 67.00 (C-4’), 63.50 (C-3’), 55.20 (O-**CH$_3$**), 45.69 (**CH$_2$**-Et$_3$N), 44.78 (C-1’), 8.87 (**CH$_3$**-Et$_3$N); MS-ESI$^-$ *m/z* (%): 722 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{38}H_{37}O_8N_5P$ (M-H$^+$) 722.2385, found 722.2382. $[\alpha]^{20}_D$ = -11.7 (c = 0.301 g/100ml, DMSO).

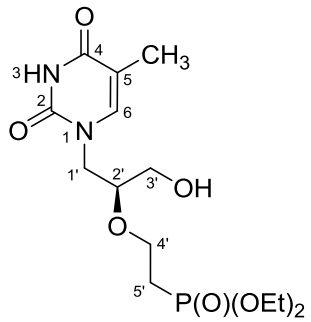


2-{[([2-([(*S*)-1-(6-Benzamido-*9H*-purin-9-yl)-3-(bis(4-methoxyphenyl)(phenyl)methoxy)propan-2-yl]oxy)ethyl](hydroxy)phosphoryl)oxy]methyl}-4-methoxypyridine 1-oxide (triethylammonium salt) (181)

Compound **179** (926 mg, 1 mmol) was treated by **method O** to give 462 mg (as a salt) (yield 48%) of compound **181** as a white solid; ^{1}H NMR (DMSO-d$_6$) δ: 11.20 (bs, 1H, NH), 8.65 (s, 1H, H-2), 8.40 (s, 1H, H-8), 8.12 (d, $J_{6py\text{-}5py}$ = 7.2 Hz, 1H, H-6py), 8.05 (m, 2H, H-2Bz), 7.64 (m, 1H, H-4Bz), 7.55 (m, 2H, H-3Bz), 7.38 (m, 2H, H-2’’), 7.29 (m, 2H, H-3’’), 7.18 – 7.24 (m, 5H, H-4’’, H-2’’’), 7.04 ($J_{3py\text{-}5py}$ = 3.6 Hz, 1H, H-3py), 6.94 (dd, $J_{5py\text{-}6py}$ = 7.2 Hz, $J_{5py\text{-}3py}$ = 3.6 Hz, 1H, H-5py), 6.86 (m, 4H, H-3’’’), 4.75 (m, 2H, **CH$_2$**-py), 4.43 (dd, J_{gem} = 14.2 Hz, $J_{1'a\text{-}2'}$ = 4.3 Hz, 1H, H-1’a), 4.36 (dd, J_{gem} = 14.2 Hz, $J_{1'b\text{-}2'}$ = 7.3 Hz, 1H, H-1‘b), 3.89 (m, 1H, H-2’), 3.77 (s, 3H, **CH$_3$**-O-py), 3.72 (s, 6H, O-**CH$_3$**), 3.66 (m, 1H, H-4’a), 3.46 (m, 1H, H-4’b), 3.07 (q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 6H, **CH$_2$**-Et$_3$N), 3.04 (dd, J_{gem} = 10.2 Hz, $J_{3'a\text{-}2'}$ = 4.6 Hz, 1H, H-3’a), 2.98 (dd, J_{gem} = 10.2 Hz, $J_{3'b\text{-}2'}$ = 5.3 Hz, 1H, H-3’b), 1.56 (m, 2H, H-5’), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 9H, **CH$_3$**-Et$_3$N); ^{13}C NMR (DMSO-d$_6$) δ: 165.70 (C=O), 158.23 (C-4’’’), 156.76 (C-

4py), 152.69 (C-4), 151.37 (C-2), 150.80 (d, $J_{C\text{-}P}$ = 6.4 Hz, C-2py), 150.10 (C-6), 145.41 (C-8), 144.90 (C-1''), 139.56 (C-6py), 135.66 (C-1'''), 135.62 (C-1'''), 133.76 (C-1Bz), 132.48 (C-4Bz), 129.75 (C-2'''), 128.59 (C-3Bz), 129.58 (C-2Bz), 127.97 (C-3''), 127.80 (C-2''), 126.83 (C-4''), 125.21 (C-5), 113.33 (C-3'''), 110.37 (C-5py), 108.57 (C-3py), 85.76 (C-6'), 76.33 (C-2'), 67.00 (C-4'), 63.51 (C-3'), 60.29 (d, $J_{C\text{-}P}$ = 3.6 Hz, **CH_2**-py), 56.15 (**CH_3**-O-py), 55.17 (O-**CH_3**), 45.65 (**CH_2**-Et_3N), 44.73 (C-1'), 29.50 (d, $J_{C\text{-}P}$ = 129.5 Hz, C-5'), 8.73 (**CH_3**-Et_3N); MS-ESI$^+$ *m/z* (%): 861 (10, M+H$^+$), 883 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{45}H_{45}O_{10}N_6NaP$ (M+Na$^+$) 883.2827, found 883.2830. $[\alpha]^{20}_D$ = -6.9 (c = 0.317 g/100ml, DMSO).

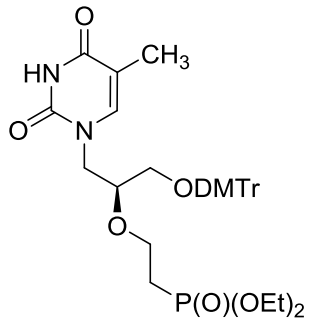
5.8.2. Synthesis of the thymine monomer



Diethyl (*S*)-{2-[(1-hydroxy-3-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl]propan-2-yl)oxy]ethyl} phosphonate (182)

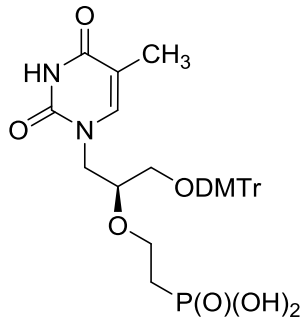
Compound **138** (568 mg, 1.5 mmol) was dissolved in 75% aqueous trifluoroacetic acid (20 mL) and heated at 50 °C overnight. Volatiles were evaporated and the residue was codistilled with water (3 x 20 mL) and neutralized with ammonia (1:10 with water) to pH 6-7. After evaporation the residue was purified by silica gel chromatography (gradient from 0-7% methanol in chloroform) to afford (after evaporation) 520 mg (yield 95%) of compound **182** as a colorless oil; ^{1}H NMR (DMSO-d_6) δ: 11.25 (s, 1H, NH), 7.43 (q, $J_{6\text{-}CH3}$ = 1.2 Hz, 1H, H-6), 4.79 (t, $J_{OH\text{-}3'a}$ = $J_{OH\text{-}3'b}$ = 5.7 Hz, 1H, 3'-OH), 3.90 – 4.02 (m, 4H, P-O-**CH_2**-CH_3), 3.84 (m, 1H, H-1'a), 3.68 (m, 1H, H-4'a), 3.50 – 3.59 (m, 3H, H-2', H-1'b, H-4'b), 3.43 (ddd, J_{gem} = 11.6 Hz, $J_{3'a\text{-}OH}$ = 5.8 Hz, $J_{3'a\text{-}2'}$ = 4.1 Hz, 1H, H-3'a), 3.37 (ddd, J_{gem} = 11.6 Hz, $J_{3'b\text{-}OH}$ = 5.7 Hz, $J_{3'b\text{-}2'}$ = 4.8 Hz, 1H, H-3'b), 1.93 – 2.01 (m, 2H, H-5'), 1.75 (d, $J_{CH3\text{-}6}$ = 1.2 Hz, 3H, 5-**CH_3**), 1.21 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 6H, P-O-CH_2-**CH_3**); ^{13}C NMR (DMSO-d_6) δ: 164.47 (C-4), 151.24 (C-2), 142.61 (C-6), 108.07 (C-5), 78.32 (C-2'), 64.02 (C-4'), 61.23 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**CH_2**-CH_3), 61.19 (d, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz, P-O-**CH_2**-CH_3), 61.13 (C-3'), 48.57 (C-1'), 25.53 (d, $J_{C\text{-}P}$ = 136.5 Hz, C-5'), 16.42 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.7 Hz, P-O-CH_2-**CH_3**), 16.41 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.7 Hz, P-O-CH_2-**CH_3**), 12.04 (5-**CH_3**); MS-ESI$^+$ *m/z* (%):

365 (10, M+H$^+$), 387 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{25}O_7N_2NaP$ (M+Na$^+$) 387.1292, found 387.1292; FTIR (KBr, cm^{-1}) ν: 3056, 2984, 1684, 1471, 1432, 1387, 1367, 1249, 1164, 1099, 1051, 1028, 963. $[\alpha]^{20}_D$ = -55.7 (c = 0.281 g/100mL, DMSO).



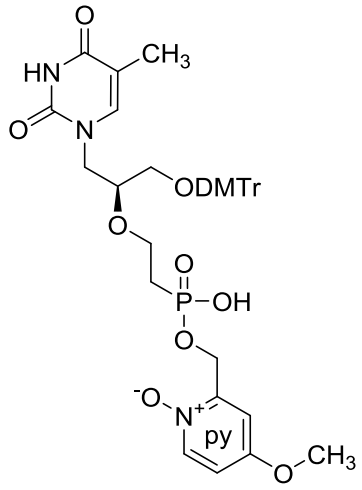
Diethyl (*S*)-{2-[(1-[bis(4-methoxyphenyl)(phenyl) methoxy]-3-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl]propan-2-yl)oxy]ethyl}phosphonate (183)

Compound **182** (729 mg, 2 mmol) was treated by **method M** to give 1.13 g (yield 85%) of compound **183** as a white foam; ^{1}H NMR (DMSO-d$_6$) δ: 11.22 (bs, 1H, NH), 7.35 – 7.41 (m, 3H, H-6, H-2''), 7.31 (m, 2H, H-3''), 7.23 – 7.28 (m, 4H, H-2'''), 7.22 (m, 1H, H-4''), 6.86 – 6.90 (m, 4H, H-3'''), 3.96 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.81 (dd, J_{gem} = 13.3 Hz, $J_{1'a\text{-}2'}$ = 4.4 Hz, 1H, H-1'a), 3.73 (s, 6H, O-**CH$_3$**), 3.67 – 3.76 (m, 2H, H-2', H-4'a), 3.64 (dd, J_{gem} = 13.3 Hz, $J_{1'b\text{-}2'}$ = 7.4 Hz, 1H, H-1'b), 3.51 – 3.60 (m, 1H, H-4'b), 3.07 (dd, J_{gem} = 10.4 Hz, $J_{3'a\text{-}2'}$ = 3.8 Hz, 1H, H-3'a), 2.94 (dd, J_{gem} = 10.4 Hz, $J_{3'b\text{-}2'}$ = 5.0 Hz, 1H, H-3'b), 2.00 (dt, $J_{5'\text{-}P}$ = 18.3 Hz, $J_{5'\text{-}4'}$ = 7.5 Hz, 2H, H-5'), 1.69 (d, $J_{CH3\text{-}6}$ = 1.2 Hz, 3H, 5-**CH$_3$**), 1.19 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-**CH$_3$**), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 164.39 (C-4), 158.27 (C-4'''), 151.10 (C-2), 144.98 (C-1''), 142.32 (C-6), 135.62 (C-1'''), 135.58 (C-1'''), 129.83 (C-2'''), 128.02 (C-3''), 127.79 (C-2''), 126.87 (C-4''), 113.36 (C-3'''), 108.08 (C-5), 85.78 (C-6'), 76.64 (C-2'), 64.48 (C-4'), 63.39 (C-3'), 61.19 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 55.20 (O-**CH$_3$**), 48.65 (C-1'), 26.62 (d, $J_{C\text{-}P}$ = 136.5 Hz, C-5'), 16.40 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**), 12.01 (5-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 667 (5, M+H$^+$), 689 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{35}H_{43}O_9N_2NaP$ (M+Na$^+$) 689.2598, found 689.2597. $[\alpha]^{20}_D$ = -37.2 (c = 0.285 g/100ml, DMSO).



(*S*)-{2-[(1-[Bis(4-methoxyphenyl)(phenyl)methoxy]-3-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl] propan-2-yl)oxy]ethyl}phosphonic acid [bis(triethylammonium) salt] (184)

Compound **183** (1.07 g, 1.6 mmol) was treated by **method N** to give 976 mg (as a salt) (yield 75%) of compound **184** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 7.40 (m, 2H, H-2''), 7.34 (m, 1H, H-6), 7.30 (m, 2H, H-3''), 7.25 (m, 4H, H-2'''), 7.21 (m, 1H, H-4''), 6.88 (m, 4H, H-3'''), 3.82 (m, 1H, H-1'a), 3.72 (s, 6H, O-**C**$\mathbf{H_3}$), 3.58 – 3.71 (m, 3H, H-1'b, H-2', H-4'a), 3.52 (m, 1H, H-4'b), 3.02 (m, 1H, H-3'a), 2.98 (q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 12H, **C**$\mathbf{H_2}$-Et_3N), 2.88 (m, 1H, H-3'b), 1.67 (d, $J_{CH3\text{-}6}$ = 0.9 Hz, 3H, 5-**C**$\mathbf{H_3}$), 1.60 – 1.66 (m, 2H, H-5'), 1.14 (t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 18H, **C**$\mathbf{H_3}$-Et_3N); ^{13}C NMR (DMSO-d_6) δ: 164.44 (C-4), 158.24 (C-4'''), 151.08 (C-2), 144.99 (C-1''), 142.60 (C-6), 135.69 (C-1'''), 135.65 (C-1'''), 129.80 (C-2'''), 128.03 (C-3''), 127.79 (C-2''), 126.85 (C-4''), 113.37 (C-3'''), 107.83 (C-5), 85.63 (C-6'), 75.86 (C-2'), 67.20 (C-4'), 63.30 (C-3'), 55.21 (O-**C**$\mathbf{H_3}$), 49.12 (C-1'), 45.61 (**C**$\mathbf{H_2}$-Et_3N), 31.00 (d, $J_{C\text{-}P}$ = 131.2 Hz, C-5'), 12.03 (5-**C**$\mathbf{H_3}$), 8.91 (**C**$\mathbf{H_3}$-Et_3N); MS-ESI$^-$ *m/z* (%): 609 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{31}H_{34}O_9N_2P$ (M-H$^+$) 609.2007, found 609.2004. $[\alpha]^{20}_D$ = -25.4 (c = 0.297 g/100ml, DMSO).

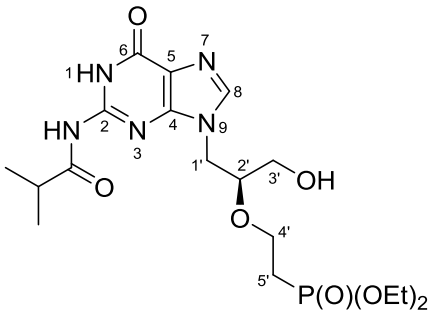


2-{[([2-([(*S*)-1-(Bis(4-methoxyphenyl)(phenyl) methoxy)-3-(5-methyl-2,4-dioxo-3,4-dihydro pyrimidin-1(*2H*)-yl)propan-2-yl]oxy)ethyl] (hydroxy)phosphoryl)oxy]methyl}-4-methoxy pyridine 1-oxide (triethylammonium salt) (186)

Compound **184** (813 mg, 1 mmol) was treated by **method O** to give 433 mg (as a salt) (yield 51%) of compound **186** as a white solid; ^{1}H NMR (DMSO-d_6) δ: 11.22 (s, 1H, NH), 8.13 (d, $J_{6py\text{-}5py}$ = 7.2 Hz, 1H, H-6py), 7.37 – 7.41 (m, 3H, H-6, H-2''), 7.29 (m, 2H, H-3''), 7.22 – 7.27 (m, 4H, H-2'''), 7.20 (m, 1H, H-4''), 7.06 (bd, $J_{3py\text{-}5py}$ = 3.6 Hz, 1H, H-3py), 6.95 (dd, $J_{5py\text{-}6py}$ = 7.2 Hz, $J_{5py\text{-}3py}$ = 3.6 Hz, 1H, H-5py), 6.84 – 6.89 (m, 4H, H-3'''), 4.81 (d, $J_{CH2\text{-}P}$ = 8.3 Hz, 2H, **C**$\mathbf{H_2}$-py), 3.81 (dd, J_{gem} = 13.4 Hz, $J_{1'a\text{-}2'}$ = 4.4 Hz, 1H, H-1'a), 3.77 (s, 3H, **C**$\mathbf{H_3}$-O-py), 3.72 (s, 6H, O-**C**$\mathbf{H_3}$), 3.50 – 3.76 (m, 4H, H-1'a, H-2', H-4'), 3.05

(q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 6H, **CH$_2$**-Et$_3$N), 3.03 (dd, J_{gem} = 10.2 Hz, $J_{3'a\text{-}2'}$ = 3.9 Hz, 1H, H-3'a), 2.90 (dd, J_{gem} = 10.2 Hz, $J_{3'b\text{-}2'}$ = 4.8 Hz, 1H, H-3'b), 1.72 (m, 2H, H-5'), 1.67 (d, $J_{CH3\text{-}6}$ = 1.2 Hz, 3H, 5-**CH$_3$**), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 9H, **CH$_3$**-Et$_3$N); ^{13}C NMR (DMSO-d$_6$) δ: 164.39 (C-4), 158.24 (C-4'''), 156.67 (C-4py), 151.09 (C-2), 150.65 (bd, $J_{C\text{-}P}$ = 5.1 Hz, C-2py), 144.98 (C-1''), 142.53 (C-6), 139.53 (C-6py), 135.73 (C-1'''), 135.67 (C-1'''), 129.78 (C-2'''), 127.99 (C-3''), 127.80 (C-2''), 126.83 (C-4''), 113.33 (C-3'''), 110.45 (C-5py), 108.65 (C-3py), 107.88 (C-5), 85.66 (C-6'), 76.15 (C-2'), 67.12 (C-4'), 63.47 (C-3'), 60.33 (bd, $J_{C\text{-}P}$ = 4.1 Hz, **CH$_2$**-py), 56.16 (**CH$_3$**-O-py), 55.18 (O-**CH$_3$**), 48.97 (C-1'), 45.58 (**CH$_2$**-Et$_3$N), 29.67 (d, $J_{C\text{-}P}$ = 129.2 Hz, C-5'), 8.65 (**CH$_3$**-Et$_3$N); MS-ESI$^-$ *m/z* (%): 746 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{38}H_{41}O_{11}N_3P$ (M-H$^+$) 746.2484, found 746.2483. $[\alpha]^{20}_D$ = -21.8 (c = 0.285 g/100ml, DMSO).

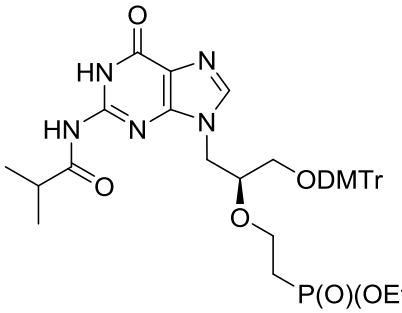
5.8.3. Synthesis of the guanine monomer



Diethyl (*S*)-{2-[(1-hydroxy-3-[2-isobutyramido-6-oxo-1,6-dihydro-*9H*-purin-9-yl]propan-2-yl)oxy]ethyl}phosphonate (187)

Compound **123** (1.17 g, 3 mmol) was dissolved in dry pyridine (30 mL) in a round-bottomed flask with a drying tube (protecting the reaction from moisture), and the reaction mixture was cooled in an ice bath. TMSCl (2.67 mL, 21 mmol) was added to the reaction mixture and after 30 min isobutyryl chloride (1.58 mL, 15 mmol) was added. The reaction flask was removed from the ice bath and the mixture was stirred for 4 h at room temperature. Then the reaction mixture was cooled in ice bath and cold water (4 mL) was added, followed after 15 min of stirring by concentrated aqueous ammonia (4 mL). The mixture was stirred at room temperature for another 30 min and solvents were removed *in vacuo* to give crude oil. The crude oil was subjected to silica gel chromatography (gradient from 0-8% methanol in chloroform) to afford (after evaporation) 1.14 g (yield 83%) of compound **187** as a yellowish foam; ^{1}H NMR (DMSO-d$_6$) δ: 12.06 (s, 1H, NH-1), 11.66 (s, 1H, NH-CO), 7.95 (s, 1H, H-8), 4.90 (bs, 1H, 3'-OH), 4.22 (dd, J_{gem} = 14.4 Hz, $J_{1'a\text{-}2'}$ = 3.8 Hz, 1H, H-1'a), 4.07 (dd, J_{gem} = 14.4 Hz, $J_{1'b\text{-}2'}$ = 7.9 Hz, 1H, H-1'b),

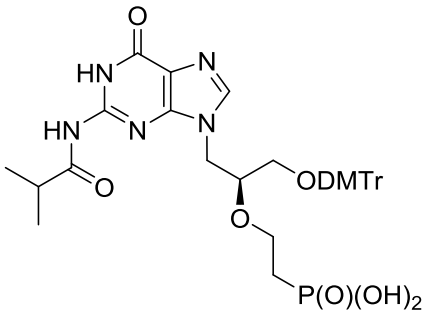
3.87 – 3.98 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.68 (m, 1H, H-2'), 3.62 (m, 1H, H-4'a), 3.39 – 3.52 (m, 3H, H-3', H-4'b), 2.78 (sept, J_{CH-CH3} = 6.9 Hz, 1H, **CH**-iPr), 1.85 – 1.94 (m, 2H, H-5'), 1.18 (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-**CH$_3$**), 1.12 (d, J_{CH3-CH} = 6.9 Hz, 6H, **CH$_3$**-iPr); ^{13}C NMR (DMSO-d$_6$) δ: 180.34 (C=O), 155.09 (C-6), 149.07 (C-4), 147.98 (C-2), 140.67 (C-8), 119.96 (C-5), 78.53 (C-2'), 63.80 (C-4'), 61.26 (d, J_{C-O-P} = 6.3 Hz, P-O-**CH$_2$**-CH$_3$), 61.20 (d, J_{C-O-P} = 6.3 Hz, P-O-**CH$_2$**-CH$_3$), 60.81 (C-3'), 44.48 (C-1'), 34.88 (**CH**-iPr), 26.32 (d, J_{C-P} = 136.5 Hz, C-5'), 19.07 (**CH$_3$**-iPr), 16.38 (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**), 16.37 (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 460 (25, M+H$^+$), 482 (100, M+Na$^+$), 504 (30, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{18}H_{31}O_7N_5P$ (M+H$^+$) 460.1956, found 460.1954; FTIR (KBr, cm^{-1}) ν: 3403, 3198, 1684, 1612, 1559, 1480, 1470, 1406, 1375, 1293, 1221, 1197, 1120, 1051, 1027, 962, 783. $[\alpha]^{20}_D$ = -29.1 (c = 0.292 g/100ml, MeOH).



Diethyl (*S*)-{2-[(1-[bis(4-methoxyphenyl) (phenyl)methoxy]-3-[2-isobutyramido-6-oxo-1,6-dihydro-*9H*-purin-9-yl]propan-2-yl)oxy]ethyl}phosphonate (188)

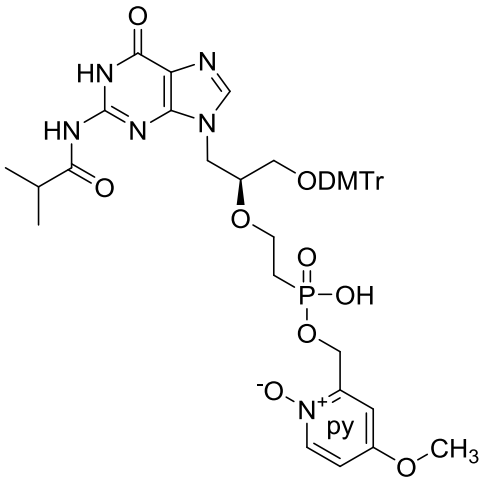
Compound **187** (919 mg, 2 mmol) was treated by **method M** to give 1.45 g (yield 95%) of compound **188** as a white foam; ^{1}H NMR (DMSO-d$_6$) δ: 7.91 (s, 1H, H-8), 7.35 (m, 2H, H-2''), 7.29 (m, 2H, H-3''), 7.18 – 7.24 (m, 5H, H-4'', H-3'''), 6.84 – 6.88 (m, 4H, H-2'''), 4.19 (dd, J_{gem} = 14.3 Hz, $J_{1'a-2'}$ = 7.0 Hz, 1H, H-1'a), 4.15 (dd, J_{gem} = 14.3 Hz, $J_{1'b-2'}$ = 4.6 Hz, 1H, H-1'b), 3.90 – 3.97 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.88 (m, 1H, H-2'), 3.68 – 3.77 (m, 1H, H-4'a), 3.73 (s, 6H, O-**CH$_3$**), 3.49 – 3.59 (m, 1H, H-4'b), 3.04 (dd, J_{gem} = 10.3 Hz, $J_{3'a-2'}$ = 3.8 Hz, 1H, H-3'a), 2.94 (dd, J_{gem} = 10.3 Hz, $J_{3'b-2'}$ = 5.7 Hz, 1H, H-3'b), 2.77 (sept, J_{CH-CH3} = 6.9 Hz, 1H, **CH**-iPr), 1.91 – 1.99 (m, 2H, H-5'), 1.17 (t, $J_{CH3-CH2}$ = 7.0 Hz, 3H, P-O-CH$_2$-**CH$_3$**), 1.16 (t, $J_{CH3-CH2}$ = 7.0 Hz, 3H, P-O-CH$_2$-**CH$_3$**), 1.11 (d, J_{CH3-CH} = 6.9 Hz, 6H, **CH$_3$**-iPr); ^{13}C NMR (DMSO-d$_6$) δ: 180.32 (C=O), 158.27 (C-4'''), 155.05 (C-6), 148.98 (C-4), 147.90 (C-2), 144.83 (C-1''), 140.39 (C-8), 135.46 (C-1'''), 135.45 (C-1'''), 129.85 (C-2'''), 129.84 (C-2'''), 129.02 (C-3''), 128.80 (C-2''), 126.89 (C-4''), 119.92 (C-5), 113.32 (C-3'''), 113.31 (C-3'''), 85.82 (C-6'), 77.10 (C-2'), 64.41 (C-4'), 63.59 (C-3'), 61.24 (d, J_{C-O-P} = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 61.23 (d, J_{C-O-P} = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 55.20 (O-**CH$_3$**), 47.49 (C-1'), 34.90 (**CH**-iPr), 26.43 (d, J_{C-P} = 136.2 Hz, C-5'), 19.08

(**CH$_3$**-iPr), 19.06 (**CH$_3$**-iPr), 16.39 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**), 16.38 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 762 (15, M+H$^+$), 784 (100, M+Na$^+$), 806 (20, M+2Na$^+$); HRMS-ESI$^+$: *m/z* calcd for $C_{39}H_{48}O_9N_5NaP$ (M+Na$^+$) 784.3082, found 784.3077. $[\alpha]^{20}_D$ = -1.4 (c = 0.291 g/100ml, MeOH).



(*S*)-{2-[(1-[Bis(4-methoxyphenyl)(phenyl) methoxy]-3-[2-isobutyramido-6-oxo-1,6-dihydro-*9H*-purin-9-yl]propan-2-yl)oxy]ethyl} phosphonic acid [bis(triethylammonium) salt] (189)

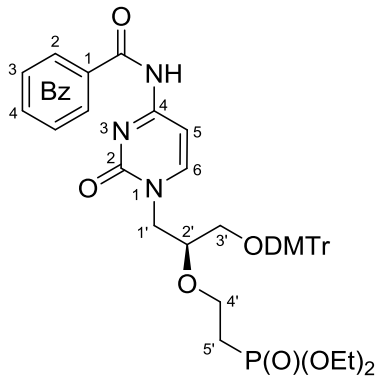
Compound **188** (1.22 g, 1.6 mmol) was treated by **method N** to give 1.06 g (as a salt) (yield 73%) of compound **189** as a white solid; ^{1}H NMR (DMSO-d$_6$) δ: 12.06 (bs, 1H, NH), 7.64 (s, 1H, H-8), 7.36 (m, 2H, H-2''), 7.28 (m, 2H, H-3''), 7.19 – 7-23 (m, 4H, H-2'''), 7.19 (m, 1H, H-4''), 6.82 – 6.86 (m, 4H, H-3'''), 4.23 (dm, J_{gem} = 14.6 Hz, 1H, H-1'a), 4.16 (bdd, J_{gem} = 14.6 Hz, $J_{1'b\text{-}2'}$ = 4.8 Hz, 1H, H-1'b), 3.87 (m, 1H, H-4'a), 3.76 (pent, $J_{2'\text{-}1'a} = J_{2'\text{-}1'b} = J_{2'\text{-}3'a} = J_{2'\text{-}3'b}$ = 5.2 Hz, 1H, H-2'), 3.71 (s, 6H, O-**CH$_3$**), 3.70 (m, 1H, H-4'a), 3.03 (q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 12H, **CH$_2$**-Et$_3$N), 2.80 – 3.01 (m, 3H, **CH**-iPr, H-3'), 1.47 – 1.77 (m, 2H, H-5'), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 18H, **CH$_3$**-Et$_3$N), 1.09 (d, $J_{CH3\text{-}CH}$ = 6.8 Hz, 3H, **CH$_3$**-iPr), 1.08 (d, $J_{CH3\text{-}CH}$ = 6.8 Hz, 3H, **CH$_3$**-iPr); ^{13}C NMR (DMSO-d$_6$) δ: 180.84 (C=O), 158.26 (C-4'''), 158.25 (C-4'''), 155.11 (C-6), 149.35 (C-4), 148.06 (C-2), 144.89 (C-1''), 140.07 (C-8), 135.61 (C-1'''), 135.50 (C-1'''), 129.78 (C-2'''), 129.76 (C-2'''), 128.04 (C-3''), 127.76 (C-2''), 126.87 (C-4''), 119.78 (C-5), 113.36 (C-3'''), 113.34 (C-3'''), 85.82 (C-6'), 75.62 (C-2'), 65.70 (C-4'), 63.26 (C-3'), 55.19 (O-**CH$_3$**), 45.66 (**CH$_2$**-Et$_3$N), 43.48 (C-1'), 34.56 (**CH**-iPr), 30.51 (d, $J_{C\text{-}P}$ = 128.5 Hz, C-5'), 19.26 (**CH$_3$**-iPr), 19.01 (**CH$_3$**-iPr), 8.85 (**CH$_3$**-Et$_3$N); MS-ESI$^-$ *m/z* (%): 704 (100, M-H$^+$), 726 (30, M+Na$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{35}H_{39}O_9N_5P$ (M-H$^+$) 704.2491, found 704.2493. $[\alpha]^{20}_D$ = -3.2 (c = 0.349 g/100ml, DMSO).



2-{[([2-([(*S*)-1-(Bis(4-methoxyphenyl) (phenyl)methoxy)-3-(2-isobutyramido-6-oxo-1,6-dihydro-*9H*-purin-9-yl)propan-2-yl]oxy)ethyl](hydroxy)(phosphoryl)oxy] methyl}-4-methoxypyridine 1-oxide (triethylammonium salt) (191)

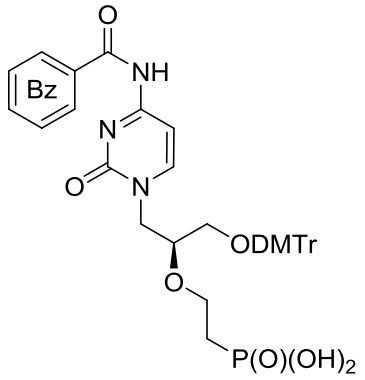
Compound **189** (908 mg, 1 mmol) was treated by **method O** to give 444 mg (as a salt) (yield 47%) of compound **191** as a white solid; ^{1}H NMR (DMSO-d$_6$) δ: 12.70 (bs, 1H, NH), 12.06 (bs, 1H, NH), 8.15 (d, $J_{6py\text{-}5py}$ = 7.2 Hz, 1H, H-6py), 7.66 (s, 1H, H-8), 7.36 (m, 2H, H-2''), 7.28 (m, 2H, H-3''), 7.17 – 7.23 (m, 5H, H-2''', H-4''), 7.03 (d, $J_{3py\text{-}5py}$ = 3.6 Hz, 1H, H-3py), 6.97 (dd, $J_{5py\text{-}6py}$ = 7.2 Hz, $J_{5py\text{-}3py}$ = 3.6 Hz, 1H, H-5py), 6.81 – 6.83 (m, 4H, H-3'''), 4.83 (d, $J_{CH2\text{-}P}$ = 8.0 Hz, 2H, **CH$_2$**-py), 4.24 (dd, J_{gem} = 14.5 Hz, $J_{1'a\text{-}2'}$ = 4.9 Hz, 1H, H-1'a), 4.13 (dd, J_{gem} = 14.5 Hz, $J_{1'b\text{-}2'}$ = 9.5 Hz, 1H, H-1'b), 3.90 (m, 1H, H-4'a), 3.77 (s, 3H, **CH$_3$**-O-py), 3.67 – 3.79 (m, 2H, H-2', H-4'b), 3.71 (s, 6H, O-**CH$_3$**), 3.06 (q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 6H, **CH$_2$**-Et$_3$N), 3.02 (m, 1H, H-3'a), 2.91 (sept, $J_{CH\text{-}CH3}$ = 6.9 Hz, 1H, **CH**-iPr), 2.87 (m, 1H, H-3'b), 1.76 (m, 1H, H-5'a), 1.60 (m, 1H, H-5'b), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 9H, **CH$_3$**-Et$_3$N), 1.08 (d, $J_{CH3\text{-}CH}$ = 6.9 Hz, 3H, **CH$_3$**-iPr), 1.07 ($J_{CH3\text{-}CH}$ = 6.9 Hz, 3H, **CH$_3$**-iPr); ^{13}C NMR (DMSO-d$_6$) δ: 180.84 (C=O), 158.26 (C-4'''), 156.78 (C-4py), 155.13 (C-6), 150.49 (d, $J_{C\text{-}P}$ = 6.1 Hz, C-2py), 149.38 (C-4), 148.05 (C-2), 144.90 (C-1''), 140.30 (C-8), 139.64 (C-6py), 135.66 (C-1'''), 135.53 (C-1'''), 129.75 (C-2'''), 129.73 (C-2'''), 128.03 (C-3''), 127.76 (C-2''), 126.87 (C-4''), 119.86 (C-5), 113.35 (C-3'''), 113.32 (C-3'''), 110.40 (C-5py), 108.66 (C-3py), 85.87 (C-6'), 75.54 (C-2'), 65.33 (C-4'), 63.47 (C-3'), 60.33 (d, $J_{C\text{-}P}$ = 3.9 Hz, **CH$_2$**-O-P), 56.22 (py-O-**CH$_3$**), 55.20 (O-**CH$_3$**), 45.67 (**CH$_2$**-Et$_3$N), 43.51 (C-1'), 34.57 (**CH**-iPr), 29.06 (d, $J_{C\text{-}P}$ = 129.2 Hz, C-5'), 19.21 (**CH$_3$**-iPr), 19.02 (**CH$_3$**-iPr), 8.71 (**CH$_3$**-Et$_3$N); MS-ESI$^-$ *m/z* (%): 841 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{42}H_{46}O_{11}N_6P$ (M-H$^+$) 841.2968, found 841.2968. $[\alpha]^{20}_D$ = -2.6 (c = 0.385 g/100ml, DMSO).

5.8.4. Synthesis of the cytosine monomer



Diethyl (*S*)-{2-[(1-[4-benzamido-2-oxopyrimidin-1(*2H*)-yl]-3-[bis(4-methoxyphenyl)(phenyl) methoxy]propan-2-yl)oxy]ethyl}phosphonate (192)

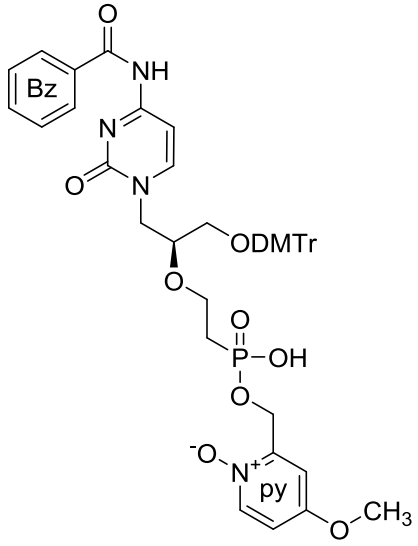
Compound **135** (907 mg, 2 mmol) was treated by **method M** to give 1.32 g (yield 87%) of compound **192** as a white foam; ^{1}H NMR (DMSO-d$_6$) δ: 11.21 (bs, 1H, NH), 7.98 – 8.02 (m, 3H, H-2Bz, H-6), 7.62 (m, 1H, H-4Bz), 7.51 (m, 2H, H-3Bz), 7.41 (m, 2H, H-2’’), 7.32 (m, 2H, H-3’’), 7.20 – 7.29 (m, 6H, H-5, H-4’’, H-2’’’), 6.89 (m, 2H, H-3’’’), 4.07 (m, 1H, H-1’a), 3.88 – 4.00 (m, 4H, P-O-**CH$_2$**-CH$_3$), 3.76 – 3.84 (m, 2H, H-1’b, H-2), 3.73 (s, 3H, O-**CH$_3$**), 3.72 (s, 3H, O-**CH$_3$**), 3.71 (m, 1H, H-4’a), 3.48 (m, 1H, H-4’b), 3.13 (bdd, J_{gem} = 10.2 Hz, $J_{3'a\text{-}2'}$ = 3.7 Hz, 1H, H-3’a), 2.98 (bdd, J_{gem} = 10.2 Hz, $J_{3'b\text{-}2'}$ = 4.8 Hz, 1H, H-3’b), 1.92 – 2.03 (m, 2H, H-5’), 1.17 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-**CH$_3$**), 1.16 (t, $J_{CH3\text{-}CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-**CH$_3$**); ^{13}C NMR (DMSO-d$_6$) δ: 167.60 (C=O), 163.26 (C-4), 158.30 (C-4’’’), 155.31 (C-2), 151.35 (C-6), 144.39 (C-1’’), 135.60 (C-1’’’), 135.56 (C-1’’’), 133.42 (C-1Bz), 132.88 (C-4Bz), 129.88 (C-2’’’), 128.64 (C-3Bz), 128.58 (C-2Bz), 128.07 (C-3’’), 127.81 (C-2’’), 126.89 (C-4’’), 113.40 (C-3’’’), 95.64 (C-5), 85.80 (C-6’), 76.16 (C-2’), 64.61 (C-4’), 63.49 (C-3’), 61.22 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 61.20 (d, $J_{C\text{-}O\text{-}P}$ = 6.1 Hz, P-O-**CH$_2$**-CH$_3$), 55.19 (O-**CH$_3$**), 51.30 (C-1’), 26.56 (d, $J_{C\text{-}P}$ = 136.2 Hz, C-5’), 16.40 (d, $J_{C\text{-}C\text{-}O\text{-}P}$ = 5.8 Hz, P-O-CH$_2$-**CH$_3$**); MS-ESI$^+$ *m/z* (%): 756 (5, M+H$^+$), 778 (100, M+Na$^+$); HRMS-ESI$^+$: *m/z* calcd for C$_{41}$H$_{46}$O$_9$N$_3$NaP (M+Na$^+$) 778.2864, found 778.2860. $[\alpha]^{20}_{D}$ = -30.1 (c = 0.282 g/100ml, MeOH).



(*S*)-{2-[(1-[4-Benzamido-2-oxopyrimidin-1(*2H*)-yl]-3-[bis(4-methoxyphenyl)(phenyl)methoxy]propan-2-yl)oxy]ethyl}phosphonic acid [bis(triethyl-ammonium) salt] (193)

Compound **192** (1.21 g, 1.6 mmol) was treated by **method N** to give 1.10 g (as a salt) (yield 76%) of title compound as a white solid; ^{1}H NMR (DMSO-d$_6$) δ: 8.00 (m, 2H, H-2Bz), 7.97 (bd, $J_{6\text{-}5}$ = 7.5 Hz, 1H, H-6), 7.61 (m, 1H, H-4Bz), 7.50

(m, 2H, H-3Bz), 7.40 (m, 2H, H-2''), 7.30 (m, 2H, H-3''), 7.26 (m, 4H, H-2'''), 7.17 – 7.24 (m, 2H, H-5, H-4''), 6.88 (m, 4H, H-3'''), 4.06 (m, 1H, H-1'a), 3.72 – 3.80 (m, 2H, H-1'b, H-2'), 3.71 (s, 3H, O-**CH$_3$**), 3.70 (s, 3H, O-**CH$_3$**), 3.69 (m, 1H, H-4'a), 3.47 (m, 1H, H-4'b), 3.08 (m, 1H, H-3'a), 2.96 (q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 12H, **CH$_2$**-Et$_3$N), 2.93 (m, 1H, H-3'b), 1.55 – 1.71 (m, 2H, H-5'), 1.13 (t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 18H, **CH$_3$**-Et$_3$N); ^{13}C NMR (DMSO-d$_6$) δ: 167-58 (C=O), 163.20 (C-4), 158.25 (C-4'''), 155.29 (C-2), 151.41 (C-6), 144.97 (C-1''), 135.67 (C-1'''), 135.61 (C-1'''), 133.44 (C-1Bz), 132.84 (C-4Bz), 129.83 (C-2'''), 128.61 (C-3Bz), 128.58 (C-2Bz), 128.06 (C-3''), 127.80 (C-2''), 126.84 (C-4''), 113.40 (C-3'''), 95.51 (C-5), 85.67 (C-6'), 75.37 (C-2'), 67.04 (C-4'), 63.40 (C-3'), 55.18 (O-**CH$_3$**), 51.60 (C-1'), 45.56 (**CH$_2$**-Et$_3$N), 30.60 (m, C-5'), 9.00 (**CH$_3$**-Et$_3$N); MS-ESI$^-$ *m/z* (%): 698 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{37}H_{37}O_9N_3P$ (M-H$^+$) 698.2273, found 698.2269. $[\alpha]^{20}_D$ = -20.1 (c = 0.364 g/100ml, DMSO).



2-{[([2-([(*S*)-1-(4-Benzamido-2-oxopyrimidin-1(*2H*)-yl)-3-(bis(4-methoxyphenyl)(phenyl) methoxy)propan-2-yl]oxy)ethyl](hydroxy) phosphoryl)oxy]methyl}-4-methoxypyridine 1-oxide (triethylammonium salt) (195)

Compound **193** (902 mg, 1 mmol) was treated by **method O** to give 478 mg (as a salt) (yield 51%) of compound **195** as a white solid; ^{1}H NMR (DMSO-d$_6$) δ: 11.18 (vbs, 1H, NH), 10.39 (vbs, 1H, Et$_3$NH$^+$), 8.12 (d, $J_{6py\text{-}5py}$ = 7.2 Hz, 1H, H-6py), 8.02 (bd, $J_{6\text{-}5}$ = 7.2 Hz, 1H, H-6), 8.00 (m, 2H, H-2Bz), 7.62 (m, 1H, H-4Bz), 7.50 (m, 2H, H-3Bz), 7.40 (m, 2H, H-2''), 7.30 (m, 2H, H-3''), 7.20 – 7.29 (5H, H-5, H-2'''), 7.20 (m, 1H, H-4''), 7.03 (bd, $J_{3py\text{-}5py}$ = 3.6 Hz, 1H, H-3py), 6.93 (bdd, $J_{5py\text{-}6py}$ = 7.2 Hz, $J_{5py\text{-}3py}$ = 3.6 Hz, 1H, H-5py), 6.85 – 6.90 (m, 4H, H-3'''), 4.81 (d, $J_{CH2\text{-}P}$ = 8.3 Hz, 2H, **CH$_2$**-py), 4.06 (dd, J_{gem} = 12.5 Hz, $J_{1'a\text{-}2'}$ = 3.4 Hz, 1H, H-1'a), 3.68 – 3.82 (m, 3H, H-1'b, H-2', H-4'a), 3.74 (s, 3H, **CH$_3$**-O-py), 3.71 (s, 6H, O-**CH$_3$**), 3.49 (m, 1H, H-4'b), 3.09 (dd, J_{gem} = 10.1 Hz, $J_{3'a\text{-}2'}$ = 3.8 Hz, 1H, H-3'a), 3.01 (q, $J_{CH2\text{-}CH3}$ = 7.3 Hz, 6H, **CH$_2$**-Et$_3$N), 2.95 (dd, J_{gem} = 10.1 Hz, $J_{3'b\text{-}2'}$ = 4.6 Hz, 1H, H-3'b), 1.64 – 1.77 (m, 2H, H-5'), 1.14 (t, $J_{CH3\text{-}CH2}$ = 7.3 Hz, 9H, **CH$_3$**-Et$_3$N); ^{13}C NMR (DMSO-d$_6$) δ: 167.44 (C=O), 163.25 (C-4), 158.26 (C-4'''), 156.79 (C-4py), 155.37

(C-2), 151.50 (C-6), 150.46 (d, $J_{C\text{-}P}$ = 5.8 Hz, C-2py), 144.98 (C-1''), 139.60 (C-6py), 135.71 (C-1'''), 135.65 (C-1'''), 133.43 (C-1Bz), 132.83 (C-4Bz), 129.82 (C-2'''), 128.61 (C-3Bz), 128.58 (C-2Bz), 128.04 (C-3''), 127.82 (C-2''), 126.86 (C-4''), 113.38 (C-3'''), 110.51 (C-5py), 108.63 (C-3py), 95.51 (C-5), 85.69 (C-6'), 75.59 (C-2'), 67.13 (C-4'), 63.64 (C-3'), 60.35 (d, $J_{C\text{-}P}$ = 4.0 Hz, **CH_2**-py), 56.18 (**CH_3**-O-py), 55.18 (O-**CH_3**), 51.64 (C-1'), 45.47 (**CH_2**-Et_3N), 29.52 (d, $J_{C\text{-}P}$ = 129.8 Hz, C-5'), 8.63 (**CH_3**-Et_3N); MS-ESI$^-$ *m/z* (%): 835 (100, M-H$^+$); HRMS-ESI$^-$: *m/z* calcd for $C_{44}H_{44}O_{11}N_4P$ (M-H$^+$) 835.2745, found 835.2747. $[\alpha]^{20}_D$ = -22.4 (c = 0.371 g/100ml, DMSO).

6. References

1. Sneader, W. (2005). *Drug discovery: a history*. New York: Wiley. 258; b) Benesch, M.; Urban, C. Liposomal cytarabine for leukemic and lymphomatous meningitis: recent developments. *Expert. Opin. Pharmaco.* **2008**, *9*, 301-309. c) Pigneux, A.; Perreau, V.; Jourdan, E.; Vey, N.; Dastugue, N.; Huguet, F.; Sotto, J. J.; Salmi, L. R.; Ifrah, N.; Reiffers J. Adding lomustine to idarubicin and cytarabine for induction chemotherapy in older patients with acute myeloid leukemia: the BGMT 95 trial results. *Haematol. - Haematol. J.* **2007**; *92*, 1327-1334.
2. a) Honma, Y.; Niitsu, N. Vidarabine and 2-Deoxycoformycin as Antileukemic Agents Against Monocytic Leukemia. *Leukemia Lymphoma.* **2000**, *39*, 57-66; b) Jackson, W. B.; Breslin, C. W.; Lorenzetti, D. W.; Michaud, R.; Dubé, I. Treatment of herpes simplex keratitis: comparison of acyclovir and vidarabine. *Can. J. Ophthalmol.* **1984,** *19*, 107-111; c) Miwa, N.; Kurosaki, K.; Yoshida, Y.; Kurokawa, M.; Saito, S.; Shiraki, K. Comparative efficacy of acyclovir and vidarabine on the replication of varicella-zoster virus. *Antiviral Res.* **2005**, *65*, 49-55.
3. Longley, D. B.; Harkin, D. P.; Johnston, P. G. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nat. Rev. Cancer* **2003**, *3*, 330-338.
4. a) Malet-Martino, M.; Martino, R. Clinical Studies of Three Oral Prodrugs of 5-Fluorouracil (Capecitabine, UFT, S-1): A Review. *Oncologist* **2002**, *7*, 288-323; b) Lamont, E. B.; Schilsky, R. L. The Oral Fluoropyrimidines in Cancer Chemotherapy. *Clin. Cancer Res.* **1999**, *5*, 2289-2296; c) Li, F.; Maag, H.; Alfredson, T. Prodrugs of Nucleoside Analogues for Improved Oral Absorption and Tissue Targeting. *J. Pharm. Sci.* **2008**, *97*, 1109-1134.
5. a) Estlin, E. J. Continuing therapy for childhood acute lymphoblastic leukaemia: clinical and cellular pharmacology of methotrexate, 6-mercaptopurine and 6-thioguanine. *Cancer Treat. Rev.* **2001**, *27*, 351–363; b) Schwab, M,; Herrlinger, K.; Schaeffeler, E.; Stange, E. F. Clinical pharmacology of azathioprine, 6-mercaptopurine and 6-thioguanine in the

treatment of inflammatory bowel diseases. *Dtsch. med. Wochenschr.* **2003**, *128*, 378-385; c) Sahasranaman, S.; Howard, D.; Roy, S. Clinical pharmacology and pharmacogenetics of thiopurines. *Eur. J. Clin. Pharmacol.* **2008**, *64,* 753–767.

6. a) Taylor, A. L.; Watson, C. J. E.; Bradley, J. A. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. *Crit. Rev. Oncol. Hematol.* **2005**, *56*, 23-46; b) Pearson, D. C.; May, G. R.; Fick, G. H.; Sutherland, L. R. Azathioprine and 6-Mercaptopurine in Crohn Disease. A Meta-Analysis. *Ann. Intern. Med*. **1995**, *123*, 132-142.
7. Seth, A. K.; Misra, A.; Umrigar, D. Topical liposomal gel of idoxuridine for the treatment of herpes simplex: pharmaceutical and clinical implications. *Pharm. Dev. Technol.* **2004**, *9*. 277–289.
8. a) Carmine, A. A.; Brogden, R. N.; Heel, R. C.; Speight, T. M.; Avery, G. S. Trifluridine: A Review of its Antiviral Activity and Therapeutic Use in the Topical Treatment of Viral Eye Infections. *Drugs* **1982**, *23*, 329-353; b) De Clercq, E. Antiviral drugs: current state of the art. *J. Clin. Virol.* **2001**, *22*, 73-89.
9. Hamuy, R.; Berman, B. Topical antiviral agents for herpes simplex virus infections". *Drugs Today* **1998**, *34*, 1013–1025.
10. Rabasseda, X. Brivudine: A herpes virostatic with rapid antiviral activity and once-daily dosing. *Drugs Today* **2003**, *39*, 359-371.
11. a) Horwitz, J. P.; Chua, J.; Noel M. Nucleosides. V. The Monomesylates of 1-(2′-Deoxy-β-D-lyxofuranosyl)thymine. *J. Org. Chem.* **1964**, *29*, 2076-2078; b) De Clercq E. New Perspectives for the Treatment of HIV Infections. *Collect. Czech. Chem. Commun.* **1998**, *63*, 449-479; c) Langtry, H. D.; Campoli-Richards, D. M. Zidovudine - a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. *Drugs* **1989**, *37,* 408-450.
12. Wright, K. AIDS therapy: First tentative signs of therapeutic promise. *Nature* **1986**, *323*, 283.
13. Broder, S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic *Antiviral Res.* **2009**, *85,* 1–18.
14. a) Horwitz, J. P.; Chua, J.; Da Rooge, M. A.; Noel, M.; Klundt, I. L. Nucleosides. IX. The formation of 2',3'-Unasaturated Pyrimidine Nucleosides

via a Novel β-Elimination Reaction. *J. Org. Chem.* **1966**, *31*, 205-211; b) Lea, A. P.; Faulds, D. Stavudine. *Drugs* **1996**, *51*, 846-864.

15. Adkins, J. C.; Peters, D. H.; Faulds, D. Zalcitabine - An update of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in the management of HIV infection. *Drugs* **1997**, *53*, 1054-1080.
16. Perry, C. M.; Noble, S. Didanosine - An updated review of its use in HIV infection. *Drugs* **1999**, *58*, 1099-1135.
17. a) Doong, S. L.; Tsai, C. H.; Schinazi, R. F.; Liotta, D. C; Cheng, Y. -C. Inhibition of the replication of hepatitis B virus in vitro by 2'-deoxy-3'-thiacytidine and related analogs. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 8495-8499; b) Coates, J. A.; Cammack, N.; Jenkinson, H. J.; Jowett, A. J.; Jowett, M. I.; Pearson, B. A.; Penn, C. R.; Rouse, P. L.; Viner, K. C.; Cameron, J. M. (-)-2'-Deoxy-3'-Thiacytidine Is a Potent, Highly Selective Inhibitor of Human Immunodeficiency Virus Type 1 and Type 2 Replication In Vitro *Antimicrob. Agents Chemother.* **1992***, 36*, 733-739.
18. a) De Clercq, E. Emtricitabine. *Drugs* **2003**, *63*, 2425-2426; b) Ortu, F.; Weimer, L. E.; Floridia, M.; Marconi, P. E. Raltegravir, Tenofovir and Emtricitabine in an HIV-infected patient with HCV chronic hepatitis, NNRTI intolerance and protease inhibitors-induced severe liver toxicity. *Eur. J. Med. Res.* **2010**, *15*, 81-83.
19. a) Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. Broad-Spectrum Antiviral Activity of Virazole: 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science* **1972**, *177*, 705-706; b) Witkowski, J. T.; Robins, R. K.; Sidwell, R. W.; Simon, L. N. Design, Synthesis, and Broad Spectrum Antiviral Activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide and Related Nucleosides. *J. Med. Chem.* **1972**, *15*, 1150-1154; c) De Clercq, E. The clinical potential of the acyclic (and cyclic) nucleoside phosphonates. The magic of the phosphonate bond. *Biochem. Pharmacol.* **2011**, *82*, 99-109.
20. Plosker, G. L.; Keating, G. M. Peginterferon-alpha-2a (40kD) plus ribavirin - A review of its use in hepatitis C virus and HIV co-infection. *Drugs* **2004**, *64*, 2823-2843.
21. a) Kwo, P. Y.; Vinayek, R. The Next Step for Taribavirin. *Hepatology* **2010**, *52*, 1185-1188; b) Poordad, F. F.; Chee, G. Taribavirin: a potential alternative

to ribavirin. *Future Virol.* **2009**, *4*, 113-120; c) Gish, R. G.; Arora, S.; Reddy, K. R.; Nelson, D. R.; O'Brien, C.; Xu, Y.; Murphy, B. Virological response and safety outcomes in therapy-naïve patients treated for chronic hepatitis C with taribavirin or ribavirin in combination with pegylated interferon alfa-2a: A randomized, phase 2 study. *J. Hepatol.* **2007**, *47*, 51-59.

22. Koszytkowska-Stawinska, M.; Mironiuk-Puchalska, E.; Sas, W. Synthesis of 1-pyrroline 1-oxides analogous to pseudouridine. *Tetrahedron Lett.* **2011**, *52*, 1866-1870.
23. Parry, R. J.; Jiang, Y. The Biosynthesis of Aristeromycin. Conversion of Neplanocin A to Aristeromycin by a Novel Enzymatic Reduction. *Tetrahedron Lett.* **1994**, *35*, 9665-9668.
24. a) Wagstaff, A. J.; Faulds, D.; Goa, K. L. Aciclovir: A reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* **1994**, *47*, 153-205; b) O'Brien, J. J.; Campoli-Richards, D. M. Aciclovir. An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* **1989**, *37,* 233–309.
25. a) Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthew, T. R.; Verheyden, J. P. H. 9-[(1,3-Dihydroxy-2-Propoxy)Methyl]-Guanine: A New Potent and Selective Antiherpes Agent. *J. Med. Chem.* **1988**, *26*, 769-761; b) Field, A. K.; Davies, M. E.; DeWitt, C.; Perry, H. C.; Liou, R.; Germemhausen, J.; Karkas, J. D.; Ashton, W. T.; Johnston, D. B. R.; Tolman, R. L. 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine: A Selective Inhibitor of Herpes Group Virus Replication**.** *Proc. Natl. Acad. Sci. USA.* **1988**, *80*, 4139-4143; c) McGavin, J. K.; Goa, K. L. Ganciclovir - An update of its use in the prevention of cytomegalovirus infection and disease in transplant recipients. *Drugs* **2001**, *61*, 1153-1183.
26. Weinberg, A.; Bate, B. J.; Masters, H. B.; Schneider, S. A.; Clark J. C.; Wren, C. G.; Allaman, J. A.; Levin, M. J. In Vitro Activities of Penciclovir and Acyclovir against Herpes Simplex Virus Types 1 and 2. *Antimicrob. Agents Chemother.* **1992**, *36*, 2037-2038; b) Bacon, T. H.; Boyd, M. R. Activity of Pencyclovir against Epstein-Barr Virus. *Antimicrob. Agents Chemother.* **1995**, *39*, 1599-1602.
27. Simpson, D.; Lyseng-Williamson, K. A. Famciclovir - A review of its use in herpes zoster and genital and orolabial herpes. *Drugs* **2006**, *66*, 2397-2416.

28. a) De Clercq, E.; Descamps, J.; De Somer, P.; Holý, A. (*S*)-9-(2,3-Dihydroxy propyladenine): an aliphatic nucleoside analog with broad spectrum antiviral activity. *Science* **1978**, *200*, 563-565; (b) De Clercq, E.; Holý, A. Antiviral activity of aliphatic nucleoside analogues: structure-function relationship. *J. Med. Chem.* **1979**, *22*, 510-513.

29. De Clercq, E. Antivirals and antiviral strategies. *Nat. Rev. Microbiol.* **2004**, *2*, 704-720.

30. De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P.C. A novel selective broad spectrum anti-DNA virus agent. *Nature* **1986**, *323*, 464-467.

31. a) Holý, A.; Rosenberg, I. Synthesis of isomeric and enantiomeric O-phosphonylmethyl derivatives of 9-(2,3-dihydroxypropyl)adenine. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2775-2791; b) Rosenberg, I.; Holý, A. Synthesis of potential prodrugs and metabolites of 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2792-2800; c) Krečmerová, M.; Masojídková, M.; Holý, A. Synthesis of N^9- and N^7-[2-Hydroxy-3-(phosphonomethoxy)propyl] Derivatives of N^6-Substituted Adenines, 2,6-Diaminopurines and Related Compounds. *Collect. Czech. Chem. Commun.* **2004**, *69*, 1889-1913; d) Janeba, Z.; Masojídková, M.; Holý, A. Alternative synthesis of 9-{3-[(diisopropoxyphosphoryl)methoxy]-2-hydroxy- propyl}adenine and its free phosphonates substituted at the C-8 position of purine base. *Collect. Czech. Chem. Commun.* **2010**, *75*, 371-381.

32. a) Balzarini, J.; Holý, A.; Jindřich, J.; Dvořáková, H.; Hao, Z.; Snoeck, R.; Herdewijn, P.; Johns, D. G.; De Clercq, E. 9-[(2*RS*)-3-fluoro-2-phosphonyl-methoxypropyl] derivatives of purines: a class of highly selective antiretroviral agents *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4961-4965; b) Jindřich, J.; Holý, A.; Dvořáková, H. Synthesis of *N*-(3-fluoro-2-phosphonomethoxypropyl) (FPMP) derivatives of heterocyclic bases. *Collect. Czech. Chem. Commun.* **1993**, *58*, 1645-1667.

33. Hartmann, K.; Kuffer, M.; Balzarini, J.; Naesens, L.; Goldberg, M.; Erfle, V.; Goebel, F. D.; De Clercq, E.; Jindřich, J.; Holý, A.; Bischofberger, N.; Kraft, W. Efficacy of the acyclic nucleoside phosphonates (*S*)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine (FPMPA) and 9-(2-phosphonylmethoxy-

ethyl)adenine (PMEA) against feline immunodeficiency virus. *J. Acquir. Immune Defic. Synd. Hum. Retrovirol.* **1998**, *17*, 120-128.

34. a) De Clercq, E. The discovery of antiviral agents: Ten different compounds, ten different stories. *Med. Res. Rev.* **2008**, *28*, 929-953; b) De Clercq, E. Antiviral drug discovery and development: Where chemistry meets with biomedicine. *Antiviral Res.* **2005**, *67*, 56-75; c) De Clercq, E. The acyclic nucleoside phosphonates from inception to clinical use: Historical perspective. *Antiviral Res.* **2007**, *75*, 1-13.
35. Balzarini, J.; Holý, A.; Jindřich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. Differential antiherpesvirus and antiretrovirus effects of the (*S*) and (*R*) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo of (*R*)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine. *Antimicrob. Agents Chemother.* **1993**, *37*, 332-338.
36. De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holý, A. Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. *Antiviral Res.* **1987**, *8*, 261-272.
37. a) Holý, A.; Votruba, I.; Masojídková, M.; Andrei, G. Snoeck, R.; Naesens, L.; De Clercq, E.; Balzarini, J. 6-[2-(Phosphonomethoxy)alkoxy]pyrimidines with Antiviral Activity. *J. Med. Chem.* **2002**, *45*, 1918-1929; b) Balzarini, J.; Pannecouque, C.; De Clercq, E.; Aquaro, S.; Perno, C.-F.; Egberink, H.; Holý, A. Antiretrovirus Activity of a Novel Class of Acyclic Pyrimidine Nucleoside Phosphonates. *Antimicrob. Agents Chemother.* **2002**, *46*, 2185-2193; c) Hocková, D.; Holý, A.; Masojídková, M.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. 5-Substituted-2,4-diamino-6-[2-(phosphonomethoxy)ethoxy] pyrimidines - Acyclic Nucleoside Phosphonate Analogues with Antiviral Activity. *J. Med. Chem.* **2003**, *46*, 5064-5073; d) Ying, C.; Holý, A.; Hocková, D.; Havlas, Z.; De Clercq, E.; Neyts, J. Novel Acyclic Nucleoside Phosphonate Analogues with Potent Anti-Hepatitis B Virus Activities. *Antimicrob. Agents Chemother.* **2005**, *49*, 1177-1180.
38. Wagner, C. R.; Iyer, V. V.; MCIntee, E. J. Pronucleotides: Toward the *in vivo* delivery of antiviral and anticancer nucleotides. *Med. Res. Rev.* **2000**, *20*, 417-451.
39. Hecker, S. J.; Erion, M. D. Prodrugs of Phosphates and Phosphonates. *J. Med. Chem.* **2008**, *51*, 2328-2345.

40. a) De Clercq, E.; Holý, A. Alkyl Esters of 3-Adenin-9-yl-2-hydroxypropanoic Acid: A New Class of Broad Spectrum Antiviral Agents. *J. Med. Chem.* **1985**, *28*, 282-287; b) Holý, A.; Votruba, I.; De Clerq, E. Structure-activity studies on open-chain analogues of nucleosides: Inhibition of *S*-adenosyl-L-homocysteine hydrolase and antiviral activity 2. Acid open-chain analogues. *Collect. Czech. Chem. Commun.* **1985**, *50*, 262-279.

41. De Clercq, E. John Montgomery's Legacy: Carbocyclic Adenosine Analogues as Sah Hydrolase Inhibitors with Broad-Spectrum Antiviral Activity. *Nucleosides, Nucleotides Nucleic Acids* **2005**, *24*, 1395-1415.

42. a) Votruba, I.; Holý, A. Eritadenines – Novel type of potent inhibitors of S-adenosyl-L-homocysteine hydrolase. *Collect. Czech. Chem. Commun.* **1982**, *47*, 167-172; b) Votruba, I.; Hasobe, M.; Holý, A.; Borchardt, R. T. 2-methylpropyl ester of 3-(adenine-9-yl)-2-hydroxypropanoic acid: Mechanism of antiviral action in vaccinia virus-infected L929 cells. *Biochem. Pharmacol*. **1990**, *39*, 1573-1580.

43. Holý, A. Synthesis of new mono- and disubstituted hydroxyalkyl and aminoalkyl derivatives of heterocyclic bases. *Collect. Czech. Chem. Commun.* **1978**, *43*, 3444-3465.

44. Holý, A. Preparation and synthetic utilization of 3-adenin-9-yl-2-hydroxyalkanoic acids and their derivatives. *Collect. Czech. Chem. Commun.* **1984**, *49*, 2148-2166.

45. Holý, A.; Votruba, I.; De Clercq, E. Synthesis and antiviral activity of stereoisomeric eritadenines. *Collect. Czech. Chem. Commun.* **1982**, *47*, 1392-1407.

46. Chibata, I.; Okumura, K.; Takeyama, S.; Kodera, K. Lentinacin: a new hypocholesterolemic substance in *Lentinus edodes*. *Experientia* **1969**, *25*, 1237-1238.

47. Matsuo, M.; Hashimoto, M. *Nippon Yakurigaku Zasshi* **1971**, *67*, 11.

48. Krečmerová, M.; Buděšínský, M.; Masojídková, M.; Holý, A. Synthesis of optically active N^6-alkyl derivatives of (*R*)-3-(adenine-9-yl)-2-hydroxypropanoic acid and related compounds. *Collect. Czech. Chem. Commun.* **2003**, *68*, 931-950.

49. Doláková, P.; Masojídková, M.; Holý, A. Synthesis of Base Substituted 2-Hydroxy-3-(purin-9-yl)-propanoic Acids and 4-(Purin-9-yl)-3-butenoic Acids. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 2145-2160.
50. Doláková, P.; Holý, A.; Zídek, Z.; Masojídková, M.; Kmoníčková, E. Synthesis and immunobiological activity of base substituted 2-amino-3-(purin-9-yl)propanoic acid derivatives. *Bioorg. Med. Chem.* **2005**, *13*, 2349-2354.
51. Patneau, D. K.; Mayer, M. L.; Jane, D. E.; Watkins, J. C. Activation and Desensitization of AMPA/Kainate Receptors by Novel Derivatives Willardiine. *J. Neurosci.* **1992**, *12*, 595-606.
52. Dolman, N. P.; Troop, H. M.; More, J. C. A.; Alt, A.; Knauss, J. L.; Nistico, R.; Jack, S.; Morley, R. M.; Bortolotto, Z. A.; Roberts, P. J.; Bleakman, D.; Collingridge, G. L.; Jane, D. E. Synthesis and Pharmacology of Willardiine Derivatives Acting as Antagonists of Kainate Receptors. *J. Med. Chem.* **2005**, *48*, 7867-7881.
53. Nollet, A. J. H.; Hunting, C. M.; Pandit, U. K. Unconventional nucleotide analogues .1. N^9-purinyl alpha-amino acids. *Tetrahedron* **1969**, *25*, 5971-5981.
54. Moffat, J. G. The Synthesis of Orotidine-5'-phosphate. *J. A. Chem. Soc.* **1963**, *85*, 1118-1122.
55. Holý, A. Preparation of 5-ethoxycarbonyluridine, 5-carboxyuridine and their nucleotidic derivatives. *Collect. Czech. Chem. Commun.* **1972**, *37*, 1555-1576.
56. Imai, K.; Honjo, M. Synthesis of 5'-Substituted Pyrimidine Nucleosides. *Chem. Pharm. Bull.* **1965**, *13*, 7-16.
57. Prasad, R. N.; Bariana, D. S.; Fung, A.; Savic, M.; Tietje, K.; Stein, H. H.; Brondryk, H. D.; Egan, R. S. Modification of the 5' position of purine nucleosides. 2. Synthesis and some cardiovascular properties of adenosine-5'-(*N*-substituted)carboxamides. *J. Med. Chem.* **1980**, *23*, 313-319.
58. Moss, G. P.; Reese, C. B.; Schofield, K.; Shapiro, R.; Todd, L. 212. Nucleotides. Part XLVII. The catalytic oxidation of nucleosides and nucleotides: a projected stepwise degradation of polynucleotides. *J. Chem. Soc.* **1963**, *1*, 1149-1154.
59. Harper, P. J.; Hampton, A. Conversion of 2',3'-O-Isopropylidene Adenosine into Its 5',5'-Di-C-Methyl Derivative. *J. Org. Chem.* **1970**, *35*, 1688-1689.

60. Žemlička, J.; Gasser, R.; Freisler, J. V.; Horwitz, J. P. Decarboxylative Elimination of 2'-Deoxynucleoside Uronic Acid. *J. Amer. Chem. Soc.* **1972**, *94*, 3213-3218.

61. Jung, M. E.; Xu, Y. Efficient synthesis of specifically deuterated nucleosides: Preparation of 4'-deuteriothymidine. *Heterocycles* **1998**, *47*, 349-356.

62. Mackman, R. L.; Boojamra, C. G.; Prasad, V.; Zhang, L.; Lin, K.-Y.; Petrakovsky, O.; Babusis, D.; Chen, J.; Douglas, J.; Grant, D.; Hui, H. C.; Kim, C. U.; Markevitch, D. Y.; Vela, J.; Ray, A.; Cihlar, T. Synthesis, anti-HIV activity, and resistance profiles of ribose modified nucleoside phosphonates. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6785-6789.

63. Mackman, R. L.; Lin, K.-Y.; Boojamra, C. G.; Hui, H.; Douglas, J.; Grant, D.; Petrakovsky, O.; Prasad, V.; Ray, A. S.; Cihlar, T. Synthesis and anti-HIV activity of 2'-fluorine modified nucleoside phosphonates: Analogs of GS-9148. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1116-1119.

64. Henn, F. G. T.; Garnett, M. C.; Chhabra, S. R.; Bycroft, B. W.; Baldwin, R. W. Synthesis of 2'-Deoxyuridine and 5-Fluoro-2'-deoxyuridine Derivatives and Evaluation in Antibody Targeting Studies. *J. Med. Chem.* **1993**, *36*, 1570-1579.

65. a) Harmon, R. E.; Zenarosa, C. V.; Gupta, C. V. Permanganate oxidation of purine nucleosides. *Chem. Ind.* **1969**, 1141-1150; b) Hutchison, A. J.; Williams, M.; de Jesus, R.; Yokoyama, R.; Oei, H. H.; Ghai, G. R.; Webb, R. L.; Zoganas, H. C.; Stone, G. A.; Jarvis, M. F. 2-(Arylalkylamino)adenosine-5'-uronamides: A New Class of Highly Selective Adenosine A_2 Receptor Ligands. *J. Med. Chem.* **1990**, *33*, 1919-1924; c) Ha, S. B.; Nair, V. An improved approach to the synthesis of adenosine-5'-*N*-ethyluronamides of interest as adenosine receptor agonists. *Tetrahedron Lett.* **1996**, *37*, 1567-1570.

66. a) Olsson, R. A.; Kusachi, S.; Thompson, R. D.; Ukena, D.; Padgett, W.; Daly, J. W. N^6-Substituted *N*-Alkyladenosine-5'-uronamides: Bifunctional Ligands Having Recognition Groups for A1 and A2 Adenosine Receptors. *J. Med. Chem.* **1986**, *29*, 1683-1689; b) Schmidt, R. R.; Fritz, H. J. Synthese und Struktur von Inosin-5'-carbonsäure und Derivaten. *Chem. Ber.* **1970**, *103*, 1867-1871.

67. Mackman, R. L.; Ray, A. S.; Hui, H. C.; Zhang, L. Birkus, G.; Boojamra, C. G.; Desai, M. C.; Douglas, J. L.; Gao, Y.; Grant, D.; Laflamme, G.; Lin, K.-Y.; Markevitch, D. Y.; Mishra, R.; McDermott, M.; Pakdaman, R.; Petrakovsky,

O. V.; Vela, J. E.; Cihlar, T. Discovery of GS-9131: Design, synthesis and optimization of amidate prodrugs of the novel nucleoside phosphonate HIV reverse transcriptase (RT) inhibitor GS-9148. *Bioorg. Med. Chem.* **2010**, *18*, 3606-3617.

68. Djerassi, C.; Engle, R. R. Oxidations with Ruthenium Tetroxide. *J. Am. Chem. Soc.* **1953**, *75*, 3838-3840.
69. Nunez, M. T.; Martin, V. S. Efficient oxidation of phenyl groups to carboxylic acids with ruthenium tetraoxide. A simple synthesis of (*R*)-γ-caprolactone, the pheromone of Trogoderma granarium. *J. Org. Chem* **1990**, *55*, 1928-1932.
70. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. A greatly improved procedure for ruthenium tetroxide catalyzed oxidations of organic compounds. *J. Org. Chem.* **1981**, *46*, 3936-3938.
71. Singh, A. K.; Varma, R. S. Ruthenium tetraoxide: a mild reagent for the oxidation of 2',3'-O-isopropylidene purine nucleosides. *Tetrahedron Lett.* **1992**, *33*, 2307-2310.
72. Varma, R. S.; Hogan, M. E. Ruthenium tetraoxide catalyzed oxidation of nucleosides: A facile synthesis of 5'-carboxylic acid derivatives. *Tetrahedron Lett.* **1992**, *33*, 7719-7720.
73. Shakya, N.; Vedi, S.; Liang, C.; Agrawal, B.; Tyrrel, D. L.; Kumar, R. A new class of pyrimidine nucleosides: inhibitors of hepatitis B and C viruses. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6475-6480.
74. Debnath, J.; Dasgupta, S.; Pathak, T. Comparative inhibitory activity of 3'- and 5'-functionalized nucleosides on ribonuclease A. *Bioorg. Med. Chem.* **2010**, *18*, 8257-8263.
75. a) Prasad, R. N.; Fung, A.; Tietje, K.; Stein, H. H.; Brondyk, H. D. Modification of the 5' position of purine nucleosides. 1. Synthesis and biological properties of alkyl adenosine-5'-carboxylates *J. Med. Chem.* **1976**, *19*, 1180-1186; b) Henderson, J. F.; Battell, M. L. Metabolic effects of ethyl adenosine 5'-carboxylate in Ehrlich ascites tumor cells *in vitro*. *Biochem. Pharmacol*. **1976**, *25*, 1915-1916.
76. Kraupp, O. German Patent **1977**, No. 2610985; *Chem. Abstr.* **1978**, *88*, 7312d.
77. Homma, H.; Watanabe, Y.; Abiru, T.; Murayama, T.; Nomura, Y.; Matsuda, A. 2-(1-Hexyn-1-yl)adenosine-5'-uronamides: A New Entry of Selective A_2

Adenosine Receptor Agonists with Potent Antihypertensive Activity. *J. Med. Chem.* **1992**, *35*, 2881-2890.

78. a) de Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. On the use of stable organic nitroxyl radicals for the oxidation of primary and secondary alcohols. *Synthesis* **1996**, *10*, 1153-1174; b) Bobbit, J. M.; Flores, M. C. L. Organic nitrosonium salts as oxidants in organic chemistry. *Heterocycles* **1988**, *27*, 509-533.
79. De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. A versatile and highly selective hypervalent iodine (III)/2,2,6,6-tetramethyl-1-piperidinyloxyl-mediated oxidation of alcohols to carbonyl compounds. *J. Org. Chem.* **1997**, *62*, 6974-6977.
80. Epp, J. B.; Widlanski, T. S. Facile Preparation of Nucleoside-5'-carboxylic Acids. *J. Org. Chem.* **1999**, *64*, 293-295.
81. Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. Fast and selective oxidation of primary alcohols to aldehydes or to carboxylic acids and of secondary alcohols to ketones mediated by oxoammonium salts under two-phase conditions. *J. Org. Chem.* **1987**, *52*, 2559-2562.
82. Boojamra, C. G.; Mackman, R. L.; Markevitch, D. Y.; Prasad, V.; Ray, A. S., Douglas, J.; Grant, D.; Kim, C. U.; Cihlar, T. Synthesis and anti-HIV activity of GS-9148 (2'-Fd4AP), a novel nucleoside phosphonate HIV reverse transcriptase inhibitor. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1120-1123.
83. Mackman, R. L.; Zhang, L. Prasad, V.; Boojamra, C. G.; Douglas, J.; Grant, D.; Hui, H.; Kim, C. U.; Laflamme, G.; Parrish, J.; Stoycheva, A. D.; Swaminathan, S.; Wang, K.-Y.; Cihlar, T. Synthesis, anti-HIV activity, and resistance profile of thymidine phosphonomethoxy nucleosides and their bis-isopropyloxymethylcarbonyl (bisPOC) prodrugs. *Bioorg. Med. Chem.* **2007**, *15*, 5519-5528.
84. Montevecchi, P. C.; Manetto, A.; Navacchia, M. L.; Chatgilialoglu, C. Thermal decomposition of the *tert*-butyl perester of thymidine-5'-carboxylic acid. Formation and fate of the pseudo-C4' radical. *Tetrahedron* **2004**, *60*, 4303-4308.
85. Meurillon, M.; Chaloin, L.; Périgaud, C.; Peyrottes, S. Synthesis of Pyrimidine-Containing Nucleoside β-(*R/S*)-Hydroxyphosphonate Analogues. *Eur. J. Org. Chem.* **2011**, *20-21*, 3794-3802.

86. Rozantsev, E. G.; Sholle, V. D. Synthesis and reactions of stable nitroxyl radicals. 2. reactions. *Synthesis* **1971**, *8*, 401.

87. a) Golubev, V. A.; Rozantsev, E. G.; Neiman, M. B. Izv. Akad. Nauk SSSR, *Ser. Krim.* **1965**, 1927; *Chem. Abstr.* **1966**, *64*, 11164e; b) Ganem, B. Biological spin labels as organic reagents - oxidation of alcohols to carbonyl compounds using nitroxyls. *J. Org. Chem.* **1975**, *40*, 1998; c) Miyazawa, T.; Endo, T.; Siihashi, S.; Okawara, M. Selective oxidation of alcohols by oxoaminium salts. *J. Org. Chem.* **1985**, *50*, 1332; d) Miyazawa, T.; Endo, T. Oxidation of diols with oxoaminium salts. *J. Org. Chem.* **1985**, *50*, 3930.

88. Anelli, P. L.; Banfi, S.; Montanari, F.; Quici, S. Oxidation of diols with alkali hypochlorites catalyzed by oxammonium salts under two-phase conditions. *J. Org. Chem.* **1989**, *54*, 2970-2972.

89. Zhao, M.; Li, J.; Mano, E.; Song, Z.; Tschaen, D. M.; Grabowski, E. J. J.; Reider, P. J. Oxidation of Primary Alcohols to Carboxylic Acids with Sodium Chlorite Catalyzed by TEMPO and Bleach. *J. Org. Chem.* **1999**, *64*, 2564-2566.

90. a) Mancuso, A. J.; Huang, S.-L.; Swern, D. Oxidation of long-chain and related alcohols to carbonyls by dimethyl sulfoxide “activated” by oxalyl chloride. *J. Org. Chem.* **1978**, *43*, 2480-2482; b) Mancuso, A. J.; Brownfain, D. S.; Swern, D. Structure of the dimethyl sulfoxide-oxalyl chloride reaction product. Oxidation of heteroaromatic and diverse alcohols to carbonyl compounds. *J. Org. Chem.* **1979**, *44*, 4148-4150; c) Ireland, R. E.; Norbeck, D. W. Application of the Swern oxidation to the manipulation of highly reactive carbonyl compounds. *J. Org. Chem.* **1985**, *50*, 2198-2200.

91. a) Lindgren, B. O.; Nilsson, T. Preparation of Carboxylic Acids from Aldehydes (Including Hydroxylated Benzaldehydes) by Oxidation with Chlorite. *Acta Chem. Scand.* **1973**, *27*, 888-890; b) Dalcanale, E.; Montanari, F. Selective oxidation of aldehydes to carboxylic acids with sodium chlorite-hydrogen peroxide. *J. Org. Chem.* **1986**, *51*, 567-569.

92. Zhao, M. M.; Li, J.; Mano, E.; Song, Z. J.; Tschaen, D. M. Oxidation of primary alcohols to carboxylic acids with sodium chlorite catalyzed by TEMPO and bleach: 4-methoxyphenylacetic acid. *Org. Synth.* **2005**, *81*, 195-203.

93. Tuske, S.; Sarafianos, S. G.; Clark, A. D., Jr.; Ding, J.; Naeger, L. K.; White, L. K.; Miller, M. D.; Gibbs, C. S.; Boyer, P. L.; Clark, P.; Wang, G.; Gaffney, B. L.; Jones, R. A.; Jerina, D. M.; Hughes, S. H.; Arnold, E. Structures of HIV-1 RT-DNA complexes before and after incorporation of the anti-AIDS drug tenofovir. *Nat. Struct. Mol. Biol.* **2004**, *11*, 469.

94. a) Webb, R. R.; Martin, J. C. A convenient synthesis of (*S*)-HPMPA. *Tetrahedron Lett.* **1987**, *28*, 4963; b) Holý, A. Syntheses of Enantiomeric *N*-(3-Hydroxy-2-phosphonomethoxypropyl) Derivatives of Purine and Pyrimidine Bases. *Collect. Czech. Chem. Commun.* **1993**, *58*, 649.

95. Modern Oxidation Methods; Bäckval, J.-E., Ed.; WILEY-VCH: Weinheim, 2004.

96. Mori, N.; Togo, H. Facile oxidative conversion of alcohols to esters using molecular iodine. *Tetrahedron* **2005**, *61*, 5915-5925.

97. Holý, A.; Rosenberg, I. Preparation of 5'-O-phosphonylmethyl analogs of nucleoside-5'-phosphates, 5'-diphosphates and 5'-triphosphates. *Collect. Czech. Chem. Commun.* **1982**, *47*, 3447-3463.

98. a) Jansa, P.; Holý, A.; Dračínský, M.; Baszczyňski, O.; Česnek, M.; Janeba, Z. *Collect. Symp. Ser.* **2011**, *12*, 347; b) Jansa, P.; Holý, A.; Dračínský, M.; Baszczyňski, O.; Česnek, M.; Janeba, Z. Efficient and 'green' microwave-assisted synthesis of haloalkylphosphonates via the Michaelis-Arbuzov reaction. *Green. Chem.* **2011**, *13*, 882-888.

99. a) Cundy, K. C. Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir. *Clin. Pharmacokinet.* **1999**, *36*, 127-143; b) Cundy, K. C.; Bidgood, A. M.; Lynch, G.; Shaw, J. P.; Griffin, L.; Lee, W. A. Pharmacokinetics, bioavailability, metabolism, and tissue distribution of cidofovir (HPMPC) and cyclic HPMPC in rats. *Drug Metab. Dispos.* **1996**, *24*, 745-752.

100. a) Shaw, J. P.; Sueoka, C. M.; Oliyai, R.; Lee, W. A.; Arimilli, M. N.; Kim, C. U.; Cundy, K. C. Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(*R*)-2-(phosphonomethoxy)propyl]adenine (PMPA) in dogs. *Pharm. Res.* **1997**, *14*, 1824-1829; b) Arimilli, M. N.; Kim, C. U.; Dougherty, J.; Mulato, A.; Oliyai, R.; Shaw, J. P.; Cundy, K. C.; Bischofberger, N. Synthesis, in vitro biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)

propyl]adenine (PMPA) prodrugs. *Antiviral Chem. Chemother.* **1997**, *8*, 557-564.

101. Hostetler, K. Y. Alkoxyalkyl prodrugs of acyclic nucleoside phosphonates enhance oral antiviral activity and reduce toxicity: Current state of the art. *Antiviral Res.* **2009**, *82*, A84-A98.
102. Lanier, E. R.; Ptak, R. G.; Lampert, B. M.; Keilholz, L.; Hartman, T.; Buckheit, R. W.; Mankowski, M. K.; Osterling, M. C.; Almond, M. R.; Painter, G. R. Development of hexadecyloxypropyl tenofovir (CMX 157) for treatment of infection caused by wild-type and nucleoside/nucleotide-resistant HIV. *Antimicrob. Agents Chemother.* **2010**, *54*, 2901-2909.
103. Painter, G. R.; Almond, M. R.; Trost, L. C.; Lampert, B. M.; Neyts, J.; De Clercq, E.; Korba, B. E.; Aldern, K. A.; Beadle, J. R.; Hostetler, K. Y. Evaluation of hexadecyloxypropyl-9-R-[2-(phosphonomethoxy)propyl]adenine CMX 157, as a potential treatment for human immunodeficiency virus type 1 and hepatitis B virus infections. *Antimicrob. Agents Chemother.* **2007**, *51*, 3505-3509.
104. a) Reiser, H.; Wang, J. Y.; Chong, L.; Watkins, W. J.; Ray, A. S.; Shibata, R.; Birkus, G.; Cihlar, T.; Wu, S.; Li, B.; Liu, X. H.; Henne, I. N.; Wolfgang, G. H. I.; Desai, M.; Rhodes, G. R.; Fridland, A.; Lee, W. A.; Plunkett, W.; Vail, D.; Thamm, D. H.; Jeraj, R.; Tumas, D. B. GS-9219 - A novel acyclic nucleotide analogue with potent antineoplastic activity in dogs with spontaneous non-Hodkin's lymphoma. *Clin. Cancer Res.* **2008**, *14*, 2824-2832; b) Tsai, C. Y.; Ray, A. S.; Tumas, D. B.; Keating, M. J.; Reiser, H.; Plunkett, W. Targeting DNA repair in chronic lymphotic leukemia cells with a novel acyclic nucleotide analogue, GS-9219. *Clin. Cancer Res.* **2009**, *15*, 3760-3769.
105. Wolfgang, G. H. I.; Shibata, R.; Wang, J.; Ray, A. S.; Wu, S.; Doerrfler, E.; Reiser, H.; Lee, W. A.; Birkus, G.; Christensen, N. D.; Andrei, G.; Snoeck, R. *Antimicrob. Agents Chemother.* **2009**, *53*, 2777-2784.
106. Ballatore, C.; McGuian, C.; De Clercq, E.; Balzarini, J. Synthesis and Evaluation of Novel Amidate Prodrugs of PMEA and PMPA. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1053-1056.
107. Cihlar, T.; Ray, A. S. Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine. *Antiviral Res.* **2010**, *85*, 39-58.

108. Lee, W. A.; He, G. X.; Eisenberg, E.; Cihlar, T.; Swaminathan, S.; Mulato, A.; Cundy, K. C. Selective Intracellular Activation of a Novel Prodrug of the Human Immunodeficiency Virus Reverse Transcriptase Inhibitor Tenofovir Leads to Preferential Distribution and Accumulation in Lymphatic Tissue. *Antimicrob. Agents Chemother.* **2005**, *49*, 1898-1906.

109. Jansa, P.; Baszczyňski, O.; Dračínský, M.; Votruba, I.; Zídek, Z.; Bahador, G.; Stepan, G.; Cihlar, T.; Mackman, R.; Holý, A.; Janeba, Z. A novel and efficient one-pot synthesis of symmetrical diamide (bis-amidates) prodrugs of acyclic nucleoside phosphonates and evaluation of their biological activites. *Eur. J. Med. Chem.* **2011**, *46*, 3748-3754.

110. Gallo-Rodriguez, C.; Ji, X.; Melman, N. Siegman, B. D.; Sanders, L. H.; Orlina, J.; Fischer, B.; Pu, Q.; Olah, M. E.; Van Galen, P. J. M.; Stiles, G. L.; Jacobson, K. A. Structure-Activity Relatioships of N^6-Benzoyladenosine as A_3-Selective Adenosine Agonists. *J. Med. Chem.* **1994**, *37*, 641.

111. Ducati, R. G.; Namanja-Magliano, H. A.; Schramm, V. L. Transition-state inhibitors of purine salvage and other prospective enzyme targets in malaria. *Future Med. Chem.* **2013**, *5*, 1341-1360.

112. Baird, J. K. Resistance to Therapies for Infection by *Plasmodium vivax*. *Clin. Microbiol. Rev.* **2009**, *22*, 508.

113. Nahlen, B. L.; Korenromp, E. L.; Miller, J. M.; Shibuya, K. Malaria risk – Estimating clinical episodes of malaria. *Nature* **2005,** *437*, E3.

114. De Jersey, J.; Holý, A.; Hocková, D.; Naesens, L.; Keough, D. T.; Guddat, L. W. 6-Oxopurine Phosphoribosyltransferase: A Target for the Development of Antimalarial Drugs. *Curr. Top. Med. Chem.* **2011,** *11*, 2085-2102.

115. Raviña, E. (2011) *The Evolution of Drug discovery: From Traditional Medicines to Modern Drugs.* Weinheim: Wiley, 124-139.

116. a) Keough, D. T.; Hockova, D.; Holy, A.; Naesens, L. M. J.; Skinner-Adams, T. S.; de Jersey, J.; Guddat, L. W. Inhibition of Hypoxanthine-Guanine Phosphoribosyltransferase by Acyclic Nucleoside Phosphonates: A New Class of Antimalarial Therapeutics. *J. Med. Chem.* **2009**, *52*, 4391-4399. b) Hockova, D.; Holy, A.; Masojidkova, M.; Keough, D. T.; de Jersey, J.; Guddat, L. W. Synthesis of branched 9-[2-(2-phosphonoethoxy)ethyl]purines as a new class of acyclic nucleoside phosphonates which inhibit *Plasmodium falciparum*

hypoxanthine-guanine-xanthine phosphoribosyltransferase. *Bioorg. Med. Chem.* **2009,** *17*, 6218-6232.

117. Keough, D. T.; Ng, A. L.; Winzor, D. J.; Emmerson, B. T.; de Jersey, J. Purification and characterization of *Plasmodium falciparum* hypoxanthine-guanine-xanthine phosphoribosyltransferase and comparison with the human enzyme. *Mol. Biochem. Parasitol.* **1999,** *98*, 29-41.
118. Keough, D. T.; Skinner-Adams, T.; Jones, M. K.; Ng, A. L.; Brereton, I. M.; Guddat, L. W.; de Jersey, J. Lead compounds for antimalarial chemotherapy: Purine base analogs discriminate between human and *P-falciparum* 6-oxopurine phosphoribosyltransferases. *J. Med. Chem.* **2006,** *49*, 7479-7486;
119. a) Cesnek, M.; Hockova, D.; Holy, A.; Dracinsky, M.; Baszczynski, O.; de Jersey, J.; Keough, D. T.; Guddat, L. W. Synthesis of 9-phosphonoalkyl and 9-phosphonoalkoxyalkyl purines: Evaluation of their ability to act as inhibitors of *Plasmodium falciparum*, *Plasmodium vivax* and human hypoxanthine-guanine-(xanthine) phosphoribosyltransferases. *Bioorg. Med. Chem.* **2012**, *20*, 1076-1089; b) Hockova, D.; Keough, D. T.; Janeba, Z.; Wang, T. H.; de Jersey, J.; Guddat, L. W. Synthesis of Novel *N*-Branched Acyclic Nucleoside Phosphonates As Potent and Selective Inhibitors of Human, *Plasmodium falciparum* and *Plasmodium vivax* 6-Oxopurine Phosphoribosyltransferases. *J. Med. Chem.* **2012**, *55*, 6209-6223; c) Keough, D. T.; Hockova, D.; Krecmerova, M.; Cesnek, M.; Holy, A.; Naesens, L.; Brereton, I. M.; Winzor, D. J.; de Jersey, J.; Guddat, L. W. *Plasmodium vivax* hypoxanthine-guanine phosphoribosyl- transferase: A target for anti-malarial chemotherapy. *Mol. Biochem. Parasitol.* **2010**, *173*, 165-169.
120. a) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, *1*, 1-28; b) Ludek, O. R.; Meier, C. Synthesis of the carbocyclic pyrimidine nucleosides, III. Influence of the N3-protection group on N1-vs. O^2-alkylation in the Mitsunobu reaction. *Eur. J. Org. Chem.* **2006**, *4*, 941-946.
121. Zhou, D.; Lagoja, I. M.; Van Aerschot, A.; Herdewijn, P. Synthesis of aminopropyl phosphonate nucleosides with purine and pyrimidine bases. *Collect. Czech. Chem. Commun.* **2006**, *71*, 15-34.

122. Cristau, H. J.; Virieux, D. Anomalous reactivity of diphenylhydroxymethyl-phosphine oxide in the synthesis of a phosphorylated ether by oxa-Michael reaction. *Tetrahedron Lett.* **1999**, *40*, 703-706.

123. Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. Facile cleavage of carbohydrate benzyl ethers and benzylidene acetals using the $NaBrO_3/Na_2S_2O_4$ reagent under two-phase conditions. *Tetrahedron Lett.* **1999**, *40*, 8439-8441.

124. Ikehara, M; Muneyama, K. Studies of Nucleosides and Nucleotides. XXXIV. Purine Cyclonucleosides. 4. Synthesis of a Cyclonucleoside Having an *O*-Cyclo Linkage Derived from Guanosine. *J. Org. Chem.* **1967**, *32*, 3039-3042.

125. a) Mackman, R. L.; Cihlar, T. Prodrug strategies in the design of nucleoside and nucleotide antiviral therapeutics. *Annu. Rep. Med. Chem.* **2004**, *39*, 305-321; b) Hecker, S. J.; Erion, M. D. Prodrugs of phosphates and phosphonates. *J. Med. Chem.* **2008**, *51*, 2328-2345.

126. Hazleton K. Z.; Ho, M. C.; Cassera, M. B.; Clinch, K.; Crump, D. R.; Rosario, I.; Merino, E. F.; Almo, S. C.; Tyler, P. C.; Schramm, V. L. Acyclic immucillin phosphonates: Second generation inhibitors of *Plasmodium falciparum* hypoxanthine-guanine-xanthine phosphoribosyltransferase. *Chem. Biol.* **2012**, *19*, 721-730.

127. Li, C. M.; Tyler, P. C.; Furneaux, R. H.; Kicska, G.; Xu, Y. M.; Grubmeyer, C.; Girvin, M. E.; Schramm, V. L. Transition-state analogs as inhibitors of human and malarial hypoxanthine-guanine phosphoribosyltransferases. *Nat. Struct. Biol.* **1999**, *6*, 582-587.

128. Keough, D. T.; Hockova, D.; Rejman, D.; Spacek, P.; Vrbkova, S.; Krecmerova, M.; Eng, W. S.; Jans, H.; West, N. P.; Naesens, L. M. J.; de Jersey, J.; Guddat, L. W. Inhibition of the *Escherichia coli* 6-oxoprine phosphoribosyltransferases by nucleoside phosphonates: potential for new antibacterial agents. *J. Med. Chem.* **2013**, *56*, 6967-6984.

129. Eads, J. C.; Scapin, G.; Xu, Y. M.; Grubmeyer, C.; Sacchettini, J. C. The crystal structure of human hypoxanthine-guanine phosphoribosyltransferase with bound GMP. *Cell* **1994**, *78*, 325-344.

130. Shoshani, I.; Laux, W. H. G.; Périgaud, C.; Gosselin, G.; Johnson, R. A. Inhibition of Adenylyl Cyclase by Acyclic Nucleoside Phosphonate Antiviral Agents. *J. Biol. Chem.* **1999**, *274*, 34742-34744.

131. Shen, Y.; Zhukovskaya, N. L.; Zimmer, M.; Soelaiman, S.; Bergson, P.; Wang, C. R.; Gibbs, C. S.; Tang, W. J. Selective inhibition of anthrax edema factor by adefovir, a drug for chronic hepatitis B virus infection. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3242-3247.

132. Weiss, B. (ed.): Antisense Oligodeoxynucleotides and Antisense RNA: Novel Pharmacological and Therapeutic Agents, CRC Press, Boca Raton, FL, **1997**.

133. Hannon, G. J.; Rossi, J. J. Unlocking the potential of the human genome with RNA interference. *Nature* **2004**, *431,* 371–378.

134. Ellington, A.D.; Szostak, J. W. In vitro selection of RNA molecules that bind specific ligands. *Nature* **1990**, *346,* 818–822.

135. Zamecnik P. C., Stephenson M. L. Inhibition of Rous-sarcoma virus-replication and cell transformation by a specific oligodeoxynucleotide. *Proc. Natl. Acad. Sci. U.S.A*. **1978**, *75*, 280-284.

136. Perry, C. M.; Balfour, J. A. B. Fomivirsen. *Drugs* **1999**, *57*, 375-380.

137. Pirollo K. F., Rait A. R., Sleer L. S., Chang E. H. Antisense therapeutics: from theory to clinical practice. *Pharmacol. Ther*. **2003**, *99*, 55-77.

138. Kurreck J. Antisense technologies - Improvement through novel chemical modifications. *Eur. J. Biochem*. **2003,** *270*, 1628-1644.

139. Ricotta, D. N.; Frishman, W. Mipomersen: A Safe and Effective Antisense Therapy Adjunct to Statins in Patients With Hypercholesterolemia. *Cardiol. Rev.* **2012**, *20*, 90-95.

140. Humeau, L. M.; Binder, G. K.; Lu, X. B.; Slepushkin, V.; Merling, R.; Echeagaray, P.; Pereira, M.; Slepushkina, T.; Barnett, S.; Dropulic, L. K.; Carroll, R.; Levine, B. L.; June, C. H.; Dropulic, B. Efficient lentiviral vector-mediated control of HIV-1 replication in CD4 lymphocytes from diverse HIV+ infected patients grouped according to CD4 count and viral load. *Mol. Ther.* **2004**, *9*, 902-913.

141. Jaschinski, F.; Rothhammer, T.; Jachimczak, P.; Seitz, C.; Schneider, A.; Schlingensiepen, K. H. The Antisense Oligonucleotide Trabedersen (AP 12009) for the Targeted Inhibition of TGF-beta 2. *Curr. Pharm. Biotech.* **2011**, *12*, 2203-2213.

142. Geisbert, T. W.; Lee, A. C. H.; Robbins, M.; Geisbert, J. B.; Honko, A. N.; Sood, V.; Johnson, J. C.; de Jong, S.; Tavakoli, I.; Judge, A.; Hensley, L. E.; MacLachlan I. Postexposure protection of non-human primates against a lethal

Ebola virus challenge with RNA interference: a proof-of-concept study. *Lancet* **2010**, *375*, 1896-1905.

143. Siddiqui, M. A. A.; Keating, G. M. Pegaptanib - In exudative age-related macular degeneration. *Drugs* **2005**, *65*, 1571-1577.
144. a) Drabovich, A. P.; Berezovski, M.; Okhonin, V.; Krylov, S. N. Selection of smart aptamers by methods of kinetic capillary electrophoresis. *Anal. Chem.* **2006**, *78,* 3171-3178; b) Cho, E. J.; Lee, J. W.; Ellington, A. D. Applications of Aptamers as Sensors. *Annu. Rev. Anal. Chem.* **2009**, *2,* 241-264.
145. Rejman D., Snášel J., Liboska R., Točík Z., Pačes O., Králíková S., Rinnová M., Koiš P., Rosenberg I. Oligonucleotides with isopolar phosphonate internucleotide linkage: A new perspective for antisense compounds? *Nucleosides Nucleotides* **2001**, *20*, 819-823.
146. Giannaris P. A., Damha M. J. Oligoribonucleotides containing 2',5'-phosphodiester linkages exhibit binding selectivity for 3',5'-RNA over 3',5'-SSDNA. *Nucleic Acids Res*. **1993**, *21*, 4742-4749.
147. Snášel J., Rejman D., Liboska R., Točík Z., Ruml T., Rosenberg I., Pichová I. Inhibition of HIV-1 integrase by modified oligonucleotides derived from U5' LTR. *Eur. J. Biochem*. **2001**, *268*, 980-986.
148. ArgusLab 4.0, M. A. Thompson, Planaria Software LLC, Seattle, http://www.ArgusLab.com.
149. Páv, O.; Košiová, I.; Barvík, I.; Pohl, R.; Buděšínský, M.; Rosenberg, I. Synthesis of oligoribonucleotides with phosphonate-modified linkages. *Org. Biomol. Chem.* **2011**, *9*, 6120-6126.
150. Hocek, M.; Masojídková, M.; Holý, A.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. Synthesis and Antiviral Activity of Acyclic Nucleotide Analogues Derived from 6-(Aminoethyl)purines and Purine-6-carboxamidines. *Collect. Czech. Chem. Commun.* **1996**, *61*, 1525-1537.